

**धान में प्रजनन अवस्था पर ताप तनाव सहनशीलता हेतु
क्यू.टी.एल मानचित्रण**

**Mapping quantitative trait loci governing heat stress
tolerance at reproductive stage in rice**

**रविकिरण के टी
Ravikiran K T**



**आनुवंशिकी संभाग
भा.कृ.अ.प. - भारतीय कृषि अनुसंधान संस्थान
नई दिल्ली ११००१२**

**DIVISION OF GENETICS
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI 110 012
2022**

Mapping quantitative trait loci governing heat stress tolerance at reproductive stage in rice

By

Ravikiran K T

A thesis submitted to the Faculty of Post Graduate School,
Indian Agricultural Research Institute, New Delhi,
in partial fulfillment of the requirements
for the award of degree of

Doctor of Philosophy

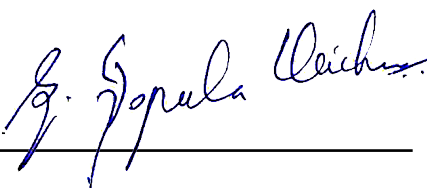
in

GENETICS AND PLANT BREEDING

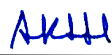
2022

Approved by the Advisory Committee:

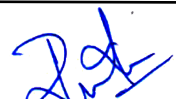
Chairperson: Dr. Gopala Krishnan S



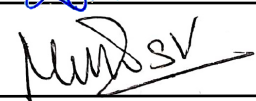
Co-chairman: Dr. Ashok Kumar Singh



Member: Dr. Ranjit Kumar Ellur



Member: Dr. S.V. Amitha C.R. Mithra



Member: Dr. Madan Pal





आनुवंशिकी संभाग
भा.कृ.अ.प. - भारतीय कृषि अनुसंधान संस्थान
नई दिल्ली ११००१२, भारत
Division of Genetics
ICAR-Indian Agricultural Research Institute
New Delhi 110 012, India



Dr. Gopala Krishnan S
Principal Scientist

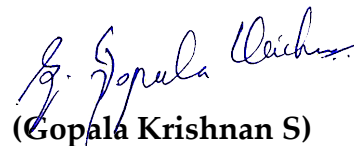
CERTIFICATE

This is to certify that the thesis entitled **Mapping quantitative trait loci governing heat stress tolerance at reproductive stage in rice** submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of requirement of degree of **Doctor of Philosophy in Genetics and Plant Breeding**, embodies the result of a *bona-fide* research work carried out by **Mr. Ravikiran KT** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed of in this work, has been duly acknowledged.

Date: 30-12-2022

Place: New Delhi


(Gopala Krishnan S)

Chairman
Advisory Committee

Acknowledgement

It took an army of people and a God to put me at this pedestal and ensure that I don't ruin this. I would like to take this opportunity to acknowledge all, who supported me directly or indirectly, bringing this dissertation to a really good shape.

*Fervently and modestly, I extol the genuine and constant encouragement, immaculate guidance and every suggestive help offered to me by the Chairman of my Advisory Committee **Dr. Gopala Krishnan, S**, Principal Scientist, Division of Genetics, IARI. I should be highly obliged for his wise counsel, concrete suggestions, his inspiring, meticulous, and affectionate guidance, constant help, and persistent encouragement during my study and research work. I take it as a great privilege and pride in having an opportunity of working under his unending, motivating, and indomitable leadership. Despite his significant achievements in research and cornucopia of knowledge, his humble demeanour always reminds me of Ratan Tata's words in one of his speeches "Let humility be your best defence" which I took home from IARI.*

*I deem it my honour in expressing my deepest sense of reverence, gratitude and indebtedness to Co-chairman of my Advisory Committee **Dr. A.K. Singh**, Director, IARI, and the esteemed Basmati Rice breeder of this country for his sincere technical guidance, learned counsel, unstinted attention during the formulation of the research work and during its progress. His keen interest, patient hearing, invincible quest for science and constructive criticism is unparalleled.*

*I also express my sincere gratitude to **Dr. Ranjit Kumar** who had been more a learned friend and senior than a member of advisory committee for his nuanced guidance in practical aspects of my research work and data analysis. I owe my flow of thanks to other members of my advisory committee **Dr. Madan Pal** and **Dr. Amita Mitra** for their worthy suggestions and providing facilities for conducting certain part of my research work. Special thanks are due to **Dr. K.K. Vinod**, Rice Genetics for assistance in research work, data analysis, reporting and publication. I am also thankful to **Dr. P.K. Bhowmick** and **Dr. Haritha** for their assistance in carrying out various research activities in Rice Genetics. Thanks are also due for **Dr. M. Nagarajan**, RBGRC, Aduthurai, for providing facility and generous manpower for offseason heat stress evaluation of crop and kind hospitality. I also want to thank **Dr. Padmini Swain**, **Dr. Meera Kumari Kar**, **Dr. Prasant Hanjagi** and **Gautam Dash** of ICAR-NRRI, Cuttack for supporting off-season evaluation of RILs for heat stress.*

*I place on record my profound thanks to **Professor (Dr Vinod & Dr Anju M Singh)**, **Division of Genetics**, and his/her staff **Mr. Ishwar** and **Lalji** for their timely help in successful completion of my course. It's an immense pleasure to express great, heartfelt respect to my beloved teachers of courses I have registered from Division of Genetics **Dr. Akshay**, **Dr. Lal**, **Dr. Firoz**, **Dr. Ganapati**, **Dr. Neelu Jain**, **Dr. Aski**, **Dr. Shilender** and **Dr. Hari Krishna**.*

*I feel to express my heartfelt, sincere thanks to lab mates **Gaurav**, **Pankaj**, **Vijay**, **Vikram**, **Gagan**, **Ankit**, **Neha**, **Neeraj**, **Anurag**, **Sarves**, **Ashutosh** and **Nitasha** and technical staff **Devender**, **Sunil**, **Yogendar**, **Mahendran** and **Bivek** for their immense help, and constant*

support during my work at Rice Genetics, which will be remembered by me everlastingly.

I would heartily acknowledge the constant encouragement, assistance and constructive suggestions received from seniors, **Dr. Rajkumar Zhunjare, Dr. Kyaw Swar Oo, Dr. Bhuvaneshwari, Dr. Hari Krishna and Dr. Shilender Jha**. I fondly thank my beloved juniors **Abhijith, Manoranjan, Omkar, Amaresh, Amarkant, Prem, Reddiah, Vijay Kamal, Nanda Kumar**, and others for their help.

It will be ignorance on my part to praise the immense support, moral help, constant encouragement, and sustained camaraderie bestowed upon me during tough times by my soul mates **C. Hruday Ranjan and A. Lakshmi Prasad**. Words are not enough to express my heartfelt thanks to my friends **Vamsi Krishna, Sunil Kumar, Sai Prasad**, and others for their unparalleled affection, encouragement, and cooperation. Special thanks go to my dear friend **Dr. Natraj**, alumni of this division for opening the doors of IARI for me through his guidance for entrance examination.

It is rather difficult to express in words my deep sense of boundless affection towards my dearest batchmates **Tribhuvan, Krishna Kumar, Ratan, Mandeep, Aman, Jorben, Ranjith, Jeet Ram, Neethu, Archana, Seema, Reddy Sir and Mukesh Sir** for their lovely friendship, help and care and for making this three-year outing very much enjoyable and memorable.

It is time to surface out genuflect love and affectionate gratitude to my dearest parents, **Smt. K. Hari Priya and Late Sri. K.T. Sreenivasan** for their blessings, unparalleled love and affection, inspiration, persistent encouragement, and moral support throughout my educational career. I feel it a rare opportunity to express my bountiful regards, affection and gratitude to my beloved Uncle **Mr. Kiran Kumar** and cousin **Mrs. Madhavi** for supporting me during the toughest time of my life.

I also thank Director, ICAR-Central Soil Salinity Research Institute, **Dr. P.C. Sharma** for permitting me to pursue Ph.D. at IARI timely and for all the administrative support during this period. I also thank my dearest colleague friends from ICAR-CSSRI **Mr. Vineeth, Mr. Aslam, Dr. Parul, and Dr. Arjun Singh** for their help and assistance.

I also thank each and every one, whose names are missing here and who are indirectly involved in the development of this thesis. The financial assistance provided by IARI and NAHEP-CAAST is greatly acknowledged.

Finally, I thank **Sri Venkateshwara Perumal** for having my back at every stage of my life.

Date: 30-12-2022
Place: New Delhi



(K. T. RAVI KIRAN)

CONTENTS

No.	CHAPTERS	Page No.
1.	INTRODUCTION	1-3
2.	REVIEW OF LITERATURE	5-40
3.	MATERIALS AND METHODS	41-58
4.	RESULTS	59-112
5.	DISCUSSION	113-126
6.	SUMMARY AND CONCLUSION	127-131
	ABSTRACT	133-134
	सारांश	135-136
	REFERENCES	137-165
	ANNEXURES	167-175

LIST OF TABLES

Table No.	TITLE	Page No.
2.1	List of reports on genotypes screened for reproductive stage high temperature tolerance in rice	30-32
2.2	QTLs reported for traits associated with heat stress tolerance in rice	33-38
2.3	Genes associated with heat stress tolerance in rice	40
3.1	List of rice genotypes characterized for their tolerance to reproductive stage heat stress	42-44
3.2	List of Basmati germplasm characterized for their tolerance to reproductive stage heat stress	44
3.3	Score scales for alkali spreading value	50
4.1	Summary statistics of grain yield plant ⁻¹ (g) and spikelet fertility (%) under control and RSHS conditions	61
4.2	Adjusted means of grain yield per plant and spikelet fertility and their corresponding indices of 192 genotypes (along with checks) evaluated for reproductive stage heat stress during off-season, 2018-19 at Aduthurai, Tamil Nadu	62-66
4.3	Summary statistics of grain yield and spikelet fertility recorded on 35 Basmati rice genotypes under control and RSHS during off-season, 2018-19 and 2019-20	68
4.4	Mean performance and stress indices of genotypes for grain yield per plant (g) and spikelet fertility (%) under control and RSHS situations during off-seasons, 2019-20 and 2020-21	69-70
4.5	Significant marker trait associations (MTAs) identified for grain yield plant ⁻¹ (GYPP) and spikelet fertility (SF) under RSHS and their respective stress tolerance index	73-74
4.6	Genotypes exhibiting superior performance and highest number of favorable alleles of 29 MTAs identified for tolerance to RSHS	75
4.7	Summary statistics for grain yield plant ⁻¹ , spikelet fertility (%) and pollen fertility (%) recorded in the 127 RILs and parents under timely sown (unstressed) and late sown (high temperature stressed) conditions	79
4.8	Summary of heat stress indices measured for grain yield plant ⁻¹ and spikelet fertility among the RILs from PB1/NL44 cross	79
4.9	Markers showing significant association with the respective traits revealed through single marker analysis	80

Table No.	TITLE	Page No.
4.10	Stress tolerance index (STI) values of parents and RILs from BLUP values of grain yield and spikelet fertility in two locations and seasons	89
4.11	Variance and heritability for grain yield and its contributing traits under control and RSHS situations across two locations and two off-seasons	98
4.12	Performance of the parents, PB 1, NL 44 and the RILs from the biparental cross between PB1/ NL44, under non-stress and RSHS conditions for grain yield and its contributing traits at Aduthurai and Cuttack during off-seasons, 2018-19 and 2019-20	99-100
4.13	Characterization of the parents, PB 1, NL 44 and the RILs from the biparental cross between PB1/ NL44, for various grain and cooking quality attributes from the produce of off-season, 2018-19 at Aduthurai and <i>Kharif</i> , 2019 at New Delhi	101
4.14	Details of parental polymorphism survey and linkage maps developed using SSR and SNP markers	108
4.15	QTLs identified for grain yield and spikelet fertility (under control and RSHS) and their respective STI values using individual and combined environment analysis in QTL IciMapping software	109
4.16	QTLs identified for other agronomic traits (under control and RSHS) using individual and combined environment analysis in QTL IciMapping software	110
4.17	Performance of F ₆ families under RSHS at IARI-RBGRC, Aduthurai during off-season, 2021-22	111

LIST OF FIGURES

Figure No.	TITLE	Page No.
2.1	Summary of effect of heat stress on various growth stages of rice plants	9
2.2	Effect of heat stress on reproductive development of rice plants	10
2.3	Effect of heat stress on grain development of rice plants	15
2.4	Mechanisms (escape, avoidance and true tolerance) of heat stress tolerance in rice	23
2.5	Chromosomal locations (physical in Mb) of QTLs reported so far for reproductive stage heat stress tolerance in rice. Distinct clusters of QTLs can be observed on chromosomes 1 (38.4 – 43.3 Mb), 2 (18.9 – 25.5 Mb), 4 (26.8 – 33.6 Mb), 5 (0.1 – 3.1 Mb), 8 (24.2 – 27.6 Mb), 10 (18.7 – 20.8 Mb)	39
4.1	Boxplots depicting the distribution of (A) grain yield plant ⁻¹ (g) and (B) spikelet fertility (%) under control and RSHS conditions	60
4.2	Scree plot showing the proportion of total variance accumulated by each principal component (A) and PCA 3D plot depicting the distribution of genotypes across three principal components (B)	68
4.3	Circular Manhattan plot representing marker trait associations (MTAs) obtained through MLM (A), FamCPU (B) and BLINK (C) models. Circles from outside to inside depict grain yield plant ⁻¹ (GYPP) under RSHS, spikelet fertility (SF) under RSHS, STI estimated for GYPP, and for SF	72
4.4	Physiological characterization of parents of mapping populations. (A) Canopy temperature depression between ambient and foliage temperatures; (B) Membrane stability index values of various genotypes; (C) SPAD meter readings of genotypes under control and heat stress treatments; (D) Hydrogen peroxide accumulated in various genotypes under different treatments; (E) Lipid peroxidation using TBARS as proxy; (F) Total antioxidant capacity estimated using FRAP assay	77
4.5	Performance comparison of F ₁ between PB1 and NL44 under control and RSHS conditions during Offseason 2018-19 at IARI-RBGRC, Aduthurai	78
4.6	Performance of RILs under control (C) and heat stress (HT) in various environments (E1: Off-season, 2019, Aduthurai; E2: Off-season, 2019, Cuttack, E3: Off-season, 2020, Aduthurai; E4: Off-season, 2020, Cuttack) in terms of grain yield (GY; A), spikelet fertility (SF; B), plant height (PH; C), panicle length (PL; D), tiller number (TN; E), and panicle number (PN; F)	88

Figure No.	TITLE	Page No.
4.7	GGE biplots of environment view (A: Grain yield; C: Spikelet fertility) and genotype view (B: Grain yield; B: Spikelet fertility) of 127 RILs evaluated under four environments (E1: Off-season, 2018-19, Aduthurai; E2: Off-season, 2018-19, Cuttack, E3: Off-season, 2019-20, Aduthurai; E4: Off-season, 2019-20, Cuttack) under heat stress	90
4.8	Linkage map constructed using 94 polymorphic SSR markers analyzed over 127 RILs	103
4.9	SNP based linkage map constructed using 10,924 homozygous polymorphic markers	104
5.1	Co-localization of QTLs identified through biparental mapping (in brown) and QTNs found through GWAS (in green)	126

LIST OF PLATES

Plate No.	TITLE	Page No.
4.1	Representative gel images showing parental polymorphism survey between PB1 (S) and NL44 (T) (A) and genotyping of RILs (1-22) using SSR markers linked to QTLs reported for RSHS tolerance (B)	80
4.2	Representative photographs showing the trials conducted during offseason at Aduthurai (A) and Cuttack (B)	96
4.3	Phenotypic contrast between the parents, NL44 (Tolerant) and PB 1 (Sensitive) for filled grain under RSHS conditions	97
4.4	Variation among the RILs from PB1/NL44 for spikelet fertility under RSHS conditions	97
4.5	Representative gel images showing parental polymorphism between NL44 (T), PB1 (S1) and PB1509 (S2) (A), genotyping results of RILs using markers RM493 (B)	102
4.6	Representative picture depicting the performance of a subset of F ₆ families along with checks under heat stress evaluated during off-season, 2021-22 at Aduthurai, Tamil Nadu	112
4.7	Representative gel image depicting the genotyping of PB1509 (S) and NL44 (T) F ₆ progenies (1-20) for validating putative QTLs using SSRs (RM247) present near their physical locations	112

Abbreviations used

μL	Micro litre
μM	Micro Molar
AC	Apparent amylose content
ANOVA	Analysis of variance
ASV	Alkaline Spreading Value
BLINK	Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway
BLUP	Best Linear Unbiased Predictor
BLUE	Best Linear Unbiased Estimate
CI	Confidence Interval
CIM	Composite Interval Mapping
cM	Centi Morgan
CRD	Completely Randomized Design
CTD	Canopy Temperature Depression
C.V.	Co-efficient of Variation
DEB	DNA Extraction Buffer
FarmCPU	Fixed and random model Circulating Probability Unification
FRAP	Ferric Reducing Antioxidant Power
FW	Fresh Weight
g	grams
GAPIT	Genome Association and Prediction Integrated Tool
GC	Gel Consistency
GCV	Genotypic Coefficient of Variation
GGE	Genotype plus Genotype by Environment Interaction
GLM	General Linear Model
GYPP	Grain Yield Per Plant
GWAS	Genome Wide Association Study
HP	Hulling Percentage
HRR	Head Rice Recovery
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
ICIM	Inclusive Composite Interval Mapping
IPCC	Inter-governmental Panel on Climate Change
KI	Potassium Iodide
KLBC	Kernel length before cooking
KBRC	Kernel breadth before cooking
KLAC	Kernel length after cooking
KBAC	Kernel breadth after cooking
KER	Kernel Elongation Ratio
LD	Linkage Disequilibrium
LOD	Logarithm of Odds
LSD	Least Significant Difference
M	Molar
ml	Milli litre
MLM	Mixed Linear Model
mM	Milli molar

mm	Millimetre
MP	Milling Percentage
MSI	Membrane Stability Index
N22	Nagina 22
NL44	NERICA-L-44
NRRI	National Rice Research Institute
PB1	Pusa Basmati 1
PB1121	Pusa Basmati 1121
PB1509	Pusa Basmati 1509
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
PVE	Phenotypic Variance Explained
QTL	Quantitative Trait Locus
QTN	Quantitative Trait Nucleotide
R ²	Co-efficient of determination
RCBD	Randomized Complete Block Design
RGBRC	Rice Genetics and Breeding Research Centre
RIL	Recombinant Inbred Line
ROS	Reactive Oxygen Species
RSHS	Reproductive Stage Heat Stress
SCMR	SPAD Chlorophyll Meter Reading
S.D.	Standard Deviation
S.E.	Standard Error
SF	Spikelet Fertility
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
STI	Stress Tolerance Index
TAE	Tris base, Acetic acid and EDTA
TBARS	Thiobarbituric acid reactive substance
TE	Tris EDTA
USA	United States of America
UV	Ultraviolet

Chapter I

INTRODUCTION

Global warming caused by indifferent anthropogenic activities is a major challenge for sustaining agricultural production. Since the beginning of 20th century, the earth's average surface temperature has risen by 0.8°C, with about 0.6°C of this abnormal spike encountered, since 1980 (IPCC, 2007). Estimates suggests global mean surface temperatures are likely to increase from 0.3°C up to 4.8°C by 2081–2100 considering different greenhouse gas emission settings in comparison with 1986–2005 (IPCC, 2013). It is highly certain that, in most places, there will be episodes of extreme temperatures. These projected changes in temperatures will be more vividly felt in tropical and sub-tropical regions which harbour the major rice growing regions of the world. The situation is equally alarming in India. The temperatures of the warmest day have risen by 0.63°C and the coldest night by about 0.63°C and 0.4°C in the last three decades (1986-2015) and it is predicted to further increase by 4.7°C and 5.5°C, respectively towards the end of 2100 (Krishnan et al., 2020). The fertile swathe of Indo-Gangetic plains, considered food bowl of India, is mostly likely to face the brunt of this supra-optimal temperatures in the nearest future (Im et al., 2017).

Rice is the principal cereal feeding more than three billion people across the globe, contributing about 20% of the total human calorie intake. Asia tops in its production and consumption, where it accounts for more than 80% of the calorie intake (Mahajan et al., 2010). It is the principal staple crop of India, where it is grown in 45.07 m ha with a production of 122.27 m tonnes during 2020-21 (GoI, 2021). Among abiotic stress, high temperature stress severely affects rice yield along with drought and salinity. Rice is sensitive to heat stress with a diurnal temperature threshold of 33°C to 35°C (Day) /29°C (night) (primarily in tropical rice) (Yoshida et al., 1981). The predicted changes, cited above, in current and future climate would severely affect global rice production (Teixeira et al., 2013) and grain quality (Lin et al., 2010) debilitating both it's economic (Lyman et al., 2013) and nutritional value. Previous reports suggest a 7–8% yield decline in rice for every 1°C increase in maximum/minimum temperature from 28/21°C to 34/27°C (Baker et al., 1992). Rice yield penalties due to heat stress have already been documented in many Asian countries including India, Bangladesh, Thailand, Pakistan, China, Japan, and others such as Australia and U.S.A. (Hossain et al., 2021; Tian et al., 2009; Hasegawa et al., 2009, 2011; Yang et al., 2004; Li et al., 2004; Matsushima et al., 1982; Osada et al., 1973). It is

predicted that by 2030 about 16% of global rice area will be exposed to temperatures $> T_{crit}$ and this is expected to shoot up to 27% by 2050 (Gourdji et al., 2013).

Flowering followed by microsporogenesis stage (visible externally as booting) is the most sensitive stage to heat stress in the life cycle of rice (Satake and Yoshida, 1978; Yoshida et al., 1981). Susceptibility at reproductive stage is particularly important as it can directly result in yield declines. High temperature exceeding 35°C at the flowering stage results in inviable pollen, poor anther dehiscence, low pollen production and sterile spikelets, resulting in a significant decline in grain yield, grain quality and harvest index (Xu et al., 2021). Hence, spikelet sterility/fertility is considered an ideal proxy in differentiating heat tolerant and sensitive genotypes (Prasad et al., 2006). However, the female reproductive system is considered more resilient to extreme temperatures compared to male reproductive organs. Heat stress post fertilization affects grain quality by increasing grain filling rate while causing a significant decrease in its duration, thereby disturbing starch synthesis and resulting in chalky grains (Sreenivasulu et al., 2015; Ishimaru et al., 2016; Mitsui et al., 2016; Morita et al., 2016).

Rice plants utilize different mechanisms to survive through heat stress such as true tolerance (Jagadish et al., 2010a), avoidance (Julia and Dingkuhn, 2013) and escape through the early morning flowering (EMF) trait (Ishimaru et al., 2010; Julia and Dingkuhn, 2013; Hirabayashi et al., 2015). In general, true tolerance has been largely determined on the basis of spikelet sterility of the test genotypes in many reports. Literature is replete with genotypic variation for spikelet sterility at high temperatures (Pradhan et al., 2016; de Brito et al., 2019; Paul et al., 2020; Thuy et al., 2020; Chidambaranathan et al., 2021; Ezin et al., 2022). Nagina22 (N22), an *aus* variety, has been frequently identified to be highly heat tolerant at the flowering stage (Satake and Yoshida, 1978; Yoshida et al., 1981, Prasad et al., 2006, Jagadish et al., 2008, 2010b, 2011). Recently, a new genotype NERICA-L-44 (NL44) has reportedly shown high seedling survival (52%) and superior growth and greater reproductive plasticity even at 42.2°C under field conditions among 71 rice accessions tested which were almost at par with N22 (Bahuguna et al., 2015). NL44 was proposed as an alternative donor for heat stress tolerance at reproductive stage in rice. However, the genetic and molecular basis of high temperature tolerance in NL44 hadn't been elucidated yet.

Heat tolerance at flowering stage in rice is a quantitative trait governed by a multitude of genes with cumulative effects on trait expression, often referred to as quantitative trait

loci (QTL). From the first report of Cao et al. (2002), based on per cent spikelet fertility using doubled haploid population derived from IR64/Azucena, there were many mapping studies involving F₂, back cross inbred lines (BIL) and recombinant inbred lines (RIL) populations, evaluated primarily at the time of heading in both controlled environments and actual field conditions (Ye et al., 2012, 2015a, b; Tazib et al., 2015; Zhao et al., 2016; Shanmugavadivel et al., 2017; Li et al., 2018; Seo et al., 2019; Cao et al., 2020). RFLP or SSR markers, in majority of cases and SNP in few reports (Ye et al., 2012, 2015a, b; Shanmugavadivel et al., 2017; Nubankoh et al., 2020; Chen et al., 2021) were utilized to delineate genome regions associated with heat tolerance. QTLs identified in these studies have been found to be distributed across the rice genome, almost all the chromosomes. Further, these QTLs were not validated in other backgrounds (except by Ye et al., 2015a); nor the significant QTLs fine mapped (except by Ye et al., 2015b; Cao et al., 2020) and hence their reliability for use in marker assisted selection has been under question.

A recombinant inbred line (RIL) population from cross, between the heat stress tolerant rice genotype, NL44 and a susceptible cultivar Pusa Basmati 1 has been developed at the Division of Genetics, ICAR-IARI, New Delhi. It had been hypothesized that NL44 may be a putative novel donor possessing novel genomic regions conferring tolerance to heat stress at reproductive stage. To test this research hypothesis, the present investigation entitled “Mapping quantitative trait loci governing heat stress tolerance at reproductive stage in rice” was formulated with the following objectives:

1. To assess the phenotypic variation in the rice germplasm for heat stress tolerance at reproductive stage.
2. To map QTLs governing heat stress tolerance at reproductive stage in the NERICA rice cultivar, NL44.
3. To validate the molecular markers associated with QTLs governing heat stress tolerance in rice.

Chapter II

REVIEW OF LITERATURE

The global environment is becoming less congenial to crop plants due to human inflicted climate change resulting in extreme weather events like severe cyclones, forest fires, heat waves etc (Sun et al., 2019). Rice is the second most important staple crop across the world catering to the calorie intake of more than 50% of the world's populace (FAOSTAT, 2014). Rice yields and productivity are severely plagued by several abiotic stresses such as drought, heat, salinity, submergence etc. Heat stress is an emerging abiotic stress which is predicted to seriously affect rice yields in future. The area under rice in South Asia is already under the threat of high temperatures and it is predicted that other rice growing countries (Central and Eastern Asia, Central North America, Southeast Brazil, and Southern Australia) are going to experience frequent heat episodes in coming years (Teixeira et al., 2013). The temperatures of most of the tropical and sub-tropical rice growing countries are already hitting 33°C during rice growing season and any further increase will have catastrophic effects on rice production (Prasad et al., 2006). Under such conditions, yields of rice were estimated to decline by 41% towards the end of this 21st Century (Ceccarelli et al., 2010). With the ever-increasing world population, to safeguard food security, knowledge of mechanisms of heat stress tolerance in rice is imperative for breeding resilient rice varieties.

2.1. Historical records of high temperature induced losses in rice

Though heat stress induced yield penalties were observed in various rice growing countries, only a few systematic reports documented these. China suffered rice yield losses due to heat stress in 1959, 1966, 1967, 1978, 1994 and 2003 (Tian et al., 2009). In 1978, the seed set percentage of some varieties dropped to 10-50% in the Hubei and Sichuan provinces of China. Another extreme heat stress event occurred in 1994 when certain sensitive varieties recorded as low as 10% seed set. However, the most severe heat shock in the recent past was encountered in 2004 which was well documented. In the Anhui province and Wuhan area of Hubei, 0.33 million ha and 27,000 ha of rice growing area (with 50% of yield loss relative to the normal average) were affected due to heat stress (Li et al., 2004; Yang et al., 2004). Overall, in 2003 an area of 3 million ha of rice encountered heat stress injury with a yield loss of about 5.18 million tonnes. Thus, rice yield shrank between 1.5% to 4.6% due to post-heading high temperature exposure in South China

during 1981-2010 (Shi et al., 2015a). A significant increase in empty grains was also observed in the central plains of Thailand during the dry season in 1969 which was attributed to heat stress during flowering stage (Osada et al., 1973). A declining trend in the rice grain quality was observed over the years in the western part of Japan (Hasegawa et al., 2009). Similarly, an extreme heat wave in the mid-summer of 2007 resulted in spikelet sterility from 2% to 23% in the Kanto and Tokai regions of Japan (Hasegawa et al., 2011). In 2010, a severe reduction in milling recovery of rice grown was documented in Arkansas in the U.S. due to extreme night-time air temperatures (Laborte et al., 2012). Similar evidence is obtained in Sudan (Matsushima et al., 1982), the Philippines and South India (Ishimaru et al., 2016). Extensive damage to rice planted on 68,000 hectares occurred most recently culminating in a loss of \$39 million by the farmers of Bangladesh when temperatures rose to 36°C instead of the average 33°C during April 2021 for a mere two consecutive days (Hossain, 2021).

2.2. Effects of heat stress on rice reproductive development

Heat stress affects every stage of crop growth – vegetative, reproductive and maturity stages and responses of rice towards heat stress are stage specific (Figure 2.1). Among the plant organs, leaves are more tolerant to high temperatures than panicles (Shi et al., 2016; Schaarschmidt et al., 2020). High temperatures at the seedling stage lead to seedling mortality and the resultant poor plant stand in the main field. Though rice is tolerant at the vegetative stage, there will be a reduction in the number of tillers which will be translated into a reduced number of panicles per plant (Xu et al., 2020). Sometimes, heat stress at the vegetative stage (45 days before heading) was found to be more damaging than the booting stage (15 days before heading) in terms of seed-setting rate and yield (Cheabu et al., 2018). A poor correlation was observed between tolerance at different growth stages signifying different genes govern tolerance at different growth stages (Hsuan et al., 2019). Sensitivity at the reproductive stage is more severe and significant since it will directly deter the ultimate yield potential of rice.

2.2.1. Panicle characteristics vis-à-vis heat stress

The effects of heat stress on rice panicles seem to be far from uniform. It was observed that the fertility and matured grain weight of superior spikelets suffered more than the inferior spikelets when exposed to high temperatures. This was ascribed to the position of superior spikelets which exposes them more to higher temperatures and illumination

intensities than the inferior spikelets which are partially shaded by the leaves (Fu et al., 2016). This may be explained by the ‘thermal burden’ concept. The top spikelets of the canopy have the least thermal burden (5°C) during midday in the tropics with a boundary layer resistance of 17 s m⁻¹ while those within the panicle have a thermal burden of 17°C and boundary layer resistance of 100 s m⁻¹ unless these spikelets can offset this through efficient transpirational cooling (Sheehy et al., 1998). However, the spikelets present at the bottom of the panicle were affected more by the high night temperature (Mohammed and Tarpley, 2010). High temperatures can also jeopardize normal panicle development. For instance, heat stress suppressed differentiation of secondary branches and florets resulting in their degeneration in susceptible cultivars. Further failure of panicle exertion in response to elevated temperatures was also found responsible for poor spikelet fertility in sensitive genotypes (Julia and Dingkuhn, 2013; Das et al., 2014; Wu et al., 2016). On the other hand, it was demonstrated that the variety Sathi with >90% sheathed panicle could maintain 67 and 40% higher spikelet fertility than IR64 and N22 respectively which seems an exception (Lawas et al., 2018).

2.2.2. Effect of heat stress on floral morphology

Externally, High temperatures lead to deformed floral organs with a simultaneous reduction in their number and size. Reproductive organogenesis under high temperatures investigated using SEM and stereo microscopy revealed pistil hyperplasy (multiple stigma and/or ovaries, outgrowth of parenchymatous tissues from ovule and differentiation of trichomes from ovary epidermis) followed by stamen hypoplasia (decline in the number of stamens) are the primary causes of sexual failure in rice (Takeoka et al., 1991). The shape of anthers, lemma and palea are also altered in spikelets exposed to high temperatures (Takeoka et al., 1991).

2.2.3. Effect of heat stress on male reproductive system of rice

The optimal temperature for flower development in rice ranges between 23–26°C, beyond which various stages of reproductive process will be seriously affected (Baker et al., 1992). Heading (panicle emergence), followed by microsporogenesis stage (visible as booting), is the most sensitive stage to heat stress in rice (Satake and Yoshida, 1978; Yoshida et al., 1981). A high temperature of over 35°C during anthesis reduces pollen viability, decreases spikelet fertility, and grain weight resulting in significant obliteration of yield, low grain quality and harvest index (Osada et al., 1973; Matsushima et al., 1982;

Matsui et al., 1997; Prasad et al., 2006; Jagadish et al., 2007, 2008, 2010a&b; Sánchez et al., 2014; Wu et al., 2016) whereas heat stress at booting stage reduces spikelet number (Chaturvedi et al., 2017; Soda et al., 2018; Wu et al., 2016) (Figure 2.2). Beyond 37°C there shall be a dramatic decline in grain yield (Sánchez et al., 2014). Reduction in the seed set per cent is the primary cause of decreased yields under high temperatures closely followed by reduced spikelet number per panicle and grain weight (Xiong et al., 2017). Male reproductive organs and pre-fertilization events are comparatively more vulnerable to extreme temperatures than the female reproductive tissues and post-fertilization events. Pollen viability is most sensitive to high temperature followed by pistil viability, fertilization, and embryogenesis (Jagadish et al., 2010b; Jagadish, 2020). Externally, spikelets with visibly exerted anthers but with no ovule enlargement were found to be the most heat stress sensitive stage (Shi et al., 2015b).

2.2.3.1. *Anther characteristics and its dehiscence*

Anther dehiscence is the most sensitive reproductive event to high temperature in the entire rice reproductive process which was reported to be driven by rapid swelling of pollen grains leading to rupture of the septum (Matsui et al., 1999a, b, 2000). In tolerant cultivars, the cavities inside the anthers are well developed with thick locule walls enabling easy rupture of the septa as the pollen grains swell. The thick locule walls also facilitate swelling of pollen grains by retaining adequate moisture inside the locules and preventing pollen desiccation (Matsui et al., 1999a; Matusi et al., 2001). This is attested by a significant correlation observed between the pollen grain diameter and percentage of dehiscent anthers in florets (Matsui et al., 1999a). A decrease in the moisture content of pollen was observed in plants exposed to high temperatures (Das et al., 2014). This might result in a decrease in the size of pollen (Kumar et al., 2015b). Further, tight closure of locules by multiple cell layers defers locule opening and decreases pollen fertility at high temperatures in sensitive cultivars (Matsui and Osama, 2002). Thus, the anthers of tolerant genotypes dehisce well and early during anthesis even under high temperature conditions (Satake and Yoshida, 1978; Mackill et al., 1982; Matsui et al., 1997; Malumpong et al., 2019). In addition to this, length of the basal theca dehiscence purportedly resulted in higher pollen landing on stigma and was proposed as a selection criterion for high temperature tolerance since the basal pore is immediately above the stigma (Matsui et al., 2005). This will eventually increase the number of pollen grains reaching the stigma (Mackill et al., 1982; Matsui et al., 1997; Matsui et al., 2001; Prasad et al., 2006). There should be a minimum of 10 – 20 pollen

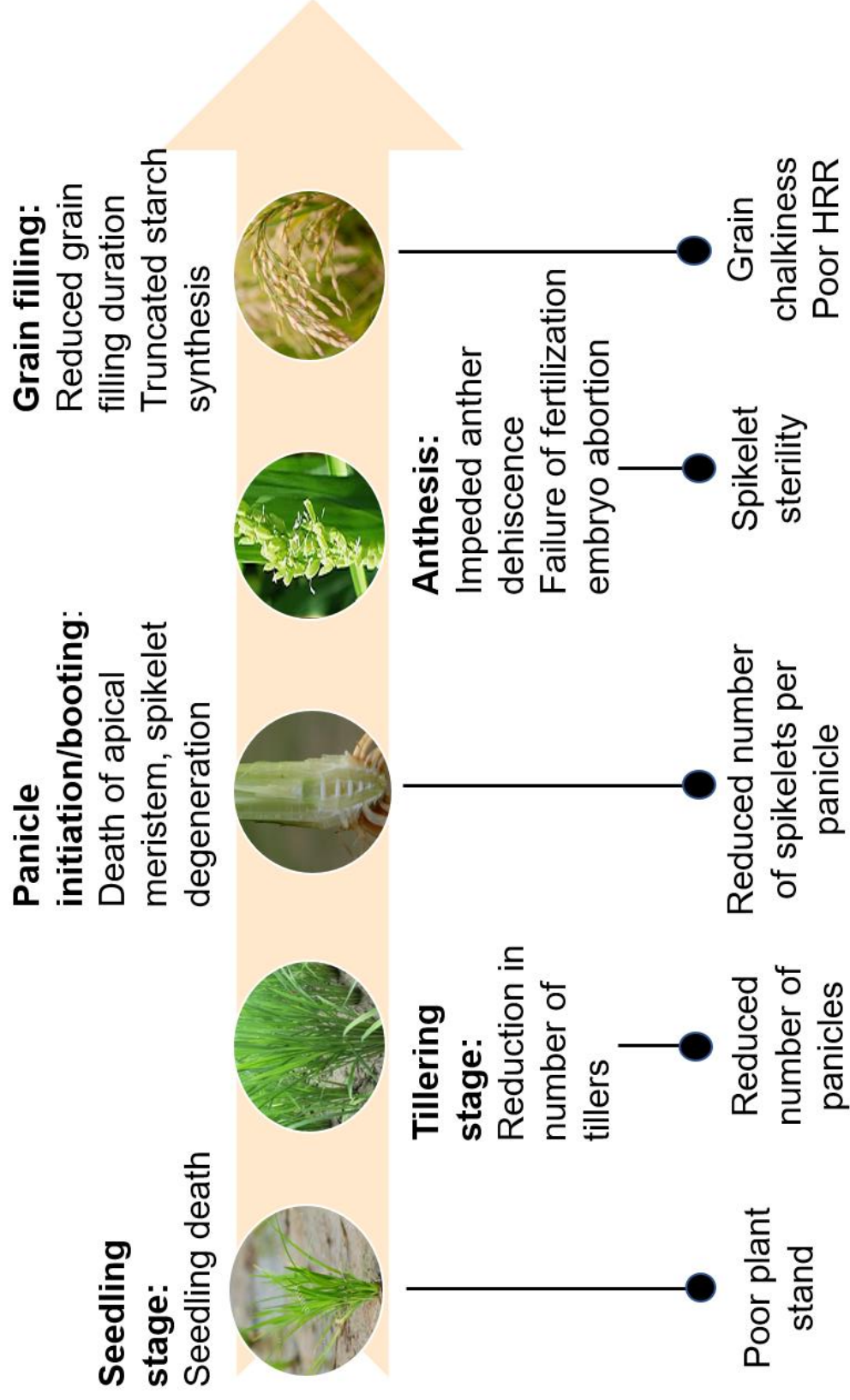


Figure 2.1 Summary of effect of heat stress on various growth stages of rice plants

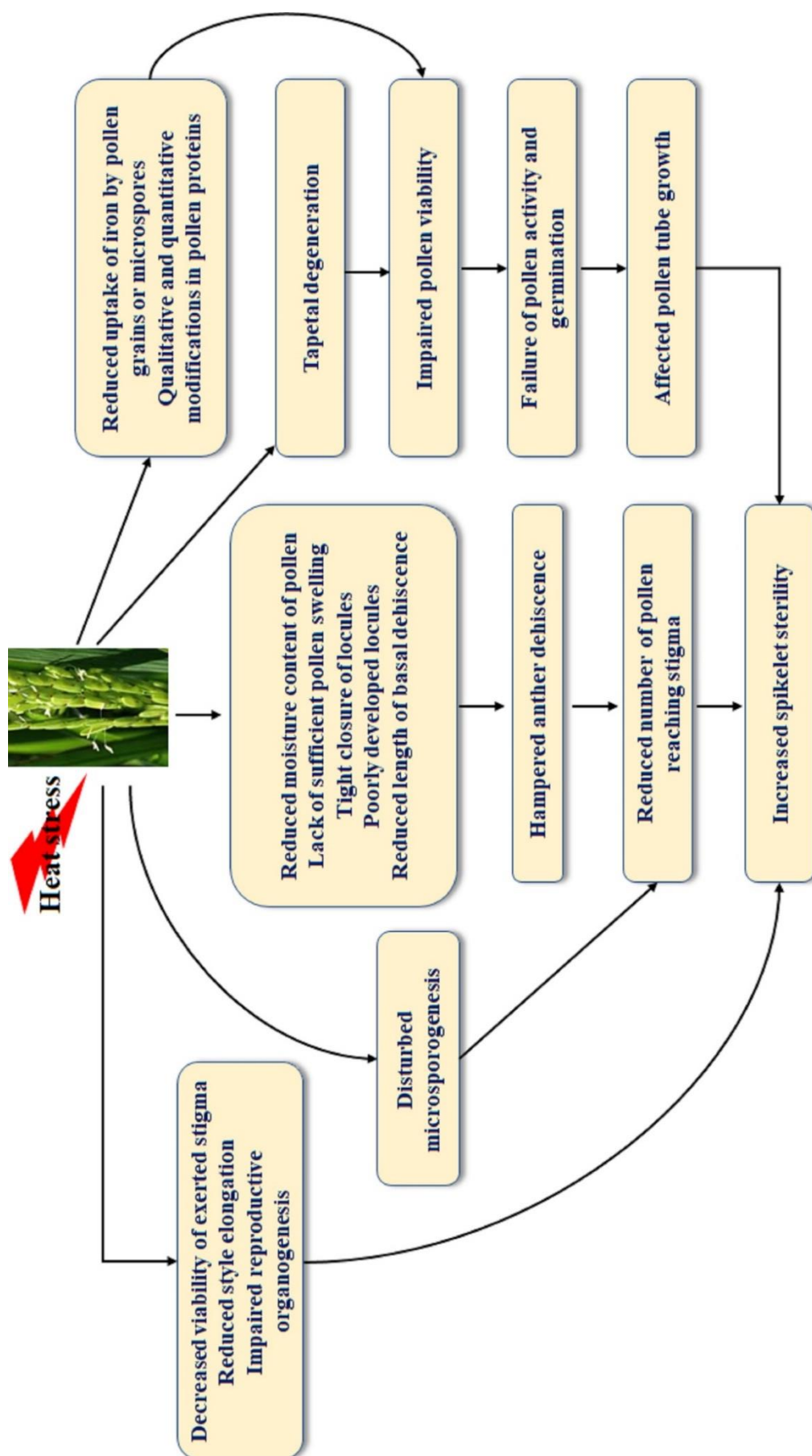


Figure 2.2 Effect of heat stress on reproductive development of rice plants

grains landing on the stigma to effect successful fertilization of ovules (Yoshida et al., 1981; Kobayashi et al., 2011). Furthermore, basal and apical pore length of anthers increased under high temperature, however, showed no correlation with pollen number on stigma contradicting the above observation (Jagadish et al., 2010a). Furthermore, genotypes with larger anthers are supposed to harbour a greater number of pollen grains than the ones with small anther sizes under high temperatures (Matusi and Osama, 2002). The morphological and structural development of the anther was impeded and microsporogenesis attenuated. Recently it was reported that this restricted anther dehiscence is due to thick anther wall, thicker cuticle of anther epidermis, un-degraded septum, uneven distribution of Ubisch bodies, and inhibited confluent locule in sensitive genotype (Hu et al., 2021).

2.2.3.2. Pollen characteristics and their viability

The pollen viability was lost within 10 minutes of exposure to high temperatures (Song et al., 2001). This lower pollen production at extreme temperature may, in part, be attributed to disturbed division of microspore mother cells and poor development of microspores (Takeoka et al., 1991; Cao et al., 2008). Pollen activity and their germination will also be affected by high temperatures (Tang et al., 2006; Endo et al., 2009; Coast et al., 2016; González-Schain et al., 2016; Shi et al., 2018; Satake and Yoshida, 1978). This is important since a strong correlation was observed between spikelet fertility and the number of pollen germinating on stigmas (Rang et al., 2011). Reduced iron uptake by pollen tubes or microspores during heat stress was also implicated in pollen sterility (Jagadish et al., 2010a). Apart from this, heat stress incited quantitative and qualitative alterations in pollen proteins that are also detrimental to pollen viability (Das et al., 2014). Once germinated, the pollen tube growth through the style towards ovule may also be impeded by heat stress (Yamada, 1964; Das et al., 2014; Coast et al. 2016; Shi et al., 2018). The early pollen development stages such as tetrad formation and early microspore stages are also sensitive to high temperatures (Jagadish et al., 2015). Additionally, heat stress leads to malformation of pollen including, thinner exine, lack of nexine, distorted sporopollenin deposition and disturbed tectum which lead to loss of viability (Hu et al., 2021).

2.2.3.3. Impaired tapetal function

The nutritive cell layer, tapetum gets degenerated with reduced activity of the invertase enzyme (Ku et al., 2003) and developing microspores/pollen grains are starved of

nutrients which eventually leads to their unviability (Endo et al., 2009). A subset of tapetal cell functions seems to be sensitive to high temperature. Tapetal cells' shrinkage, nuclear condensation, fragmentation, programmed cell death and tissue degeneration were also observed under heat stress (Fang et al., 2018). A delayed tapetal degeneration also resulted in decreased pollen viability under heat stress (Deng et al., 2010).

2.2.4. Effect of heat stress on the female reproductive system of rice

The effect of heat stress on female reproductive system is poorly understood compared to the male counterpart. This is in part due to the ease in accessibility of male organs (Wang et al., 2021). Female reproductive tissues seem more resilient to heat stress compared to their male counterparts (González-Schain et al., 2015). Logically, a good stigma exertion should enhance pollen grain reception leading to higher spikelet fertility under heat stress. Conversely, it was established that the presence of enclosed or 'hidden' stigma contributed to higher spikelet fertility and heat stress tolerance in rice (Wu et al., 2019). Further, the viability of the exerted stigma was faltered by high temperature. Stigma elongation is also affected by high temperature (Wu et al., 2019). The heat stress sensitivity of carpels is species dependent, where in some species female reproductive system is more sensitive than the male organs. There are no studies in rice which details the effect of heat stress on development of megagametophyte except the effect on pistil morphology as mentioned in the succeeding section.

2.2.5. Effect of timing of heat stress on spikelet fertility

The variation in the timing of high temperature exposure seems to have varied effects on male reproductive biology. For instance, spikelet fertility is not affected by high temperatures (even at 38°C and 41°C), an hour before or after anthesis (Yoshida et al., 1981; Shi et al., 2018). Nor was there a significant yield reduction when high temperature coincided with the grain filling stage (Cao et al., 2008; Aghamolki et al., 2014; Shi et al., 2016; Ali et al., 2019). However, exposure of spikelets a day before anthesis resulted in a significant reduction of spikelet fertility in contrast to the above. A stark linear dip in the spikelet fertility on consecutive flowering days reflected a cumulative effect of heat stress (Rang et al., 2011). Furthermore, high temperature before anthesis affects the anther dehiscence later; whilst heat stress within 45 minutes of spikelet opening affects pollination, pollen germination and pollen tube growth (Prasad et al., 2006). Temperatures

>35°C during anthesis for five days resulted in spikelet sterility and total failure of seed set and for >1 h considerably reduced the spikelet fertility (Jagadish et al., 2007).

2.3. Effect of heat stress on rice grain quality

Though maintenance of optimum yield under high temperature is the ultimate objective from farmers' viewpoint, sustaining grain quality is equally important from miller's and consumer's perspectives (Lyman et al., 2013). High temperature adversely affects the rice grain quality when encountered during grain filling stage since it hastens rate of grain maturity, but decreases its duration leading to hastened endosperm development, decreased grain weight, grain dimensions, head rice recovery, grain cracking, immature grains, grain chalkiness and poor palatability (Sato & Inaba, 1973; Tashiro and Wardlaw, 1991; Okamoto, 1994; Zakaria et al., 2002; Wakamatsu et al., 2007; Matsue, 2012; Zhao and Fitzgerald, 2013; Chun et al., 2015; Sreenivasulu et al., 2015; Shi et al., 2016; Cheabu et al., 2018) (Figure 2.3). These effects, physiologically, are due primarily to muddled source-sink carbohydrate balance (Morita, 2008). This is more apparent in the sensitive genotypes. The effect of heat stress on rice grain quality was extensively researched and documented in Japan (Terashima et al., 2001; Morita, 2008; Okada et al., 2009). Temperature around 24°C during ripening was found optimum for temperate *japonica* rice since at this temperature grain weight was found maximum (Wakamatsu et al., 2007). High temperatures 15 days after flowering remarkably decreased 1000 grain weight (Sato, 1979). This was mainly due to the increased rate of grain ripening but its early cessation (Nagato and Ebata, 1965; Sato et al., 1971, 1979; Feng et al., 2006). Added to this, heat stress makes rice plants more vulnerable to insufficient radiation resulting in further deterioration of grain quality (Okada et al., 2009).

2.3.1. Effect of heat stress on grain dimensions

The endosperm cell divisions continue till 10 days after anthesis followed by the accumulation of metabolites. The effect of heat stress on endosperm cell division and development affects grain dimensions (Tashiro and Wardlaw, 1991). This was corroborated by the decreased expression of elongation factor 1 β involved in cell cycle regulation (Lin et al., 2005). High temperature affects grain length (within 2 weeks after anthesis) early in the development while there will be reduced grain width and breadth later (6 weeks after anthesis) (Tashiro and Wardlaw, 1991). High temperature induced grain fissuring or cracking is particularly due to more permeable hull in sensitive genotypes which loses

moisture at higher rates during early grain filling stages (Nagata et al., 2004; Abayawickrama et al., 2017). This poses serious bottlenecks in rice milling which results in low head rice recovery (Takahashi et al., 2002; Nagata et al., 2013; Chun et al., 2015). Cultivar differences exist for this trait; for instance, Yan Xuang 203 showed consistently lower grain cracking under high temperature (Nagata et al., 2013). Immature thin grain (low grain weight) also reduces overall yield and milling efficiency due to deep creases on the surface, impeding removal of the bran (Yonemaru and Morita, 2012).

2.3.2. Grain chalkiness – one of the significant effects of heat stress

For every 1% decrease in grain chalkiness, there will be a ~1% increase in head rice recovery (Zhao and Fitzgerald, 2013). Grain chalkiness is the most frequently reported repercussion of heat stress which results from impeded starch synthesis and accumulation. In terms of grain chalkiness, developing caryopses are the most sensitive targets of heat stress (Morita et al., 2004). The chalky grain is categorized into various types based on the location of chalky areas within the grain. Among these, milky-white, basal-white, white-back grain and belly white are induced by high temperature. Differential effects of temperature were observed on different chalky grain types because of source-sink modulation (Nagato and Ebata, 1965; Tashiro and Ebata, 1975; Nagahata and Yamamoto, 2005; Tsukaguchi and Iida, 2008). The milky white kernels can be further classified into those with white centre and others with white periphery (ring type). These two kernel types were found to behave differently vis-à-vis temperature and management practices (thinning and leaf clipping) (Tsukaguchi et al., 2012). Taste deterioration of cooked rice stems more from white-back kernel than milky white kernel (Wada et al., 2019). High temperature 28 days before anthesis also resulted in increased white back and milky white grains and degraded brown rice (Wada et al., 2010). But elevated CO₂ seems to revive the grain quality damaged by heat stress which needs to be validated in further studies (Usui et al., 2014).

The temperature thresholds of development of these chalky grains were worked out by Masutomi et al. (2015). Temperatures of 27.28°C, 25.13°C, and 25.05°C are critical limits for white-back and white-belly, white-based and milky-white and white-core respectively. Furthermore, the type of chalky grains depends on the stage at which high temperature is encountered. Milky-white grains are formed when the starch accumulation at the centre is affected at the beginning of grain filling (4 to 12 days after heading), while the remaining chalky grain types are formed when it is affected in the later stages (16 days after heading) (Tashiro and Wardlaw, 1991). Chalkiness was also attributed to the early

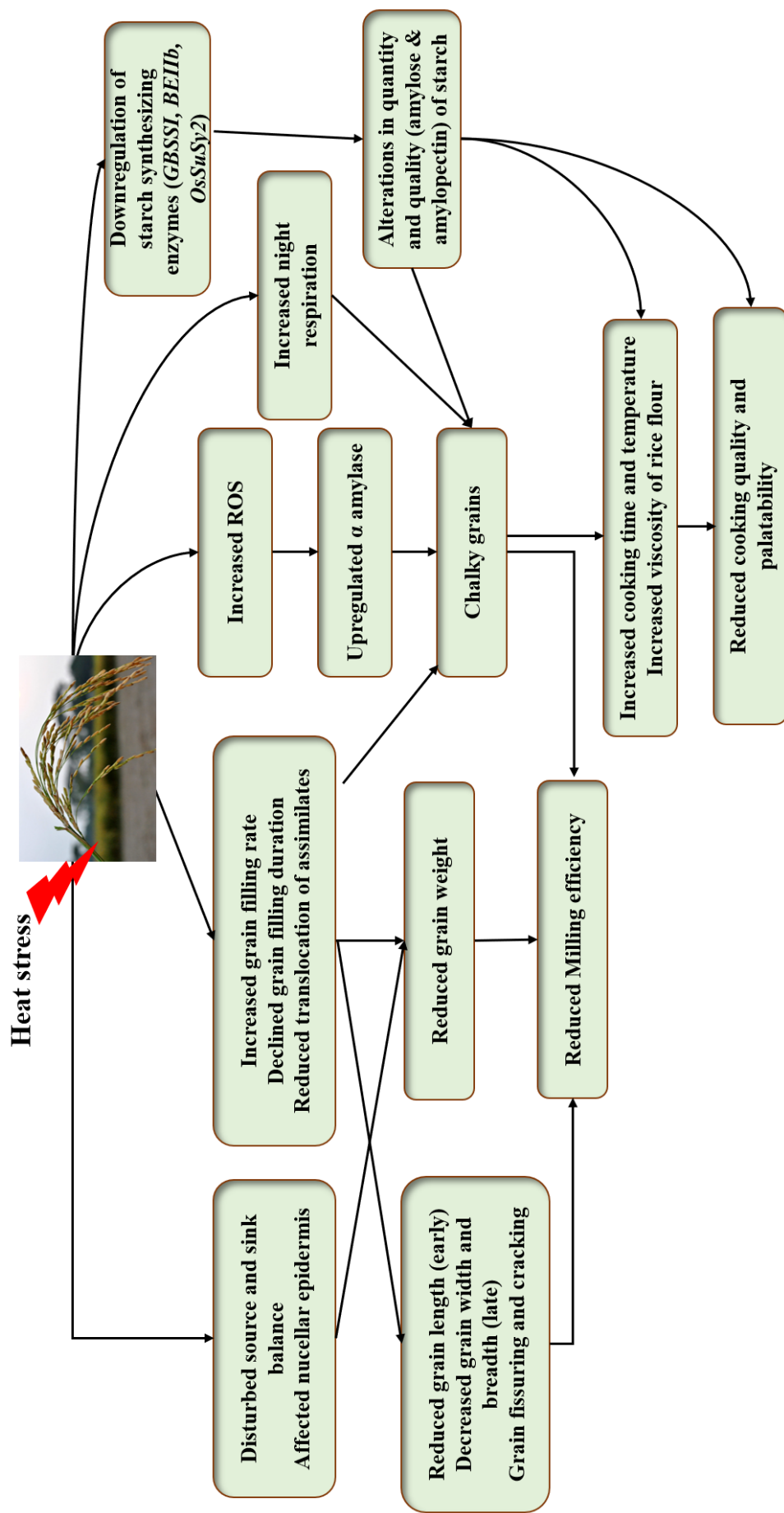


Figure 2.3 Effect of heat stress on grain development of rice plants

decline in the water content of the grains under high temperature leading to disorganization of amyloplasts (Lisle et al., 2000; Funaba et al., 2006; Ishimaru et al., 2009). However, it was demonstrated that this will be managed by the cell *in situ* by accumulating osmolytes such as cysteine, ascorbic acid and glutathione (Wada et al., 2019). All the above chalky grain types are more frequent in inferior spikelets than the superior ones (Lisle et al., 2000).

2.3.3 Disrupted trade-off between source and sink under heat stress

Another reason for milky white grains is due to the paucity of assimilates to feed the accelerated grain filling under high temperatures (Kobata et al., 2004a&b; Tsukaguchi et al., 2011; Xiong et al., 2017). This decrease in the substrate might be due to an increase in dark respiration triggered by high night temperatures. Genotypes differ in the way they handle decreased substrate supply during heat stress. For instance, IR60 was found to reduce (almost half) the number of spikelets leading to optimum accumulation of photosynthates and significantly lesser chalky grains (Fitzgerald and Ressureccion, 2009). In contrast, the decreased grain filling was proposed mainly due to lack of sink activity, but not due to the lack of assimilates, since active photosynthetic activity even after the cessation of grain filling could be observed from the delayed flag leaf senescence (Kim et al., 2011). Furthermore, nucellar epidermis was also affected under high temperature indicating a clear disruption in the assimilate transit to the developing grains (Zakaria et al., 2002; Tanaka et al., 2009; Tanamachi et al., 2016). This effect on mobility of assimilates was due to the downregulation of sucrose transporter genes, *SUT1* and *SUT2*; ADP-glucose translocator, and *BT1-2* expression (Yamakawa and Hakata, 2010). A negative relationship was observed between the protein content (nitrogen content) of brown rice and the incidence of white-back kernels (Wakamatsu et al., 2007). Application of nitrogen indirectly enhances protein synthesis including enzymes of starch synthesis since disrupted accumulation of protein bodies in protein-storage vacuoles also contribute to chalky or floury appearance of rice grains under high temperature (Wada et al., 2019).

2.3.4. Derailed starch synthesis and its structural characteristics

Starch occupies a significant portion of the rice seed (~80%) and any change in the structural characteristics of starch will seriously compromise rice grain quality. Scanning electron microscopy of chalky grains reveals loosely packed, rounded starch granules with intermittent small pits or airspaces (particularly at 21 days after heading/milky stage; Abayawickrama et al., 2017; Tashiro and Wardlaw, 1991). These loosely packed areas look

opaque at maturity leading to a chalky appearance. This is attributed to disappearance or disturbance of enzymes catalysing various steps of starch synthesis in the maturing grains, thus affecting the starch deposition in rice grains leaving pits or airspaces. And starch is the deciding factor guiding the eating and cooking quality of rice (Asaoka et al., 1984, 1985, 1989; Inouchi et al., 2000; Lisle et al., 2000; Umemoto and Terashima, 2002; Cheng et al., 2005a & b; Yamakawa et al., 2007; Abayawickrama et al., 2017; Shi et al., 2017; Fan et al., 2020). The effects of heat stress on amylose content are variety dependent (Cheng et al., 2000; Zhong et al., 2005). The amylose contents of high amylose varieties increase under high temperature and vice-versa (Zhong et al., 2005). Transcriptomic analysis revealed a significant downregulation of key genes – granule-bound starch synthase (GBSSI) (amylose), starch branching enzyme (BEIIb) (amylopectin), *OsSuSy2* which disturbs the synthesis of starch (Yamakawa et al., 2007; Tanamachi et al., 2016). The sensitivity of starch branching enzymes to high temperature is of the order BEIIb > BEIIa > BEI (Ohdan et al., 2010). The decreased activity of GBSSI is due to the downregulation of the *Wx* gene under high temperature (Ahmed et al., 2015). This sensitivity to high temperature stems from heat sensitive SNP (G to T polymorphism) within this *Wx* locus which leads to disturbed alternative splicing (Larkin and Park, 1999). Using CSSLs derived from heat stress tolerant *indica* 9311 in the background heat stress sensitive *japonica* Nipponbare, four QTLs were identified which could restore and enhance the splicing efficiency of the *Wx* gene transcript (Zhang et al., 2014). In addition to these, other enzymes such as AGPase, UGPase, sucrose synthase (SuSy), ADP-glucose pyrophosphorylase (ADPG-Ppase), soluble starch synthase (SSS), starch de-branching enzyme (SDBE) and starch phosphorylase (SPase) are equally important in sustaining starch synthesis and accumulation under high temperature (Cheng et al., 2005b; Yamakawa and Hakata, 2010). However, Jiang et al. (2003) reported that SSSI was unaffected by high temperature (29/35°C) in a non-waxy genotype which could ensure the extension of A and B chains of amylopectin (Jiang et al., 2003).

Heat stress disrupted the delicate balance in amylose and amylopectin synthesis (Cheng et al., 2005a; Fan et al., 2020). Heat stress reduced the amylose content and slightly altered the fine structure (reduced short chains but increased long B chains) of amylopectin (Asaoka et al., 1984; Inouchi et al., 2000; Jiang et al., 2003; Liu et al., 2010; Chun et al., 2015; Fan et al., 2020). These conformational changes in amylopectin structure are mainly attributed to declined activity of starch branching enzyme (Zhang et al., 2006). This is not

true in the case of basmati rice as reported by Ahmed et al. (2015) who found that effect of GBSS was the key player in maintaining the grain quality of basmati rice. There was a significant decrease in compactness of amylopectin structure and crystallinity of rice flour. This coupled with increased prolamins (Lin et al., 2005) in turn resulted in higher gelatinization temperature (due to higher longer chains in amylopectin), higher cooking temperature, and longer cooking time, higher viscosity (increased peak and trough viscosities, but decreased setback) of rice flour. Increased gelatinization also stems from dense packing of starch granules under high temperature which restricts their hydration and swelling (Zhong et al., 2005). This ultimately resulted in deteriorated cooking quality and palatability of rice (Zhong et al., 2005; Chun et al., 2015). Thus, high temperature has a comparatively higher effect on starch composition than starch accumulation in developing rice grains (Cheng et al., 2005b).

Further, the grain chalkiness seems to be induced by ROS which triggers α amylase (and β amylase; Wang et al., 2019) activity (Yamakawa et al., 2007; Liu et al., 2010; Hakata et al., 2012; Tanamachi et al., 2016; Mitsui et al., 2016) mediated by GA/ABA which disturbs amylose/amylopectin ratio in rice grain (Suriyasak et al., 2017). Increased α amylase activity was predicated by enhanced expression of the corresponding genes *Amy1A*, *Amy1C*, *Amy3A*, *Amy3D* and *Amy3E* under high temperature (Yamakawa et al., 2007; Yamakawa and Hakata, 2010) and reduced ABA accumulation, a repressor of α amylase (Hakata et al., 2012). However, the α amylase triggered starch degradation seems to occur towards the later stages of grain maturity (Ishimaru et al., 2009). Finally, the initiation and packaging of amyloplast with starch will also be affected by high temperature (Lisle et al., 2000). In addition to these, energy starvation for starch synthesis via downregulation of cytochrome respiration chain was observed under high temperature (Yamakawa and Hakata, 2010). Thus, heat stress tilts the balance between starch synthesis and hydrolysis more towards starch hydrolysis.

2.3.5. Effect on grain protein content

In general, high temperature affects protein homeostasis resulting in protein denaturation, ROS mediated protein oxidation, protein misfolding and increased accumulation of heat shock proteins (HSPs). The effect of high temperature on protein content of the grain seems variable with an increase in content early while a decline particularly of prolamins was observed at a later stage of grain maturation (Lin et al., 2010).

The decline in protein synthesis, except HSPs, was also evident from the decreased synthesis of aminoacyl tRNA synthesis (Yamakawa and Hakata, 2010).

2.4. Physiology of heat stress tolerance/sensitivity in rice

2.4.1. Regulation of stomatal conductance and transpirational cooling

Maintenance of lower panicle temperatures vis-à-vis air temperatures is one of the important physiological mechanisms underpinning high temperature avoidance (Yoshimoto et al., 2012; Sailaja et al., 2015). This results from greater stomatal conductance and enhanced transpirational cooling. The *indica* genotypes show relatively higher tolerance compared to *japonica* genotypes attributable to greater stomatal conductance in *indica* genotypes (Shimoda 2012; Maruyama et al., 2013).

High stomatal conductance helps *indica* genotypes to maintain wide panicle and air temperature difference and keep panicles at a lower temperature. Superior transpirational cooling of flag leaf resulted in 4°C lower temperature in comparison with panicles reflecting relatively more avoidance capacity of flag leaf than panicle. (Zhang et al., 2016). The temperature of rice organs during the grain filling stage was negatively correlated with spikelet fertility (Chuan et al., 2008). However, this lower spikelet temperature relative to ambient air temperature seemingly plays trivial role in safeguarding plants against high temperatures; the reproductive resilience of the genotypes is the one that matters the most (Shi et al., 2018).

2.4.2. Effect on chlorophyll content and photosynthesis

Decreased photosynthesis is one of the important repercussions of heat stress (Herman et al. 2013; Sailaja et al., 2015). This was attributed to the deactivation of Rubisco due to the inactivation of Rubisco activase, which is highly thermolabile (Salvucci and Crafts-Brander, 2004a and b; Sharkey, 2005; Kurek et al., 2007) and/or injury to the electron transport chain (ETC) (Wise et al., 2004; Cen and Sage, 2005). Further, thermal damage to the PSII is also one of the reasons for the inhibition of photosynthesis under heat stress. The D1 subunit of PSII, encoded by chloroplast gene *psbA* is particularly vulnerable to heat stress (Chen et al. 2020). However, PSII seems to have become vulnerable mostly to higher temperatures (>45°C) (Čajánek et al., 1998, Yamane et al., 1998). Light reaction was affected more than the dark reaction, but carbon assimilation was maintained in certain genotypes with strong upregulation of Rubisco activase (Scafaro et al., 2010). Heat stress also decreased the chlorophyll content due to its effect on the biosynthesis of 5-

aminolevulinic acid (ALA) and protoporphyrin LX (PPIX) and LAI, hence net photosynthetic rate. However, the decrease in leaf photosynthesis and stomatal conductance under high temperatures imposed at panicle primordial initiation and grain filling stages did not match SPAD and Fv/Fm values which are relatively unaffected (Restrepo-Diaz and Garces-Varon, 2013).

2.4.3. ROS production and antioxidant mechanism

The reduction in photosynthesis translates into over reduction of ETC leading to the accumulation of reactive oxygen species (ROS) (Allakhverdiev et al., 2008). Improved scavenging of ROS through the antioxidant machinery was frequently reported to confer high temperature tolerance in rice (Bahuguna et al., 2015; Sailaja et al., 2015; Zhang et al., 2016; Karwa et al., 2020). Superior antioxidant mechanism evidenced by the lower accumulation of H₂O₂, TBARS (proxy of lipid peroxidation) and higher accumulation of polyamines (putrescine, spermidine, and spermine) and antioxidant enzyme activity (ascorbate peroxidase (APX) and guaiacol peroxidase (GPX)) was found responsible for high temperature tolerance (Karwa et al., 2020). Negating this, polyamine accumulation (supported by increased expression level of ADC2 and ODC1) was found more in sensitive genotypes (Glaubitz et al., 2015). Thus, the role of polyamines in high temperature responses of rice should be elucidated in future studies. Enhanced activity of antioxidant enzymes and photosynthesis in leaves, ATPase activity in grains, lower leaf temperature and higher root activity were found to be the reasons for higher tolerance in the Huanghuazhan and T226 than their sensitive counterparts Shuanggui 1 and T129 (Yun-Ying et al., 2009).

2.4.4. Role of plant growth regulators

Modulation of phytohormones under heat stress was elucidated in few studies. Heat stress decreased active cytokinins, gibberellin A1 (GA1), and indole-3-acetic acid (IAA) and increased abscisic acid (ABA) and bound cytokinins in young panicles. Cytokinin was found to be a key player in maintaining reproductive biology of rice plants under heat stress through its influence on panicle exertion, spikelets per panicle, spikelet fertility, grain weight and grain yield. This was evident from the improved spikelet fertility of sensitive genotype Liangyoupei9 on exogenous application of 6-benzyl amino purine (6-BA) (Wu et al., 2016). Reduced exertion of panicle was also attributed to a localised increase in the levels of ethylene and decreased GA1 and IAA levels (Panda et al., 2009; Rang et al., 2011).

A similar decline in the contents of IAA, ZR and GA and an increase in ABA were reported during the early grain filling stage as well. Decrease in IAA and GA is detrimental to pollen function leading to its abortion; whilst ABA ensures proper anther development and may also incite pollen abortion if necessary (Tang et al., 2008). Thus, the exogenous application of auxin reportedly alleviates the heat stress induced damage to the reproductive organs in rice (Sakata et al., 2010). Application of NAA completely reversed high temperature (40°C) induced spikelet sterility by resuming the ceased pollen tube growth in style in the heat stress sensitive mutant of Nipponbare (Zhang et al., 2018). Further, a possible crosstalk between ROS and auxins in ensuring pollen germination was also proposed. The plausible role of auxins in pollen germination and pollen tube growth was also previously suggested (Wu et al., 2008). Exogenous application of another growth regulator, salicylic acid was demonstrated to safeguard rice plants against high temperatures by alleviating pollen abortion through preventing ROS induced programmed cell death (PCD) of tapetal cells and enhancing the accumulation of auxins (IAA), zeatin (ZR) and sugars. This response is mediated by H₂O₂ which acts as a basal trigger for antioxidant machinery (Fu et al., 2015; Zhang et al., 2017; Feng et al., 2018). Role of ethylene in conferring heat stress tolerance in rice seedlings was briefly studied wherein it was demonstrated that exogenous application of ethylene precursor (ACC) successfully ameliorated high temperature induced damage to rice seedlings in terms of fresh weight, chlorophyll a/b ratio and antioxidant activity. This was attributed to ethylene induced increase in the expression of various heat shock transcription factors such as *HSA1a* and *HSF2a*, c, d, e and f reflecting the role of ethylene in heat stress signalling (Wu et al., 2019). Further, higher ROS production and decreased Q enzyme and IAA levels in superior spikelets resulted in reduced grain filling in superior spikelets than in the inferior spikelets (Fu et al., 2016).

2.4.5. Disruption of membrane integrity

Cell membrane structure and function were destroyed under heat stress resulting in the relaxation of membrane conductivity (Xie et al., 2011). The elongation rate of third leaf was decreased even in extremely thermo-resilient *O. meridionalis* by 47% over 24 hr period at 45°C. A decrease in MSI and proteins and an increase in sugar content were documented in pollen grains when exposed to high temperatures (Das et al., 2014). Though high temperature affects membrane fluidity and permeability, the heat stress tolerance or sensitivity of cultivars during reproductive stage showed no relation with cell membrane thermostability of mature flag leaves (Prasad et al., 2006).

Thus, efficient scavenging of ROS, maintenance of photosynthesis coupled with efficient transpirational cooling of leaves (and panicles), synthesis and accumulation of protective cellular components like proline, glycine betaine, heat shock proteins and plant growth regulators seems to be the important physiological mechanisms contributing to the survival of rice plants under high temperature.

2.5. Mechanisms of high temperature tolerance in rice

Rice plants utilize different mechanisms to overcome heat stress such as true tolerance by maintaining appreciable economic yield under heat stress conditions (Jagadish et al., 2010), avoidance through transpirational cooling and long growth duration (Matsui et al., 2007; Fukuoka et al., 2012; Julia and Dingkuhn, 2013; Sathishraj et al., 2016; Huang et al., 2016) and escape through the time of day of flowering or the early morning flowering (EMF) trait (Ishimaru et al., 2010; Julia and Dingkuhn, 2012; Hirabayashi et al., 2015) (Figure 2.4). Transpirational cooling of panicle and canopy also serves as an avoidance mechanism for high temperature induced post-harvest losses in rice (Zhao and Fitzgerald, 2013). However, this is practically more feasible in low land paddy ecologies with a luxurious supply of water. But the utilization of this trait is questionable since there is an increasing emphasis these days on water-saving paddy technologies such as directed seeded or aerobic rice (Jagadish et al., 2015). This mechanism can be efficiently exploited by indirectly selecting genotypes with superior canopy architecture with a greater number of leaves in the vicinity of the panicle so that panicles are provided with cooler microclimate. Cleistogamy was also proposed as an avoidance mechanism wherein the closed spikelets registered $\sim 2^{\circ}\text{C}$ lesser temperature than outside (Koike et al., 2015). Even the transpirational conductance of rice panicle, apart from the leaves, was also reported to contribute to heat stress avoidance in rice (Fukuoka et al., 2012). Other avoidance mechanisms include asynchronous tiller and panicle development and staggered flower opening of different spikelets within the same panicle (Shah et al., 2011). In general, true tolerance has been largely adjudged through spikelet sterility of the test genotypes under high temperature in many reports. Such tolerance is achieved by adopting mechanisms like efficient scavenging of ROS, chaperoning native proteins by eliciting heat shock response etc. At a molecular level, several heat shock proteins and the corresponding heat shock factors were reported in rice which chaperone high temperature induced protein modification and confer heat stress tolerance (Hu et al., 2009; Jagadish et al., 2011; Chen et al., 2014b; Jung et al., 2013; Zhang et al., 2016, 2017). An eclectic combination of all

the above three mechanisms will be more sustainable and lucrative than relying solely on a single mechanism to breed heat stress resilient rice genotypes.

2.6. Genetic variation for heat stress tolerance at reproductive stage in rice

Presence of genetic variation is an important prerequisite for any plant breeding activity. There are numerous reports on the genetic variation in rice germplasm for various traits related to high temperature tolerance. Though geographical origin of the genotype played no role in tolerance or sensitivity of genotypes (Shi et al., 2015b & Prasad et al., 2006) and Weerakoon et al. (2008) and Xu et al. (2020) did find such sub-type specific sensitivities for heat stress; *indica* subspecies can be safely considered more tolerant to high temperature than other ecotypes, because of their adaptation to tropical and subtropical climates. *Indica* varieties showed higher T₇₅ (temperature at which spikelet fertility is 75%) values than *japonica* varieties reflecting the greater tolerance of *indica* genotypes to high temperatures (Maruyama et al., 2013). However, Matsui et al. (2005) proposed more tolerance of *japonica* genotypes than the *indica* genotypes; though, their sample was significantly skewed towards *japonica* types. Further, it was observed by Baker (2004) that US rice varieties were more sensitive to high temperatures than the Asian *indica* and *japonica* cultivars. Upland rice cultivars are reported to tolerate high temperatures than the low land paddy genotypes (Das et al., 2014).

Genetic variability for heat stress tolerance in rice was reported as early as the 1980s. For instance, Jennings et al. (1979), found the variety, Hoveyzeh from southern Iran to be tolerant at temperature > 45°C while other varieties were completely sterile. Yoshida et al. (1981) demonstrated the tolerance of varieties such as Agbede, Carreon, Dular, N22, OS4, P1215936, and Sintiane Diofor with superior per cent spikelet fertility (84 to 90%) even at high temperatures. This was closely followed by other seminal reports of Satake and Yoshida (1978) and Mohammad and Avesi (1985) who further elucidated the heat stress tolerance mechanisms of tolerant genotypes. N22 is the most frequently proposed as high temperature (both day and night) tolerant variety at all growth stages – seedling, vegetative and reproductive. This extreme resilience was attributed to its efficient reproductive success even under extremely high temperature, effective physiological malleability including the invocation of antioxidant machinery, higher photosynthesis and transpiration. This genotype was also reported to suffer less during grain filling, efficiently sustaining grain quality.

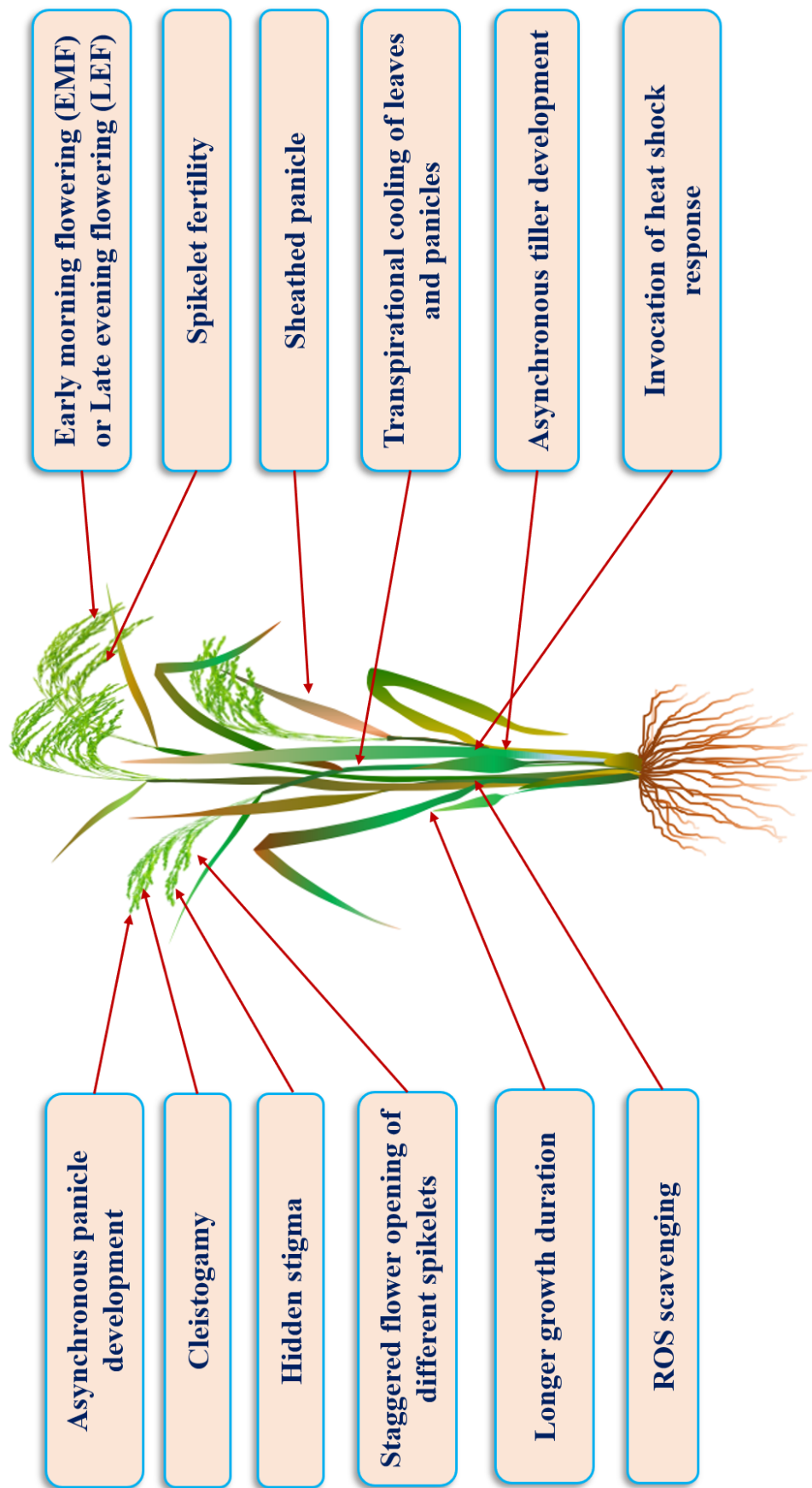


Figure 2.4 Mechanisms (escape, avoidance and true tolerance) of heat stress tolerance in rice

Though conflicting reports emerged regarding its seedling stage heat stress tolerance (Hsuan et al., 2019), it is the most popularly utilized genotype for basic and applied heat stress research in rice (Yoshida et al., 1981; Jagadish et al., 2008; Madan et al., 2012; Sailaja et al., 2015; Coast et al., 2016; Kilasi et al., 2018). However, N22 was found sensitive to high temperatures at early reproductive stage coinciding with panicle initiation/differentiation (Wu et al., 2016) in stark contrast to its tolerance at flowering stage which need further validation. Barring those reported in basic research studies, the genetic variability reported for high temperature tolerance (for reproductive stage) was summarized in Table 2.1.

Hybrids seem to be more resilient to high temperature compared to corresponding purelines or inbreds. This may be due to their greater buffering capacity to environment. When a rice hybrid, IR75217H was compared for its high temperature tolerance with N22 and IR64, the rice hybrid showed moderate tolerance better than IR64. Presence of a greater number of spikelets could offset the reduced spikelet fertility in hybrid (Madan et al., 2012). Conversely, Shi et al. (2018) found hybrids to be more vulnerable to heat stress than the high yielding inbreds. Wild relatives are rich sources of abiotic stress tolerant genes as they are adapted to adverse environmental ecologies and survived tests of the time. The heat stress tolerance of *O. meridonalis*, an Australian wild race has been established in comparison with *O. sativa* ssp. *japonica* var Amaro when exposed to temperature of 45°C (Scafaro et al., 2010). A variety, Sathi with sheathed panicle phenotype recorded higher tolerance to high temperature than IR64 and N22 in terms of spikelet fertility, number of pollen grain landing on the stigma, normal grain thickness, low chalkiness, high starch synthase and invertase activity making it a potential heat stress tolerant donor for future studies (Lawas et al., 2018). Matsui et al. (2007) reported the heat resilience of the variety Langi despite extremely high temperatures of Australia (~40°C) was due to low panicle temperature rendered by evaporative cooling under high VPD conditions and long basal dehiscence of thecae. The number of genotypes studied in various studies never crossed 500 and it can be averaged around 80 genotypes indicating huge variability for high temperature tolerance remains untapped.

2.7. Phenotyping for high temperature tolerance: screening protocols and selectable phenotypic markers

The phenotypic screening for heat stress tolerance has been well elaborated and documented in numerous studies (Jagadish et al., 2007; Jagadish et al., 2008; Ye et al.,

2012; Jagadish et al., 2014; Hakata et al., 2017). Temperature treatment of 39°C is suitable for screening at the booting stage, while 38°C is suitable for flowering-stage assessment (Tenorio et al., 2013). Natural field conditions with seasonal and local high temperature hotspots were utilized in many studies to evaluate genotypes. In several other studies, controlled environment structure such as walk-in chamber, high temperature tunnels, growth chambers, phytotrons etc was utilized to mimic natural field conditions (Table 2.1). Few studies reported intricate designs of artificial structures erected in the field to increase the temperatures of crop microclimate (Chiba and Terao, 2014; Hakata et al., 2017). Spikelet fertility is the most frequently used trait for assessing heat stress tolerance of genotypes, though yield and several physiological traits were utilized for assessing heat stress tolerance. However, Prasanth et al. (2017b) maintained that both spikelet fertility and grain yield are equally important since some genotypes may have similar spikelet fertility under control and high temperature treatments but show superior yield under stress treatment compared to sensitive genotypes. Size of the anthers and basal pore size are easily observable traits and can also be used for selecting tolerant genotypes. Jagadish et al. (2013) proposed a phenotypic marker where a distance of -8 to -9 cm between the base of the last fully opened leaf and the flag leaf collar, which coincides with microsporogenesis in majority of the genotypes, can be utilized to identify the stage for imposing stress. Infra-red thermal imaging was used to track canopy temperature and chlorophyll fluorescence for photochemical quantum yield of PSII was used as surrogate to screen genotypes for high temperature tolerance (de Brito et al., 2019). Heat stress susceptibility index and cumulative stress responsive index were also used to judge heat stress tolerant and sensitive genotypes (Karwa et al., 2020). Leaf elongation was measured to compare heat tolerance stress between genotypes (*O. meridionalis* and *O. sativa* ssp. *japonica* var. Amaro) (Scafaro et al., 2010). To track the tissue temperature in panicles the use of thermocouples in leaf sheath and spikelets was suggested by Shi et al. (2015b). Pollen fertility was also proposed to be a suitable marker for judging heat stress tolerance (Das et al., 2014).

Chalkiness is the most frequently used trait for assessing rice grain quality under high temperature which showed significant variability among the genotypes (Iida et al., 2002). It reflects the disturbed starch synthesis and impaired accumulation and inadequate protein accumulation in grains and subsequent losses in hulling and milling and the final palatability. Membrane injury and canopy temperature depression (CTD), the physiological traits indicating susceptibility and tolerance respectively, can be utilized for selecting heat stress tolerant genotypes (Bahuguna et al., 2015; Sailaja et al. 2015; Vivitha et al. 2017;

Hsuan et al., 2019). Other physiological traits such as photosynthetic parameters, ROS and antioxidant enzymes were also employed to understand the tolerance mechanism of genotypes (Bahuguna et al., 2015; Sailaja et al., 2015; Karwa et al., 2020).

2.8. QTL mapping for high temperature tolerance

2.8.1. DNA based marker systems

Several types of DNA or molecular markers were developed and tested in rice, starting from Restricted Fragment Length Polymorphism (RFLP) markers to the most recent single nucleotide polymorphism (SNP) markers. RFLP utilizes a restriction enzyme and a labelled probe combination to detect polymorphism among the tested genotypes. With the invention of polymerase chain reaction (PCR), several PCR based markers became popular such as rapid amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR), among others. RFLP dominated fingerprinting and mapping studies in the initial years which was later replaced by PCR based markers. Using a restriction enzyme and hybridization probe combination RFLP detects polymorphism among the genotypes. RFLP markers played significant role in classification of several rice species and the construction of initial linkage maps (Wang et al., 1992; Jain et al., 2010). RAPD markers were used to check genetic diversity among a set of rice genotypes (Yu and Nguyen, 1994; Mackill, 1995; Raghunathachari et al., 2000) and for tagging and mapping of genomic regions governing target traits (Monna et al., 1994, 1995; Subudhi and Huang, 1999). RAPD uses a random set of primers (known as decamers) to amplify regions between two aligned primers in a polymerase chain reaction (PCR). AFLP is selective amplification (through PCR) of restricted fragments. AFLP markers were primarily used for understanding genetic diversity and DNA fingerprinting of rice genotypes (Zhu et al., 1998). The evolution of SSR markers has brought in a paradigm shift in the mapping studies in rice. SSRs have replaced all other markers and become the markers of choice for linkage mapping, QTL mapping, DNA fingerprinting and genetic diversity studies. An elaborate SSR based linkage map of rice was published by McCouch et al (2002). The generation and compilation of the rice genome (*japonica* rice variety Nipponbare) by the international rice genome sequencing project (IRGSP) in 2005 further added 18, 828 SSR markers. This has extended the utilization of SSRs to wild relatives of rice. SSR markers are extensively used

even today for marker assisted selection in rice owing to ease in handling and scoring system.

Next generation sequencing (NGS) techniques popularized another sequence-based marker – single nucleotide polymorphism (SNP) markers. SNP genotyping either through resequencing (by for instance genotyping by sequencing (GBS)) or chip-based sequencing has gained significant traction in the genomics and post-genomics era. Several SNP chips are available in rice such as 1M (McCouch et al., 2010), 44K (Tung et al., 2010), 50K (Chen et al., 2014a; Singh et al., 2015), 6K (Yu et al., 2014), 5K (Kumar et al., 2015a), and the most recent 80K (Daware et al., 2022). These SNP genotyping arrays have become widely popular these days due to simple analysis procedures and low computational burden. Array based genotyping is currently being used for genome wide association studies, biparental mapping and marker assisted selection (background selection in MAS) in rice.

2.8.2. QTLs for tolerance

Genetics of heat stress tolerance in rice was elucidated by evaluating parents and hybrids under high temperatures. It was proposed that high temperature tolerance in rice is under recessive gene control. But there are contradictory reports specifying the role of male and female parents in contributing to the tolerance in hybrids (Hu et al., 2012; Guan-fu et al., 2015). Heat stress tolerance in rice is a quantitative trait governed by a multitude of genes with cumulative effects on a trait popularly known as Quantitative Trait Loci (QTL). Several studies have reported distinct genomic regions governing high temperature tolerance in rice, primarily at three growth stages (1) seedling stage and/or vegetative stage; (2) reproductive and (3) post-reproductive stage. However, in line with present research work, only reports on reproductive stage were discussed below.

The earliest report was of Cao et al. (2002), who described a QTL governing spikelet fertility/seed setting from IR64, an elite *indica* cultivar released by International Rice Research Institute (IRRI, Manila, Philippines). Another report came from the same group (Cao et al., 2003) where a doubled haploid population derived from IR64/Azucena was evaluated under both field and greenhouse conditions for heat stress tolerance. Six additive effect QTLs on chromosomes 1, 3, 4, 8 and 11 were identified with QTL *qhr11-2* reportedly explaining 23% of the phenotypic variance and eight pairs of epistatic QTLs were detected. These are subsequently followed by reports from Chang-Lan et al. (2005), Zhao et al. (2006), Qing-quan et al. (2008), Tao et al. (2008), Zhang et al. (2009), Jagadish et al. (2010b) and Xiao et al. (2011a & b). Zhang et al. (2009) utilized bulked segregant analysis

(BSA) and tagged the genomic region governing seed set rate under high temperatures with two markers RM3735 and RM3586. Majority of these studies utilized either RFLP or SSR markers for genotyping respective populations.

SNP markers were first utilized for QTL mapping of heat stress tolerance by Ye et al. (2012) who could delineate two major QTLs on chromosome 1 (*qHTSF1.1*) and 4 (*qHTSF4.1*) in BC₁F₁ and F₂ populations generated from a cross IR64 / N22, which could explain 12.6% and 17.6% of the variation in spikelet fertility under high temperature respectively. Furthermore, they could later validate *qHTSF4.1* in the BC₁F₂ population generated from the same cross and in different backgrounds (IR64/Giza178 and IR64/Milyang23/Giza178; Ye et al., 2015a) and fine map it to a narrow interval of 1.2 Mb (Ye et al., 2015b). *qHTSF4.1* was also found to be a major QTL in an F₂ population derived from the cross between Takanari and IR64 (Takai et al., 2020). Similarly using a custom-made 5K SNP chip and indices (tolerance and sensitivity) Shanmugavadivel et al. (2017) reported Several novel QTLs were reported on chromosomes 3 (*qSSiY3.1*), 5 (*qSTiY5.1*, *qSSiY5.1*), 9 (*qSTiPSS9.1*) and 12 (*qSSiPSS12.1*) in a RIL population derived from IR64 / N22. Between these two studies, many other independent reports were published utilizing primarily SSR markers and unique traits like pollen viability, anther characteristics etc (Cheng et al., 2012; Poli et al., 2013; Buu et al., 2015; Cao et al., 2015; Tazib et al., 2015; Zhao et al., 2016; Prasanth et al., 2017a; Zhu et al., 2017; Li et al., 2018). Recently, another major QTL, *qHTB1-1* which explained 13.1 to 17.8% of phenotypic variation in different generations were identified using *O. rufipogon* accession HHT4 (Cao et al., 2020). It was fine mapped to a region of 47.1 kb between RM11633 and RM11642. Using BSA-Seq four QTLs were identified for spikelet fertility on chromosomes 1 (*qSF1*), 2 (*qSF2*) and 3 (*qSF3.1* and *qSF3.2*) using an F₂ derived from Sinlek (sensitive) / M9962 (tolerant) (Nubankoh et al. 2020). Similarly, another QTL, *qHTT8* was reported on chromosome 8 governing spikelet fertility under heat stress in the F₂ population developed from a cross between HHZ (heat-tolerant) and 9311 (heat-sensitive) using BSA-Seq (Chen et al., 2021). Spikelet fertility, either directly or indirectly (filled grains per panicle) is the commonly used proxy for heat stress tolerance QTL mapping. Further details of these studies are summarized in Table 2.2 and physical locations of these reported QTLs are depicted in Figure 2.4.

Table 2.1 List of reports on genotypes screened for reproductive stage high temperature tolerance in rice

Trait	Temperature stress	Phenotyping methodology	Growth stage	No. of genotypes screened	Tolerant genotypes identified	Reference
Anther characteristics, Spikelet fertility	37.5 & 40°C	Phytotron	Reproductive	9	Nipponbare and Akitakomachi	Matusui et al., 2001; Matsui and Osama, 2002
Yield, spikelet fertility and physiological traits	~38°C	Greenhouse	Reproductive	14	N22	Prasad et al., 2006
Spikelet fertility	35 & 38°C	Controlled environment	Reproductive	18	N22	Jagadish et al., 2008
Flowering period, peduncle length and panicle exertion	38°C for 6 hr	Growth chamber	Reproductive	5	N22	Rang et al., 2011
Green leaf area, shoot biomass and panicle biomass	31-34°C	Growth chamber	Vegetative and reproductive	18	IR64, IR72, N22, Vandana, Takanaari and Koshihikari	Pasuquin et al., 2013
Spikelet fertility	38-39°C	Growth chamber	Reproductive	455	booting – Dular and Todorokiwase; flowering – Milyang23 and IR2006-P12-12-2-2; Giza178 - both the stages	Tenorio et al., 2013
Pollen morphology, physiology and biochemistry	35/25, 40/30, 45/35, 50/40 °C	Greenhouse	Reproductive	6	-	Das et al., 2014
Spikelet fertility, grain yield and its contributing traits and quality	38 to 42°C	Controlled environment	Reproductive	71	N22 and NL44	Bahugana et al., 2015

Trait	Temperature stress	Phenotyping methodology	Growth stage	No. of genotypes screened	Tolerant genotypes identified	Reference
Grain yield and its attributes, physiological and biochemical traits	5°C higher than ambient	Polyhouse	Reproductive	11	N22, Jaya, MTU1010 and Rasi	Sailaja et al., 2015
Spikelet fertility	38°C	Greenhouse	Reproductive	18	Ciherang	Shi et al., 2015
Sterility %, flowering date and flowering period	~38°C	Field conditions		292	-	Bheemanahalli et al. (2016)
Grain yield and quality related traits	~33°C	Field conditions	Reproductive	11	WYG24, YG4227, and NG44	Huang et al., 2016
Spikelet fertility	38°C	Both field and controlled conditions	Reproductive	240	CR3621-6-1-3-1-1, CR3820-2-1-5-1-2, CR3813-4-4-2-2, CR3820-4-5-3-1-3, AC.39890, AC.39973, AC.39790 and N22	Pradhan et al., 2016
Growth and yield parameters	34-37°C	Growth chamber	Reproductive	11	IRGC 105491	Wang et al., 2016b
Spikelet fertility, grain yield and its contributing traits	34 to 37°C	Field conditions	Reproductive	7	SPT1, NP1, R258 and SKN	Sukkeo et al., 2017
Yield and its contributing traits	35/25°C	Glasshouse	Reproductive	11	YRM 67, Koshihikari and Opus	Ali et al., 2019
Yield and grain quality	40°C to 45°C for 6 hr	Greenhouse	Vegetative and reproductive	169	N22, AUS17, M9962, SONALEE and AUS16	Cheabu et al., 2018
Spikelet fertility	40 - 45°C	Field and greenhouse	Reproductive	10000 mutant lines	M9962	Cheabu et al., 2019

Trait	Temperature stress	Phenotyping methodology	Growth stage	No. of genotypes screened	Tolerant genotypes identified	Reference
Spikelet fertility, grain yield and its contributing traits	35 to 40°C	Field conditions	Reproductive	182	LTB 14301 and BRS Pampa	de Brito et al., 2019
Yield, physiological and biochemical traits	39 - 44°C	Controlled environment	Reproductive	36	IET22218	Karwa et al., 2020
Pollen fertility and germination and spikelet fertility	24, 28, 30, and 33°C	Phytotron	Reproductive	11	-	Thuy et al., 2020
Yield contributing characters	≥35°C	Field conditions	Reproductive	198	JR345, Varun Dhan, PC19, Ratnagiri-4, N22 and Annapurna	Chidambaranathan et al., 2021
Morpho-physiological and agronomic traits	36 - 39°C	Field conditions	Reproductive	6	BRIZ-8B and BRIZ-10B	Ezin et al., 2022
Morpho-physiological and agronomic traits	40–45 °C	Field conditions	Reproductive	16	NGSR-16 and NGSR-18	Zafar et al., 2022

Table 2.2 QTLs reported for traits associated with heat stress tolerance in rice

Population	Donor	Population	Mapping trait	QTL	Chr	Marker type	LOD	R ²	Reference
IR64 / Azucena	Azucena	DH	Heat tolerance	<i>qhr1</i>	1, 3,	RFLP	10.2	-	Cao et al., 2003
				<i>qhr3-1</i>	4, 8,		6.4		
				<i>qhr4-3</i>	11, 11		6.4		
				<i>qhr8-1</i>			5.2		
				<i>qhr11-1</i>			7.0		
				<i>qhr11-2</i>			10.7		
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Grain weight heat susceptibility index	<i>qHT1</i>	1, 4, 7	RFLP	8.2	-	Chang-lan et al., 2005
				<i>qHT4</i>			11.1		
				<i>qHT7</i>			12.9		
USSR5/ Guangjie 9// USSR5	Guangjie 9	BCs	Spikelet fertility in nature Spikelet fertility in high temperature Relative spikelet fertility	<i>qss-4</i>	4, 5	SSR	4.6	18.6	Zhao et al., 2006
				<i>qss-5</i>			5.3	19.7	
				<i>qhs-2</i>	2, 4, 5		2.8	6.4	
				<i>qhs-4</i>			5.1	15.8	
				<i>qhs-5</i>			3.3	9.1	
				<i>qhs-4</i>	4, 8		3.8	16.8	
<i>Oryza sativa</i> indica Zhongyouzao No.8 and <i>Oryza sativa</i> japonica Fengjin T226 / T219	ZY28	RIL	Heat tolerance in tassal period	<i>qhs-8</i>	5	SSR	2.9	9.9	Zhang et al., 2008
				<i>qhts-5</i>			4.6	10.7	
	T226	RIL	Heat tolerance at flowering	<i>qHt3</i>	3, 9,	SSR	14.0	11.4	Qing-quan et al., 2008
				<i>qHt9a</i>	12, 2,		5.0	7.6	
				<i>qHt12</i>	3, 8, 9		5.3	10.0	
996 / 4628	996	F ₂	Seed set rate	<i>qHt2</i>			5.2	7.4	Zhang et al., 2009
				<i>qHt3</i>			7.1	10.9	
				<i>qHt8</i>			4.5	7.0	
				<i>qHt9b</i>			6.3	12.0	
					4, 3	SSR			

Population	Donor	Population	Mapping trait	QTL	Chr	Marker type	LOD	R ²	Reference
Bala / Azucena	Azucena	RILs (F ₆)	Absolute spikelet fertility - HT	<i>qtl_1.1</i> <i>qtl_2.2</i> <i>qtl_8.2</i> <i>qtl_1.1</i> <i>qtl_8.1</i> <i>qtl_10.1</i> <i>qtl_1.1</i> <i>qtl_3.4</i> <i>qtl_8.3</i> <i>qtl_10.1</i>	1, 2, 8, 1, 8, 10, 1, 3, 8, 10	RFLP	6.2 3.7 2.7 3.1 3.4 3.6 5.4 3.3 3.4 3.6	17.6 7.4 7.0 8.6 7.5 13.5 14.7 11.4 7.4 13.0	Jagadish et al., 2010b
996 / 4628	996	RILs	Relative spikelet fertility	<i>qtl_1.1</i> <i>qtl_11.1</i> <i>qtl_10.1</i> <i>qtl_1.1</i> <i>qtl_10.1</i> <i>qtl_11.1</i>	1, 11, 10, 1, 10, 11	SSR	2.9 3.2 3.5 2.6 3.0 2.8	9.1 8.0 13.4 8.6 8.1 7.4	Xiao et al., 2011a
996 / 4628	996	RILs	Seed set percentage	<i>SSPF4</i> <i>SSPF10</i> <i>SSPC4</i> <i>SSPC10</i>	4, 10, 4, 10	SSR		21.3 11.5 25.8 11.6	
Xiushui 09 / IR2061	Xiushui 09	RILs	Pollen fertility	<i>qPF4</i> <i>qPF6</i>	4, 6	SSR		15.1 9.3	Xiao et al., 2011b
		BC ₂ F ₆	Spikelet fertility	<i>qSF4</i> <i>qSF6</i>	4, 6	SSR	4.9 4.9	10.3 14.6	Cheng et al., 2012
Nipponbare / Koshihikari	Koshihikari	BILs	38 traits	122 QTLs	All but 5 and 9	SNP			Hori et al., 2012
IR64 / N22	N22	BC ₁ F ₁ and F ₂	Spikelet number	<i>qGN1.1</i> <i>qGN4.1</i> <i>qGN7.1</i>	1, 4, 7	SNP	6.0 4.5 7.4	16.1 12.4 19.4	Ye et al., 2012

Population	Donor	Population	Mapping trait	QTL	Chr	Marker type	LOD	R ²	Reference
IR64 / N22	N22	BC ₁ F ₁ and F ₂	Spikelet fertility (F ₂)	<i>qHTSF1.1</i>	1, 4,		6.7	12.6	Ye et al., 2012
				<i>qHTSF4.1</i>	9, 11		2.4	17.6	
				<i>qHTSF9.1</i>			2.4	6.8	
				<i>qHTSF11.1</i>			2.3	6.4	
			Spikelet fertility (BC ₁ F ₁)	<i>qHTSF1.1</i>	1, 4,		13.3		
IR64 / NH219 NH219 / IR64	NH219	F ₂	Leaf width	<i>qHTSF4.2</i>	5, 7		5.7		Poli et al., 2013
				<i>qHTSF5.1</i>			11.5		
				<i>qHTSF7.1</i>			7.8		
			6-day senescence		6, 2,	SSR			
			Number of tillers		5, 5,				
<i>Oryza sativa</i> ssp. <i>Indica</i> (Shuhui 527) / <i>O. rufipogon</i> Griff. (Yuanjiang common wild rice Hehuatang 3) OM5930 / N22	<i>O. rufipogon</i>	F _{2:3}	Yield per plant						Cao et al., 2015
			Heat tolerance at heading stage	<i>qHTH5</i>	5	SSR	8.9	19.4	
OM5930 / N22	N22	BC ₂ F ₂	HT score		3, 4,	SSR	36.2		Buu et al., 2015
					3, 8		32.6		
							17.1		
							28.0		
			Filled grain no. / panicle		10		15.3		
					11		13.1		
			Unfilled grain no. / panicle		3		25.0		
					3		11.2		
			1000 grain weight		6		30.6		
			Grain yield		4, 8,		10.8		
			Filled grain no. / panicle		3, 4		21.7		
			Grain yield		4				
			HT score		4, 3				

Population	Donor	Population	Mapping trait	QTL	Chr	Marker type	LOD	R ²	Reference
IR64/Giza178 Milyang23/Giza178 IR64/Milyang23/Giza178	Giza178	F ₂	Spikelet fertility	<i>qHTSF1.2</i>	1, 2,	SNP	2.9	14.6	Ye et al., 2015b
				<i>qHTSF2.1</i>	3, 4,		4.6	21.8	
				<i>qHTSF3.1</i>	6, 11,		3.5	17.3	
				<i>qHTSF4.1</i>	1, 2,		4.1	19.5	
				<i>qHTSF6.1</i>	4, 6,		4.8	20.6	
				<i>qHTSF11.2</i>	11		3.4	15.2	
				<i>qHTSF1.2</i>			5.2	13.3	
				<i>qHTSF2.2</i>			4.8	12.5	
				<i>qHTSF4.1</i>			5.0	13.0	
				<i>qHTSF6.1</i>			4.5	11.6	
				<i>qHTSF11.3</i>			5.4	13.9	
				<i>qAL09-1</i>	1, 2, 3	RFLP	4.3	12.0	
				<i>qAL09-2</i>			7.8	23.0	
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Anther length (2009)	<i>qAL09-3</i>			4.3	12.0	Tazib et al., 2015
				<i>qAL10-2</i>	2, 3, 7		5.1	16.0	
				<i>qAL10-3</i>			4.6	15.0	
				<i>qAL10-7</i>			3.6	11.0	
				<i>qADL09-5</i>	5, 11		7.3	21.0	
				<i>qADL09-11</i>			3.2	11.0	
				<i>qADL10-5</i>	5, 8,		3.2	11.0	
				<i>qADL10-8</i>			4.1	14.0	
				<i>qBDL09-3</i>	3, 8, 9		3.5	13.0	
				<i>qBDL09-8</i>			3.8	13.0	
				<i>qBDL09-9</i>			3.1	10.0	
				<i>qBDL10-2</i>	2, 8		4.6	17.0	
				<i>qBDL10-8</i>			3.2	11.0	
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Basal dehiscence length (2009)	<i>qADL10-5</i>	5, 8,		3.2	11.0	
				<i>qADL10-8</i>			4.1	14.0	
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Basal dehiscence length (2010)	<i>qBDL09-3</i>	3, 8, 9		3.5	13.0	
				<i>qBDL09-8</i>			3.8	13.0	
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Basal dehiscence length (2009)	<i>qBDL09-9</i>			3.1	10.0	
				<i>qBDL10-2</i>	2, 8		4.6	17.0	
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Basal dehiscence length (2010)	<i>qBDL10-2</i>	2, 8		4.6	17.0	
				<i>qBDL10-8</i>			3.2	11.0	

Population	Donor	Population	Mapping trait	QTL	Chr	Marker type	LOD	R ²	Reference
(Nipponbare / Kasalath) / Nipponbare	Kasalath	BILs	Longitudinal dehiscence (2011)	<i>qLD10-11</i>	11		5.5	25.0	Tazib et al., 2015
Sasanishiki / Habataki	Habataki	CSSL	spikelet fertility under HT	<i>qSFht2</i> <i>qPSLht4.1</i> <i>qSFht4.2</i> <i>qPSLht5</i> <i>qDFT3</i> <i>qDFT8</i> <i>qDFT10.1</i> <i>qDFT11</i> <i>qPSLht1</i> <i>qPSLht7</i> <i>qPSLht7</i>	2, 4, 4, 5, 3, 8, 10, 11, 1, 7, 7	SSR			Zhao et al., 2016
IR64 / N22	N22	RIL	STI for % spikelet fertility SSI for % spikelet fertility STI for yield per plant	<i>qSTIPSS9.1</i> <i>qSSIPSS12.1</i> <i>qSTIY5.1</i>	9 12 5	SNP	4.2 3.9 4.6	16.1 6.4 9.0	Shanmugavadivel et al., 2017
IR64 / Koshihikari	Koshihikari	BILs	SSI for yield per plant Spikelet fertility	<i>qSSIY3.1</i> <i>qSSIY5.1</i> <i>qSF1.1</i> <i>qSF1.2</i> <i>qSF1.3</i> <i>qSF7</i> <i>qSF8.1</i> <i>qSF8.2</i> <i>qSF10</i> <i>qSF12</i>	3 5 1, 1, 1, 7, 8, 8, 10, 12	SNP	4.0 3.5 2.6 4.2 3.2 5.0 2.7 4.3 7.1/4.3 4.4	6.5 21.3 9.3 6.2 6.8 6.8 6.2 6.3 21.5/4.1 6.1	Seo et al., 2019

Population	Donor	Population	Mapping trait	QTL	Chr	Marker type	LOD	R ²	Reference
IR64 / Koshihikari	Koshihikari	BILs	Grain yield	<i>qGY1</i> <i>qGY5</i> <i>qGY6</i> <i>qGY10</i>	1, 5, 6, 10		3.4/3.1 6.3 4.2 3.8/5.9	16.6/1.4 21.1 17.6 16.1/21.0	Seo et al., 2019
R53 / HHT4 (<i>O. rufipogon</i> Griff accession) M9962 / Sinlek	HHT4	BC	Spikelet fertility	<i>qHTB1-1</i>	1	SSR	5.6	13.1	Cao et al., 2020
Tanakari / IR64 Koshihikari / IR64	M9962 Tanakari Koshihikari	F ₂ population F ₂ CSSLs	Spikelet fertility Spikelet fertility Spikelet fertility	<i>qSF1</i> , <i>qSF2</i> , <i>qSF3</i> <i>qHTSF4.1</i> 12 QTLs	1, 2, 3 4 1, 2, 3, 4, 5, 6, 8, 9, 12	BSA- Seq SNP SNP	- 11.9	- 19.4	Nubankoh et al., 2020 Takai et al., 2020
Huanghuazhan / 9311 N22 / 9311	Huanghuazhan N22	F _{2:3} population CSSL	Spikelet fertility Seed setting rate	<i>qHTT8</i> <i>qSSR6-1</i> <i>qSSR7-1</i> <i>qSSR8-1</i> <i>qSSR9-1</i> <i>qSSR11-1</i>	8 6 7 8 9 11	BSA- Seq SSR	- 2.81 10.75 2.95 3.63 6.37	- 5.83 26.5 6.11 7.66 14.2	Chen et al., 2021 Nguyen et al., 2022

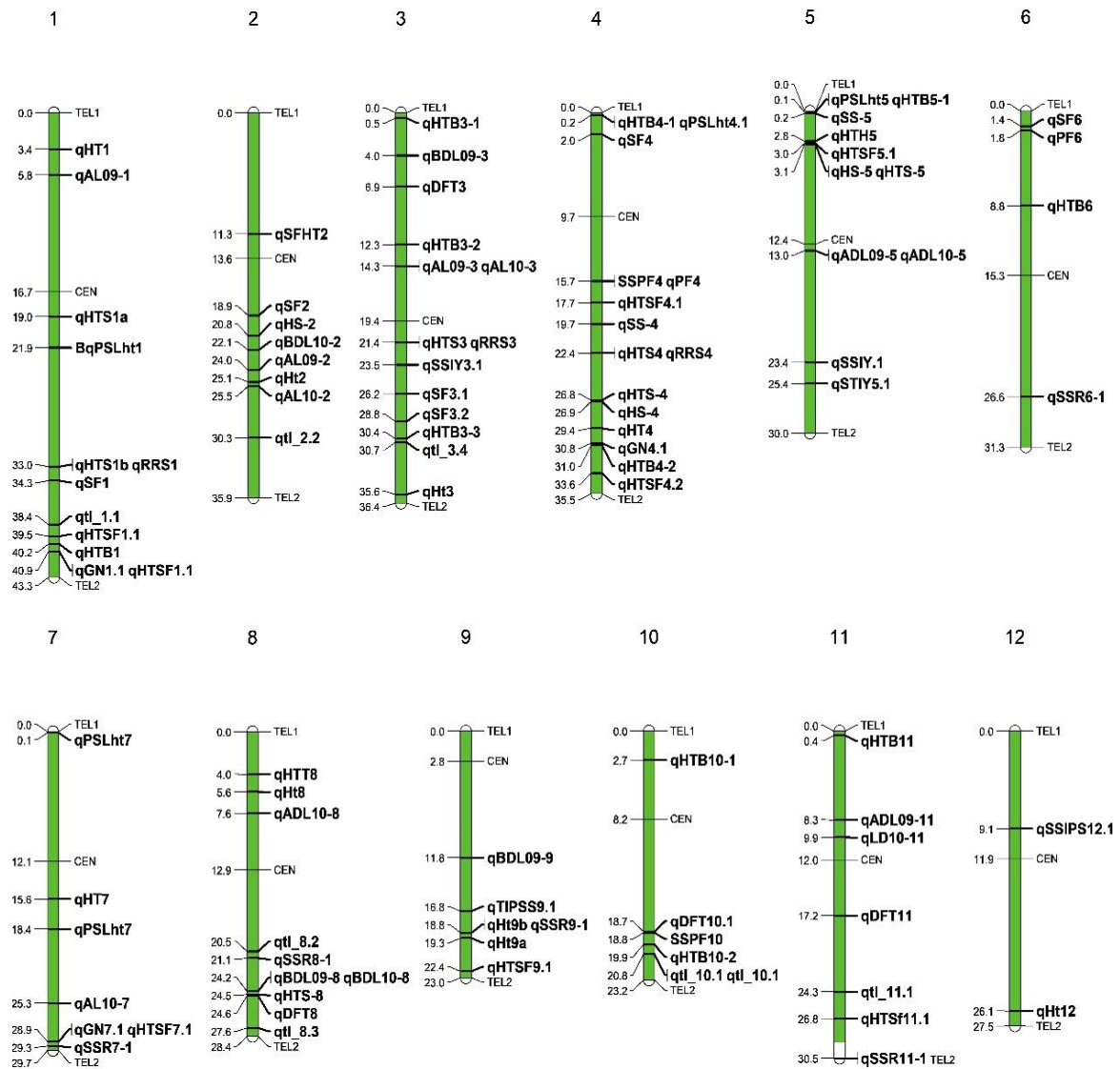


Figure 2.5 Chromosomal locations (physical in Mb) of QTLs reported so far for reproductive stage heat stress tolerance in rice. Distinct clusters of QTLs can be observed on chromosomes 1 (38.4 – 43.3 Mb), 2 (18.9 – 25.5 Mb), 4 (26.8 – 33.6 Mb), 5 (0.1 – 3.1 Mb), 8 (24.2 – 27.6 Mb), 10 (18.7 – 20.8 Mb)

2.9. Genes with established roles in heat stress tolerance in rice

A total of 27 HSP proteins were reported in rice including three Hsp60s, six Hsp70s, three Hsp90s, three Hsp100s and 12 small Hsps with a direct role in heat stress (Wang et al., 2014). Around 25 heat shock factors (HSFs), the regulators of heat shock proteins, were found in rice with varied functions under several abiotic stresses among which *OsHsfA2a* was particularly heat sensitive (Guo et al., 2008; Chauhan et al., 2011). Apart from these few other genes were also implicated in heat stress in rice. Neutralization of denatured proteins generated due to heat stress is indispensable for normal cellular activities. A QTL named *Thermo-tolerance 1 (TT1)* was identified from *O. glaberrima*, which encodes the

26S proteasome $\alpha 2$ subunit associated with the degradation of ubiquitinated denatured proteins (Li et al., 2015) (Table 2.3). *TOGRI* (*Thermotolerant Growth Required 1*), a nucleolar DEAD-box RNA helicase located on chromosome 3 maintains rRNA homeostasis by acting as a pre-rRNA chaperone and confers heat stress tolerance to rice plants (Wang et al., 2016a). Overexpression of *SLG1* (*Slender guy 1*), which encodes the cytosolic tRNA 2-thiolation protein 2 (RCTU2), leads to heat stress tolerance. There was clear differentiation in the haplotypes of promoter and coding sequences of *indica* and *japonica* cultivars and the resultant high temperature tolerance distinction between these two subspecies of rice (Xu et al., 2020). As mentioned earlier, PSII is more sensitive to heat stress. The damage to PSII is repaired through *de novo* synthesis of the D1 subunit protein encoded by *psbA*. Heat stress responsive promoter driven *psbA* could protect PSII appreciably and enhance the survival of transgenic plants under heat stress (Chen et al., 2020). Recently, *HTG3* (heat-tolerance gene on chromosome 3) was identified through GWAS. The expression of this gene was controlled by a transposon, miniature inverted-repeat transposable element (MITE) in its promoter (Wu et al., 2022). Further, an alternative splice variant of this gene, *HTG3a* functions as Hsf and confers heat stress tolerance during both vegetative and reproductive stages in rice.

Table 2.3 Genes associated with heat stress tolerance in rice.

S. No.	Gene	Protein	Function	Reference
1.	<i>TTI</i> (<i>Thermo-tolerance 1</i>)	26S proteasome $\alpha 2$ subunit	Degradation of ubiquitinated denatured proteins	Li et al., 2015
2.	<i>TOGRI</i> (<i>Thermotolerant Growth Required 1</i>)	Nucleolar DEAD-box RNA helicase	rRNA homeostasis	Wang et al., 2016a
3.	<i>SLG1</i> (<i>Slender guy 1</i>)	tRNA 2-thiolation protein 2 (RCTU2)	tRNA thiolation	Xu et al., 2020
4.	<i>psbA</i>	PSII D1 subunit	Repair of PSII	Chen et al., 2020
5.	<i>HTG3</i>	Heat shock factor	Heat stress tolerance	Wu et al., 2022

Chapter III

MATERIALS AND METHODS

Heat waves have become a regular phenomenon these days due to climate change induced global warming. Many a time these heat stress episodes coincide with sensitive stages of crops (particularly reproductive stage) resulting in drastic yield decline. Rice is one such crop which is sensitive to heat stress at reproductive stage and there is an urgent need to develop heat stress tolerant rice varieties to be prepared for the future climate catastrophes. In this regard, the present study was aimed at mapping the genomic regions governing reproductive stage heat stress (hereafter abbreviated as RSHS) tolerance in novel donor, NL44 and validate the markers linked to the QTLs in a segregating population of different genomic background. The materials utilized and protocols adopted to achieve this are presented in the following sections:

3.1. Experimental locations

The heat stress screening of germplasm and the validation population (F₆ families) was done at ICAR-IARI, IARI-Rice Breeding and Genetics Research Centre (IARI-RBGRC), Aduthurai, Tamil Nadu (1°00'N; 79°28'E, 19.5 m), while mapping populations (RILs) were evaluated at two locations, ICAR-IARI, RBGRC, Aduthurai, Tamil Nadu and ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack, Odisha (20°46'N; 85°88'E, 28.46 m). The multiplication of genotypes, generation advancement of mapping population and experiments in controlled environments were done both at ICAR-IARI, New Delhi (28°38'N; 77°09'E, 228.61 M) and RBGRC, ICAR-IARI, Aduthurai, Tamil Nadu.

3.2. Plant materials

Objective 1. To assess the phenotypic variation in rice germplasm for heat stress tolerance at reproductive stage

A set of 192 rice germplasm from the collection maintained at Rice section, Division of Genetics, ICAR-IARI, New Delhi was assessed for their RSHS tolerance. The collection comprised of several popular rice varieties of India along with certain land races and exotic lines. The list of rice germplasm taken up for the assessing the phenotypic variation for heat stress tolerance at reproductive stage is presented in Table 3.1. Additionally, a set of 35 diverse Basmati germplasm was also characterized for their response to RSHS along with checks, NL44, PB1, Nagina 22 and IR64 (Table 3.2).

Table 3.1 List of rice genotypes characterized for their tolerance to reproductive stage heat stress

S. No.	Genotype	S. No.	Genotype
1	CR 2461-9	42	Kataktara
2	UPRI 2003-45	43	VOH-PCR-3110
3	PNR 381	44	Karuppunel
4	Punjab Mehak 1	45	Kasturi
5	PNR 381	46	C 1268-7-10
6	Jayati	47	BJ 1
7	CT 10006-7-2M-5-1 P3-M	48	VL 88-97-1-7
8	Chandrasahini	49	Kamlesh
9	HPR 2143	50	IR 78908
10	MAS 946-1	51	B6144-MR-6-0-0
11	PRR 126 (NPP- 27/KH-2010)	52	Seond Basmati
12	Ajay	53	HPR 2104
13	RNRM 7	54	Shah Pasand
14	Falguna	55	Chimbalate Basmati
15	VLT-6	56	PMK 1
16	RIL 10	57	Sitwa Dhan
17	Pant Dhan 19	58	Gouri
18	NDR 8015-1	59	Ananga
19	Pusa Sugandh 3	60	Pant Dhan 4
20	Bala	61	OYR 69
21	Pant Sankar Dhan 3	62	Khuch
22	Samanta	63	CR 2499
23	Tapaswani	64	Pusa 1460
24	HPR 2083	65	GP325
25	Birupa	66	UPRI 2003-24
26	Bhubana	67	Poornima
27	Pant Dhan 10	68	UPRI 2003-18
28	OYR 128	69	Narendra Usar Dhan III
29	OYC 183	70	NDR 359
30	BJ 1 (Red Kl, Purple awns)	71	NDR 97
31	Chandana	72	Bhadrakali
32	Urvashi	73	Shiva
33	Pant Dhan 16	74	IR 77384-12-35-3-6-7-2-B
34	P 1490-03	75	Narendra Usar Dhan II
35	CSR 27	76	SKAU 220
36	China 988	77	PRR 105 (NPP-Kh-2010)
37	DV 85	78	PRR 117 (NPP- 94/KH-2010)
38	IRAT 240(IREM950)	79	PRR 103 (NPP-43/Kh-2010)
39	CR 2363-26	80	PRR 123 (NPP-56 /KH-2010)
40	Selected Sabarmati	81	PRR 114 (NPP-15/kh-2010)
41	MTU 1010	82	PRR 120 (NPP-71 /KH-2010)

S. No.	Genotype	S. No.	Genotype
83	PRR 115 (NPP-85/kh-2010)	127	Tompha Khau
84	PRR 104 (NPP-75/Kh-2010)	128	CN-1268-7
85	PRR 108 (NPP-108/KH-2010)	129	WGL 14
86	PRR 121 (NPP- 70/KH-2010)	130	Hema
87	Pant Dhan 18	131	UPRVS 8-26
88	Indravati	132	Super Basmati
89	Pant Sugandh Dhan 17	133	Pusa 1301
90	HUR 200-57-1	134	Kanak
91	Pant dhan 15	135	PRR 109 (NPP-29/Kh-2010)
92	JGL 11727	136	PRR 110 (NPP-57/Kh-2010)
93	Mahanadi	137	PRR 127 (NPP-47 /KH-2010)
94	Pant Sugandh Dhan15	138	PRR 122 (NPP-97/KH-2010)
95	Bhuman San	139	PRR 118 (NPP- 93/KH-2010)
96	JR 75	140	HUR 36
97	CO 37	141	RR 166-645
98	Sumati	142	Nirri
99	SAF 1221-83	143	C 22
100	P 1447	144	DHMAS-70G-164-29
101	P 1447-00-5-1 (P1324xAJAY)	145	Ranbir Basmati
102	HUR 105	146	T23
103	Manaswini	147	Langphou
104	RR 8585	148	ASD 19
105	CSR 23	149	Basmati 370
106	Kudrat-3	150	BPT 5204
107	RAU 3061	151	IC 2127
108	NDR 625	152	VOH-PCR-3113
109	Sagar Dambha	153	TYPE 3
110	JGL 3828	154	Nagina 12
111	P 1460	155	Sonasal
112	Muskan	156	Khara Munga
113	Pusa 1342	157	IR 70
114	MR 219	158	ADT 39
115	UPRI 2003-15	159	Haldimuri
116	Samba Mahsuri	160	MTU 7029
117	Sharbati	161	PDKV Chinoor 2
118	HKR 200-57-1	162	Swarna Sub 1
119	WGL 23985	163	Tilak Chandan
120	Rajendra basmati	164	Pusa 33
121	MR 220	165	Pratikshya
122	Raja Vaellu	166	Jhulhat
123	CR 246-16	167	NDR 9830144
124	Phunchi	168	MTU 1001 (Vijetha)
125	Pusa Basmati 1121	169	Bamleshwari
126	Improved Sabarmati	170	Ramachandi

S. No.	Genotype	S. No.	Genotype
171	GP584	182	Kew
172	Apo	183	Mehvan (purple)
173	Aziz Beoul	184	Mehvan (green)
174	Begum	185	Mir Zug
175	Bala Koun	186	CO 50
176	Buta Baber	187	CO 51
177	Baber	188	Arupathaam Kuruvai
178	Baber Safed	189	Improved Samba Mahsuri
179	Budgi	190	Sabour Surbhit (RAU 3036)
180	Gull Baber	191	North Andaman 2
181	Kaw Qudder	192	GP975

Table 3.2 List of Basmati germplasm utilized for validation of QTL linked markers.

S. No.	Genotype	S. No.	Genotype
1	Taraori Basmati	19	Hasan Serai
2	CSR 30	20	Haryana Basmati 1
3	Basmati 370	21	Punjab Basmati 4
4	Ranbir Basmati	22	Punjab Basmati 2
5	Super Basmati	23	Punjab Basmati 3
6	Type 3	24	Vallabh Basmati 21
7	Type 23	25	Vallabh Basmati 22
8	Basmati 386	26	Vallabh Basmati 23
9	Punjab Basmati 5	27	Pusa Basmati 6
10	Kasturi Basmati	28	Pusa Basmati 1121
11	Palam Basmati	29	Pusa Basmati 1509
12	Sanwal Basmati	30	Pusa Basmati 1728
13	Basmati Kota	31	Pusa Basmati 1637
14	Basmati Jamuna	32	Pusa Basmati 1718
15	Seond Basmati	33	Punjab Basmati 1
16	Chimbalate Basmati	34	Haryana Basmati 2
17	Domsiah	35	Pusa Basmati 1
18	Shah Pasand		

Objective 2. To map QTLs governing heat stress tolerance at reproductive stage in the NERICA rice cultivar, NL44.

A population of 127 RILs developed from Pusa Basmati 1/NL44 through single seed descent method (SSD) which were in F₆ generation was available at Division of Genetics, ICAR-IARI, New Delhi. Pusa Basmati 1 is the world's first high yielding semi-dwarf Basmati rice variety released by ICAR-IARI, New Delhi with extra-long grain type, superior grain and cooking quality and mild aroma (Siddiq et al., 2012).

However, this variety is highly sensitive to reproductive stage heat stress (RSHS). NL44 is one of the lowland NERICA (NEw RiCe for Africa) lines evolved in Africa by crossing Asian rice (*O. sativa*) with African rice (*O. glaberrima*) to combine high yield potential of Asian rice with stress tolerance of African rice at AfricaRice, a CGIAR research center based at Ivory Coast, Africa. This genotype shows significant RSHS tolerance with higher seedling survival rate, superior growth, and greater reproductive resilience, even when exposed to 42.2°C under field conditions (Bahuguna et al., 2015). The F₆ generation from the cross, Pusa Basmati 1/NL44 were advanced to F₁₀ generation. The F₈ and F₁₀ generations from this cross were phenotyped for RSHS at two locations (Aduthurai and Cuttack) during off-season 2018-19 and 2019-20, which was utilized for QTL mapping.

Objective 3. To validate the molecular markers associated with QTLs governing heat stress tolerance in rice.

A set of F₆ families was developed from the cross between PB1509 and NL44 for validation of the linked markers.

3.3. Phenotyping for reproductive stage heat stress tolerance

3.3.1. Parental characterization

The parents, PB 1 and NL44, along with checks, N22, IR64 and PB1509 were characterized for physiological attributes through a pot experiment. N22 and IR64 were taken as positive and negative controls of heat stress tolerance whilst PB1509 is the parent used for the construction of F₆ population utilized for validation of identified QTLs. Each genotype is grown in triplicates under two treatments – control (~33°C) and heat stress (~39°C) in CRD design. Initially, pots designated for both control and treatment were kept at normal temperatures. However, when the plants started booting (coinciding with microsporogenesis), the plants considered for heat stress were shifted to high temperature chambers. The plants were exposed to such high temperatures till maturity. Flag leaves and panicles were sampled at maximum anthesis stage in both control and heat stress treatments for further biochemical analysis. The following parameters were recorded

3.3.1.1. Hydrogen peroxide

About 0.2 g of plant tissue (flag leaf and panicle) was homogenized in 2ml of 0.1% trichloroacetic acid. To this equal volume of 100 mM K-phosphate buffer and double the volume of 1 M KI was added and the absorbance of the final solution was read at 390 nm

(Alexieva et al., 2001). Trichloroacetic acid (0.1%) was taken as blank. The concentration of H₂O₂ ($\mu\text{mol g}^{-1}$ fresh weight (FW)) in the test sample was calculated from the standard curve plotted using different concentrations of H₂O₂.

3.3.1.2. *Lipid peroxidation*

About 500 mg of frozen plants tissue was pulverized in liquid nitrogen and taken in a 1.5 ml of centrifuge tubes. To this 0.5 mL of 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid and 0.5 mL of buffer (175 mM NaCl in 50 mM Tris-HCl, pH 8) was added. The samples were then incubated at 95°C for 25 min and then centrifuged at full speed for 20 min. The absorbance of the supernatant was measured at 532 nm (Larkindale and Knight 2002). The amount of thiobarbituric acid reactive substances (TBARS) was calculated using an extinction coefficient of $155 \text{ mM}^{-1}\text{cm}^{-1}$.

3.3.1.3. *Total antioxidant capacity*

Total antioxidant capacity was measured through Ferric Reducing Antioxidant Power (FRAP) Assay (Benzie and Strain, 1996). The reducing property of antioxidants is exploited in this method. In a redox-linked colorimetric reaction, Fe³⁺ is reduced to Fe²⁺ by antioxidants at low pH, which causes the formation of a colored ferrous-probe complex from a colorless ferric-probe complex. About 0.1 g of sample was processed in liquid nitrogen using a pestle and mortar. To this 1 ml of 70% ethanol was added and the mixture is centrifuged at 12000 rpm for 20 min. Later, 100 μL of supernatant was mixed with 3 mL of FRAP reagent in dark and incubated in a water bath for 10 min at 37°C and absorbance was recorded at 593 nm. Ascorbic acid standards (100 μM to 1000 μM) were processed in a similar way to generate standard curve.

3.3.1.4. *Chlorophyll content (SCMR)*

The relative greenness of the leaves (chlorophyll content) was measured using SPAD chlorophyll meter (Spectrum Technologies Inc., USA). Readings are taken at three different positions in each leaf (flag leaf and 2nd leaf from the top) and the average was taken per each plant for each genotype.

3.3.1.5. *Canopy temperature depression (CTD)*

Leaf-temperature depression (TD) was calculated from leaf surface temperature and chamber air temperature. Leaf surface temperature was measured using hand-held infrared crop temperature meter (Spectrum Technologies, Inc., Aurora, Illinois, USA) between

1330 and 1430 h. The chamber temperature was obtained from data loggers installed in the chamber. CTD was estimated by subtracting leaf surface temperature from chamber air temperature according to Ayeneh et al. (2002).

3.3.1.6. *Membrane Stability Index (MSI)*

The plant tissues are cut into pieces of about 15 to 25 cm² and placed in test tubes that can accommodate a conductivity electrode. The samples were washed for 2-3 times with de-ionized water. For each genotype, tubes containing samples from normal temperature are treated as control (C), while tubes containing samples from stressed plants are named as treatment (T). Around 20 ml of deionized water was added to each test tube and were incubated at about 10°C for 24h. After incubation, the samples were allowed to come to room temperature and the conductivity of the medium was measured by inserting a conductivity electrode into each test tube. Conductivity of samples in “C” test tubes are recorded as “C1”, while that of “T” test tubes are recorded as “T1”. Later all the test tubes were properly sealed and autoclaved for 15 min to kill all tissues. Again, the test tubes were brought to room temperature and the conductivity of all samples was measured. Conductivity of samples in “C” tubes were recorded as “C2”, while that of “T” tubes were recorded as “T2”. The cell membrane stability was calculated as follows (Tripathy et al., 2000):

$$\text{CMS (\%)} = \{1 - [T1/T2]\} / \{1 - [C1/C2]\} \times 100$$

3.3.2. *Characterization of RILs, F6 populations and germplasm*

3.3.2.1. *Field experimentation for reproductive stage heat stress tolerance*

The screening of various populations (mapping populations and germplasm) was done under natural field conditions by following staggered method of sowing during off-season for two consecutive seasons, 2018-19 and 2019-20 at two locations namely, IARI-RBGRC, Aduthurai and ICAR-NRRI, Cuttack. In addition, to test the presence of previously reported QTLs the RILs were initially also screened during off-season during 2017-18 at RGBRC, Aduthurai. Two sowings were taken up one in mid-December and the other in second fortnight of January. The sowings are timed in such a way that the genotypes of first staggering set came to flowering when the ambient temperatures are optimum during early to mid-March (~33°C); whereas the second staggering flowered when the temperatures were extreme during mid to late April (~38°C or more). The plot sizes were kept uniform across each population. Two experimental designs were followed

to evaluate different populations. RILs and germplasm (192 utilized for characterization) were evaluated in an augmented RCB design, while the Basmati germplasm was transplanted in RCBD. The adjusted means from the augmented design were utilized for QTL mapping.

3.3.2.2. *Yield and its related attributes recorded*

The following observations were taken on five randomly tagged plants during flowering for each genotype except the days to 50% flowering for which observations were recorded on plot basis.

(A) *Days to 50% flowering (days)*: Number of days from sowing to 50% flowering was recorded on plot basis.

(B) *Plant height (cm)*: Length of plant was measured from the base of the plant to the tip of the primary panicle excluding awns.

(C) *Panicle length (cm)*: Length of primary panicle measured from the base of peduncle to the tip excluding awns.

(D) *Productive tiller number*: Number of tillers bearing panicles with filled grains were counted.

(E) *Pollen fertility (%)*: This was determined using I₂-KI staining method. The florets from primary tiller that were exposed to heat stress were fixed in Carnoy's fixative (three parts of 70% ethanol mixed with one part of glacial acetic acid) taken in 10 ml polyvinyl vials. In laboratory, three anthers from each spikelet were processed in the stain taken on a microscope slide. The stained pollens were observed under a Nikon Alphaphot-2 YS2 compound microscope (Tokyo, Japan) through 100x magnification. The pollen grains which imbibed stain and appeared dark brown were considered fertile, while the unstained ones were treated sterile. The proportion of fertile to total number of pollen grains per microscopic field was used to calculate pollen fertility and expressed in percentage. Three such readings were taken and averaged for further analysis.

(F) *Spikelet fertility (%)*: Number of filled and unfilled grains in a primary panicle were counted by pressing the grains with fingers and the per cent of filled grain to the total number of grains per panicle was expressed as spikelet fertility (%).

(G) *Grain yield (g)*: Single plants were threshed separately after drying to optimum moisture level around 14% and the grain was weighed on the electronic balance.

3.3.2.3. Grain and cooking quality traits recorded (RILs)

(A) *Hulling percentage*: 200 g of paddy of each genotype was dehusked using laboratory paddy husker (Model: THU35B, Satake Corporation, Hiroshima, Japan) and quantity of dehusked grain was measured. The proportion of output (brown rice) to the total input (paddy/ rough rice) was expressed in per cent as hulling percentage.

(B) *Milling percentage*: After taking the weight of brown rice, the samples were polished in a laboratory paddy miller (Model: TM05, Satake Corporation, Hiroshima, Japan) and the quantity of milled rice was measured. The proportion of milled rice to the total initial paddy was expressed in per cent as milling percentage.

(C) *Head rice recovery (%)*: The milled rice was passed through a metal tray with 2 mm depressions where the broken grains get trapped. This was done thrice till the broken grain was completely separated from the whole grain and the weight of whole rice grain was measured. The proportion of whole rice grain to the paddy/ rough rice was expressed as head rice recovery per cent.

(D) *Kernel dimensions before cooking*: Grain dimensions such as kernel length before cooking (KLBC), Kernel breadth before cooking (KBBC) were measured on 10 milled grains of each genotype using image analyzer and length to breadth ratio (L/B) was calculated as ratio of the observations recorded for KLBC and KBBC.

(E) *Kernel dimension traits after cooking*: The cooked kernel dimensions such as kernel length after cooking (KLAC) and kernel breadth after cooking (KBAC) were measured as follows:

- Ten whole milled grains were soaked in a test tube containing 10 ml of distilled water for twenty minutes and placed in a water bath containing boiling water (100°C) for 8 to 10 minutes.
- The tubes were then removed from water bath and the cooked grain samples were transferred to separate petri-plates. The lids of the petri-plates were closed for fifteen minutes until the samples reach room temperature.
- The kernel length after cooking (KLAC) and breadth after cooking (KBAC) were later measured using image analyzer.

(F) *Kernel elongation ratio (KER)*: The kernel elongation ratio was calculated using the formula:

$$\text{KER} = \frac{\text{Kernel length after cooking (mm)}}{\text{Kernel length before cooking (mm)}}$$

(G) *Alkali spreading value (ASV)*: ASV was tested as per the protocol described by Little et al. (1958). Six whole milled grains per each entry, equally spaced, were soaked in two replicates in 10 ml of 1.7% KOH taken in a Petri plate. The petri-plates were incubated at 30°C for 24 hours. Thereafter, the grains were visually scored on a standard scale of 1-7. The spreading and clearing values of each of the six grains were noted individually and the average of them was considered as the final value. The interpretation of the score is as follows.

Table 3.3. Score scales for alkali spreading value

ASV score	Spreading value	Clearing value	ASV	GT
1	Kernel not affected	Kernel chalky	Low	High (>74°C)
2	Kernel swollen	Kernel chalky, collar powdery		
3	Kernel swollen, collar incomplete and narrow	Kernel chalky, collar cottony or cloudy	Low, intermediate	High, intermediate
4	Kernel swollen, collar complete and wide	Centre cottony, collar cloudy	Intermediate	Intermediate (70°C - 74°C)
5	Kernel split or segmented, collar complete and wide	Centre cottony, collar clearing		
6	Kernel dispersed, merging with collar	Centre cottony, collar cleared	High	Low (55°C - 69°C)
7	All kernel dispersed and intermingled	Centre and collar cleared		

Scoring of ASV and correlation with GT (Lanceras et al., 2000)

(H) *Gel consistency*: Milled rice flour (100 mg) was taken in 11×100 mm test tubes to which 0.2 ml of thymol blue (0.025% (w/v)) was dissolved in 95% ethanol and 2 ml of 0.2N KOH were added. The sample was mixed vigorously using a vortex. Tubes were covered with glass marbles and placed in boiling water bath for 5 min. Later the tubes were removed and vortexed again, followed by cooling in ice water bath for 20 min. The cooled tubes were then laid horizontally against a ruled graph sheet and gel length was measured after 1 hour. The gel consistency values were classified as soft (61-100 mm), medium (41-60 mm) or hard (26-40 mm) (Lanceras et al., 2000)

(I) *Amylose content*: About 100 mg of rice flour is suspended in 1 ml of rectified spirit and 9 ml of 1.0N sodium hydroxide in long test tubes. The tubes were shaken vigorously and incubated in boiling water bath for 15 minutes. The contents of the test tube were decanted into a 100 ml volumetric flask and volume was made up with distilled water. About 5 ml of this solution was pipetted out into three separate 100 ml volumetric flasks each. To this 1 ml of acetic acid and 2 ml of I₂ – KI solution was added, and the volume was made up with distilled water. This step was performed under dark, and the absorbance values were taken at 620 nm. A standard curve was simultaneously prepared by taking absorbance values of known concentrations of amylose. The sample absorbance values were substituted in the equation of standard curve to arrive at sample amylose content after accounting for dilution.

3.3.3. Statistical analysis of phenotypic data

The statistical analysis of phenotypic data was done using PBTools (<http://bbi.irri.org/products>). The augmented RCB ANOVA for individual locations and pooled ANOVA was generated by considering genotypes as random effects to estimate variance components and BLUPs of genotype trait values. The ANOVA model for single environment is as follows:

$$Y_{ij} = \mu + b_i + c_j + g_{ik} + \varepsilon_{ijk}$$

Where Y_{ij} is the mean trait value; μ is grand mean of environment; b_i is the fixed effect of i^{th} block; c_j is the random effect of j^{th} replicated check; g_{ik} is the random effect of k^{th} test entry in i^{th} block and ε_{ijk} is the residual error. The ANOVA model for pooled analysis across locations is as follows:

$$Y_{ij} = \mu + b_i + c_j + g_{ik} + e_l + be_{il} + ce_{jl} + ge_{ikl} + \varepsilon_{ijkl}$$

Where Y_{ij} is the mean trait value; μ is the grand mean; b_i is the fixed effect of i^{th} block; c_j is the random effect of j^{th} replicated check; g_{ik} is the random effect of k^{th} test entry in i^{th} block, e_l fixed effect of l^{th} environment, be_{il} interaction fixed effect between i^{th} block and l^{th} environment, ce_{jl} interaction random effect between j^{th} check and l^{th} environment, ge_{ikl} is the interaction random effect of k^{th} test entry within i^{th} block and l^{th} environment and ε_{ijkl} is the residual error. These BLUPs were further used for calculating both stress tolerance index (STI) and quantitative trait loci (QTL) analysis. A modified heritability value (H^2_c) in the context of mixed model was estimated as proposed by Cullis et al. (2006).

3.4. Analysis of molecular markers (SSR genotyping)

3.4.1. Isolation of genomic DNA

DNA isolation protocol of Murray and Thompson (1980) was adopted with little customization. The genotypes were grown in germination trays till two leaf stage and the genomic DNA was extracted from them through following steps:

- The leaves of young seedlings were chopped into fine bits and collected in 2ml polypropylene tubes
- Just before sample processing, small (~2mm diameter) stainless steel beads were added to the tubes which were then arranged in sample blocks of TissueLyser, tightly secured and tumbled into liquid nitrogen
- After two minutes, the blocks were removed from liquid nitrogen and mounted in TissueLyser and the samples were pulverized for 4 minutes at a frequency of 25 oscillations per second.
- To these tubes, warm DNA extraction buffer (DEB) was added, and the samples were incubated at 65°C in water bath for 30 minutes with intermittent inverting to facilitate optimum extraction.
- The samples were brought to reach room temperature before adding 500µl of chloroform: isoamyl alcohol (24:1) to each tube.
- The tubes were properly inverted for 10 mins, after which they were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was eluted to fresh Eppendorf Tubes using micro-pipette.
- Equal volume of chloroform: isoamyl alcohol (24:1) was added to the supernatant and the previous step was repeated.
- To this, 3M sodium acetate maintained at pH – 5 and ice cold – isopropanol was added at one tenth volume and equal volume of the supernatant respectively. The tubes were inverted properly and incubated overnight at 4°C.
- The next day tubes were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was discarded, and 70% ethanol was added on to the pellet.
- Again, the tubes were centrifuged at 10,000 rpm for 5 min at 4°C. This was repeated once or twice till a clear white pellet was obtained. The ethanol was finally discarded, and pellet was dried at room temperature.

- After drying, the pellet was re-suspended in 100µl of TE buffer (pH 8.0). To this, 2 µl RNaseA (10mg/ml) was added and incubated at 37°C for half an hour.
- Initially, the DNA concentration and quality were checked using a Nano spectrophotometer (Microdigital Co. Ltd., South Korea). Later, for further confirmation, the DNA of randomly selected samples was quantified using 0.8% agarose gel in 1x TAE buffer. λ uncut genomic DNA was used as the standard to compare the concentrations of the DNA samples.

3.4.2. PCR conditions

The reaction volume for polymerase chain reaction (PCR) was pegged at 10µl with 20-30 ng template DNA, 5 pmol of each primer, and 2 × ready-to-use PCR master mix (Genei, Bangalore). The amplification program of PCR is as follows:

Step 1. Initial denaturation at 94°C for 4 min.

Step 2. Denaturation at 94°C for 30 sec followed by annealing at 55-56°C (depending on the marker) for 30 sec and extension at 72°C for 1 min. This step constitutes one cycle and 35 such cycles were run for sizeable amplification of DNA

Step 3. Final extension at 72°C for 7-10 min.

The above program was executed on a thermal cycler (Bio-Rad Laboratories Inc., Hercules, California, United States)

3.4.3. Resolution of amplified PCR products

The amplified PCR products were resolved on 3.5% agarose gel, taking 17.5 g of agarose powder in 500 ml of 1× TAE buffer and heating in microwave oven till agarose dissolved completely giving a clear solution. The solution was brought to room temperature and 25 µl (@0.05 µl/ml of 1× TAE) of ethidium bromide (EtBr) was added. The gel was then gently stirred and poured into the gel casting tray after properly securing the stoppers. The combs were then placed to create wells for loading PCR product. After proper solidification, the gel slab was placed in the electrophoresis tank containing 1× TAE buffer and the combs were carefully removed from gel. The PCR product of 10 µl re-suspended with 1.5 µl of 6x loading dye (0.25% bromophenol blue; 0.25% xylene cyanol FF; 40% sucrose) was loaded in individual wells along with a 50bp ladder (Fermentas, Lithuania, USA). The power pack was set to 5 Volts/cm of run and the electrophoresis was run for 2 to 3 hours. After optimum run of samples, the gel slabs were visualized under UV trans-

illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., Hercules, California, United States).

3.5. Genome wide association mapping for reproductive stage heat stress tolerance

3.5.1. SNP genotyping of rice germplasm

The rice germplasm selected for characterization of heat stress tolerance was already genotyped using 50k Affymetrix GeneChip (Thermo Fisher Scientific, United States) and described in Bollinedi et al. (2020). Briefly, DNA was extracted from 15-day old seedlings using method described in the preceding section (3.4.1). The purified DNA with appropriate concentration was sent for SNP genotyping. The gene chip used for genotyping contained 50,051 SNPs strategically selected from 18,980 genes spanning across 12 chromosomes with an average inter-marker interval of 1 kb. A few pre-mapping exercises were done to improve the quality of genotype data. Markers with minor allele frequency of 5% and with >20% missing reads were dropped, and the downstream analysis was restricted to only 32,712 SNPs.

3.5.2. Pre-association and association analyses

As mentioned earlier, population structure and linkage disequilibrium (LD) decay were already described in Bollinedi et al. (2020). All the pre-association and association analyses were done using the R package genome association, and prediction integrated tool (GAPIT) Version 3 (Lipka et al., 2012). PCA technique in built in GAPIT was used to check population structure and generate LD decay plot. The association analysis was done by executing one single locus model – mixed linear model (MLM) and two multi-locus models – Fixed and random model Circulating Probability Unification (FarmCPU) and Bayesian information and Linkage-disequilibrium Iteratively nested Keyway (BLINK). The phenotypic data (grain yield and spikelet fertility) generated under both control and heat stress and respective stress tolerance indices (STI) were utilized for genome-wide association studies (GWAS). The threshold for declaring significant marker-trait associations were set at $-\log_{10}$ p value of 5.0, though the Bonferroni threshold being 5.8, to avoid false negatives. Accordingly, the significant SNP and trait associations identified were considered QTNs (quantitative trait nucleotides). The R script used for GWAS in GAPIT is as follows:

```
#install necessary package
library(multtest)
library("gplots")
```

```

library("LDheatmap")
library("genetics")
library(MASS)
library("compiler")
library(RColorBrewer)
library("scatterplot3d")
#load gapit
source("http://www.zzlab.net/GAPIT/emma.txt")
source("http://www.zzlab.net/GAPIT/gapit_functions.txt")
#LOAD THE FILES
pheno<-read.table(file = "HT_TASSEL.txt",head=TRUE)
geno<-read.delim("ravifilter.hmp.txt", head=FALSE)
#RESULTS- MLM
results_MLM<-GAPIT(Y=pheno,G=geno,PCA.total = 3)
#RESULTS- FARM CPU
results_fc<-GAPIT(Y=pheno, G=geno, PCA.total = 3, model = "FarmCPU")
#RESULTS- BLINK
results_bk<-GAPIT(Y=pheno, G=geno, PCA.total = 3, model = "Blink")

```

3.6. Validation of previously reported QTLs

A preliminary exercise was carried out to test the presence of any reported QTLs in the present mapping population contributed by NL44. An exhaustive literature survey was done and a list of QTLs reported for reproductive stage heat stress tolerance in rice was prepared. A set of 116 SSR markers linked to 58 previously reported QTLs were finally selected for validation. Initially, the markers were tested on parents for polymorphism and the polymorphic markers were analysed across the entire RIL population. The RILs were scored for each marker according to the allelic pattern of parents, PB1 type allele and NL44 type allele and heterozygotes, if any. A single marker analysis was done using a simple linear regression by considering trait values as dependent variable and marker score as independent variable. A significant regression co-efficient was considered as putative association and co-efficient of determination as phenotypic variance explained.

3.7. QTL mapping

3.7.1. Genotyping of parents and RILs

A total of 1301 simple sequence repeat (SSR) markers were initially analyzed between parents, PB1 (sensitive) and NL44 (tolerant) for polymorphism. A set of 127 RILs were later genotyped using only the polymorphic markers. For this, the genomic DNA of parents as well as RILs were extracted using the protocol of Murray and Thompson (1980) as detailed above. PCR reactions were set up with a total volume of 10 µl and the reactions were carried out in a thermal cycler (Agilent, USA) using the program mentioned before. The amplified PCR products were resolved on 3.5% agarose gel by adding 0.1 mg/ml of

ethidium bromide (Amresco, Solon, OH, USA) along with a 50bp DNA ladder (MBI, Fermentas) and visualized on UV transilluminator. The gel images were captured as JPEG files in a gel documentation system (Bio-Rad Laboratories Inc., Hercules, California, United States) and saved for scoring the alleles later. The RILs were scored for each marker according to the allelic pattern of parents, PB1 type allele and NL44 type allele and heterozygotes, if any.

3.7.2. Genotyping of RILs and parents through 80k SNP array and refinement of linkage map

Since there are significant gaps in the linkage map constructed using SSR markers, the RILs along with the parents were genotyped using high density 80k SNP chip (Daware et al., 2022). The genotyping was outsourced to Thermo Fisher Scientific, USA. Genotyping calls could be obtained for a total of 80,504 SNP markers of which 60,026 SNPs are anchored to 12 chromosomes while the remaining were unanchored. Based on the SNP calls of parents, polymorphic and homozygous SNPs were filtered and the same was used for construction of linkage map. Some pre-mapping exercises were done using QTL IciMapping. The SNP functionality of the software was used to convert the SNP calls into the format suitable for downstream analyses. Next using BIN functionality redundant markers, the ones with too many missing values and/or showing segregation distortion were identified and discarded. The missing rate and p -value for distortion were pegged at 20% and 0.01, respectively. The markers remaining were used for linkage map construction as described for SSR based linkage map. Furthermore, to combine the two linkage maps, CMP function was utilized. The consensus maps were constructed for each chromosome separately using the default parameters and thus updated map was used for final QTL mapping.

3.7.3. Construction of genetic linkage map

The linkage map was constructed using the software QTL IciMapping software (Meng et al., 2015). Since the physical positions of markers were already known, they were pre-anchored to their respective chromosomes before calculating genetic distances. Markers which were showing significant segregation distortion were culled out and the final genetic linkage map was constructed by executing Kosambi mapping function (Kosambi, 1944). This mapping function allows some interference unlike its predecessor, the Haldane mapping function. Kosambi function follows the equation given below:

$$m_k = 25 \ln[1+2r] / (1-2r)$$

Where m_k represents Kosambi genetic distance and r indicates recombination frequency. The values obtained will be in the units of centiMorgan (cM).

3.7.4. QTL mapping

The linkage map and the phenotype were then used to perform QTL mapping using the software QTL IciMapping software (Meng et al., 2015). Inclusive composite interval mapping was utilized for this purpose. Composite interval mapping is an extension of interval mapping where certain selected markers were utilized as co-factors to control background noise generated by other linked or unlinked QTLs/intervals on the interval/QTL under study thus rendering increased precision of QTL detection. The mathematical model for CIM is as follows

$$y_i = \mu + \sum_{j=1}^{m+1} b_j x_{ij} + e_i$$

Where y_i is the phenotypic or trait value of the i^{th} RIL; μ is the grand mean; x_{ij} is a dummy variable for the genotype of i^{th} RIL at the j^{th} marker, its values being 1 for homozygote and -1 for heterozygote; b_j is the regression coefficient of the phenotype on the j^{th} marker; and e_i is the residue assumed to follow normal distribution.

However, there is a certain arbitrariness in this co-factor selection, since there are different methods to do this. Further, CIM cannot ensure the deflation of QTL effect at an interval in question by the effect of markers considered as co-factors. To address these two caveats of CIM, a modification of CIM, Inclusive Composite Interval Mapping (ICIM) was proposed (Li et al., 2007). In ICIM, the marker selection is done only once using stepwise regression analysis simplifying the co-factor selection factor. The phenotype is adjusted through the effects of markers retained in the model (except the markers in the current testing interval) which is used for final QTL mapping. ICIM has robust detection power, reduced false positives and reduced biasedness in estimation of QTL effects based on various simulation models. For a given testing position in interval $(k, k + 1)$, the phenotype values in the CIM model are adjusted as:

$$\Delta y_i = y_i - \sum_{j \neq k, k+1} b'_j x_{ij}$$

Where b'_j is the estimate of b_j in the CIM model, Δy_i is the adjusted phenotype value. Only additive model was run on IciMapping and logarithm of odds (LOD) threshold

was fixed through a permutation test (~1000 iterations) to declare significant QTLs. The additive effect and phenotypic variance explained were extracted for these significant QTLs. QTLs were generated for individual locations in individual seasons for each character and QTE function was used to extract QTE variance. The QTLs thus obtained named according to the guidelines laid out by McCouch et al. (1997).

3.8. Validation of putative QTLs

The SSR markers near the physical positions of putative QTLs were validated on a subset of F₆ families generated from PB1509/NL44. The genotypes were scored as '0' with the allele from sensitive parent, '1' for heterozygotes and '3' with allele from tolerant parent. A simple linear regression was done by regressing phenotype values on marker genotype scores. The markers which showed significant regression co-efficient was considered valid in resolving the tolerance of NL44, irrespective of genetic background.

The present study was undertaken to assess the variability present in the rice germplasm for RSHS tolerance and to map the genomic regions controlling RSHS tolerance in novel RSHS tolerant donor NL44. The results obtained in various experiments were presented in the succeeding sections.

4.1. Characterization of rice germplasm for tolerance to reproductive stage heat stress (RSHS)

4.1.1. Experiment 1: Screening of rice germplasm for tolerance to RSHS

A total of 192 rice genotypes were assessed for heat stress tolerance at reproductive stage under natural field conditions at IARI-Rice breeding and genetics research centre (RGBRC), Aduthurai, Tamil Nadu. Two staggered sowing windows were adopted so as to ensure the heat stress tolerance of genotypes showed a clear distinction in the diurnal mean temperatures during peak anthesis stage of the germplasm. The temperatures during anthesis for the first staggered sown set was 33° - 35°C, while it is around 38° - 40°C for the late sown set (Annexure II). Accordingly, the performance of the rice genotypes exhibited significant reduction in traits measured for late sown set (Figure 4.1). Trait-wise differences observed in different rice genotypes are presented in the following section.

4.1.1.1 Grain yield per plant

Grain yield per plant ranged from 4.47 g to 38.13 g under controlled conditions, while it was only 1.04 g to 26.23 under late sown conditions which experienced RSHS (Table 4.1). Accordingly, there was a shift in the mean from 20.41 g under control to 15.14 g under RSHS. In terms of percentage reduction, the mean yield decline under stress was observed to be 26% relative to control. Some genotypes were found to superior *per se* grain yield under RSHS which includes Bhuban (26.24 g), Indravati (25.56 g), PRR127 (25.42 g) and PRR122 (24.93 g). Stress tolerance index for grain yield (STI_{gy}) varied from 0.01 to 2.14. The genotypes with high STI_{gy} values included Ajay (2.14), HUR105 (2.07), PNR381 (1.96), Bhuban (1.93) and PRR127 (1.92) (Table 4.2). Taking both *per se* grain yield and STI_{gy} together, Bhuban and PRR127 were found to be promising for tolerance to reproductive stage high temperature stress. Overall, the heritability under RSHS was found to be higher relative to control and most of the variation observed was primarily genetic as can be deduced from the difference between PCV and GCV.

4.1.1.2. Spikelet fertility

Spikelet fertility ranged from 33.85% to 97.52% under timely sown non-stress situation, while under RSHS it ranged between 5.30% to 91.46% (Table 4.1). The mean showed significant shift from 82.49% under control to 66.82% under RSHS. Considering percentage reduction, the mean dip in the spikelet fertility under stress relative to control is around 19%, which is comparatively less when compared to grain yield. The genotypes N22 (95.87), DV85 (91.46%), Improved Samba Mahsuri (90.43%) and BJ1 (90.18%), were found superior in terms of *per se* spikelet fertility. Furthermore, the stress tolerance index for spikelet fertility (STI_{sf}) ranged from 0.03 to 1.27 with a mean of 0.82. In terms of STI_{sf} , N22 (1.34), improved Samba Mahsuri (1.28), DV85 (1.26), Haldimuri (1.26) and North Andaman 2 (1.24) were found promising (Table 4.2). Considering both *per se* spikelet fertility and STI_{sf} , DV85 and Improved Samba Mahsuri were found to be the best performing genotypes. The heritability for spikelet fertility was higher under control compared to RSHS in contrast to the observation in grain yield. Corroborating this, the difference between PCV and GCV was found to be higher under RSHS than the control.

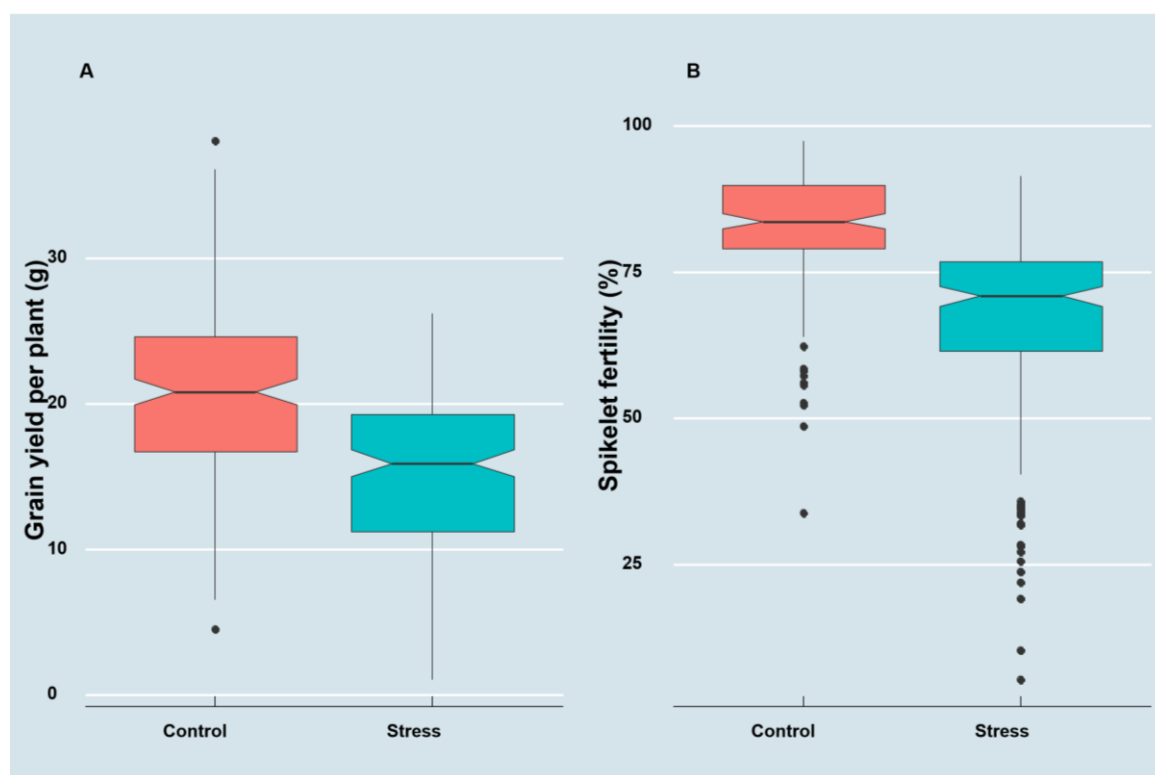


Figure 4.1 Boxplots depicting the distribution of (A) grain yield plant⁻¹ (g) and (B) spikelet fertility (%) under control and RSHS conditions

Table 4.1 Summary statistics of grain yield plant⁻¹ (g) and spikelet fertility (%) under control and RSHS conditions

Statistics	Grain yield plant ⁻¹ (g)		Spikelet fertility (%)		STI _{sf}	STI _{gy}
	Control	RSHS	Control	RSHS		
Mean	20.41	15.14	82.49	66.82	0.82	0.81
Min	4.47	1.04	33.85	5.30	0.03	0.01
Max	38.13	26.23	97.52	91.46	1.27	2.14
S.D.	6.60	5.77	9.87	16.85	0.25	0.47
S.E.	0.47	0.42	0.72	1.22	0.01	0.03
C.V. (%)	24.05	16.28	6.30	23.09		
PCV	31.93	37.62	11.72	24.75		
GCV	20.89	33.94	9.89	16.54		
h ² (broad sense)	42.83	81.36	71.19	65.91		
GA	5.78	9.58	14.21	10.08		

S.D., Standard deviation; S.E., Standard error mean; C.V., Co-efficient of variation; PCV, Phenotypic coefficient of variation; GCV, Genotypic coefficient of variation; h², Heritability; GA, Genetic advance, STI_{sf}, Stress tolerance index calculated for spikelet fertility; STI_{gy}, Stress tolerance index calculated for grain yield per plant

4.1.2. Experiment 2: Screening of Basmati rice germplasm for tolerance to RSHS

A set of 35 Basmati genotypes were characterized for heat stress behavior at reproductive stage for two consecutive seasons at IARI-RBGRC, Aduthurai, Tamil Nadu. Grain yield and spikelet fertility were primarily used for delineating the heat stress tolerance of these genotypes. The two staggered sowings provided adequate contrast in ambient temperature for exposing the genotypes to optimum and high temperatures. The set sown during second fortnight of December came to flowering during early to mid-March, while those sown in second fortnight of January flowered during mid-April. The temperature during anthesis of genotypes in the timely sown condition was ~33°C (hence considered normal or non-stress) whereas the late sown materials were ~39°C (heat stress) (Annexure II). This was clearly reflected as differences in the genotypic performance under the respective treatments. The grain yield ranged from 4.93 g to 24.45 g and 5.95 g to 25.83 g under non-stress condition during off-seasons 2018-19 and 2019-20. The mean grain yield of the Basmati rice germplasm was almost similar in both seasons, i.e., 16.62 g during 2018-19 and 14.22 g during 2019-20. However, significant reduction in grain yield was observed across all genotypes under RSHS varying between 1.0 g to 12.2 g during 2018-19 and 3.59 to 13.03 during 2019-20 and their respective means being 7.61 g and 8.74 g. In terms of mean per cent reduction of grain yield (g) under stress, there was a decline of 54% in 2018-19 and 38.53% during 2019-20 (Table 4.3).

Table 4.2 Adjusted means of grain yield per plant and spikelet fertility and their corresponding indices of 192 genotypes (along with checks) evaluated for reproductive stage heat stress during off-season, 2018-19 at Aduthurai, Tamil Nadu^s

S. No.	Genotype	Grain yield plant ⁻¹ (g)			Spikelet fertility (%)		
		Control	RSHS	STI	Control	RSHS	STI
1	CR 2461-9	19.50	17.80	0.83	91.32	67.35	0.90
2	UPRI 2003-45	23.32	21.50	1.20	90.86	83.32	1.11
3	PNR 381	25.55	16.75	1.02	87.21	67.32	0.86
4	Punjab Mehak 1	21.41	15.80	0.81	86.25	63.14	0.80
5	PNR 381	35.49	23.17	1.96	77.08	71.25	0.81
6	Jayati	33.54	18.25	1.46	80.21	76.76	0.90
7	CT 10006-7-2M-5-1 P3-M	30.37	9.67	0.70	72.63	55.19	0.59
8	Chandrasahini	38.13	14.63	1.33	80.91	75.80	0.90
9	HPR 2143	17.82	13.25	0.56	83.16	43.32	0.53
10	MAS 946-1	23.45	17.67	0.99	87.96	65.25	0.84
11	PRR 126	17.00	15.18	0.62	83.33	72.03	0.88
12	Ajay	36.18	24.88	2.15	90.38	73.98	0.98
13	RNRM 7	24.62	20.25	1.19	90.63	84.11	1.12
14	Falguna	22.18	19.38	1.03	85.89	70.03	0.88
15	VLT-6	18.85	18.67	0.84	85.82	77.01	0.97
16	RIL 10	29.87	23.50	1.68	90.59	86.23	1.14
17	Pant dhan 19	33.05	23.50	1.85	83.00	67.78	0.82
18	NDR 8015-1	23.68	22.00	1.24	80.61	69.99	0.83
19	Pusa Sugandh 3	25.08	15.40	0.92	85.52	76.29	0.96
20	Bala	9.77	9.00	0.21	79.30	68.65	0.80
21	Pant sankar dhan 3	19.39	16.30	0.75	80.47	70.00	0.83
22	Samanta	26.00	24.90	1.55	84.39	74.98	0.93
23	Tapaswani	25.82	19.13	1.18	86.37	69.75	0.88
24	HPR 2083	24.57	22.50	1.32	76.02	34.30	0.38
25	Birupa	20.88	19.87	0.99	87.72	74.62	0.96
26	Bhuban	30.83	26.24	1.93	94.70	75.35	1.05
27	Pant dhan 10	21.09	18.33	0.92	92.22	75.78	1.02
28	OYR 128	23.25	18.99	1.05	82.82	79.84	0.97
29	OYC 183	20.31	19.80	0.96	94.02	80.22	1.11
30	BJ 1	16.50	14.73	0.58	88.96	66.67	0.82
31	Chandana	11.59	9.50	0.26	79.74	33.83	0.40
32	Urvashi	15.46	14.10	0.52	88.78	61.12	0.80
33	Pant dhan 16	18.17	17.73	0.77	91.94	53.91	0.73
34	P 1490-03	15.02	7.00	0.25	48.74	27.12	0.19
35	CSR 27	24.10	16.80	0.97	93.10	76.13	1.04
36	China 988	13.83	10.14	0.33	85.26	77.02	0.96
37	DV 85	25.47	17.13	1.04	94.09	91.46	1.26

S. No.	Genotype	Grain yield plant ⁻¹ (g)			Spikelet fertility (%)		
		Control	RSHS	STI	Control	RSHS	STI
38	IRAT 240	25.91	18.33	1.13	89.30	83.47	1.09
39	CR 2363-26	13.75	7.38	0.24	73.88	44.34	0.48
40	Selected Sabarmati	21.98	20.38	1.07	86.80	86.79	1.10
41	MTU 1010	25.83	20.60	1.27	95.07	78.79	1.10
42	Kataktara	19.61	16.50	0.77	75.59	74.45	0.82
43	VOH-PCR-3110	23.98	19.30	1.11	84.05	76.13	0.94
44	Karuppunel	16.50	12.75	0.50	88.25	75.76	0.98
45	Kasturi	15.33	11.41	0.42	81.98	80.67	0.97
46	C 1268-7-10	23.42	20.63	1.15	94.23	54.01	0.75
47	BJ 1	26.49	16.75	1.06	90.54	90.18	1.20
48	VL 88-97-1-7	18.09	14.00	0.60	77.41	75.75	0.86
49	Kamlesh	15.47	13.70	0.51	92.04	87.71	1.18
50	IR 78908	15.20	8.44	0.31	76.93	69.84	0.79
51	B6144-MR-6-0-0	16.81	15.33	0.62	83.59	82.60	1.01
52	Seond Basmati	6.98	6.43	0.11	52.36	28.11	0.22
53	HPR 2104	12.45	10.74	0.32	68.12	46.26	0.46
54	Shah Pasand	10.09	9.70	0.23	56.01	31.77	0.26
55	Chimbalate Basmati	20.78	19.71	0.98	85.68	68.16	0.86
56	PMK 1	29.74	21.14	1.50	78.18	64.00	0.73
57	Sitwa Dhan	26.15	24.66	1.54	77.49	75.02	0.85
58	Gouri	27.44	24.90	1.63	78.06	76.81	0.88
59	Ananga	17.62	13.61	0.57	87.73	61.24	0.79
60	Pant dhan 4	20.10	17.90	0.86	86.27	71.30	0.90
61	OYR 69	8.49	2.14	0.04	55.61	43.53	0.35
62	Khuch	8.85	8.65	0.18	75.83	60.96	0.68
63	CR 2499	26.90	18.48	1.19	91.97	87.03	1.17
64	Pusa 1460	21.26	17.69	0.90	79.33	69.66	0.81
65	GP325	22.09	19.20	-	77.51	66.98	-
66	UPRI 2003-24	24.36	17.71	1.03	90.79	77.41	1.03
67	Poornima	12.42	11.67	0.35	67.27	61.84	0.61
68	UPRI 2003-18	20.14	19.96	0.96	89.27	79.68	1.04
69	Narendra Usar Dhan III	25.23	21.54	1.30	91.96	79.78	1.08
70	NDR 359	32.02	23.49	1.80	90.35	82.57	0.82
71	NDR 97	27.22	16.85	1.10	90.99	82.95	1.11
72	Bhadrakali	25.50	21.54	1.31	90.73	89.81	1.19
73	Shiva	29.98	10.27	0.74	85.34	73.49	0.92
74	IR 77384-12-35-3-6-7-2-B	17.23	15.20	0.81	87.58	83.88	1.08
75	Narendra Usar Dhan II	28.73	22.19	1.52	74.15	73.68	0.80
76	SKAU 220	12.59	8.54	0.26	85.52	73.77	0.92
77	PRR 105	19.33	18.00	0.83	68.15	59.23	0.59
78	PRR 117	16.74	14.53	0.58	87.13	85.58	1.09

S. No.	Genotype	Grain yield plant ⁻¹ (g)			Spikelet fertility (%)		
		Control	RSHS	STI	Control	RSHS	STI
79	PRR 103	21.12	18.60	0.94	81.18	68.34	0.81
80	PRR 123	21.49	17.74	0.91	79.47	68.08	0.79
81	PRR 114	20.80	14.66	0.73	91.28	73.37	0.98
82	PRR 120	17.55	15.18	0.64	78.89	78.58	0.91
83	PRR 115	18.57	17.37	0.77	86.78	71.02	0.90
84	PRR 104	23.11	17.01	0.94	89.91	73.76	0.97
85	PRR 108	21.63	12.89	0.67	80.18	62.52	0.73
86	PRR 121	20.84	19.26	0.96	87.74	74.77	0.96
87	Pant dhan 18	19.98	19.27	0.92	91.15	88.72	1.19
88	Indravati	27.88	25.56	1.70	83.64	82.81	1.02
89	Pant Sugandh Dhan 17	16.11	14.30	0.55	86.46	70.71	0.90
90	HUR 200-57-1	23.37	11.11	0.62	85.75	53.93	0.68
91	Pant dhan 15	26.08	5.54	0.35	81.76	25.54	0.31
92	JGL 11727	23.64	15.37	0.87	83.65	73.67	0.90
93	Mahanadi	33.65	14.21	1.14	80.29	63.08	0.74
94	Pant Sugandh Dhan15	18.06	6.64	0.29	79.95	23.65	0.28
95	Bhuman San	21.49	7.59	0.39	84.51	35.71	0.44
96	JR 75	23.58	19.88	1.12	93.18	87.06	1.19
97	CO 37	14.12	12.25	0.41	72.13	71.11	0.75
98	Sumati	18.45	6.83	0.30	78.21	19.17	0.22
99	SAF 1221-83	8.68	7.34	0.15	75.87	62.49	0.70
100	P 1447	17.94	7.48	0.32	76.82	65.17	0.73
101	P 1447-00-5-1	19.61	15.22	0.71	87.00	67.87	0.87
102	HUR 105	35.42	24.42	2.07	87.48	65.65	0.84
103	Manaswini	16.36	13.23	0.52	67.07	61.19	0.60
104	RR 8585	21.97	21.90	1.15	88.14	76.62	0.99
105	CSR 23	29.13	20.66	1.44	79.93	71.71	0.84
106	Kudrat-3	22.37	12.31	0.66	93.41	84.01	1.15
107	RAU 3061	28.67	21.23	1.45	82.57	65.72	0.82
108	NDR 625	23.60	14.07	0.79	80.10	60.98	0.72
109	Sagar Dambha	15.15	14.18	0.51	71.88	64.27	0.68
110	JGL 3828	23.45	18.45	1.03	94.23	82.76	1.14
111	P 1460	23.64	11.57	0.65	85.71	69.62	0.87
112	Muskan	23.97	19.28	1.10	90.47	76.29	1.01
113	Pusa 1342	11.24	6.66	0.18	66.85	50.82	0.50
114	MR 219	23.99	17.17	0.98	90.80	74.71	0.99
115	UPRI 2003-15	20.01	19.73	0.94	91.49	79.40	1.06
116	Samba Mahsuri	24.23	22.32	1.29	88.64	78.24	1.02
117	Sharbati	26.69	16.75	1.07	80.39	74.13	0.87
118	HKR 200-57-1	22.39	20.23	1.08	93.44	73.28	1.00
119	WGL 23985	23.37	18.12	1.01	84.05	75.97	0.94

S. No.	Genotype	Grain yield plant ⁻¹ (g)			Spikelet fertility (%)		
		Control	RSHS	STI	Control	RSHS	STI
120	Rajendra Basmati	24.99	14.25	0.85	82.57	60.92	0.82
121	MR 220	24.13	23.09	1.33	78.77	69.27	0.80
122	Raja Vaellu	24.01	15.52	0.89	78.95	69.00	0.80
123	CR 246-16	20.19	17.28	0.83	81.04	73.87	0.88
124	Phunchi	19.07	10.45	0.48	80.81	70.48	0.83
125	Pusa Basmati 1121	19.48	8.68	0.40	58.13	51.76	0.44
126	Improved Sabarmati	26.30	16.27	1.02	78.92	68.63	0.79
127	Tompha Khau	9.06	8.44	0.18	82.36	74.34	0.90
128	CN-1268-7	18.08	16.83	0.73	84.24	62.17	0.77
129	WGL 14	30.34	19.61	1.42	78.58	72.43	0.83
130	Hema	24.88	22.22	1.32	86.79	86.01	1.09
131	UPRVS 8-26	20.47	18.36	0.81	82.57	73.58	0.82
132	Super Basmati	17.37	12.16	0.50	75.29	62.85	0.69
133	Pusa 1301	10.30	5.32	0.13	91.57	40.34	0.54
134	Kanak	18.38	14.96	0.66	84.55	79.72	0.99
135	PRR 109	18.88	15.95	0.72	70.94	66.67	0.82
136	PRR 110	19.03	12.78	0.58	58.44	46.24	0.40
137	PRR 127	31.57	25.42	1.92	89.96	71.46	0.94
138	PRR 122	26.20	24.93	1.56	91.97	88.52	1.19
139	PRR 118	6.53	3.06	0.05	33.85	5.30	0.03
140	HUR 36	19.85	17.64	0.84	89.59	54.77	0.72
141	RR 166-645	7.23	6.23	0.11	52.70	51.21	0.40
142	Nirri	25.89	20.46	1.26	74.49	63.99	0.70
143	C 22	21.35	19.27	0.98	92.75	73.71	1.00
144	DHMAS-70G-164-29	16.74	5.60	0.22	75.46	53.36	0.59
145	Ranbir Basmati	18.73	11.31	0.51	82.42	28.45	0.34
146	T23	26.89	24.64	1.58	87.92	75.62	0.97
147	Langphou	22.79	15.03	0.82	77.55	61.81	0.70
148	ASD 19	25.35	20.20	1.22	91.05	45.39	0.61
149	Basmati 370	15.78	13.39	0.50	80.98	70.83	0.84
150	BPT 5204 (?)	18.87	13.26	0.60	82.31	69.28	0.84
151	IC 2127	22.84	15.11	0.82	88.91	79.89	1.04
152	VOH-PCR-3113	18.53	16.62	0.74	82.49	67.39	0.81
153	TYPE 3	16.04	13.86	0.53	90.40	78.40	1.04
154	Nagina 12	22.61	17.13	0.92	82.64	70.68	0.86
155	Sonasal	16.81	8.88	0.36	71.20	65.44	0.68
156	Khara Munga	14.76	11.65	0.41	91.99	74.15	1.00
157	IR 70	25.04	22.48	1.34	81.31	80.29	0.96
158	ADT 39	29.21	23.57	1.64	89.85	79.38	1.05
159	Haldimuri	25.14	23.38	1.40	97.52	88.35	1.26
160	MTU 7029	23.77	21.75	1.23	91.73	79.82	1.07

S. No.	Genotype	Grain yield plant ⁻¹ (g)			Spikelet fertility (%)		
		Control	RSHS	STI	Control	RSHS	STI
161	PDKV Chinoor 2	21.62	7.71	0.40	81.73	65.37	0.78
162	Swarna Sub 1	21.96	17.16	0.90	79.67	71.31	0.83
163	Tilak Chandan	11.67	8.08	0.23	86.69	53.46	0.68
164	Pusa 33	22.95	19.65	1.08	76.91	75.74	0.85
165	Pratikshya	22.85	17.57	0.96	92.26	74.31	1.00
166	Jhulhat	21.16	11.74	0.59	82.57	72.42	0.82
167	NDR 9830144	20.33	13.88	0.67	89.83	76.62	1.01
168	MTU 1001	29.12	17.97	1.25	90.40	83.06	1.10
169	Bamleshwari	30.65	11.25	0.82	82.57	83.28	0.82
170	Ramachandi	24.27	18.67	1.08	91.43	86.57	1.16
171	GP584	20.35	8.31	0.40	88.08	75.20	0.97
172	Apo	17.30	16.03	0.66	91.41	73.60	0.99
173	Aziz Beoul	11.76	7.88	0.22	82.80	67.42	0.82
174	Begum	9.17	3.17	0.07	83.52	10.26	0.45
175	Bala Koun	6.82	5.49	0.09	57.28	53.37	0.45
176	Buta Baber	7.24	5.00	0.09	81.28	57.67	0.69
177	Baber	13.16	5.09	0.16	87.13	57.09	0.73
178	Baber Safed	12.52	9.18	0.27	84.61	34.75	0.43
179	Budgi	13.15	4.99	0.16	83.74	21.88	0.27
180	Gull Baber	10.75	8.80	0.23	64.00	46.46	0.44
181	Kaw Qudder	8.23	8.03	0.16	73.53	54.58	0.59
182	Kew	8.65	7.00	0.14	62.22	35.16	0.32
183	Mehvan (purple)	10.67	5.29	0.13	75.58	33.43	0.37
184	Mehvan (green)	9.72	6.82	0.16	83.19	31.98	0.39
185	Mir Zug	4.47	1.04	0.01	77.98	44.86	0.51
186	CO 50	14.79	6.71	0.24	90.25	45.03	0.60
187	CO 51	16.11	15.41	0.59	95.95	83.46	1.17
188	Arupathaam Kuruvai	19.35	12.53	0.58	79.16	70.58	0.82
189	Improved Samba Mahsuri	17.08	16.84	0.69	96.54	90.43	1.28
190	Sabour Surbhit	17.70	13.56	0.57	70.13	66.51	0.68
191	North Andaman 2	17.58	15.87	0.67	94.92	89.38	1.24
192	GP975	10.73	5.82	0.15	84.73	61.57	0.76
C1	NL44	25.21	20.94	1.24	90.53	87.92	1.16
C2	Pusa Basmati 1	23.04	13.76	0.75	88.57	56.85	0.74
C3	N22	21.89	20.16	1.04	96.21	95.87	1.34
C4	IR64	29.72	18.27	1.28	87.51	75.96	0.97
C5	PB1509	25.57	12.15	0.73	86.24	49.89	0.63
C.D. (5%) Checks		3.50	2.74	-	3.67	11.03	-
C.D. (5%) Genotypes of same block		7.00	3.49	-	7.34	22.06	-
C.D. (5%) Genotypes of different blocks		7.67	3.82	-	8.04	24.17	-
C.D. (5%) Checks vs test genotypes		5.86	2.92	-	6.14	18.46	-

*The mean of checks across blocks was given

Similarly, spikelet fertility ranged between 52.96 % to 90.51 % and 28.31 % to 86.72 % under control situation during off-season, 2018-19 and 2019-20, respectively. However, the spikelet fertility under RSHS varied between 8.14 to 65.5% and 10.31 to 61.86% in 2018-19 and 2019-20, respectively. The shift in mean spikelet fertility from control to RSHS was from 76.50 to 41.40 in 2019, whilst it was from 61.58 to 44.08 during 2020-21. In percentage terms, this reduction was higher in 2018-19 (46%) than in 2019-20, (28.42%). This trend is also observed for grain yield indicating the higher level of RSHS during off-season, 2018-19. The distributions of genotypes were skewed towards sensitive side showing that most of them were highly susceptible to RSHS. The variation (S.D.) for the traits was narrow in both control and RSHS. Particularly under heat stress S.D. showed further shrinkage.

In order to classify the genotypes into various tolerance categories stress tolerance index (for grain yield) and relative spikelet fertility (RSF) were calculated for individual seasons (Table 4.4). During both 2019-20 and 2020-21, highest STI was recorded in Basmati370 (0.97 & 0.96) closely followed by Type23 (0.92 & 0.89) while the lowest was observed in Seond Basmati (0.07 & 0.08). However, in terms of RSF, Haryana Basmati 1 showed highest value (0.61 & 0.77) and PB1718 recorded the lowest value (0.31 & 0.48) during off-seasons, 2019-20 and 2020-21. Considering both STI and RSF, Basmati 370, Basmati Jamuna, Chimbale Basmati, and Type 23 were found to be moderately tolerant while Seond Basmati and PB 1718 were highly sensitive to RSHS. However, these categories are relative, not exclusive since there was significant reduction in both the traits across genotypes in general.

4.2. Genome wide association mapping for tolerance to RSHS

4.2.1. Population structure and linkage disequilibrium

Population structure analysis using PCA showed that there are three distinct sub-populations in the panel. PC1 accounted for 29% of total variation followed by PC2 (7%) and PC3 (6%) (Figure 4.2A). These three PCs are considered for deciphering the number of sub-populations. The first sub-population contained the highest number of genotypes i.e., 139, whereas the second and third amassed 35 and 15 accessions, respectively (Figure 4.2B). The first sub-population comprised of mostly popular varieties released in India such as Improved Sabarmti, MTU1001, ADT39 and some advanced breeding lines. Temperate

landraces originating from Jammu and Kashmir were grouped into second sub-population, whereas the third sub-population comprised of remaining miscellaneous genotypes.

Table 4.3 Summary statistics of grain yield and spikelet fertility recorded on 35 Basmati rice genotypes under control and RSHS during off-season, 2018-19 and 2019-20

Statistic	Off-season, 2018-19		Off-season, 2019-20	
	Control	RSHS ^{\$}	Control	RSHS ^{\$}
Grain yield plant⁻¹ (g)				
Mean	16.62	7.61 (54.21)	14.22	8.74 (38.53)
Maximum	24.45	12.2 (50.10)	25.83	13.03 (49.55)
Minimum	4.93	1.00 (79.71)	5.95	3.59 (39.66)
Standard Error	0.68	0.49	0.77	0.41
Standard deviation	3.99	2.86	4.54	2.41
Skewness	-0.65	-0.53	0.57	0.11
Kurtosis	1.95	-0.06	0.04	-0.66
C.V. (%)	24.03	37.59	31.94	27.61
Spikelet fertility (%)				
Mean	76.50	41.40 (45.88)	61.58	44.08 (28.42)
Maximum	90.51	65.50 (27.63)	86.72	61.86 (28.66)
Minimum	52.96	8.14 (84.62)	28.31	10.31 (63.58)
Standard Error	1.73	2.83	2.56	2.08
Standard deviation	10.11	16.28	14.90	12.14
Skewness	-0.78	-0.37	-0.38	-0.81
Kurtosis	-0.08	-0.80	-0.37	0.48
C.V. (%)	13.21	39.34	24.20	27.55

^{\$}Figures in the parenthesis are the percentage reduction values over the control.

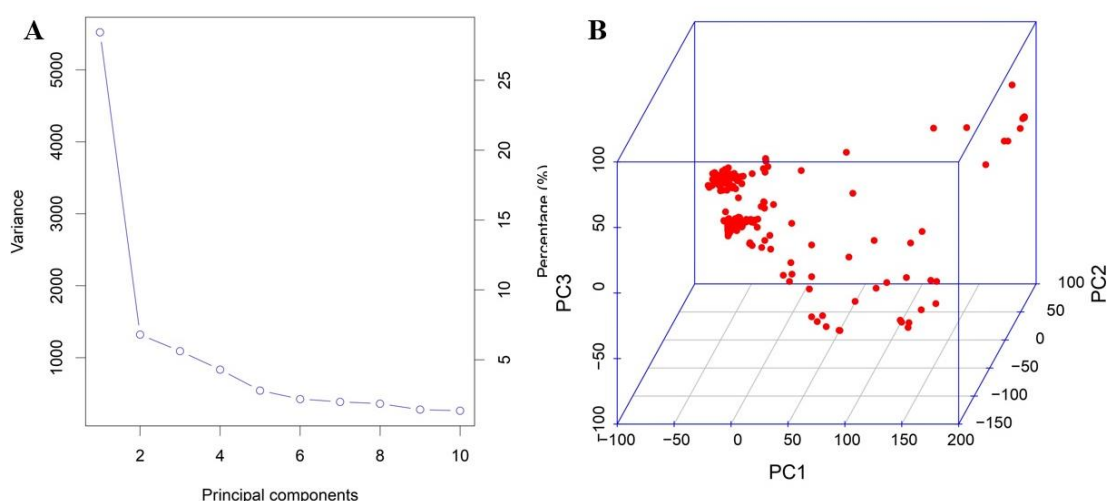


Figure 4.2 Scree plot showing the proportion of total variance accumulated by each principal component (A) and PCA 3D plot depicting the distribution of genotypes across three principal components (B)

Table 4.4 Mean performance and stress indices of genotypes for grain yield per plant (g) and spikelet fertility (%) under control and RSHS situations during off-seasons, 2018-19 and 2019-20.

Genotype	Grain yield plant ¹				Spikelet fertility (%)								Overall heat stress reaction
	Off-season, 2018-19		Off-season, 2019-20		Off-season, 2018-19		Off-season, 2019-20		Off-season, 2019-20		RSF ^{\$}		
	Control	Heat stress	STI ^{\$}	Control	Heat stress	STI ^{\$}	Control	Heat stress	Control	Heat stress			
Taraori Basmati	11.17	6.50	0.29	9.18	6.48	0.29	81.89	51.45	0.57	63.87	42.08	0.72	S
CSR 30	11.56	7.03	0.32	11.55	5.67	0.32	89.09	64.64	0.59	55.09	31.46	0.70	S
Basmati 370	24.45	12.20	0.97	23.44	7.30	0.96	90.51	53.19	0.60	80.28	55.42	0.77	MT
Ranbir Basmati	14.19	9.45	0.57	18.38	7.40	0.56	86.62	65.50	0.61	81.52	44.71	0.76	MS
Super Basmati	15.81	9.90	0.57	17.04	6.75	0.56	79.77	40.24	0.55	70.67	38.21	0.72	MS
Type 3	17.43	5.35	0.52	15.02	9.71	0.52	71.62	36.94	0.51	46.58	45.30	0.69	MS
Type 23	20.25	11.21	0.92	20.06	12.39	0.89	81.09	40.34	0.56	69.83	54.01	0.74	MT
Basmati 386	17.32	9.35	0.53	13.10	7.71	0.52	77.97	61.49	0.60	75.29	58.88	0.76	MS
Punjab Basmati 5	9.68	8.10	0.30	10.09	5.66	0.29	70.89	44.38	0.50	28.31	23.21	0.59	S
Kasturi Basmati	17.25	-	0.77	17.13	13.03	0.74	83.85	-	0.57	60.29	49.48	0.73	MS
Palam Basmati	13.32	8.30	0.41	10.04	8.55	0.40	78.57	28.44	0.51	52.27	41.93	0.69	S
Sanwal Basmati	17.28	6.75	0.48	11.07	9.49	0.47	69.26	16.43	0.47	62.51	48.63	0.71	MS
Basmati Kota	15.40	9.70	0.46	10.79	7.16	0.45	79.47	58.73	0.59	69.45	58.89	0.76	MS
Basmati Jamuna	21.75	7.94	0.85	25.83	8.07	0.85	88.23	58.35	0.61	81.93	56.04	0.77	MT
Seond Basmati	4.93	1.00	0.07	5.95	3.59	0.08	55.42	16.76	0.38	45.96	24.71	0.61	HS
Chimbalate Basmati	22.35	11.20	0.91	18.16	12.04	0.89	81.86	60.12	0.60	81.87	61.86	0.77	MT
Domsiab	17.92	11.00	0.75	18.25	9.92	0.73	83.15	54.47	0.59	71.89	50.81	0.75	MS
Shahi Pasand	21.94	8.40	0.65	13.42	9.28	0.64	82.64	32.13	0.53	53.20	38.46	0.69	MS
Hasan Serai	18.18	5.95	0.52	13.95	9.31	0.52	64.24	22.12	0.46	55.08	45.60	0.69	MS
Haryana Basmati 1	17.50	10.20	0.76	17.27	12.37	0.74	89.49	58.38	0.61	86.72	55.13	0.77	MS
Punjab Basmati 4	19.94	10.69	0.62	13.04	7.77	0.60	84.75	56.40	0.60	76.92	61.77	0.77	MS
Punjab Basmati 2	15.96	7.00	0.39	10.21	6.80	0.39	80.78	42.56	0.55	59.32	48.72	0.72	S

Genotype	Grain yield plant ¹						Spikelet fertility (%)						Overall heat stress reaction
	Off-season, 2018-19			Off-season, 2019-20			Off-season, 2018-19			Off-season, 2019-20			
	Control	Heat stress	STI ^{\$}	Control	Heat stress	STI ^{\$}	Control	Heat stress	RSF ^{\$}	Control	Heat stress	RSF ^{\$}	
Punjab Basmati 3	15.98	5.60	0.46	13.35	8.93	0.45	85.74	42.03	0.57	73.92	53.52	0.75	MS
Vallabh Basmati 21	15.66	9.95	0.73	18.00	12.18	0.71	74.81	59.49	0.58	73.02	53.23	0.75	MS
Vallabh Basmati 22	16.42	7.88	0.47	10.40	9.42	0.46	68.71	34.82	0.49	54.43	27.97	0.66	MS
Vallabh Basmati 23	15.17	11.59	0.48	10.50	6.89	0.47	67.00	47.74	0.53	56.48	43.40	0.70	MS
Pusa Basmati 6	18.69	4.76	0.46	10.69	9.71	0.45	75.41	31.90	0.52	64.39	39.47	0.71	MS
Pusa Basmati 1121	14.45	7.80	0.37	10.19	6.40	0.37	55.67	44.79	0.47	35.70	24.86	0.60	S
Pusa Basmati 1509	19.90	3.20	0.57	15.35	11.92	0.57	72.16	8.14	0.43	48.87	40.23	0.66	MS
Pusa Basmati 1728	21.63	5.95	0.68	17.86	10.09	0.67	78.40	28.71	0.52	61.55	48.95	0.72	MS
Pusa Basmati 1637	15.85	6.80	0.71	20.40	12.61	0.69	62.37	29.81	0.49	69.09	44.74	0.72	MS
Pusa Basmati 1718	10.58	1.10	0.15	7.75	5.11	0.16	52.96	10.00	0.31	30.87	10.31	0.48	HS
Punjab Basmati 1	17.44	4.30	0.49	14.43	10.05	0.49	86.13	41.76	0.55	47.80	44.35	0.70	S
Haryana Basmati 2	17.58	5.15	0.41	11.45	7.39	0.41	70.40	23.81	0.47	48.80	32.30	0.65	S
Pusa Basmati 1	11.17	6.50	0.91	9.18	6.48	0.89	81.89	51.45	0.57	63.87	42.08	0.77	MS
LSD (<i>p</i> ≤0.05)	7.75	4.22	-	7.46	NS	-	15.65	16.30	-	19.03	24.3	-	-

^{\$}Indices are calculated based on the predicted values (BLUPs) of genotypes which were given in Annexure I.

STI: Stress tolerance index; RSF: Relative spikelet fertility; S: Sensitive; MS: Moderately sensitive; MT: Moderately tolerant; T: Tolerant; LSD: Least significant difference.

4.2.2. Genome wide association analysis

GWAS was carried out using three models, MLM, FarmCPU, and BLINK for traits such as grain yield plant⁻¹ and spikelet fertility (%) under both control and RSHS conditions. No significant quantitative trait nucleotides (QTNs) were observed for control treatment for both the traits studied. Similarly, no QTNs were identified for grain yield plant⁻¹ under heat stress using MLM (Figure 4.3A). However, seven QTNs and three QTNs were detected using FarmCPU (Figure 4.3B) and BLINK (Figure 4.3C), respectively (Table 4.5). These were distributed on the chromosomes 1, 5, 7, 8, 9, 10, and 11. Interestingly one QTN, namely *qHTGY8.1* was observed under both FarmCPU and BLINK. In terms of phenotypic variance explained (PVE) calculated using simple linear regression two QTNs *qHTGY1.1*, identified through FarmCPU and *qHTGY8.1*, found through both FarmCPU and BLINK were found to be major with PVE values of 23.22% and 19.88%, respectively. Several QTNs were identified for spikelet fertility – eight through FarmCPU, seven each through MLM and BLINK located on chromosomes 1, 2, 3, 4, 5, 6, 7 and 11. One QTN, *qHTSF5.1* was identified through all the models. Furthermore, QTNs, *qHTSF1.1* and *qHTSF6.1* were found through both MLM and FarmCPU, QTNs, *qHTSF4.2* and *qHTSF1.2* were shared by FarmCPU and BLINK, and QTN, *qHTSF6.1* was identified through MLM and BLINK. Furthermore, five of these QTNs were found to be major effect QTNs explaining PVE ranging from 18.4% (*qHTSF2.1*) to 25.87% (*qHTSF4.2*). Additionally, STI calculated for grain yield and spikelet fertility (%) were also used for GWAS. However, close correspondence was observed between the QTNs identified for STI and their respective traits in terms of positions.

4.2.3. Identification of genotypes with favourable allelic combination

The allelic composition of genotypes with best mean performance (grain yield and spikelet fertility) was investigated for the QTNs identified. About 18 genotypes were identified with superior trait values and maximum number of positive alleles. Highest number of positive alleles 29 out of 29 MTAs was observed in RIL10, which also displayed high grain yield and spikelet fertility under RSHS. This genotype can be valuable donor for RSHS tolerance in rice after further validation in future studies. Similarly, six genotypes had 28 positive alleles, three genotypes showed 27 positive alleles, four genotypes possessed 26 positive alleles and two genotypes each had 25 and 24 alleles (Table 4.6).

4.3. Characterization of parents of mapping population for RSHS tolerance

The parents, NL44 and PB 1 along with checks, N22, IR64 and PB1509 were initially characterized for their heat stress tolerance under controlled environment conditions to assess the contrast between them before proceeding to final evaluation of RILs. Physiological and biochemical attributes were characterized so as to validate the tolerance or sensitive behaviour of the genotypes towards RSHS (Figure. 4.4). The difference between the ambient temperature and foliage surface measured as canopy temperature depression (CTD) was highest for NL44 under control condition closely followed by PB 1 and PB 1509 (Figure. 4.4A).

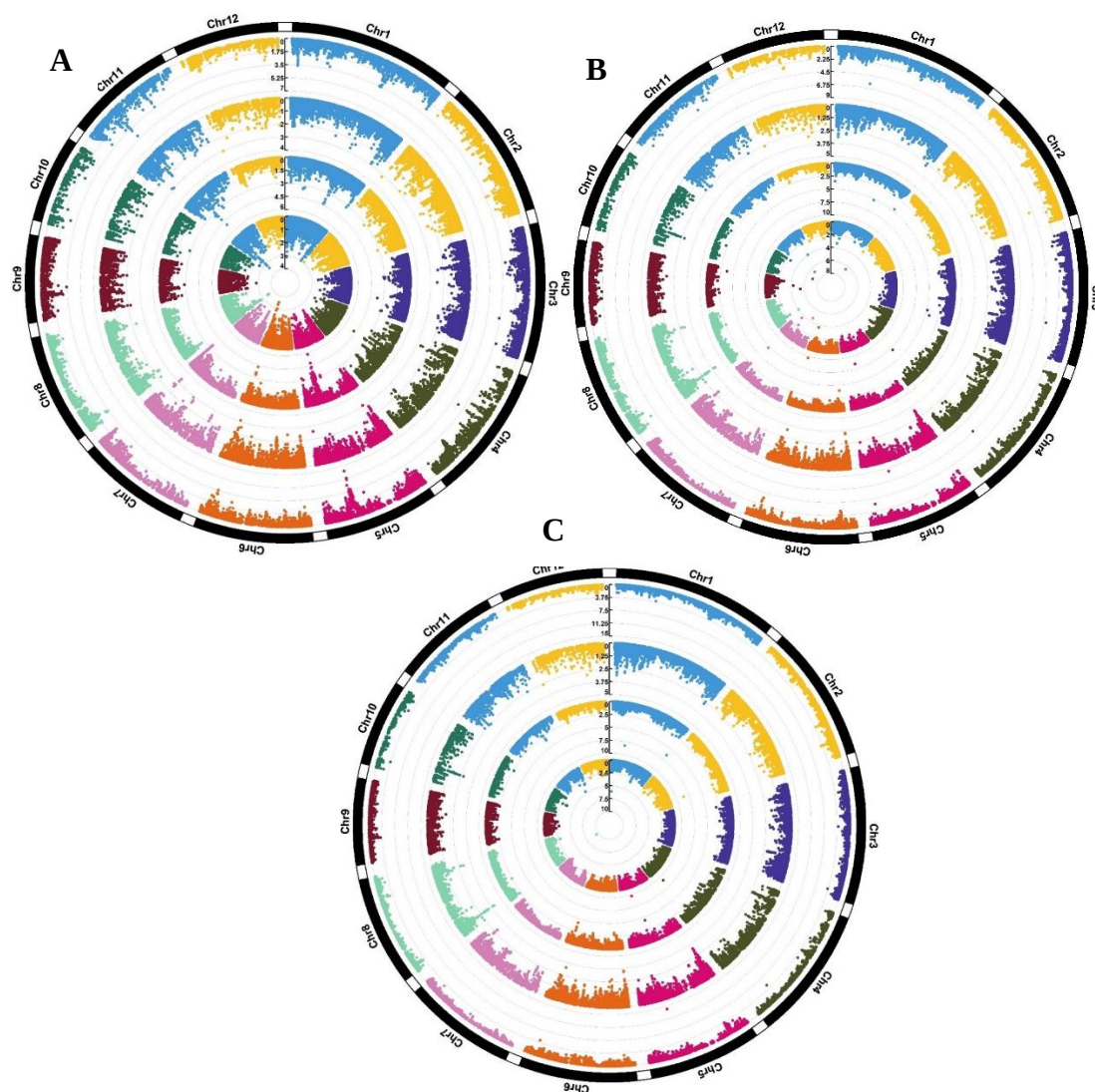


Figure 4.3 Circular Manhattan plot representing marker trait associations (MTAs) obtained through MLM (A), FamCPU (B) and BLINK (C) models. Circles from outside to inside depict grain yield plant⁻¹ (GYPP) under RSHS, spikelet fertility (SF) under RSHS, STI estimated for GYPP, and for SF.

Table 4.5 Significant marker trait associations (MTAs) identified for grain yield plant⁻¹ (GYPP) and spikelet fertility (SF) under RSHS and their respective stress tolerance index.

Trait	SNP	Model	Fav allele	Chr	Position	Probability	R ^{2#}	QTL	Previously reported QTLs ^s
GYPP	AX-95938448	FarmCPU	C	10	12691143	9.11E-08	6.13	qHTGY10.1	qhr3-1 (Cao et al., 2003)
	AX-95938539		T	11	7598615	2.03E-07	8.41	qHTGY11.1	qADL09-11 (Tazib et al., 2015)
	AX-95918021		G	1	43529356	2.74E-07	23.22	qHTGY1.1	-
	AX-95959896		C	9	6976331	4.10E-06	2.95	qHTGY9.1	-
	AX-95957982	BLINK	G	7	7144612	2.51E-05	8.29	qHTGY7.1	-
	AX-95938211		A	9	13559420	4.95E-05	1.07	qHTGY9.2	-
	AX-95937704		C	8	3014640	8.57E-05	19.88	qHTGY8.1	-
	AX-95937704		C	8	3014640	2.43E-10	19.88	qHTGY8.1	-
	AX-95916113	MLM	C	1	2051081	8.53E-07	3.65	qHTGY1.2	-
	AX-95932408		C	11	20582446	2.31E-05	5.71	qHTGY11.2	-
	AX-95926541		C	5	20542291	1.62E-06	2.29	qHTSF5.1	-
	AX-95961044		G	11	9168125	1.09E-05	3.68	qHTSF11.1	qHTSF11.2 (Ye et al., 2015)
SF	AX-95926170	FarmCPU	G	5	20547603	1.70E-05	2.89	qHTSF5.1	qLD10-11 (Tazib et al., 2015)
	AX-95927077		T	5	20540979	1.70E-05	2.89	qHTSF5.1	-
	AX-95962696		C	4	16049320	1.73E-05	0.02	qHTSF4.1	SSPF4 (Xiao et al., 2011a)
	AX-95918542		A	1	28440408	4.95E-05	18.85	qHTSF1.1	qPF4 (Xiao et al., 2011b)
	AX-95956527	FarmCPU	T	6	28019302	5.42E-05	3.69	qHTSF6.1	-
	AX-95956527		T	6	28019302	2.53E-10	3.69	qHTSF6.1	-
	AX-95926541		C	5	20542291	3.57E-09	2.29	qHTSF5.1	-
	AX-95930097		G	7	25603077	6.96E-08	25.1	qHTSF7.1	qAL10-7 (Tazib et al., 2015)
	AX-95924814	BLINK	C	4	28011513	9.78E-08	25.87	qHTSF4.2	-
	AX-95944879		T	1	28469004	6.75E-06	18.92	qHTSF1.1	-
	AX-95923670		G	3	31000572	1.45E-05	4.28	qHTSF3.1	qtl_3.4 (Jagadish et al., 2010)
	AX-95918181		G	1	43540685	2.80E-05	9.36e-03	qHTSF1.2	-
	AX-95949687	BLINK	A	3	34204030	5.32E-05	1.04	qHTSF3.2	-
	AX-95926541		C	5	20542291	2.15E-10	2.29	qHTSF5.1	-
	AX-95924814		C	4	28011513	7.73E-10	25.87	qHTSF4.2	-

Table 4.5 Contd.

Trait	SNP	Model	Fav allele	Chr	Position	Probability	R ^{2#}	QTL	Previously reported QTLs ^s
STIGY	AX-95921100	FarmCPU	T	2	30694562	2.67E-09	18.4	qHTSF2.1	qtl_2.2 (Jagadish et al., 2010)
	AX-95940947		T	1	10799807	5.56E-09	25.71	qHTSF1.3	-
	AX-95918181		G	1	43540685	3.66E-07	9.36e-03	qHTSF1.2	-
	AX-95936404		C	5	16852857	4.32E-05	5.15	qHTSF5.2	-
	AX-95956527		T	6	28019302	6.21E-05	3.69	qHTSF6.1	-
	AX-95930775		A	8	3009287	8.96E-05	16.97	qSTIGY8.1	-
	AX-95930775		A	8	3009287	8.96E-05	16.97	qSTIGY8.1	-
	AX-95926541		C	5	20542291	5.10E-07	2.29	qSTISF5.1	-
	AX-95927077		T	5	20540979	5.11E-06	2.89	qSTISF5.1	-
	AX-95926170		G	5	20547603	5.11E-06	2.89	qSTISF5.1	-
STISF	AX-95952837	MLM	A	5	20642828	8.34E-06	2.61	qSTISF5.1	-
	AX-95961044		G	11	9168125	1.53E-05	3.68	qSTISF11.1	qHTSF11.2 (Ye et al. 2015)
	AX-95927241		A	5	21126325	4.49E-05	1.19	qSTISF5.2	qLD10-11 (Tazib et al. 2015)
	AX-95962696		C	4	16049320	4.54E-05	0.02	qSTISF4.1	-
	AX-95926541		C	5	20542291	3.77E-09	2.29	qSTISF5.1	SSPF4 (Xiao et al. 2011a)
	AX-95924814		C	4	28011513	1.48E-07	25.87	qSTISF4.2	qPF4 (Xiao et al. 2011b)
	AX-95940947		G	1	10799807	1.17E-06	25.71	qSTISF1.1	-
	AX-95919771		T	2	31278025	6.76E-06	20.18	qSTISF2.1	-
	AX-95926541		C	5	20542291	8.87E-15	2.29	qSTISF5.1	-
	AX-95924814		C	4	28011513	1.50E-05	25.87	qSTISF4.2	-
	AX-95956527	BLINK	T	6	28019302	3.67E-05	3.69	qSTISF6.1	-
	AX-95919771		T	2	31278025	5.25E-05	20.18	qSTISF2.1	-

^sQTLs reported exclusively for reproductive stage heat stress tolerance in rice; [#]R² values are obtained through single marker analysis;

Fav allele, Favourable allele; Chr, Chromosome; GYPP, Grain yield per plant; SF, Spikelet fertility, STISF, Stress tolerance index calculated for spikelet fertility; STIGY, Stress tolerance index calculated for grain yield per plant

Table 4.6 Genotypes exhibiting superior performance and highest number of favourable alleles of 29 MTAs identified for tolerance to RSHS

S. No.	Genotype	GYC	GYH	STIGY	SFC	SFHT	STISF	NFA
1	RIL 10	29.87	23.5	1.68	90.59	86.23	1.14	29
2	Selected Sabarmati	21.98	20.38	1.07	86.8	86.79	1.1	28
3	Sitwa Dhan	26.15	24.66	1.54	77.49	75.02	0.85	28
4	Bhadrakali	25.5	21.54	1.31	90.73	89.81	1.19	28
5	Samba Mahsuri	24.23	22.32	1.29	88.64	78.24	1.02	28
6	Hema	24.88	22.22	1.32	86.79	86.01	1.09	28
7	Haldimuri	25.14	23.38	1.4	97.52	88.35	1.26	28
8	UPRI 2003-45	23.32	21.5	1.2	90.86	83.32	1.11	27
9	B6144-MR-6-0-0	16.81	15.33	0.62	83.59	82.6	1.01	27
10	Pant dhan 18	19.98	19.27	0.92	91.15	88.72	1.19	27
11	RNRM 7	24.62	20.25	1.19	90.63	84.11	1.12	26
12	Gouri	27.44	24.9	1.63	78.06	76.81	0.88	26
13	Indravati	27.88	25.56	1.7	83.64	82.81	1.02	26
14	ADT 39	29.21	23.57	1.64	89.85	79.38	1.05	26
15	IR 77384-12-35-3-6-7-2-B	17.23	15.2	0.81	87.58	83.88	1.08	25
16	JR 75	23.58	19.88	1.12	93.18	87.06	1.19	25
17	PRR 117	16.74	14.53	0.58	87.13	85.58	1.09	24
18	IR 70	25.04	22.48	1.34	81.31	80.29	0.96	24

GY_C, Grain yield under control; GY_H, Grain yield under heat stress; STI_GY, Stress tolerance index for grain yield; SF_C, Spikelet fertility under control; SF_H, Spikelet fertility under heat stress; STI_SF, Stress tolerance index for spikelet fertility; NFA, Number of favourable alleles.

Under heat stress also, NL44 maintained significant CTD followed by IR64 and N22. However, the membrane stability index (MSI) was highest for the tolerant check, N22 followed by NL44 and IR64, while PB1509 registered lowest MSI under RSHS (Figure. 4.4B). The damage to the chloroplasts was measured as SPAD chlorophyll meter reading (SCMR) on both flag leaf and 2nd leaf from the top which did not show a clear pattern across treatments (Figure. 4.4C). For flag leaf, SCMR under control was highest for IR64 followed by PB 1 and NL44, whereas under heat stress NL44 registered high SCMR. Similarly for 2nd leaf from the top, highest SCMR under control was observed in IR64 while under heat stress PB 1509 and PB 1 displayed highest SCMR values. Interestingly, the SCMR values under heat stress were higher than under control for all the genotypes except in case of IR64.

As proxies of ROS damage, H₂O₂ and thiobarbituric acid reactive substance (TBARS) were estimated in the genotypes using flag leaf and panicle samples. In general, a significantly higher accumulation of H₂O₂ was observed in flag leaf as compared to

panicle and the accumulation was more pronounced under RSHS than in control (Figure 4.4D). Lowest accumulation of H_2O_2 in flag leaf was observed in NL44 under both control and RSHS situations. The check N22, though considered heat stress tolerant, showed relatively higher accumulation of H_2O_2 in both the treatments. However, this contrast was not clear in panicles, where almost all genotypes showed similar concentrations of H_2O_2 with lesser values under control than in heat stress. The damage to the cell membrane rendered by ROS was estimated through TBARS assay. Under control, highest TBARS values was observed for PB 1509 in flag leaf, while N22 and NL44 recorded the lowest (Figure. 4.4E). However, under RSHS, there was a significant increase in the values with the highest observed in PB 1509 followed by PB 1 and IR64. Same trend was observed in panicle samples, however, the values obtained were on lower side in comparison to flag leaf.

The capacity of these genotypes to scavenge ROS (antioxidant capacity) was assessed through a Ferric Reducing Antioxidant Power (FRAP) assay. In general, the antioxidant capacity under control were low as expected as less amount of reduction pool is available to convert Fe^{3+} to Fe^{2+} than under RSHS (Figure. 4.4F). In congruence with the estimates of H_2O_2 and TBARS, all the genotypes could effectively contain the buildup of ROS in the panicles. Individually, all the genotypes exhibited similar values (of Fe^{2+}) under control conditions in flag leaf. However, under heat stress, highest value was observed for PB 1509 followed by PB 1, whilst the lowest values were obtained for N22 and NL44. IR64 showed moderate concentrations of Fe^{2+} . Similar trend was observed in panicles, albeit the values were significantly low. Considering all the above parameters, there are three distinct heat stress tolerant classes among the genotypes – tolerant (N22 and NL44), moderately sensitive (IR64) and highly sensitive (PB 1 and PB 1509).

4.4. Characterization of F_1 between PB1 × NL44 for tolerance to RSHS

The F_1 generated from a fresh cross between PB1 and NL44 was assessed along with parents to explore its behavior towards high temperature. Two staggered sowings were taken up to expose the F_1 to heat stress under natural field conditions during Off-season 2018-19 at IARI-RGBRC, Aduthurai, Tamil Nadu. Observations were recorded on grain yield plant⁻¹ (g) and spikelet fertility (%). The grain yield of F_1 under non-stress was significantly higher than parents, while PB 1 and NL44 were at par (Figure 4.5). However, the spikelet fertility was almost same across F_1 and parents. Under heat stress, interestingly F_1 displayed performance at par with the heat stress tolerant genotype, NL44 for both grain

yield and spikelet fertility. This alludes that the genes responsible for heat stress tolerance may be primarily dominant in nature.

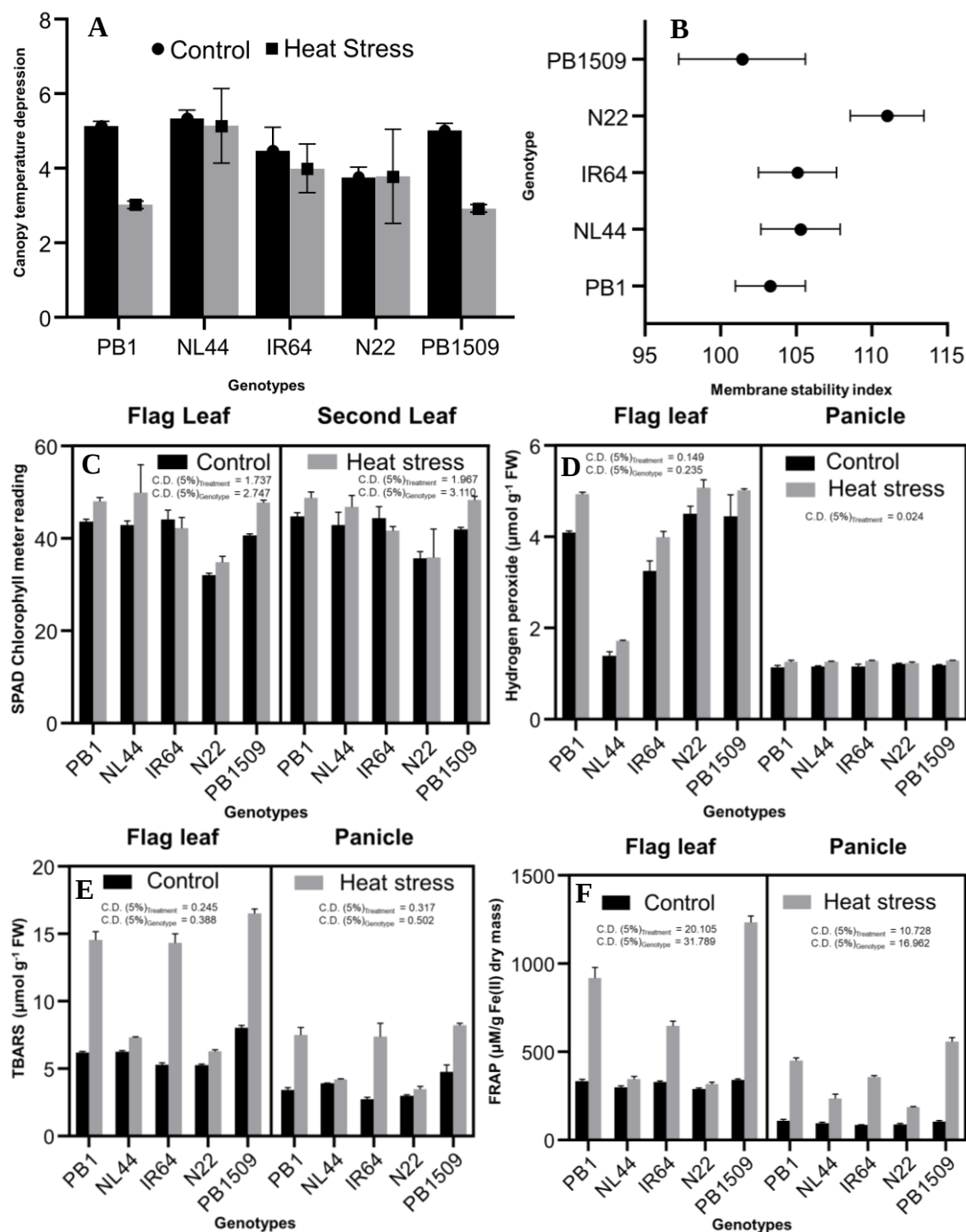


Figure 4.4 Physiological characterization of parents of mapping populations. (A) Canopy temperature depression between ambient and foliage temperatures; (B) Membrane stability index values of various genotypes; (C) SPAD meter readings of genotypes under control and heat stress treatments; (D) Hydrogen peroxide accumulated in various genotypes under different treatments; (E) Lipid peroxidation using TBARS as proxy; (F) Total antioxidant capacity estimated using FRAP assay.

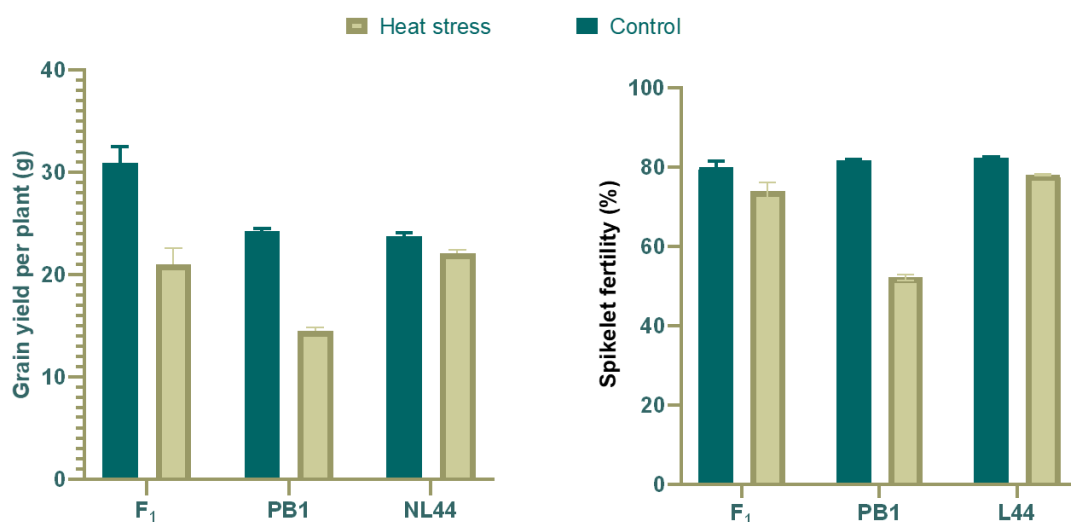


Figure 4.5 Performance comparison of F₁ between PB1 and NL44 under control and RSHS conditions during off-season 2018-19 at IARI-RBGRC, Aduthurai.

4.5. Validation of previously reported QTLs for reproductive stage heat stress tolerance in rice

4.5.1. Screening of RILs and parents for RSHS tolerance

The RILs were initially screened for RSHS tolerance during off-season, 2017-18 at IARI-RBGRC, Aduthurai with two staggered sowings, one timely sown (normal) and another late sown (RSHS) condition. There was no significant difference between the parents for both grain yield and spikelet fertility under control and the mean grain yield plant⁻¹ varied between 9.6 and 38.2 g with a mean of 22.4 g, and the spikelet fertility ranged from 64.0% to 90.8% with a mean of 79.1% (Table 4.7). However, under RSHS, there was a clear contrast between the parents. The sensitive parent, PB 1 showed grain yield and spikelet fertility of 7.5 g and 24.8%, respectively, whereas NL44 registered grain yield of 14.6 g and spikelet fertility of 77.2%. Accordingly, there was a change in the range among the RILs for the respective traits, i.e., grain yield varied between 2.1g and 22.7 g with a mean of 13.0 g and spikelet fertility between 16.6% and 82.2% with a mean of 58.9%. Additionally, stress tolerance index (STI) was calculated for grain yield plant⁻¹ and spikelet fertility which varied between 0.22 and 1.12 among RILs for spikelet fertility and between 0.10 to 1.57 for grain yield plant⁻¹ (Table 4.8). The parents also showed clear difference for tolerance indices. However, pollen fertility showed an interesting pattern. Both the parents showed high pollen fertility under both unstressed and heat stress situation with PB 1 showing marginal reduction under stress. Accordingly, the distribution of pollen fertility was significantly skewed with majority of RILs showing higher pollen fertility values under

both treatments. The mean pollen fertility was 98.7% under control whereas under stress it was 90.7%.

Table 4.7 Summary statistics for grain yield plant⁻¹, spikelet fertility (%) and pollen fertility (%) recorded in the 127 RILs and parents under timely sown (unstressed) and late sown (high temperature stressed) conditions

Statistic	Grain yield plant ⁻¹ (g)		Spikelet fertility (%)		Pollen fertility (%)	
	Unstressed	Stressed	Unstressed	Stressed	Unstressed	Stressed
Range	9.6 – 38.2	2.1 – 22.7	64.0 – 90.8	16.6 – 82.2	97.0-100.0	40.4 – 99.9
Mean	22.4	13.0	79.1	58.9	98.2	90.7
SE	0.45	0.37	0.51	1.17	0.06	0.88
SD	5.08	4.17	5.74	13.23	0.74	9.89
Kurtosis	0.09	-0.45	-0.29	-0.16	-1.01	8.50
Skewness	-0.15	0.09	-0.36	-0.52	-0.38	-2.74
NL44	22.7	14.6	77.8	77.2	100.0	93.3
PB1	23.2	7.5	71.0	24.8	99.0	86.3
CV (%)	22.7	32.0	7.3	22.5	0.73	10.9

SE, standard error; SD, standard deviation; CV (%), coefficient of variation in per cent

Table 4.8 Summary of heat stress indices measured for grain yield plant⁻¹ and spikelet fertility among the RILs from PB1/ NL44 cross.

Indices	RILs					NL44	PB1
	Minimum	Maximum	Mean	Range	SE		
STI _{sf}	0.22	1.12	0.74	0.90	0.01	0.96	0.28
STI _{gy}	0.10	1.57	0.59	1.46	0.02	0.66	0.35

STI_{sf}, Stress tolerance index for spikelet fertility; STI_{gy}, stress tolerance index for grain yield plant⁻¹; SE, standard error.

4.5.2. Single marker analysis using linked simple sequence repeat (SSR) markers of reported QTLs

A total of 54 QTLs were selected out of the 109 QTLs reported for RSHS tolerance in rice at the time of analysis depending on the marker type and their relative position on the chromosomes. Altogether, 116 SSR markers were tested for polymorphism between the parents, of which 31 markers were found to be polymorphic (Plate 4.1A). These 31 markers were analyzed across the RILs. Of these a subset of 19 markers which gave clear unambiguous bands were finally selected for scoring to avoid genotyping errors and false positives (Plate 4.1B) A single marker analysis using a simple linear regression was used to test the association between the markers and traits measured i.e., grain yield plant⁻¹, spikelet fertility (%), and their respective indices. Interestingly, only six markers showed significant associations with spikelet fertility (%). No associations were found for grain

yield plant⁻¹ (Table 4.9). The marker RM401 showed association for both spikelet fertility and its tolerance index (STI_{sf}). Highest PVE (6.9%) was accrued by RM6394 for STI_{sf}, while the lowest (3.3%) was by RM7283 for spikelet fertility (%). Thus, it was hypothesized that the genomic regions governing tolerance to RSHS in NL44 might be novel.

Table 4.9 Markers showing significant association with the respective traits revealed through single marker analysis

Marker	SF _s			STI _{sf}			Linked QTL	
	PVE	p-value	AE	PVE	p-value	AE	QTL ID	RPVE
RM3355	5.7	0.016	-3.03				<i>qhs2</i>	6.4
RM401	3.8	0.037	2.69	3.8	0.0261	0.02	<i>qSF4</i>	10.3
RM5687	4.8	0.026	3.05				<i>SSPF4</i> , <i>qPF4</i>	21.3
RM6394				6.9	0.0027	0.03	<i>qPSLht7</i>	-
RM6673	3.6	0.037	2.59				<i>qHTB10-2</i>	-
RM7283	3.3	0.047	2.49				<i>qHTB11</i>	-

SF_s, Spikelet fertility under stress; STI_{sf}, Stress tolerance index for spikelet fertility; PVE, percent variation explained (%); AE, Additive effect; RPVE, reported PVE

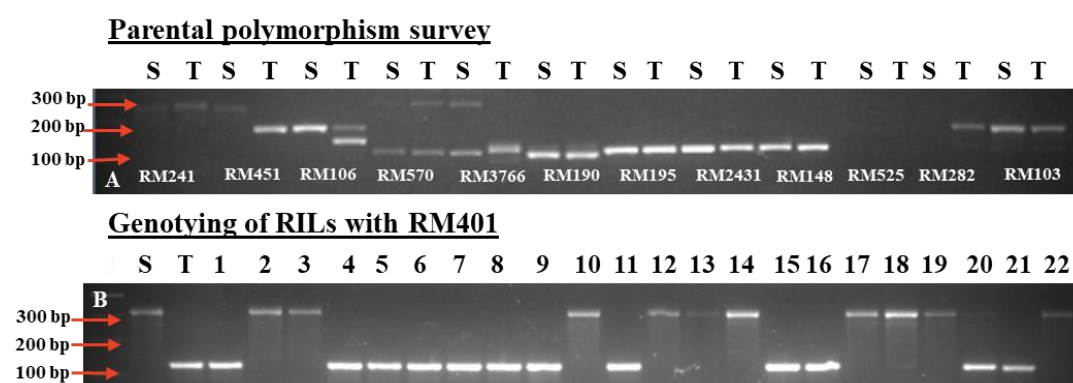


Plate 4.1 Representative gel images showing parental polymorphism survey between PB1 (S) and NL44 (T) (A) and genotyping of RILs (1-22) using SSR markers linked to QTLs reported for RSHS tolerance (B)

4.6. Multi-location and multi-seasonal evaluation of RILs for tolerance to RSHS

The set of 127 along with the parents (NL44 and PB1) and checks (N22, IR64, PB1509) was assessed for their reproductive stage heat stress tolerance at two locations namely IARI-RBGRC, Aduthurai and ICAR-NRRI, Cuttack for two consecutive off-seasons namely offseason 2018-19 and offseason 2019-20 (Plate 4.2) The data were recorded on days to 50% flowering, plant height, panicle length, total tillers, productive tillers, grain yield per plant and spikelet fertility. A mixed model analysis was executed

with genotype as random effect and environment as fixed effect to extract variance components. The BLUPs generated through the model were utilized for comparison between parents and among RILs.

4.6.1. Grain yield and it's contributing traits

4.6.1.1. Grain yield per plant (g)

Significant genotype, environment, and genotype $\times$ environment effects were observed under both control and heat stress conditions. The heritability values were higher under heat stress (71%) than under control (57%) (Table 4.11). Parents showed similar grain yield values under unstress conditions. However, under heat stress, there was a clear difference between the parents with NL44 significantly outyielding PB 1 across both the locations in both the seasons. Furthermore, there was no significant difference for grain yield of NL44 under control and RSHS situation. Individually, the grain yield of PB 1 was 26.05 g and 16.62 g under control and RSHS condition, respectively at Aduthurai, whereas it was 20.70 g and 6.72 g under unstress and RSHS situation, respectively at Cuttack during off-season, 2018-19 (Table 4.12). PB 1 registered a grain yield of 22.85 g and 13.85 g under control and RSHS situation, respectively at Aduthurai, whilst it was 23.86 g and 13.79 g under unstress and RSHS situation, respectively at Cuttack during off-season, 2019-20. At Aduthurai, NL44 displayed a grain yield of 25.75 g under control and 23.57 under RSHS, while it was 20.31 g and 14.12 g under unstress and RSHS situation, respectively at Cuttack during off-season, 2018-19. NL44 maintained a grain yield of 24.33 g and 21.11 g under control and RSHS condition at Aduthurai, whereas it was 25.66 g and 22.34 g under unstress and RSHS situation at Cuttack during off-season, 2019-20.

Wide variation was observed among the RILs with a clear shift in the mean under RSHS towards the lower side compared to unstress situation. Among the environments, high yield decline was observed during off-season, 2018-19 at Cuttack (Figure 4.6). Under control, grain yield varied between 10.89 g and 36.40 g with a mean of 22.82 g, while under RSHS it was between 6.40 g and 29.07 g with a mean of 15.26 g at Aduthurai during off-season, 2018-19. Grain yield ranged from 6.40 g to 29.07 g with a mean of 17.59 g under control and from 0.82 g to 11.44 g with a mean of 6.35 g under RSHS at Cuttack during off-season, 2018-19. The range of grain yield under unstress situation was between 10.72 g and 29.29 g with a mean of 19.53 g, while it was between 9.21 g and 32.08 g with a mean of 12.85 g under stress situation at Aduthurai during off-season, 2019-20. Grain yield

varied between 9.21 g and 32.08 g with a mean of 19.41 g under control, whereas it ranged between 3.06 g and 15.06 g with an average of 11.31 g under stress at Cuttack during off-season, 2019-20. Several transgressive segregants were identified which out-performed tolerant parent under RSHS for grain yield per plant.

4.6.1.2. *Spikelet fertility (%)*

Spikelet fertility showed highly significant genotype, environment, and genotype $\times$ environment effect across locations and seasons (Table 4.11). Moderate to high heritability values were observed under control (57%) and heat stress condition (59%). As in grain yield, highest reduction in spikelet fertility (%) was observed during off-season, 2018-19 at Cuttack (Figure 4.6). A clear differentiation was observed between the parents for spikelet fertility (%) particularly under RSHS situation with NL44 maintaining superior spikelet fertility (%) under RSHS relative to PB 1 (Plate 4.3). Individually, spikelet fertility per cent of PB1 was 74.66% and 62.85% under control and RSHS situation, respectively at Aduthurai, while it was 72.29% and 34.87% under unstress and RSHS situation, respectively at Cuttack during the off-season, 2018-19 (Table 4.12). PB 1 showed spikelet fertility of 81.34% under stress and 41.11% under RSHS at Aduthurai, whereas at Cuttack it registered a spikelet fertility of 71.27% and 34.87% under unstress and RSHS situation, respectively during the off-season, 2019-20. In contrast, NL44 maintained a spikelet fertility of 82.42% and 83.47% under control and RSHS conditions, respectively at Aduthurai while the spikelet fertility was 78.04% and 74.64% under unstress and RSHS situation, respectively at Cuttack during off-season, 2018-19. Similarly, NL44 registered a spikelet fertility of 85.62% and 78.04% under control and RSHS situation, respectively at Aduthurai, whereas at Cuttack it showed 75.70% and 74.64% under unstress and RSHS condition, respectively. Thus, NL44 showed a marginal reduction in spikelet fertility due to heat stress at reproductive stage at both the locations.

Wide variation was observed among the RILs for spikelet fertility (%) both under control and RSHS. A clear shift was observed in the range and mean of spikelet fertility (%) among the RILs under RSHS relative to control. Transgressive segregants were also observed under both unstress and RSHS situations. The spikelet fertility varied between 39.53% and 88.02% with a mean of 76.18% under control, whereas under RSHS, it ranged from 29.43% to 81.15% with a mean of 64.28% at Aduthurai during off-season, 2018-19. At Cuttack, spikelet fertility (%) varied between 45.19% and 88.12% with a mean of 71.06% under unstress situation, while it was between 14.15% and 77.53% with a mean of

41.88% under RSHS situation during off-season, 2018-19. Spikelet fertility (%) at Aduthurai during off-season, 2019-20 varied between 54.65% and 89.93% with a mean of 78.45% under control, whereas it was between 21.69% to 83.03% with a mean of 58.79% under RSHS condition. The range of per cent spikelet fertility under unstress was from 43.04% to 89.48% with a mean of 71.37%, whereas it was between 14.15% and 77.53% with a mean of 52.31% under RSHS situation at Cuttack during off-season, 2019-20.

4.6.1.3. *Plant height (cm)*

A pooled analysis of variance showed significant differences between the genotypes, environment and genotype by environment interaction effects under both control and RSHS situations with high heritability values (Table 4.11). No significant reduction in plant height was observed at Cuttack during both seasons. The difference between the parents in terms of height was lesser under control and RSHS conditions. Similarly, minimal contrast was also observed between the treatments when individual parents were taken into consideration. Overall, the sensitive parent PB 1 was found slightly taller than the tolerant parent, NL44. The height of PB 1 was 88.22 cm and 97.77 cm under control and RSHS condition, respectively at Aduthurai during off-season, 2018-19, while it was 87.82 cm and 88.70 cm under stress and RSHS situation, respectively at Cuttack during off-season 2019-20 (Table 4.12). Similarly, a height of 100.73 cm and 92.36 cm was recorded under control and RSHS situation, respectively for PB 1 at Aduthurai during off-season, 2019-20 while a height of 92.28 cm and 88.93 cm was observed under unstress and RSHS condition, respectively at Cuttack during off-season, 2019-20. NL44 registered a height of 86.51 cm and 92.01 cm under control and RSHS condition, respectively at Aduthurai during off-season, 2018-19 whilst it was 73.83 cm and 81.50 cm at Cuttack during off-season, 2018-19 under unstress and RSHS situation, respectively. Similarly, a plant height of 99.86 cm and 93.74 cm was observed for NL44 at Aduthurai under control and RSHS situation, respectively during off-season, 2019-20, whereas it was 82.37 cm and 81.85 cm under unstress and RSHS situation, respectively at Cuttack during off-season, 2019-20.

Notable variation in the plant height was registered among RILs with tallest RIL being double the height of shortest RIL on an average (Figure 4.6). Similar to parents, there was no significant difference in the range of RILs in terms of plant height with or without the exposure to RSHS. Several transgressive segregants were observed relative to both the parents. Between the locations, the mean plant height observed on RILs was relatively

higher at Aduthurai than in Cuttack. Individually, plant height ranged between 61.09 cm to 111.47 cm with a mean of 79.69 cm under control and 73.09 cm to 120.67 cm with a mean of 92.48 under RSHS at Aduthurai during off-season, 2018-19. At Cuttack, the plant height varied between 57.99 cm to 100.17 cm with a mean of 78.36 cm under unstress and from 67.04 cm to 111.42 cm with a mean of 83.57 cm under RSHS situation during off-season, 2018-19. The variation in plant height was from 81.09 cm to 127.93 cm with a mean of 99.93 cm under unstress and 68.24 cm to 119.58 cm with a mean of 86.99 cm under RSHS at Aduthurai, during off-season, 2019-20. Whereas at Cuttack, the range is from 64.40 to 112.56 with a mean of 81.70 cm under control, and between 64.06 cm and 126.12 cm with a mean of 83.72 cm under RSHS during off-season, 2019-20.

4.6.1.4. *Tiller number*

Significant differences were observed between genotypes, environments, and genotype $\times$ environment effects for tiller number under control, while only genotype and environment effects were significant under RSHS situation as in panicle length (Table 4.11). The heritability value under RSHS (70%) was significantly higher than under control (24%). Similar to panicle length, the distinction between the parents was very meagre for tiller number with a maximum of 4.85 observed under control at Aduthurai during off-season, 2019-20 (Table 4.12). In general, the number of tillers registered in PB 1 was higher than in NL44. The tiller number of PB 1 was 18.20 and 15.53 under control and RSHS, respectively at Aduthurai during off-season, 2018-19 while it was 9.64 and 12.11 under unstress and RSHS situations, respectively at Cuttack during the same season. PB 1 registered tiller number of 17.18 and 14.75 under control and RSHS condition at Aduthurai during off-season, 2019-20, while it showed 15.57 and 14.45 under unstress and RSHS situation at Cuttack during the same season. Similarly, NL44 displayed the tiller number of 13.91 and 12.81 under control and RSHS situation, respectively at Aduthurai during off-season, 2018-19, while it showed 9.72 and 11.91 under unstress and RSHS condition at Cuttack during the same season. The tiller number of NL44 was 12.33 and 13.02 under control and RSHS condition at Aduthurai during off-season, 2019-20, while it was 11.92 and 12.30 under unstress and RSHS situation at Cuttack during the corresponding season.

The variation among the RILs for tiller number was lesser as in the case of parents as reflected from low S.D. values. Highest range (high S.D.) was observed at Aduthurai during off-season, 2018-19 under control conditions (Figure 4.6). Individually, tiller number ranged between 11.01 to 22.62 with a mean of 16.93 under control, whereas it was

between 13.18 and 18.34 with a mean of 15.24 under RSHS at Aduthurai during the off-season 2018-19, while it varied between 9.10 and 16.22 with a mean of 10.78 under unstress situation and between 11.73 and 15.84 with an average of 13.61 under RSHS situation at Cuttack during the same season. During off-season, 2019-20, the tiller number ranged between 9.82 to 20.32 with a mean of 15.36 under control situation and between 12.68 and 16.61 with a mean of 14.65 under RSHS situation at Aduthurai, where it varied between 11.82 to 20.22 with an average of 15 under unstress and between 12.78 and 17.01 with an average of 14.94 under RSHS situation at Cuttack.

4.6.1.5. *Panicle number*

The environment and genotype $\times$ environment effects were significant under control condition whereas genotype, environment, and genotype $\times$ environment effects were found significant under RSHS (Table 4.11). The heritability value was higher under RSHS (77%) than under control (13%) similar to tiller number. No significant contrast was observed between the parents for panicle number with maximum being 3.49 at Aduthurai during off-season, 2018-19 under control. Overall, the number of panicles was higher in PB 1 compared to NL44. The panicle number of PB 1 was 17.11 and 14.34 under control and RSHS situations at Aduthurai during off-season, 2018-19, whereas at Cuttack during the same season it was 9.64 and 12.11 under unstress and RSHS situation, respectively (Table 4.12). Similarly, the panicle number of PB 1 was 15.95 and 13.51 under control and RSHS conditions at Aduthurai during off-season, 2019-20, whilst it was 15.01 and 13.58 under unstress and RSHS situation at Cuttack during the same season. NL44 showed a panicle number of 13.62 and 11.32 under unstress and RSHS situation at Aduthurai during off-season, 2018-19, whereas it registered 8.74 and 10.91 under control and RSHS situation at Cuttack during the same season. Similarly, NL44 produced 11.91 and 11.76 panicles under unstress and RSHS situation at Aduthurai during off-season, 2019-20, while it showed 11.92 and 12.30 under control and RSHS condition at Cuttack during the same season.

In general, the reduction in panicle number was observed only during off-season, 2018-19 at Aduthurai (E1) (Figure 4.6). The variation among the RILs was low as was the contrast between the parents. The panicle number varied between 14.25 and 20.83 with a mean of 16.74 under control, while it was between 11.07 and 17.14 with a mean of 13.65 under RSHS at Aduthurai during off-season, 2018-19. The range of panicle number was from 8.70 to 13.76 with a mean of 9.51 under control and from 9.94 to 15.57 with a mean

of 12.24 under RSHS at Cuttack during off-season, 2018-19. The RILs displayed variation for panicle number from 13.38 to 18.10 with a mean of 14.70 under unstress and from 10.61 to 16.29 with a mean of 13.34 under RSHS situation at Aduthurai during off-season, 2019-20. The panicle number was found to vary between 11.50 and 18.21 with a mean of 14.18 under control, whilst it varied between 10.84 and 17.07 with a mean of 13.91 under RSHS at Cuttack during off-season, 2019-20.

4.6.1.6. *Panicle length (cm)*

Significant genotype and environment effects were observed for panicle length under both control and heat stress situations through a pooled environment analysis (Table 4.11). However, interaction effect was non-significant under both the treatments. The heritability values under control and heat stress were observed to be 82% and 95%, respectively. There was no significant difference between the parents for panicle length across treatments and environments; nor it was observed between the treatments for individual genotypes across environments. The panicle length of PB 1 was 28.51 cm and 29.39 cm under control and RSHS conditions, respectively at Aduthurai during off-season, 2018-19, while it was 23.96 cm and 27.30 cm under unstress and RSHS situations, respectively at Cuttack during off-season, 2018-19 (Table 4.12). Similarly, the panicle length of PB 1 was 29.05 cm and 26.84 cm under control and RSHS conditions, respectively at Aduthurai during off-season, 2019-20, whilst it was 28.96 cm and 23.74 cm under unstress and RSHS situations at Cuttack during off-season, 2019-20. NL44 displayed a panicle length of 26.48 cm and 27.84 cm under control and RSHS situation, respectively at Aduthurai during off-season, 2018-19, whilst it showed 22.68 cm and 25.75 cm under unstress and RSHS condition at Cuttack during off-season, 2018-19. The panicle length of NL44 was found to be 28.45 cm and 25.29 cm under control and RSHS conditions, respectively at Aduthurai during off-season, 2019-20, whilst it was 24.38 cm and 22.19 cm under unstress and RSHS situations, respectively at Cuttack during off-season, 2019-20.

In parallel with the parents, the variation among the RILs was very low for panicle length across treatments and environments (Figure 4.6). This was corroborated by very low standard deviation observed for the trait. In general, there was a reduction in heat stress treatment at both locations during off-season, 2019-20 (E3 and E4) (Figure 4.9). The range of RILs for panicle length was from 24.14 cm to 28.65 cm with a mean of 26.78 cm under control while it was from 22.77 cm to 30.31 cm with a mean of 26.97 cm under RSHS at Aduthurai during off-season, 2018-19. Panicle length varied between 19.87 cm and 24.76

cm with mean of 22.76 cm under unstress situation, while it was between 20.68 cm and 28.21 cm with a mean of 24.89 cm under RSHS at Cuttack during off-season, 2018-19. At Aduthurai, during off-season, 2019-20 panicle length ranged between 26.02 cm and 30.46 cm with a mean of 28.46 cm under control situation, whilst it ranged between 20.22 cm and 27.76 cm with a mean of 24.43 cm under RSHS situation. The panicle length varied between 21.91 cm and 26.43 cm with a mean of 24.58 cm under unstress condition and it was between 17.12 cm and 24.65 cm with a mean of 21.33 cm under RSHS at Cuttack during off-season, 2019-20.

4.6.1.7. *Days to 50% flowering*

Days to 50% flowering was recorded only one season (off-season 2018-19) at two locations. The genotype and genotype $\times$ environment interaction effects were significant for days to 50% flowering (DFF) (Table 4.11). High heritability values were observed under both the treatments. No significant difference was observed between the parents for days to flowering particularly under RSHS. Individually, PB 1 showed DFF of 111.58 days under control, while it flowered in 104.9 days under RSHS at Aduthurai (Table 4.12). NL44 displayed DFF of 108.54 under unstress and 104.02 under RSHS at same location. At Cuttack, PB 1 registered a DFF of 113.71 under control whilst it was 104.52 under RSHS. NL44 showed DFF of 106.83 under control and 104.9 under RSHS. The RILs showed moderate variation for DFF with an average of 40 days between the earliest and latest RIL. DFF ranged between 78.59 and 118.04 with a mean of 98.9 under unstress situation whereas it varied between 74.35 and 112.04 under RSHS at Aduthurai. Similarly at Cuttack, the range of DFF was from 79.74 to 119.60 under control and from 77.90 to 116.08 under RSHS.

4.6.1.8. *Pollen fertility (%)*

As in the pilot study, pollen fertility showed an interesting result. Non-significant differences were observed between the parents under stress treatment from the samples analyzed for the trials during off-season, 2018-19 at two locations, Aduthurai and Cuttack. Further majority of RILs could maintain higher pollen fertility values under RSHS, which was also observed in both the parents, PB 1 and NL44. Hence, this trait was not considered for any further analyses.

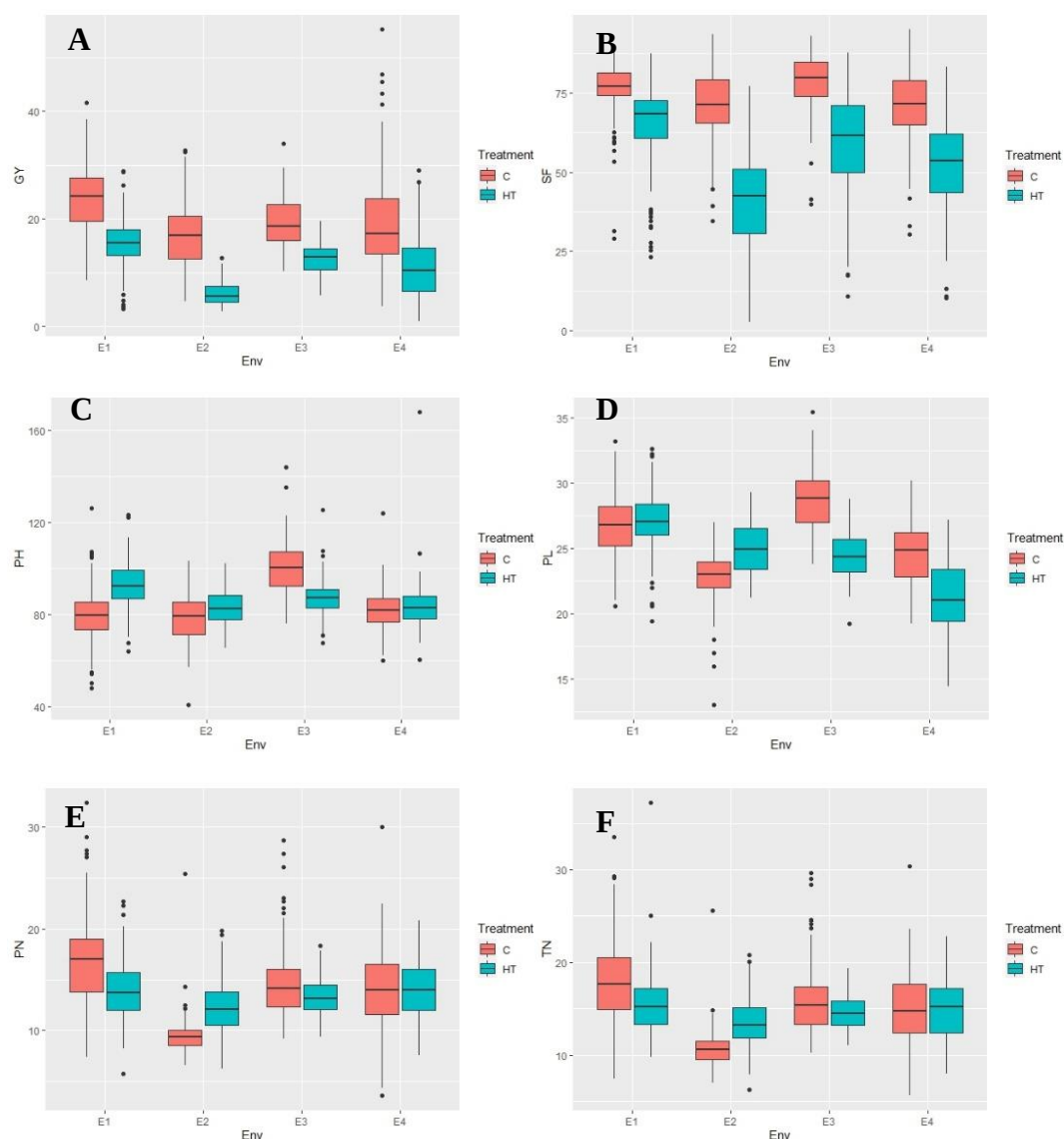


Figure 4.6 Performance of RILs under control (C) and heat stress (HT) in various environments (E1: Off-season, 2019, Aduthurai; E2: Off-season, 2019, Cuttack, E3: Off-season, 2020, Aduthurai; E4: Off-season, 2020, Cuttack) in terms of plant height (PH; A), panicle length (PL; B), tiller number (TN; C), panicle number (PN; D), grain yield (GY; E) and spikelet fertility (SF; F).

4.6.2. Stress tolerance index (STI)

In addition to *per se* trait values under stress, stress tolerance index (STI) was calculated for both grain yield (STI_GY) and spikelet fertility (STI_SF) in individual locations and seasons (Table 4.10). STI for grain yield for PB 1 varied between 0.48 at Cuttack during off-season, 2018-19 and 0.87 at Aduthurai during off-season, 2019-20. For NL44, STI for grain yield ranged from 1.00 at Cuttack during off-season, 2018-19 to 1.50 at Cuttack during off-season, 2019-20. Thus, there was clear contrast between the parents in terms of STI for grain yield. Significant variation was also observed among RILs for

STI_GY. The range of STI_GY was from 0.49 to 0.89 with a mean of 0.68 at Aduthurai, while it was from 0.55 to 1.11 with a mean of 0.79 at Cuttack during off-season, 2019-20. STI_GY varied between 0.19 to 0.45 with a mean of 0.28 at Aduthurai, whereas it was between 0.45 and 1.04 with a mean of 0.65 at Cuttack during off-season, 2018-19.

Similarly, STI_SF of PB 1 varied between 0.28 at Cuttack during off-season, 2018-19 to 0.81 at Aduthurai during the same season, whereas for NL44 STI_SF ranged from 0.87 at Cuttack during off-season, 2018-19 to 1.18 at Aduthurai during the same season. Thus, the contrast found between the parents for STI_SF was slightly higher than that observed for STI_GY. Among RILs, STI_SF was ranged from 0.33 to 1.08 with a mean of 0.75 at Aduthurai, while it ranged from 0.30 to 1.17 with a mean of 0.74 at Cuttack during off-season, 2019-20. STI_SF varied between 0.45 to 1.12 with a mean of 0.84 at Aduthurai, whereas it was between 0.24 and 1.07 with a mean of 0.58 at Cuttack during off-season, 2018-19.

Table 4.10 Stress tolerance index (STI) values of parents and RILs from BLUP values of grain yield and spikelet fertility in two locations and seasons.

Trait	Environment	PB 1	NL44	RILs	
				Range	Mean±SE
STI_GY	ADT_2019	0.75	1.06	0.19-0.45	0.28±0.06
	CTK_2019	0.48	1.00	0.45-1.04	0.65±0.12
	ADT_2020	0.87	1.41	0.49-0.89	0.68±0.09
	CTK_2020	0.86	1.50	0.55-1.11	0.79±0.13
STI_SF	ADT_2019	0.81	1.18	0.45-1.12	0.84±0.14
	CTK_2019	0.28	0.87	0.24-1.07	0.58±0.17
	ADT_2020	0.54	1.08	0.33-1.08	0.75±0.17
	CTK_2020	0.49	1.12	0.30-1.17	0.74±0.18

SE, standard error of difference

4.6.3. GGE biplot analysis

A GGE biplot analysis was done to select stable genotypes with superior performance across two locations and seasons under RSHS. Grain yield and spikelet fertility were selected, since these two traits showed optimum contrast between the parents and variability among the RILs. For grain yield, biplot captured 82% of total variation and hence can be used for interpretation. Among the environments, E3 (Aduthurai_2019-20) is highly discriminating environment followed by E2 (Cuttack_2018-19) whereas E1 (Aduthurai_2018-19) and E4 (Cuttack_2019-20) were relatively less discriminating locations. Based on mean vs stability plot, genotypes G52 (RIL52), G49 (RIL49), G57

(RIL57), G119 (RIL119), G56 (RIL56) and G50 (RIL50) were found ideal with high stability and above average yields (Figure 4.7 A & B).

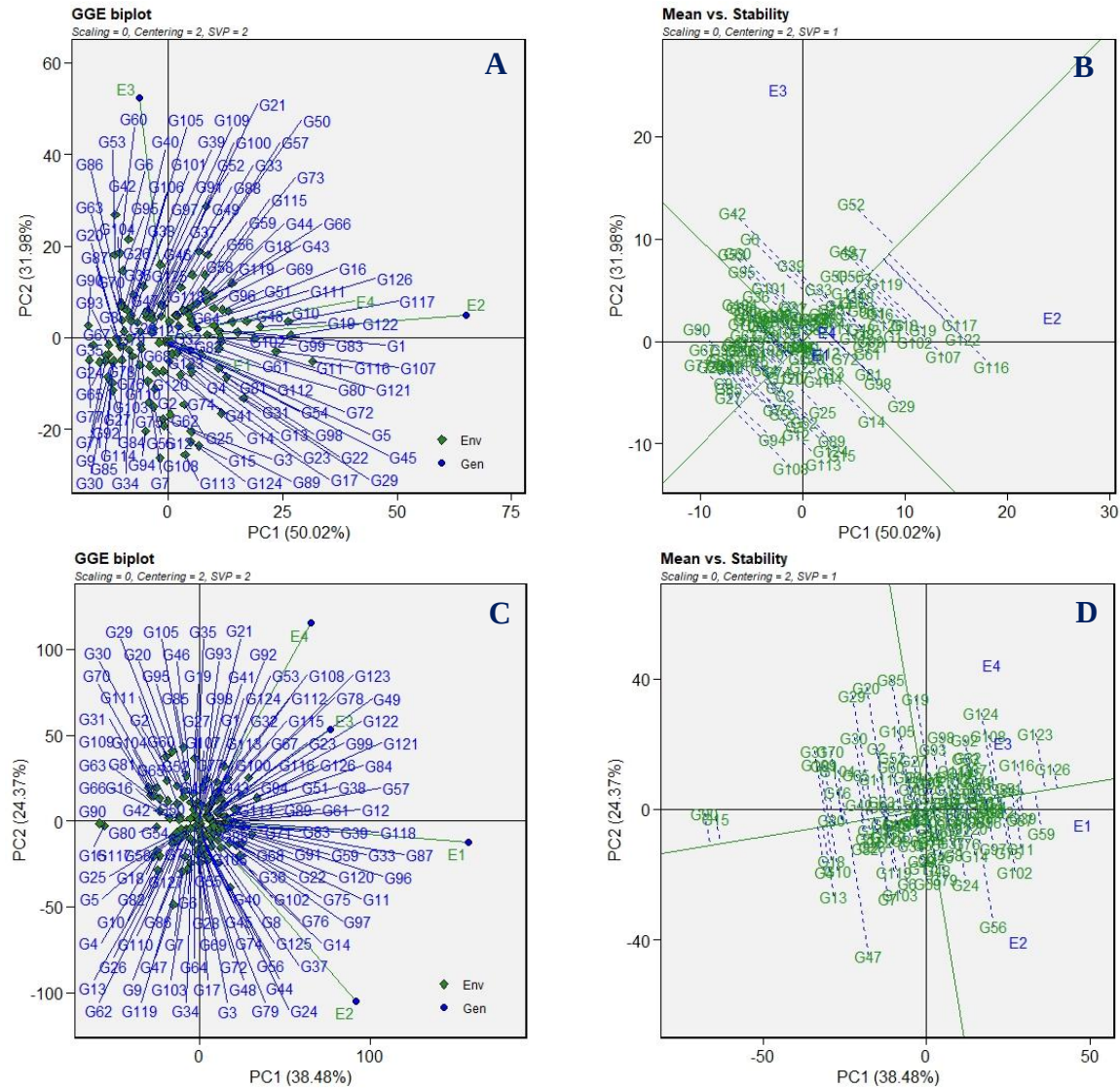


Figure 4.7 GGE biplots of environment view (A: Grain yield; C: Spikelet fertility) and genotype view (B: Grain yield; B: Spikelet fertility) of 127 RILs evaluated under four environments (E1: Off-season, 2018-19, Aduthurai; E2: Off-season, 2018-19, Cuttack; E3: Off-season, 2019-20, Aduthurai; E4: Off-season, 2019-20, Cuttack) under heat stress.

For spikelet fertility, biplot amassed 62.85% of total variation and hence could be used for interpretation. In contrast to the grain yield, all the environments showed comparable discriminating ability with highest being E1 (Aduthurai_2018-19), followed by E4 (Cuttack_2019-20) and E2 (Cuttack_2018-19) whereas E3 (Aduthurai_2019-20) displayed relatively less discriminating ability. According to means Vs stability biplot, genotypes, G126 (RIL126), G116 (RIL116), G123 (RIL123), G59 (RIL59), G87 (RIL87),

G89 (RIL89), G51 (RIL51) and G84 (RIL84) were found ideal genotypes with high stability and above average spikelet fertility (Figure 4.7 C & D).

4.6.4. Assessment of grain quality traits

The produce from Aduthurai, off-season, 2018-19 from both the two staggered sowings (abbreviated henceforth as ADTC2019 and ADTHS2019 for control and RSHS treatments, respectively) was analyzed for key grain and cooking quality traits to investigate the effect of RSHS on grain quality. The harvest of *Kharif*, 2019 from New Delhi (abbreviated as NDK2019), which was primarily a generation advancement trial was used as positive control. In general, there was a significant reduction in the key grain quality traits under RSHS relative to the grains from the plants grown under normal conditions (Table 4.13).

4.6.4.1. Hulling percentage (%)

No significant difference was observed between the parents for hulling percentage (HP) in three environments. The parents differed by 2.47 in ADTC2018-19, 1.59 in ADTHS2018-19 and 2.49 in NDK2019. The HP for PB1 was 75.84%, 73.54% and 79.31% in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively whereas for NL44 it was 78.31%, 71.95% and 81.8% in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Overall, HP of PB 1 was slightly higher than NL44. Appreciable variation was observed among the RILs for HP. The range was highest in ADTC2018-19 followed by ADTHS2018-19 and NDK2019. In ADTC2018-19, HP ranged from 48.28% to 77.62% with a mean of 72.16% while it varied between 62.86% to 80% with a mean of 76.24% in ADTHS2018-19 and between 75.81% and 84.5% with a mean of 80.02% in NDK2019.

4.6.4.2. Milling percentage (%)

Similar to hulling percentage no significant difference was observed between the parents for milling percentage (MP) particularly in ADTC2018-19 and NDK2019. The difference between the parents was 0.45 in ADTC2018-19, 5.98 ADTHS2018-19 and 1.66 in NDK2019. The MP of PB 1 was 65.89%, 64.75% and 72.43% in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively, whereas for NL44 it was 66.34%, 58.77% and 74.09% in ADTC2018-19, ADTHS2018-19 and NDK2019. Overall, in contrast to HP, MP of NL44 was slightly higher than PB 1 in ADTC2018-19 and in NDK2019, while PB1 showed higher MP than NL44 in ADTHS2018-19. Considerable variation was observed among the RILs for MP. The range was highest in ADTHS2018-19 followed by

ADTC2018-19 and NDK2019. In ADTC2018-19, MP ranged from 59.55% to 73.71% with a mean of 65.76% while it varied between 39.43% to 68.66% with a mean of 61.48% in ADTHS2018-19 and between 67.89% and 77% with a mean of 72.36% in NDK2019. Thus, milling percentage was marginally reduced under RSHS than under unstressed control.

4.6.4.3. *Head rice recovery (%)*

Parents did not differ significantly for head rice recovery (HRR, %) particularly in ADTC2018-19 and NDK2019. The contrast between the parents was 2.22 in ADTC2018-19, 3.77 in ADTHS2018-19 and 1.32 in NDK2019. PB 1 showed a HRR of 26.87%, 31.61% and 56.29% in ADTC2018-19, ADTHS2018-19 and NDK2019 respectively, whereas NL44 registered 29.09%, 27.84% and 57.61% in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Overall, in contrast to HP, HRR of NL44 was slightly higher than PB 1 in ADTC2018-19 and NDK2019, while PB1 showed higher MP than NL44 in ADTHS2018-19. Substantial variation was observed among the RILs for HRR in contrast with HP and MP. The range was highest ADTHS2018-19 followed by ADTC2018-19 and NDK2019. In ADTC2018-19, HRR ranged from 2.69% to 53.89% with a mean of 24.33% while it varied between 4.83% to 60.64% with a mean of 26.47% in ADTHS2018-19 and between 41.18% and 66.58% with a mean of 54.92% in NDK2019. Thus, the reduction in HRR was more in ADTC2018-19 followed by ADTHS2018-19 and NDK2019.

4.6.4.4. *Kernel length before cooking (cm)*

In line with the previous traits, the difference between the parents for kernel length before cooking (KLBC) did not show any significant difference across the treatments. The parents differed by 0.45 in ADTC2018-19, 0.55 in ADTHS2018-19 and 0.22 in NDK2019. KLBC of PB 1 was 6.76 cm, 6.94 cm and 7.30 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively, whereas that of NL44 was 6.31 cm, 6.39 cm and 7.08 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Limited variation was observed among the RILs for KLBC. The range was highest in NDK2019 followed by ADTC2018-19 and ADTHS2018-19. In ADTC2018-19, KLBC ranged from 4.86 cm to 7.40 cm with a mean of 6.46 cm, while it varied between 5.20 cm to 7.50 cm with a mean of 6.49 cm in ADTHS2018-19 and between 5.53 cm and 8.22 cm with a mean of 7.02 cm in NDK2019. Thus, the decrease in KLBC was higher under control (ADTC2018-19) than under RSHS (ADTHS2018-19).

4.6.4.5. *Kernel width before cooking (cm)*

Similar to KLBC the difference between the parents for kernel width before cooking (KWBC) was very less across the treatments, particularly in NDK2019. The parents differed by 0.08 in ADTC2018-19, 0.05 in ADTHS2018-19 and 0.01 in NDK2019. KWBC of PB 1 was 1.53 cm, 1.61 cm and 1.68 cm in ADTC2018-19, ADTHS2018-19 and NDK2018-19 respectively, whereas that of NL44 was 1.61 cm, 1.66 cm and 1.69 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Narrow variation was observed for KWBC among the RILs. In ADTC2018-19, KWBC ranged from 1.40 cm to 2.13 cm with a mean of 1.56 cm, while it varied between 1.46 cm to 2.23 cm with a mean of 1.62 cm in ADTHS2018-19 and between 1.53 cm and 2.33 cm with a mean of 1.68 cm in NDK2019. Thus, KWBC suffered more under control (ADTC2018-19) than under RSHS (ADTHS2018-19).

4.6.4.6. *Kernel length after cooking (cm)*

The differences for kernel length after cooking (KLAC) was very less across environments, particularly in NDK2019. The parents differed by 1.98 in ADTC2018-19, 2.37 in ADTHS2018-19 and 2.00 in NDK2019. KLAC of PB 1 was 10.55 cm, 12.19 cm and 13.12 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively, whereas that of NL44 was 8.57 cm, 9.82 cm and 11.12 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Moderate variation was observed for KLAC among the RILs. In ADTC2018-19, KLAC ranged from 7.47 cm to 13.00 cm with a mean of 10.55 cm, while it varied between 8.00 cm to 13.75 cm with a mean of 10.25 cm in ADTHS2018-19 and between 8.75 cm and 15.11 cm with a mean of 12.05 cm in NDK2019. Thus, KLAC was found to be significantly affected under RSHS (ADTHS2018-19) than under control (ADTC2018-19) in contrast to KLBC.

4.6.4.7. *Kernel width after cooking (cm)*

Similar to KWBC, the contrast between the parents for kernel width after cooking (KWAC) was very less. The parents differed by 0.19 in ADTC2018-19, 0.05 in ADTHS2018-19 and 0.12 in NDK2019. KWAC of PB 1 was 1.79 cm, 2.16 cm and 2.23 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively, whereas that of NL44 was 1.98 cm, 2.21 cm and 2.35 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Very limited variation was observed for KWAC among the RILs. In ADTC2018-19, KWAC ranged from 1.83 cm to 2.67 cm with a mean of 2.17 cm, while it

varied between 2 cm to 2.67 cm with a mean of 2.20 cm in ADTHS2018-19, and between 1.87 cm and 2.80 cm with a mean of 2.26 cm in NDK2019. Thus, KWAC suffered more under control (ADTC2018-19) than under RSHS (ADTHS2018-19) in contrast to KLAC.

4.6.4.8. *Elongation ratio*

The elongation ratio (ER) of PB 1 was higher than NL44 across environments. The difference between the parents was 0.21 in ADTC2018-19 and 0.22 in ADTHS2018-19 and NDK2019. The ER of PB 1 was 1.56, 1.76 and 1.79 in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively whereas that of NL44 was 1.35, 1.54 and 1.57 in ADTC2019, ADTHS2019 and NDK2019 respectively. The range among the RILs for ER was from 1.35 to 1.97 with a mean of 1.63 in ADTC2018-19, from 1.27 to 2.08 with a mean of 1.58 in ADTHS2018-19 and from 1.29 to 2.20 with a mean of 1.72 in NDK2019. This indicates that there is significant reduction of ER under RSHS (ADTHS2018-19) than in the control (ADTC2018-19).

4.6.4.9. *Alkaline spreading value*

There was no difference between the parents for alkaline spreading value (ASV) across the environments, particularly in NDK2019. The parents differed by 1.91 in ADTC2018-19, 1.71 in ADTHS2018-19 and 1.56 in NDK2019. ASV of PB 1 was 5.96, 6.44 cm and 6.81 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively, whereas that of NL44 was 4.05 cm, 4.73 cm and 6.81 cm in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Limited variation was observed for ASV among the RILs. In ADTC2018-19, ASV ranged from 2.5 to 7 with a mean of 5.52, while it varied between 2 to 7 with a mean of 5.31 in ADTHS2018-19, and between 4 and 7 with a mean of 5.81 in NDK2019. Thus, ASV was affected more under heat stress (ADTHS2018-19) than under control (ADTC2018-19) and PB 1 consistently showed higher ASV value across environments than NL44.

4.6.4.10. *Chalkiness (%)*

In line with all the above traits, chalkiness (%) showed meagre difference between the parents. The distinction was relatively more vivid in ADTHS2018-19 where PB1 showed a chalkiness of 11.38%, while NL44 registered 27.08% with the difference being 11.38% while in ADTC2018-19 PB1 showed chalkiness of 24.83 and NL44 recorded 27.98% with a difference of 3.15% and in NDK2019 the chalkiness of PB 1 was found to be 5.13 while that of NL44 was 8.33 with a difference of 3.2%. It is quite interesting that

chalkiness of sensitive parent was almost at par with the tolerant parent. Wide variation was observed among the RILs for grain chalkiness which ranged between 7.14% and 68.45% with a mean of 28.84% in ADTC2018-19, between 11.38% and 27.08% with a mean of 19.17% in ADTHS2018-19 and between 0.12% and 22.5% with a mean of 6.69% in NDK2019. Thus, chalkiness was observed more in ADTC2018-19 compared to ADTHS2018-19.

4.6.4.11. *Gel consistency (cm)*

A sub-sample of 34 RILs along with the parents were assessed for gel consistency to investigate the effect of RSHS on pasting characteristic of rice flour. There was significant reduction in GC values in ADTC2018-19 and ADTHS2018-19 both for parents and RILs in comparison with NDK2019. The contrast between the parents was less in ADTC2018-19 and NDK2019, while it was considerable in ADTHS2018-19. GC of PB 1 was 17.33, 20.00 and 48.00 in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively whereas the values for NL44 were 21.89, 33.39 and 40.67 in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. Thus, both the parents produced hard gels under control (ADTC2018-19) and RSHS (ADTHS2018-19) while the gels were intermediate in positive control (NDK2019). Similarly, GC values of RILs varied between 15 and 33 with a mean of 17.85 in ADC2018-19, from 15 to 39.67 with a mean of 20.35 in ADTHS2018-19 and from 14 to 64.33 with a mean of 28.24 in NDK2019.

4.6.4.12. *Apparent amylose content (%)*

As in gel consistency a sub-set of 34 RILs along with the parents were analyzed for their apparent amylose content (AAC). Slight difference was observed between the parents, NL44 and PB1 for AAC and both showed reduction of AAC in ADTC2018-19 and ADTHS2018-19 relative to NDK2019. AAC of PB 1 was 16.34, 17.79 and 23.27 in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively whereas that of NL44 was in ADTC2018-19, ADTHS2018-19 and NDK2019, respectively. In line with the values of parents, AAC of RILs was higher in NDK2019 followed by ADTHS2018-19 and ADTC2018-19. Individually, AAC varied between 9.34 to 24.49 with a mean of 14.57 in ADTC2018-19, while it ranged from 6.99 to 27.61 with a mean of 16.96 in ADTHS2018-19 and between 10.87 to 36.47 with a mean of 20.94 in NDK2019.



Plate 4.2 Representative photographs showing the trials conducted during offseason at Aduthurai (A) and Cuttack (B)



Plate 4.3 Phenotypic contrast between the parents, NL44 (Tolerant) and PB 1 (Sensitive) for filled grain under RSHS conditions



Plate 4.4 Variation among the RILs from PB1/NL44 for spikelet fertility under RSHS conditions.

Table 4.11 Variance and heritability for grain yield and its contributing traits under control and RSHS situations across two locations and two off-seasons.

Traits	Treatment	Genotype	Environment	Genotype × Environment	Block × Environment	Residual	Heritability (%)
Days to 50% flowering ^s	Control	80.27**	0.00 ^{NS}	7.21*	0.00	4.98	94
	Heat stress	79.69**	0.14 ^{NS}	5.34*	0.95	4.03	96
Plant height	Control	45.44**	100.30**	33.78**	8.42	36.59	81
	Heat stress	52.05**	18.73**	7.46*	3.01	27.19	94
Panicle length	Control	1.71**	6.29**	0.45 ^{NS}	0.39	4.28	82
	Heat stress	2.79**	5.60**	3.65e-12 ^{NS}	0.53	2.63	95
Tiller number	Control	0.68*	7.13**	5.80*	0.90	11.44	24
	Heat stress	1.35**	0.62*	0.65 ^{NS}	0.45	6.78	70
Panicle number	Control	0.22	9.42**	3.45*	0.58	9.98	13
	Heat stress	1.61**	0.59*	0.88*	0.46	4.31	77
Grain yield	Control	5.65**	5.23*	9.70**	3.71	23.39	57
	Heat stress	2.50**	14.62**	0.13*	1.27	15.61	71
Spikelet fertility	Control	18.81**	11.49**	46.29**	1.38	43.48	57
	Heat stress	47.41**	91.37**	112.93**	6.05	70.69	59

NS: Non-significant; *: significant at 5% level of probability; **: significant at 1% level of probability; ^sBased on two environment data – Aduthurai, 2018-19 and Cuttack, 2018-19.

Table 4.12 Performance of the parents, PB 1, NL 44 and the RILs from the biparental cross between PB1/ NL44, under non-stress and RSHS conditions for grain yield and its contributing traits at Aduthurai and Cuttack during off-seasons, 2018-19 and 2019-2020.

Environment	Stress	Genotypes	DFF	PH	PL	TN	PN	GY	SF
ADT_2019	C	PB1	111.58	88.22	28.51	18.20	17.11	26.05	74.66
		NL44	108.54	86.51	26.48	13.91	13.62	25.75	82.42
		RILs	78.58-118.04 (98.90±8.91)	61.09-111.47 (79.96±7.93)	24.14-28.65 (26.78±0.99)	11.01-22.62 (16.93±2.11)	14.25-20.83 (16.74±1.21)	10.89-36.40 (22.82±2.24)	39.53-88.02 (76.18±5.45)
	HS	PB1	104.90	97.77	29.39	15.53	14.34	16.62	62.85
		NL44	104.02	92.01	27.84	12.81	11.32	23.57	83.47
		RILs	74.35-112.04 (92.66±8.98)	73.09-120.67 (92.48±7.33)	22.77-30.31 (26.97±1.38)	13.18-18.34 (15.24±0.89)	11.07-17.14 (13.65±1.10)	6.40-29.07 (15.26±0.94)	29.43-81.15 (64.28±9.13)
CTK_2019	C	PB1	113.71	87.82	23.96	10.93	9.64	20.70	72.29
		NL44	106.83	73.83	22.68	9.72	8.74	20.31	78.04
		RILs	79.74-119.60 (98.84±9.38)	57.99-100.17 (78.36±6.84)	19.87-24.76 (22.76±0.95)	9.10-16.22 (10.78±0.74)	8.70-13.76 (9.51±0.52)	6.40-29.07 (17.59±2.21)	45.19-88.12 (71.60±7.08)
	HS	PB1	104.52	88.70	27.30	13.43	12.11	6.72	34.87
		NL44	104.9	81.50	25.75	11.91	10.91	14.12	74.64
		RILs	77.90-116.08 (97.93±9.34)	67.04-111.42 (83.57±6.85)	20.68-28.21 (24.89±1.38)	11.73-15.84 (13.61±0.84)	9.94-15.57 (12.24±1.09)	0.82-11.44 (6.35±0.90)	14.15-77.53 (41.88±10.15)

C: Control; HS: Heat stress; DFF: Days to 50% flowering; PH: Plant Height; PL: Panicle length; TN: Tiller number; PN: Panicle number; GY: Grain yield per plant; SF: Spikelet fertility

Table 4.12 Contd.

Environment	Stress	Genotypes	DFF	PH	PL	TN	PN	GY	SF
ADT_2020	C	PB1	-	100.73	29.05	17.18	15.95	22.85	81.34
		NL44	-	99.86	28.45	12.33	11.91	24.33	85.62
		RILs	-	81.09-127.63 (99.93±7.94)	26.02-30.46 (28.46±0.94)	9.82-20.32 (15.36±1.64)	13.38-18.10 (14.70±0.94)	10.72-29.29 (19.53±1.79)	54.65-89.83 (78.45±5.18)
	HS	PB1	-	92.36	26.84	14.75	13.51	13.85	41.11
		NL44	-	83.74	25.29	13.02	11.76	21.11	78.04
		RILs	-	68.24-119.58 (86.99±6.98)	20.22-27.76 (24.43±1.38)	12.68-16.61 (14.65±0.81)	10.61-16.29 (13.34±1.05)	9.21-32.08 (12.85±0.91)	21.69-83.03 (58.79±11.32)
	CTK_2020	PB1	-	92.28	25.96	15.57	15.01	23.86	71.27
		NL44	-	82.37	24.38	12.12	11.92	25.66	75.70
		RILs	-	64.40-112.56 (81.70±6.76)	21.91-26.43 (24.58±0.96)	11.82-20.22 (15.00±1.34)	11.50-18.21 (14.18±1.08)	9.21-32.08 (19.41±2.90)	43.04-89.48 (71.37±6.72)
	HS	PB1	-	88.93	23.74	14.45	13.58	13.79	34.87
		NL44	-	81.85	22.19	13.10	12.30	22.34	74.64
		RILs	-	64.06-126.12 (83.72±7.18)	17.12-24.65 (21.33±1.38)	12.78-17.01 (14.94±0.86)	10.84-17.07 (13.91±1.15)	3.06-15.06 (11.31±0.94)	14.15-77.53 (52.31±10.31)

C: Control; HS: Heat stress; DFF: Days to 50% flowering; PH: Plant Height; PL: Panicle length; TN: Tiller number; PN: Panicle number; GY: Grain yield per plant; SF: Spikelet fertility

Table 4.13 Characterization of the parents, PB 1, NL 44 and the RILs from the biparental cross between PB1/ NL44, for various grain and cooking quality attributes from the produce of off-season, 2018-19 at Aduthurai and *Kharij*, 2019 at New Delhi.

Quality trait	ADT_C_2019			ADT_HS_2019			DELHI_2019		
	PB1	NL44	RILs	PB1	NL44	RILs	PB1	NL44	RILs
Hulling %	75.84	78.31	48.28 – 77.62 (72.16±4.29)	73.54	71.95	62.86 – 80 (76.24±2.77)	79.31	81.8	75.81 – 84.5 (80.02±1.52)
Milling %	65.89	66.34	59.55 – 73.71 (65.76±2.53)	64.75	58.77	39.43 – 68.66 (61.48±4.73)	72.43	74.09	67.89 – 77 (72.36±1.58)
HRR (%)	26.87	29.09	2.69 – 53.89 (24.33±10.29)	31.61	27.84	4.83 – 60.64 (26.47±10.32)	56.29	57.61	41.18 – 66.58 (54.92±5.39)
KLBC (cm)	6.76	6.31	4.86 – 7.4 (6.46±0.45)	6.94	6.39	5.2 – 7.5 (6.49±0.40)	7.30	7.08	5.53 – 8.22 (7.02±0.39)
KWBC (cm)	1.53	1.61	1.4 – 2.13 (1.56±0.09)	1.61	1.66	1.46 – 2.23 (1.62±0.12)	1.68	1.69	1.53 – 2.33 (1.68±0.08)
KLAC (cm)	10.55	8.57	7.47 – 13 (10.55±0.91)	12.19	9.82	8 – 13.75 (10.25±1.36)	13.12	11.12	8.75 – 15.11 (12.05±0.98)
KWAC (cm)	1.79	1.98	1.83 – 2.67 (2.17±0.16)	2.16	2.21	2 – 2.67 (2.20±0.25)	2.23	2.35	1.87 – 2.8 (2.26±0.14)
ER	1.56	1.35	1.35 – 1.97 (1.63±0.12)	1.76	1.54	1.27 – 2.08 (1.58±0.22)	1.79	1.57	1.29 – 2.20 (1.72±0.14)
ASV	5.96	4.05	2.5 – 7 (5.52±1.04)	6.44	4.73	2 – 7 (5.31±1.29)	6.81	5.25	4 – 7 (5.81±1.06)
Chalkiness (%)	24.83	27.98	7.14 – 68.45 (28.84±13.35)	11.38	27.08	2.98 – 70.24 (19.17±11.35)	5.13	8.33	0.12 – 22.5 (6.69±4.74)
Gel consistency [§]	17.33	21.89	15 – 33 (17.85±3.42)	20.00	33.39	15 – 39.67 (20.35±5.84)	48.00	40.67	14 – 64.33 (28.24±13.91)
Apparent amylose content [§]	16.4	15.03	9.34 – 24.49 (14.57±3.05)	17.79	17.33	6.99 – 27.61 (16.96±3.45)	23.27	22.28	10.87 – 36.47 (20.94±6.09)

[§]The values of RILs are based on a sub-sample of 34 genotypes

4.7. Construction of genetic linkage map

Two linkage maps were constructed separately, one using SSR markers polymorphic between the susceptible and tolerant parent, and the other using genome-wide SNP markers. Initially, a set of 1301 SSR markers were analyzed between parents out of which 165 (12.58%) were found to be polymorphic (Plate 4.5). The number of polymorphic markers varied between 8 on chromosomes 7 & 10 and 24 on chromosome 4 with a mean of 13.8 loci (Table 4.14). The percentage of polymorphism ranged from 8.3 for chromosome 5 to 25.0 for chromosome 12 with an average of 13.5. However, out of these only 101 polymorphic markers were used for final genotyping depending on their resolution and physical location within the genome. Of these, seven markers were dropped due to high missing data and/or segregation distortion. Thus, the remaining 94 markers were used for final genotyping of RILs (Figure 4.8 & Annexure III). The linkage map measured around 1568 cM (Kosambi). Map length ranged from 23.77 cM of chromosome 12 with six polymorphic markers to 301.56 cM with 11 polymorphic markers. The average map length was 130.72 cM with a mean locus of 7.83. Marker density in terms of cM/marker varied between 3.96 (chromosome 12) to 39.96 (chromosome 5).

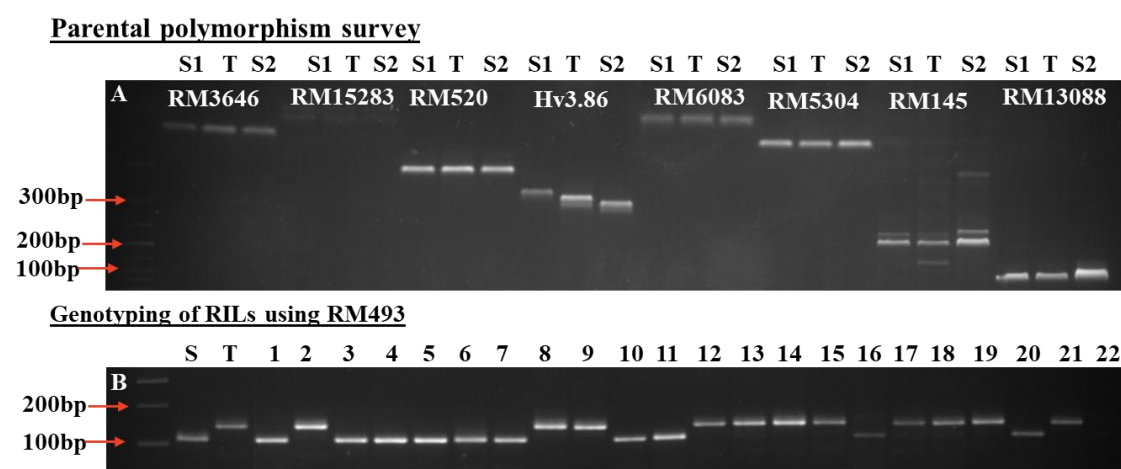


Plate 4.5 Representative gel images showing parental polymorphism between NL44 (T), PB1 (S1) and PB1509 (S2) (A), genotyping results of RILs using markers RM493 (B)

A total of 80,505 SNP markers were analyzed on RILs and parents of which 60,026 markers were aligned to chromosomes. Out of these, 17,018 SNPs were polymorphic (and homozygous) between the parents. The number of polymorphic markers ranged from 535 on chromosome 3 to 2468 on chromosome 2 with a mean of 1418 loci. The percentage polymorphism was highest for chromosome 12 (37.7%) paralleling SSR markers while the lowest was for chromosome 3 (9.48%). Of these, the markers with >20% missing data

points and segregation distortion were dropped, and a final 10,924 markers were utilized for linkage map construction (Figure 4.9). The total length of linkage map was 2977 cM (Kosambi). Map length varied between 154 cM of chromosome 9 (448 polymorphic SNP markers) and 383 cM of chromosome 2 (2187 polymorphic SNP markers) with a mean length of 248 cM and 910 loci. Marker density in terms of cM per marker ranged from 0.15 (chromosome 1) to 0.76 (chromosome 3) with a mean of 0.27. This astronomically higher than the marker density obtained through SSRs. Finally, these two maps were merged, and a consensus map was generated for QTL mapping in the next step.

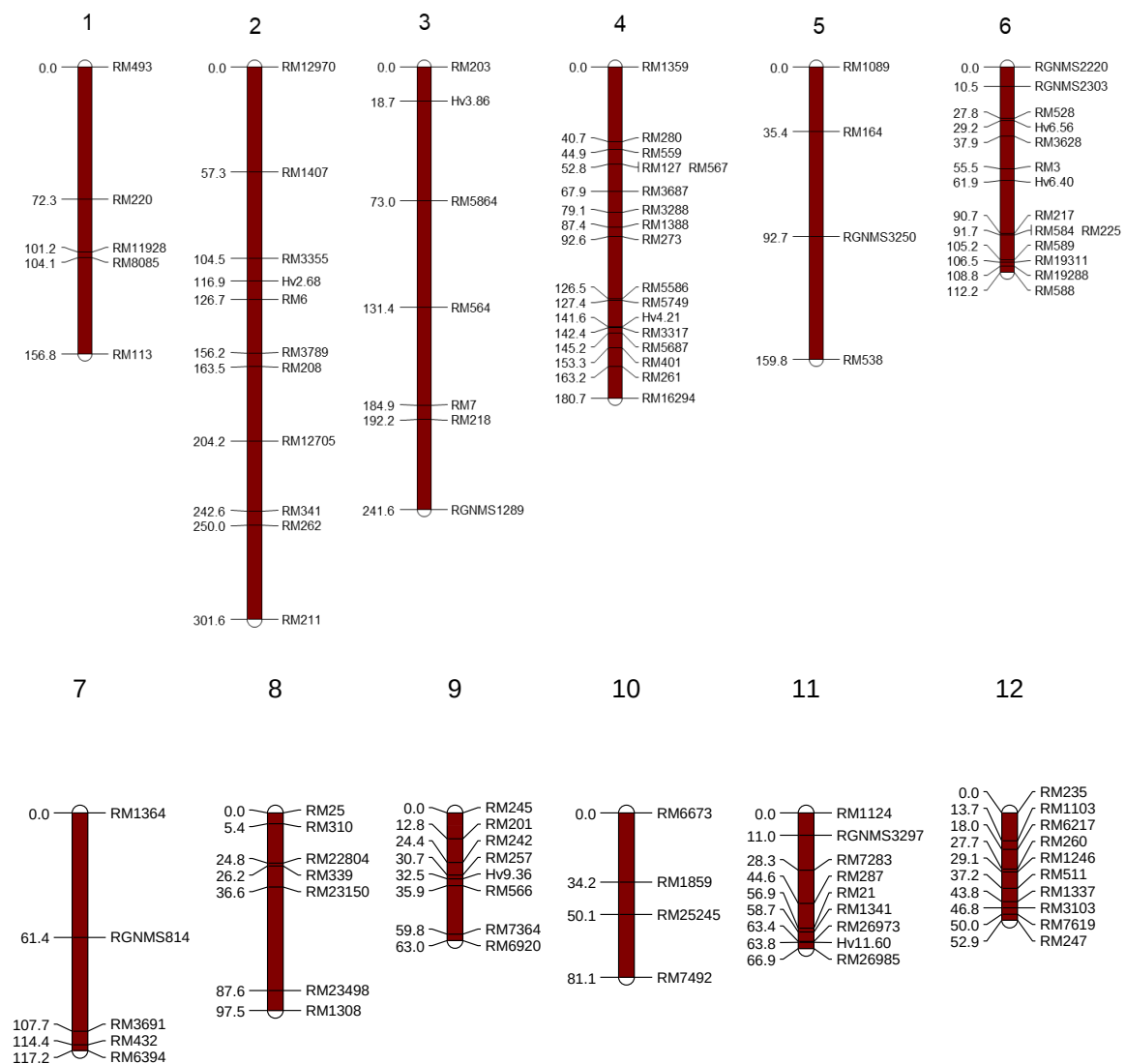


Figure 4.8 Linkage map constructed using 94 polymorphic SSR markers analyzed over 127 RILs

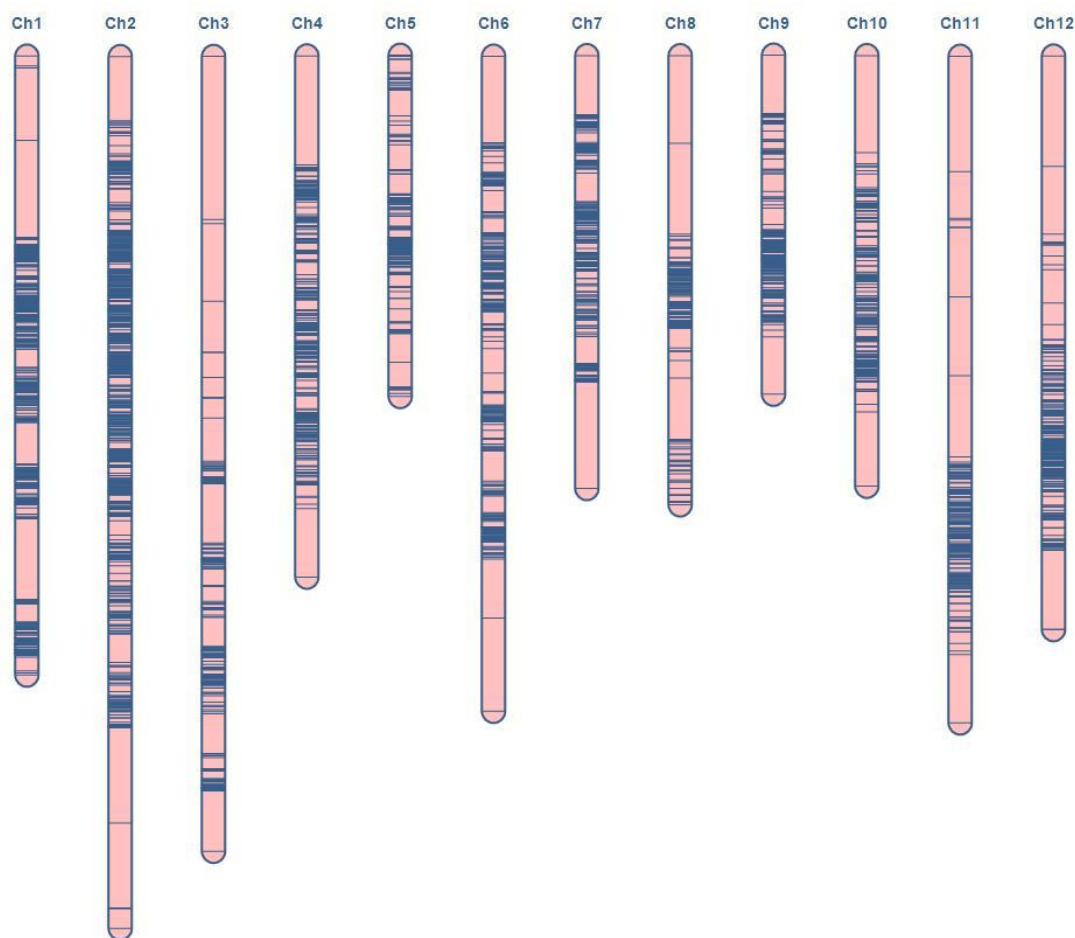


Figure 4.9 SNP based linkage map constructed using 10,924 homozygous polymorphic markers

4.8. QTL identification

QTL analysis was done using the data generated under control and heat stress and by both individual environment (by considering each environment as separate trait) using BIP option and combined environment analysis using MET option of QTL IciMapping software.

4.8.1. QTLs for Grain yield per plant and its STI

No QTL was identified for grain yield per plant under control through individual environment analysis, whereas under RSHS one QTL, i.e., *qHTGY2.1* was found on chromosome 2 (Table 4.15). However, it was a minor QTL with a PVE of 5.1% and additive effect of 0.34. Contrary to this, through combined environment analysis, two QTLs, *qCGY1.1* and *qCGY1.2* were observed, both on chromosome 1. Both were separated by 66 cM and are minor QTLs with similar PVE values, 5.6 for *qCGY1.1* and 5.8 for *qCGY1.2*. Interestingly, additive dominance environment (ADE) interaction variance of these two

QTLs is low. Similarly, one QTL was identified for STI for grain yield, *qSTIGY2.1* on chromosome 2 with a PVE of 3.2% and additive effect of 0.03 through individual environment analysis. This QTL is just 5 cM upstream to *qHTGY2.1* observed for grain yield under stress. However, combined environment analysis revealed four QTLs for the same trait, *qSTIGY1.1* and *qSTIGY1.2* on chromosome 1 with PVE values of 6.50% and 5.09%, respectively. The additive effect was 0.03 for the QTLs but from sensitive parent (negative value). Another QTL, *qSTIGY2.1* with PVE of 5.09% was found on chromosome 2 at exactly same position as that of *qHTGY2.1*. Finally, *qSTIGY8.1* was identified on chromosome 8 with a LOD of 6.4, but with very small PVE, i.e., 1.41%. Except *qSTIGY1.1*, remaining QTLs showed very low PVE (ADE) values reflecting the true genetic nature of these QTLs. Except for the QTLs, *qSTISF12.1*, *qCGY1.1*, *qCGY1.2*, *qSTIGY1.1* and *qSTIGY1.2*, positive allele of remaining QTLs was contributed by NL44.

4.8.2. QTLs for spikelet fertility and its STI

In contrast with grain yield per plant, through individual environment analysis, two major QTLs were identified for spikelet fertility under control, *qCSF10.1* on chromosome 10 with a PVE of 13.6 % and *qCSF6.1* with a PVE of 11.2% (Table 4.15). Same QTLs were unveiled through combined environment analysis, with almost identical position, albeit with deflated PVE values, *qCSF6.1* showed a PVE of 10.3% whilst *qCSF10.1* registered a PVE of 5.5%. Furthermore, significant portion of the PVE is contributed by genotype (AD) for both QTLs. Three QTLs were identified for spikelet fertility under RSHS through individual environment analysis: *qHTSF7.1*, *qHTSF12.1* and *qHTSF5.1* on chromosomes 7, 12 and 5, respectively. The PVE explained by these QTLs are 8.6% by *qHTSF7.1*, 8.5% by *qHTSF5.1* and 7.9% by *qHTSF12.1* and the additive effect of these QTLs are 3.94, 3.90 and 3.79 respectively. Two of these three QTLs, i.e., *qHTSF7.1* and *qHTSF12.1* were found through combined environment analysis at identical positions. Furthermore, the genetic component of PVE (AD) was lesser for the QTLs *qHTSF7.1* and *qHTSF12.1*, compared to *qSTIGY1.1*. Using STI values for spikelet fertility, two QTLs were identified through individual environment analysis, *qSTISF10.1* and *qSTISF12.1* on chromosomes 10 and 12, respectively. The QTL on chromosome 12 is 46 cM away from *qHTSF12.1*. The PVE of *qSTISF10.1* was 10.1% with an additive effect of 0.07 while *qSTISF12.1* displayed a PVE of 6.8% with an additive effect of -0.05. However, through combined environment analysis only one QTL, *qSTISF12.1* was identified at the same location on chromosome 12 as found through single environment analysis, but its PVE was

significantly deflated i.e., 4.18% with significant portion (3%) of it being contributed by additive dominance gene action. Except for QTLs, *qCGY1.1*, *qCGY1.2* and *qSTISF12.1* the positive alleles of all other QTLs governing spikelet fertility were contributed by NL44.

4.8.3. QTLs for other agronomic traits

In addition to grain yield and spikelet fertility, QTL mapping was also attempted for other agronomic traits such as plant height, panicle length, panicle number and tiller number recorded in both control and heat stress treatments (Table 4.16). Through individual environment analysis two QTLs were identified for plant height under control. Interestingly, of these, *qCPH8.1* mapped on chromosome 8 was a major QTL with LOD of 31.4 and PVE of 144.6%. The additive effect was 9.71 cm, and the positive allele was contributed by the sensitive parent. The second was a minor QTL, *qCPH7.1* with a LOD of 3.3 and PVE of 8.9%. Apart from this, no QTLs were identified for plant height under heat stress and for other traits either under control or RSHS.

Through combined environment analysis several QTLs were identified for agronomic traits, majority of them being minor QTLs with PVE of <10% except for plant height under control. Interestingly the positive alleles of all of these QTLs are contributed by PB 1. Coinciding with individual environment analysis, same major QTL on chromosome 8, *qCPH8.1* was unveiled, albeit, with a deflated LOD and PVE, i.e., 39.5 and 47.2%, respectively. Furthermore, more than half of this variance (27.4%) is genetic in nature. Another minor QTL, *qCPH10.1* was identified on chromosome 10 with a PVE of 4.6% and LOD of 7.0. Same QTL (now named *qHTPH8.1*) was also observed under RSHS, but with low PVE and LOD, 6.5% and 8.0, respectively. In addition, *qHTPH8.2* was found 103 cM away from *qHTPH8.1* with a LOD of 7.5 and PVE of 6.2%. However, high PVE was observed for *qHTPH10.1* identified on chromosome 10, i.e., 7.6% and LOD being 9.1. Other minor QTLs for plant height under RSHS include *qHTPH9.1* identified on chromosome 9.

Five QTLs were found for panicle length under control, all of them being minor, with PVE values ranging from 5.1% (*qCPL1.1* on chromosome 1) to 8.2% (*qCPL3.1* on chromosome 3) and LOD values varied between 3.6 (*qCPL10.1* on chromosome 10) to 8.2 (*qCPL3.2* on chromosome 3). Only QTLs were found for panicle length under RSHS on chromosomes 1, 2 and 3 with PVE values varying between 5.1% (*qCPL1.1*) to 8.9% (*qCPL2.1*) and LOD between 5.3 (*qCPL3.1*) and 10.0 (*qCPL2.1*). No QTLs were observed for panicle number under control, however, a QTL, *qHTPN6.1* was found on chromosome

6 with a PVE of 9.1% and LOD of 10.7 under RSHS. Same is the case with tiller number, for which no QTL was identified under control whereas four QTLs were found under RSHS. The PVE values ranged from 5.2% (*qHTTN2.1* and *qHTTN7.1*) to 9.4% (*qHTTN6.1*) and LOD values between 4.1 (*qHTTN7.1*) and 10.9 (*qHTTN6.1*). Furthermore, *qHTTN6.1* coincided with *qHTTN6.1* identified for panicle number. Most of phenotypic variance of majority of these QTLs are remarkably due to genotype (additive and dominance gene action).

4.9. Validation of makers associated with putatively identified QTLs

4.9.1. Characterization of a subset of F₆ families for tolerance to RSHS

A set of 20 F₆ families generated from the cross PB1509/ NL44 were evaluated under RSHS during off-season, 2021-22 at IARI-RBGRC, Aduthurai along with parents of original mapping population, NL44, PB 1 and two additional genotypes, PB 1509 and PB 1121. A significant decline was observed for both grain yield and spikelet fertility. There was a clear contrast between NL44 and PB 1 particularly for grain yield, which varied between 1.94 g to 14.48 g with a mean of 9.10 (Table 4.17). The genotypes, PB 1509 and PB 1121 were among the bottom 5 genotypes with < 3 g grain yield. Around half of the F₆ genotypes showed above average grain yields. Similarly, spikelet ranged from 12.20% to 79.47% with a mean of 58.53%. The decline in spikelet fertility in PB 1509 and PB 1121 was high significant when compared to PB1 (Plate 4.6). Interestingly, around 10 F₆ genotypes outperformed NL44 in terms of spikelet fertility (%) with spike fertility of more than 65 %. As in grain yield, PB1509 and PB1121 showed <25% spikelet fertility and among the bottom five genotypes.

Table 4.14 Details of parental polymorphism survey and linkage maps developed using SSR and SNP markers.

Chr	SSR						SNP					
	TM	PM	PP	PMA	ML	MD	TM	PM	PP	PMA	ML	MD
1	150	15	10.0	5	156.81	31.36	7348	2416	32.88	1857	274	0.15
2	88	13	14.8	11	301.56	27.41	6988	2468	35.32	2187	383	0.18
3	69	12	17.4	7	241.63	34.52	5644	535	9.48	460	350	0.76
4	149	24	16.1	17	180.72	10.63	5005	1547	30.91	794	232	0.29
5	120	10	8.3	4	159.82	39.96	3525	689	19.55	575	155	0.27
6	162	19	11.7	14	112.24	8.02	5933	1956	32.97	798	290	0.36
7	67	8	11.9	5	117.21	23.44	5014	1293	25.79	920	194	0.21
8	107	12	11.2	6	92.11	15.35	4772	1325	27.77	1106	201	0.18
9	80	12	15.0	8	63.04	7.88	4704	1506	32.02	466	154	0.33
10	59	8	13.6	4	81.15	20.29	3062	994	32.46	626	193	0.31
11	174	13	7.5	7	38.61	5.52	3871	721	18.63	442	295	0.67
12	76	19	25.0	6	23.77	3.96	4160	1568	37.69	693	255	0.37
Total	1301	165	12.7	94	1568.64	16.69	60026	17018	28.35	10924	2977	0.27
Average	108.4	13.8	13.5	7.83	130.72	16.69	5002.17	1418.17	27.95	910	248	0.27

Chr: Chromosome; TM: Total number of markers analyzed; PM: Total number of polymorphic markers; PP: Percentage polymorphism; PMA: Polymorphic markers analyzed; ML: Map length (cM); MD: Mapping density (cM/polymorphic marker)

Table 4.15 QTLs identified for grain yield and spikelet fertility (under control and RSHS) and their respective STI values using individual and combined environment analysis in QTL IciMapping software.

Trait	QTL	Ch r	Pos	Left marker	Right marker	LO D	PVE (AD)	PVE (ADE)	Add effect	Left CI	Right CI
Individual environment analysis											
Spikelet fertility under control	<i>qCSF10.1</i>	10	41	Affx-472433651	Affx-152877284	3.2	13.6	-	1.8	29.5	46.5
	<i>qCSF6.1</i>	6	212	Affx-122653726	Affx-153911802	4.4	11.2	-	2.5	211.5	212.5
Grain yield under RSHS	<i>qHTGY2.1</i>	2	245	Affx-153777400	Affx-153480602	4.1	5.1	-	0.34	244.4	245.2
	<i>qHTSF7.1</i>	7	39	Affx-154364185	Affx-93250513	6.0	8.6	-	3.94	38.5	39.5
RSHS	<i>qHTSF12.1</i>	12	143	Affx-154421492	Affx-153613789	5.9	7.9	-	3.79	142.5	144.5
	<i>qHTSF5.1</i>	5	106	Affx-152969440	Affx-155020434	3.4	8.5	-	3.90	99.5	107.5
STI for grain yield	<i>qSTIGY2.1</i>	2	240	Affx-153066045	Affx-153122012	5.7	3.2	-	0.03	238.9	241.2
STI for spikelet fertility	<i>qSTISF10.1</i>	10	166	Affx-472437559	Affx-472433557	4.3	10.1	-	0.07	158.5	177.5
	<i>qSTISF12.1</i>	12	189	Affx-153023700	Affx-153230146	4.3	6.8	-	-0.05	188.5	189.5
Combined environment analysis											
Grain yield under control	<i>qCGY1.1</i>	1	130	Affx-154977877	Affx-152864379	6.1	5.6	4.8	0.7	129.5	132.5
	<i>qCGY1.2</i>	1	196	Affx-154914113	Affx-153566562	5.5	5.8	5.0	0.8	195.5	197.5
Spikelet fertility under control	<i>qCSF6.1</i>	6	212	Affx-122653726	Affx-153911802	7.4	10.3	6.9	3.4	211.5	212.5
	<i>qCSF10.1</i>	10	45	Affx-152877284	Affx-152718582	5.4	5.5	5.2	0.3	44.5	48.5
Grain yield under RSHS	<i>qHTGY2.1</i>	2	254	Affx-155142601	Affx-153082980	5.9	8.11	6.4	1.7	253.5	257.5
	<i>qHTSF7.1</i>	7	39	Affx-154364185	Affx-93250513	6.4	8.26	2.8	5.5	38.5	39.5
RSHS	<i>qHTSF12.1</i>	12	143	Affx-154421492	Affx-153613789	6.2	7.29	2.7	4.6	142.5	144.5
	<i>qSTIGY1.1</i>	1	197	Affx-152723986	Affx-122653160	5.6	7.83	5.9	2.0	195.5	197.5
STI for grain yield	<i>qSTIGY1.2</i>	1	267	Affx-472422606	Affx-154411205	5.8	6.50	5.7	0.8	265.5	269.5
	<i>qSTIGY2.1</i>	2	245	Affx-153777400	Affx-153480602	6.4	5.09	5.0	0.1	244.5	245.5
STI for spikelet fertility	<i>qSTIGY8.1</i>	8	99	Affx-153403396	Affx-153502705	6.4	1.41	1.4	0.01	98.5	100.5
	<i>qSTISF12.1</i>	12	189	Affx-153023700	Affx-153230146	6.0	4.18	3.0	1.1	188.5	189.5

Chr: Chromosome; Pos: chromosomal position (genetic) of QTL LOD: Logarithm of odds value; PVE: Phenotypic variance explained; PVE (AD): Phenotypic variance explained by additive and dominance gene action; PVE (ADE): Phenotypic variance explained by additive and dominance by environment interaction; CI: Confidence interval.

Table 4.16 QTLs identified for other agronomic traits (under control and RSHS) using individual and combined environment analysis in QTL IciMapping software.

Trait	QTL	Chr	Pos	Left marker	Right marker	LOD	PVE (AD)	PVE (ADE)	Add effect	Left CI	Right CI
Individual environment analysis											
Plant height under control	<i>qCPH7.1</i>	7	81	Affx-153492713	Affx-152771320	3.3	8.9	-	-	2.41	80.5
	<i>qCPH8.1</i>	8	83	Affx-154164009	Affx-155522787	31.4	144.6	-	-	-9.71	82.7
Combined environment analysis											
Plant height under control	<i>qCPH8.1</i>	8	83	Affx-154164009	Affx-155522787	39.5	47.2	27.4	19.8	-3.95	82.7
	<i>qCPH10.1</i>	10	101	Affx-154284676	Affx-473173352	7.0	4.6	4.4	0.2	-1.58	100.9
Plant height under RSHS	<i>qHTPH8.1</i>	8	83	Affx-154164009	Affx-155522787	8.0	6.5	6.3	0.2	-1.80	82.7
	<i>qHTPH8.2</i>	8	190	Affx-155254327	Affx-155199733	7.5	6.2	5.8	0.4	-1.74	187.5
	<i>qHTPH9.1</i>	9	51	Affx-154464396	Affx-472432073	6.0	5.1	5.0	0.1	-1.62	47.2
	<i>qHTPH10.1</i>	10	90	Affx-472418380	Affx-153031999	9.1	7.6	7.5	0.2	-1.96	89.9
	<i>qCPL1.1</i>	1	113	Affx-154186946	Affx-472421601	6.0	5.1	5.1	0.02	-0.22	112.9
	<i>qCPL2.1</i>	2	28	Affx-472434546	Affx-155431377	6.1	5.2	5.2	0.02	-0.22	0.0
Panicle length under control	<i>qCPL3.1</i>	3	84	Affx-152944757	Affx-472438051	6.1	8.2	8.2	0.01	-0.28	73.6
	<i>qCPL3.2</i>	3	242	Affx-155431400	Affx-473172902	8.4	7.4	7.4	0.04	-0.26	241.3
	<i>qCPL10.1</i>	10	176	Affx-472437559	Affx-472433557	3.6	6.6	6.6	0.04	-0.24	160.0
	<i>qHTPL1.1</i>	1	102	Affx-472422102	Affx-472422091	6.3	5.1	5.1	0.00	-0.31	101.8
Panicle length under RSHS	<i>qHTPL2.1</i>	2	46	Affx-153094108	Affx-154938433	10.0	8.9	8.9	0.00	-0.42	45.9
	<i>qHTPL3.1</i>	3	204	Affx-155144889	Affx-153055684	5.3	8.3	8.3	0.00	-0.40	188.2
Panicle number under RSHS	<i>qHTTN6.1</i>	6	212	Affx-122653726	Affx-153911802	10.7	9.1	9.0	0.02	-0.33	211.9
	<i>qHTTN2.1</i>	2	214	Affx-154823229	Affx-154254906	6.4	5.2	5.2	0.06	-0.20	213.6
Tiller number under RSHS	<i>qHTTN6.1</i>	6	216	Affx-153111971	Affx-154984012	10.9	9.4	9.4	0.04	-0.26	214.9
	<i>qHTTN7.1</i>	7	186	Affx-472429620	Affx-472438552	4.1	5.2	5.1	0.04	-0.19	146.1
	<i>qHTTN10.1</i>	10	14	Affx-472433651	Affx-152877284	4.7	7.3	7.3	0.07	-0.23	0.0
											43.7

Table 4.17. Performance of F₆ families under RSHS at IARI-RBGRC, Aduthurai during off-season, 2021-22

Statistic	Grain yield plant ⁻¹ (g)	Spikelet fertility (%)
Mean	9.10	58.53
Maximum	14.63	79.47
Minimum	1.94	12.20
S.D.	3.72	18.76
S.E. (m)	1.46	5.92
C.V. (%)	25.39	17.54
LSD ($p \leq 0.05$)	3.81	16.92
NL44	14.63	63.48
PB1	7.84	54.55
PB1509	1.94	13.17
PB1121	2.57	22.21

4.9.2. Genotyping of F₆ families with SSR makers near the putative QTLs

The physical positions of QTLs were searched for the presence of SSRs in the nearby regions for validating these markers in the F₆ families. Polymorphic SSR markers between NL44 and PB150 were found near the QTLs *qHTGY2.1* (RM211), *qSTIGY8.1* (RM23150), and *qSTISF12.1* (RM1337 and RM247) (Plate 4.7). However, a simple linear regression done using marker scores and trait values revealed no association. Thus, there is no need to design SNP genotyping assay to validate these QTLs in future studies.



Plate 4.6 Representative picture depicting the performance of a subset of F₆ families along with checks under heat stress evaluated during off-season, 2021-22 at Aduthurai, Tamil Nadu

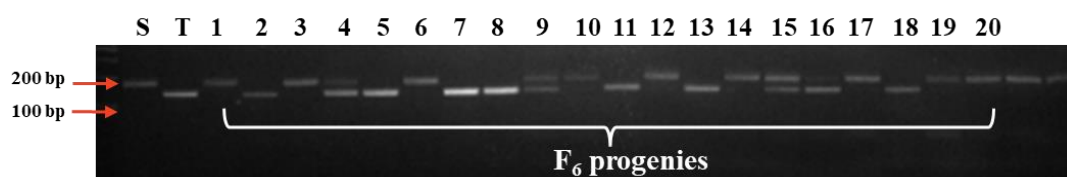


Plate 4.7 Representative gel image depicting the genotyping of PB1509 (S) and NL44 (T) F₆ progenies (1-20) for validating putative QTLs using SSRs (RM247) present near their physical locations

Temperature is one of the important environmental determinants which conditions plant growth and development, and even dictates the geographical distribution of crop species (Li et al., 2018). Every plant species requires a certain optimum temperature, where it flourishes well and a threshold temperature beyond which its normal growth and development is affected. Elevated temperatures which cause irreversible damage to the plant system is one of the abiotic stresses threatening crop productivity worldwide, termed high temperature or heat stress. High temperature stress has gained considerable attention in the recent past, due to increased frequency of climate change induced extreme weather events including episodes of heat waves across the globe. Rice, an important staple crop across the world and principal food crop of India, is highly sensitive to heat stress particularly at booting and anthesis stages (Jagadish et al., 2007). With IPCC fifth assessment report predicting a temperature increase of up to 5°C by the end of this century, it is highly likely that more and more rice growing areas (around 27% by 2050) will be exposed to heat stress (Gourdji et al., 2013; IPCC, 2014). A recent 2-day heat shock episode during April 2021 in Bangladesh affected more than 68,000 hectares (ha) of rice area resulting in losses estimated worth \$39 million to the farmers (Hossain, 2021). Thus, heat stress in rice needs urgent attention and breeding of heat stress tolerant rice varieties needs to be prioritized. Unlike other abiotic stresses such as nutrient stress, agronomic management is not possible to mitigate heat stress; use of controlled environment facility to grow field crops such as rice is not economically feasible. Hence, breeding heat stress resilient rice varieties is the only option available for managing high temperature stress.

Screening of large number of germplasm and identification of potential donors is the first step in this direction. Like any abiotic stress, screening large number of breeding populations under heat stress is quite difficult and hence needs to be supplemented with tools which can aid precise selection such as molecular markers. This requires identification of genomic regions responsible for heat stress tolerance from the tolerant germplasm(s) either through biparental QTL mapping or genome wide association study (GWAS) and molecular markers linked to the QTL(s)/ gene(s) which can aid in marker assisted selection. Validation of these QTL/ gene linked markers, can help expediting the development of heat stress tolerant varieties through marker assisted introgression of these identified genomic regions. Hence, in the present study a set of rice genotypes were screened for tolerance to

RSHS under natural field conditions to assess the extent of variability available for RSHS tolerance in the rice germplasm. A biparental mapping population comprising of 127 RILs derived from a novel RSHS tolerance donor, NL44 and sensitive parent, Pusa Basmati 1 was utilized for mapping QTLs governing tolerance to RSHS. Additionally, attempt was made to validate the QTLs identified in another population (F₆) with different sensitive parent, Pusa Basmati 1509 to check QTL $\times$ genetic background interaction. The salient findings from the study are discussed considering the current knowledge on heat stress tolerance in rice in this chapter:

5.1. Screening for tolerance to RSHS

To assess the rice germplasm and RILs, the offseason (December to May) at two locations one from South India – Aduthurai, Tamil Nadu and one from Eastern India – Cuttack, Odisha were selected. The temperatures in both these locations reach maximum during April and May months in the year and provide excellent sites for identifying heat stress tolerance in the genotypes. The two staggered sowings namely, timely sown and late sown done provided adequate differentiation in temperatures to be considered normal and heat stress. The timely sown set sown in December flowered during March, when the mean temperatures were 33°C to 35°C, whereas the late sown set sown in late January showed peak anthesis during April, when the ambient temperatures were 38°C to 40°C. Thus, the temperatures till anthesis were almost similar under both treatments. Furthermore, the mean maximum temperatures of Aduthurai was relatively higher than in Cuttack. Field based phenotyping of germplasm for tolerance to RSHS have been reported in several of previous studies (Pradhan et al., 2016; Prasanth et al., 2016; Sukkeo et al., 2017; Prasanth et al., 2018; de Brito et al., 2019; Cheabu et al., 2019; Wahab et al., 2020; Chidambaranathan et al., 2021; Ezin et al., 2022). Four staggered sowings were done by Pradhan et al. (2016) starting from mid-January at Cuttack to expose the genotypes to high temperatures during April and May, as in present study where late sowing was done so as to coincide with high temperatures during anthesis. Chidambaranathan et al. (2021) screened 198 rice genotypes by sowing them in last week of January, which was also done in the present study at Cuttack. Similar efforts have been made in Benin, West Africa, where Ezin et al. (2022) selected February to June (off-season) for imposing heat stress. Field screening of biparental mapping populations for QTL mapping of tolerance to RSHS in rice have been attempted earlier (Xiao et al., 2011a, b; Buu et al., 2014; Tazib et al., 2015; Zhao et al., 2016; Zhu et al., 2017; Seo et al., 2019; Cao et al., 2020). This justifies the use of off-season

to screen various genotypes for tolerance to RSHS in the present study as compared to *Kharif* season, wherein it is difficult to screen for heat stress tolerance unless the genotypes are tested under controlled conditions. The testing of large number of genotypes in controlled condition is very difficult due to lack of space and large facilities. Furthermore, QTLs identified through field phenotyping will have greater chance of success when deployed into popular varieties than do the ones identified using controlled environment phenotyping (Cobb et al., 2019).

5.2. Phenotypic variation in tolerance to RSHS in the rice germplasm

Two separate experiments were conducted to assess the variability for tolerance to RSHS in the rice germplasm. One experiment comprised of 192 diverse rice genotype and another a sub-set (35 genotypes) of Basmati germplasm. The 192-germplasm set was screened for tolerance to RSHS at IARI-RBGRC, Aduthurai during off-season, 2018-19 through two staggered sowing. Following IRRI Standard Evaluation System (SES; IRRI, 2002), around 32 genotypes were found to be highly sterile with SF of <50%, 92 genotypes were partially sterile with SF ranging between 50% and 74%, 62 genotypes were fertile with SF ranging from 75% to 89%, and the remaining four genotypes were highly fertile with spikelet fertility of $\geq 90\%$. As expected, the tolerant check, N22 was found to be highly fertile with an exceptional spikelet fertility of 95.87 % proving its resilience to RSHS. However, in terms of grain yield *per se* and STI, Bhuban and PRR127, and as per spikelet fertility *per se* and STI, DV85 and Improved Samba Mahsuri, were found promising. PRR127 is a medium maturing and high yielding rice restorer developed at ICAR-IARI, New Delhi. Bhuban is a medium duration rice variety of medium bold grain type released from Odisha. DV85 is a rice variety released in Bangladesh with tolerance to biotic stresses such as bacterial leaf blight (carries *xa5* and *Xa7* genes) and BPH (Sidhu et al., 1978; Wang et al., 2005; Su et al., 2008). Improved Samba Mahsuri was evolved through marker assisted introgression of three BB resistance genes *Xa21*, *xa13* and *xa5* into the background of Samba Mahsuri (BPT5204) using SS1113 as donor parent (Sundaram et al., 2008).

A separate experiment was conducted to assess the heat stress tolerance level of a sub-set of 35 Basmati genotypes including popular varieties of Basmati growing areas during two off-seasons 2018-19 and 2019-20 at IARI-RBGRC, Aduthurai. Majority of the genotypes were highly sterile with SF <50% while a few are partially sterile with a SF between 50% and 75%. Seond Basmati was highly sensitive to RSHS with 16.76% and 24.71% of spikelet fertility under RSHS during off-season, 2018-19 and 2019-20,

respectively. Based on *per se* trait values and STI, Basmati 370, Type 23 and Chimbamate Basmati was found to be moderately tolerant to RSHS. This clearly demonstrates the vulnerability of Basmati rice to heat stress and rationalizes the selection of Basmati genotypes as sensitive parents in generation of mapping populations. This is the first study, as per our knowledge, where the heat stress behavior of Basmati genotypes has been studied. Previously there were only two reports which examined the heat stress tolerance of Basmati cultivars. Ahmed et al. (2015) studied the effect of high temperature on grain quality of Basmati 2000. Wahab et al. (2020) observed a spikelet fertility of 43.59% and 69.05% for Pusa Basmati 1121 and Basmati 386, respectively under heat stress almost similar to the present study (spikelet fertility of Pusa Basmati 1121 was 55.67% and 24.86% under stress during 2018-19 and 2019-20, whereas of Basmati 386 was 61.49% and 58.88% under stress during 2018-19 and 2019-20).

5.3. Genome wide association study for mapping tolerance to RSHS

Genotype data was available for 192 rice genotypes assessed for tolerance to RSHS which was generated from another study from our lab through 50k SNP array as described by Bollinedi et al. (2020). Population structure explored through PCA revealed the presence of three sub-populations. In natural populations of rice, four sub-populations, i.e., *indica*, temperate *japonica*, *aus*/boro and Basmati/Sadri can be frequently encountered representing different ecotypes (Chen et al., 2019). However, in the present study the substructure differentiation seems to be mostly based on the history of plant breeding activity since varieties, breeding lines and landraces were categorized into distinct groups. The genetic diversity of the germplasm was further supported by near-zero kinship values. To our knowledge, ours is the only study, so far, which has used both highest number of genotypes and SNP markers. There are only two reports on GWAS for tolerance to RSHS in rice. Pradhan et al. (2016) utilized 20 SSRs linked to previously reported QTLs governing heat stress tolerance for validating them in a set of 62 rice genotypes, while Lafarge et al. (2017) carried out genotyping by sequencing of 167 rice genotypes to identify MTAs associated with tolerance to RSHS in rice. Both these studies were conducted for high day temperatures. Bheemanahalli et al. (2021) carried out GWAS for high night temperature tolerance in rice. GWAS has been attempted for mapping tolerance to RSHS in other crops such as wheat (Devate et al., 2022), *Brassica napus* (Rahaman et al., 2018), maize (Tadesse et al., 2019; Guo et al., 2020; Sharma et al., 2020; Longmei et al., 2021), and field pea (Tafesse et al., 2020).

In the present study, three GWAS models namely, MLM, FarmCPU and BLINK and two traits, spikelet fertility and grain yield per plant, and respective STIs were used for identifying MTAs for tolerance to RSHS. Highest number of QTNs were identified through FarmCPU and BLINK than MLM. Generally, MLM misses out on many minor QTNs in an effort to suppress false positives (Merrick et al., 2022). Only nine out of the 24 QTNs identified, validated the previously reported QTLs making most of them novel. For instance, *qHTST11.1* (*qSTISF11.1*) identified through MLM validated two QTLs namely, *qHTSF11.2* (Ye et al., 2015a) reported for spikelet fertility and *qLD10-11* (Tazib et al., 2015) for longitudinal dehiscence of anthers. Similarly, *qHTSF4.1* (*qSTISF4.1*) validated *SSPF4* (Xiao et al., 2011a) and *qPF4* (Xiao et al., 2011b) governing seed set percentage and pollen fertility under heat stress, respectively. The QTN, *qHTSF7.1* coincided with genomic regions associated with *qAL10-7* controlling anther length (Tazib et al., 2015) and *qHTST2.1*, validated *qtl_2.2* determining absolute spikelet fertility (Jagadish et al., 2010).

The QTN, *qHTGY8.1* for grain yield was identified through both FarmCPU and BLINK, while *qHTSF5.1* for spikelet fertility was discovered through all the three models. These QTNs are valuable candidates for further validation in future studies. A probe into the genes in the vicinity of these QTNs using genome browser of rice genome annotation project website revealed several genes involved, either directly or indirectly, in heat stress and/or other abiotic stresses in plants. For instance, among the genes in the genomic regions around the QTNs of grain yield, DUF1336 domain-containing protein, cytochrome P450 protein and OsWAK116–OsWAK receptor-like cytoplasmic kinase have established roles in heat stress. For spikelet fertility, several genes were identified with roles in proteolysis or protein ubiquitination and protein-protein interactions. It's a proven fact that heat stress results in protein degradation and misfolding. These denatured proteins need to be quickly neutralized, otherwise, they lead to cellular toxicity. Genes encoding tetratricopeptide repeat (TRP) motif containing protein, E3 ubiquitin ligase, (BTB/POX) domain-containing protein, U-box domain-containing protein and RING finger protein found near QTNs of spikelet fertility were involved in protein degradation. These genes are possible candidates for further validation in future studies for identifying suitable haplotype combinations and deployment in heat stress sensitive genotypes.

Finally, based on *per se* trait values and number of positive alleles of QTNs present, five genotypes namely RIL10, Selected Sabarmati, Sitwa Dhan, Bhadrakali and Samba

Mahsuri have been identified with favourable allelic combination for genomic regions governing tolerance to RSHS. There are contradictory reports on the behaviour of Samba Mahsuri under heat stress. It was reported to be moderately tolerant to heat stress maintaining spikelet fertility of 85.60% and grain yield of 29.31 g at 38.4°C (Wahab et al., 2020) and spikelet fertility of 88.1% at 38°C (Shi et al., 2015). However, the same genotype was reported to show high relative injury (90%) coupled with high spikelet sterility (~90%) under heat stress (Sailaja et al., 2015). Thus, tolerance of these genotypes needs to be further confirmed in future.

5.4. Verifying the presence of previously reported QTLs for tolerance to RSHS

An initial verification of the presence of previously reported, particularly frequently referred QTLs, provides valuable information on whether the tolerance in the new genotype is novel so that further studies can be undertaken for mapping the QTLs. In the present study, we first confirmed the phenotypic reaction through the data generated on grain yield and spikelet fertility under RSHS through a pilot study during off-season, 2017-18 at IARI-RGBRC, Aduthurai and genotyping with a set of 116 SSRs linked to previously reported QTLs. Of these, only 31 (26.7%) markers were found to be polymorphic between the parents, Pusa Basmati 1 and NL 44. Significant number of SSRs linked to the QTLs were rendered non-informative due to monomorphism between the parents. Further, only six markers among the 31 polymorphic markers showed significant associations, especially for spikelet fertility. However, the contribution of these QTLs was scanty indicating that they were minor QTLs with an average R^2 value (simple linear regression) of 4.6% with ≤ 3.0 additive effect. Hence, we hypothesized that the genomic regions governing tolerance to RSHS in NL44 might be novel. This validation study prompted us to carry out a systematic QTL mapping for discovering the genomic regions governing tolerance to RSHS in NL44.

5.5. Performance of parents, F₁ and RILs for tolerance to RSHS

We conducted an experiment for detailed characterization of the parental lines namely, PB 1 and NL44 for heat stress tolerance through a controlled experiment along N22, IR64 and PB 1509. Maintenance of optimum temperature of various organs is one of the mechanisms of heat avoidance adopted by rice genotypes (Chuan et al., 2008; Shimoda et al., 2012; Yoshimoto et al., 2012; Maruyama et al., 2013; Sailaja et al., 2015; Zhang et al., 2016). Even though the canopy temperature depression was higher for the sensitive genotypes, PB 1 and PB 1509, the tolerant genotypes, N22 and NL44 clearly showed

significant superiority over the sensitive genotypes considering other parameters such as Membrane stability index (MSI), H_2O_2 , lipid peroxidation and total antioxidant capacity. SPAD reading, a reflection of chlorophyll content, was not affected due to heat stress, since the plants were exposed to normal conditions till anthesis after which half of the plants were exposed to heat stress. Similar observation was made by Restrepo-Diaz and Garces-Varon (2013) where decreased photosynthesis did not correlate well with SPAD or F_v/F_m values under heat stress imposed at reproductive stage. Superior ROS scavenging have been frequently reported to be a heat stress tolerance mechanism (Bahuguna et al., 2015; Sailaja et al., 2015; Zhang et al., 2016; Karwa et al., 2020). RSHS tolerance of NL44 was previously reported to be due to reduced accumulation of H_2O_2 and low lipid peroxidation (TBARS) as found in the present study (Bahuguna et al., 2015; Karwa et al., 2020).

The study of F_1 behaviour under heat stress revealed the preponderance of dominance gene action for tolerance to RSHS in NL44. This is further supported by moderate heritability observed for spikelet fertility and lesser additive effects observed for QTLs identified later. There are no systematic studies on gene action underpinning tolerance to RSHS in rice. However, it was proposed that high temperature tolerance in rice is under recessive gene control (Fu et al., 2015). There are contradictory reports specifying the role of male and female parents in contributing to the tolerance in hybrids (Hu et al., 2012; Fu et al., 2015).

Though observations were recorded on grain yield and its contributing traits, significant contrast was observed mainly for grain yield per plant and spikelet fertility % under RSHS. This was expected since the genotypes under control and heat stress were grown under similar conditions (normal temperatures) till flowering after which they were exposed to different temperatures. For instance, Ye et al. (2012) measured days to heading, plant height, panicle neck length, panicle length, flag leaf length, spikelet number, spikelet fertility; albeit only spikelet fertility was considered for QTL mapping for tolerance to RSHS. This distinction between the parents for grain yield and spikelet fertility was observed across locations which was more pronounced for spikelet fertility under RSHS ($\geq 50\%$). Similar contrast between parents were observed for spikelet fertility by Zhao et al. (2016) between Sasanishiki and Habataki, and Shanmugavadivel et al. (2017) between N22 and IR64 but higher than that reported by Cheng et al. (2012) between Xiushui 09 and IR2061, Li et al. (2018) between Liaoyan241 and IAPAR-9, and Seo et al. (2019) between IR64 and Koshihikari. NL44 maintained consistency for both grain yield and spikelet

fertility under control and RSHS across environments compared to the sensitive parent, PB 1 reflecting its superior tolerance to RSHS. STI for grain yield of PB 1 and NL44 observed in the present study was higher than reported for IR64 and N22, respectively by Shanmugavadivel et al. (2017), while opposite was true for spikelet fertility percentage.

A wide variation was observed among the RILs in the present study for both grain yield and spikelet fertility compared to other morpho-agrological attributes. Even transgressive segregants were observed as in several of previous studies. Transgressive segregation is an indicator of optimum recombination among the RILs and complementary action of additive alleles of causal genes dispersed between the parents (Falconer, 1996; Rieseberg et al., 1999). Some of these transgressive segregants will be valuable candidates to be used as improved donors for tolerance to RSHS. Additionally, it also shows the potential of sensitive genotypes to contribute towards the trait such as tolerance. Both the traits showed near normal distributions across the seasons and treatments reflecting a polygenic inheritance and justifying QTL mapping. Normal distributions for spikelet fertility under RSHS have been reported in several earlier studies (Chen et al., 2008; Buu et al., 2014; Xiao et al., 2011a; Zhu et al., 2017; Li et al., 2018; Nubankoh et al., 2020; Chen et al., 2021). The range of grain yield among the RILs was almost similar to that observed by Seo et al. (2019) among BILs and CSSLs but higher than the range recorded by Shanmugavadivel et al. (2017). The range of spikelet fertility observed in the present study was higher than of Zhu et al. (2017), among CSSLs at par with the range observed by Seo et al. (2019) among BILs, Li et al. (2018), but lower than that observed by Xiao et al. (2011a), Cheng et al. (2012), Ye et al. (2012), Buu et al. (2014), Zhao et al. (2016), Shanmugavadivel et al. (2017), Nubankoh et al. (2020) and Chen et al. (2021). STI range observed for grain yield was higher than that observed by Shanmugavadivel et al. (2017) while it was lower for spikelet fertility. Moderate heritability values were observed for grain yield (57% and 71% under control and RSHS, respectively) and spikelet fertility (57% and 59% under control and RSHS, respectively). This was in line with the heritability observed for absolute spikelet fertility under heat stress (62 - 69%) by Jagadish et al. (2010) but lower than that reported by Xiao et al. (2011a) for seed set percentages (81.45% and 70.51% under field and growth chamber experiments, respectively).

Through GGE biplot analysis, RILs with stable and superior performance vis-à-vis grain yield and spikelet fertility were selected which can serve as improved donors for breeding reproductive stage heat stress tolerant rice varieties. Such MLT trials for

comparing terminal heat stress tolerance are highly popular in wheat. There is only one study in rice by Wu et al. (2021) which examined GEI using multi-season data (2010-14).

5.6. The non-significance of pollen fertility in determining reproductive stage heat stress tolerance

Pollen fertility presented a peculiar case, where limited contrast was observed between the parents in the initial study during off-season 2017-18 and during 2018-19 at both locations. Whereas among the RILs, distribution was significantly skewed towards fertility. This is in incongruence with several studies which had documented the loss of pollen fertility/ viability under heat stress (Das et al., 2014; Kumar et al., 2015; Thuy et al., 2020). This implies that some other structural component of pollen grains and/ or female reproductive system is getting affected under heat stress in sensitive parent and some RILs. Similar observation was made by Zafar et al. (2022) who did not find any effect of heat stress on pollen fertility in sensitive lines. This confirms the earlier reports that decrease in spikelet fertility under heat stress is more to do with the inadequate anther dehiscence than the pollen sterility *per se* (Hu et al., 2021).

5.7. Effect of heat stress on grain quality of parents and RILs

To assess the contrast between the parents and the variation in the RILs, several grain and cooking quality attributes were analysed on the produce harvested from two staggered sowings of off-season, 2018-19 along with the produce of *Kharif*, 2019 at New Delhi as a positive control. The parents showed variable performance for various grain and cooking quality traits under different environments. PB 1 showed superior values for eight out of the 12 traits studied from the late sown crop of Aduthurai, 2018-19, while NL44 displayed favourable values for seven out of 12 traits estimated from timely sown crop. This was in disagreement with Bahuguna et al. (2015) who observed chalkiness of < 20% for NL44 under heat stress. The mean performance of RILs was almost similar under two treatments of Aduthurai. However, in comparison with performance of favourable environment, i.e., New Delhi, *Kharif*, 2019, significant reduction was observed in HRR, GC and amylose. Greater chalkiness was observed in both the treatments of Aduthurai. The reduced HRR can be attributed to high grain cracking when exposed to heat stress at grain maturity stage (Nagata et al., 2013; Chun et al., 2015). Further, HRR also shows negative association with grain chalkiness (Zhao and Fitzgerald, 2013). High grain chalkiness was observed in the RILs under both treatments of Aduthurai. Grain chalkiness is frequently

reported as one of the repercussions of heat stress which is primarily due to derailed starch synthesis. As expected, reduction in amylose content was observed in both treatments of Aduthurai relative to New Delhi. Since the sensitive parent being a Basmati genotype, the locations used for evaluation are not congenial for optimum Basmati quality expression which might be the reason for the discrepancies in quality attributes at Aduthurai. The donors used for mapping grain quality attributes under heat stress are different from those which are used for mapping spikelet fertility under stress. This indicates that high spikelet fertility may not always associate with good grain quality under heat stress and vice versa as in the present study.

5.8. Linkage map construction

The introduction of molecular markers has significantly enriched the linkage maps generated for mapping. RFLPs, RAPDs, AFLPs, SSRs, etc., provided significant density for the saturation of genetic linkage maps which was not possible with phenotypic and isozyme markers. Sequence based genotyping methodologies have further improved these maps leading to a high to very high-density linkage maps and there by significantly enhancing the resolution of QTLs detected. However, SNP based QTL mapping is more popular in GWAS studies given the lack of restriction on the number of traits that can be recorded on the germplasm. Hence, SSR makers remain the marker of choice for initial QTL mapping study as in the present study. Construction of linkage map is an important pre-requisite for QTL mapping, particularly for interval mapping which begins with a parental polymorphism survey. The mean percentage of polymorphism between the parents observed was 13.5% using SSR markers and 27.95% through SNP markers. This was higher than that reported by Xiao et al. (2011a, b) (18.53%), Li et al. (2018) (21%), almost at par with that observed by Shanmugavadivel et al. (2017) (28%), but lesser than that documented by Buu et al. (2014) (52.69%), Yeo et al. (2012) (72.9%), Seo et al. (2019) (93.4%). Nevertheless, these higher polymorphism values are resultant of recruiting strategically selected markers rather than genome wide markers as utilized in the present study. The per cent polymorphism may also be dependent on how diverse the parental lines are. The coverage of the initial SSR based linkage map constructed with 94 SSR markers was found inferior to many of the previous studies. Hence, it was later upgraded to SNP based map. To our knowledge, this is the high-density linkage map ever constructed to map QTLs for tolerance to RSHS. Previously linkage maps with only 824 (Shanmugavadivel et al., 2017) and 280 SNP markers (Ye et al., 2012) were utilized for QTL mapping for

tolerance to RSHS. The percentage of segregation distortion registered in the present study was 6.93% for SSR markers and 32.33% for SNP markers. This high segregation distortion is attributable to the genetic divergence between the parents – PB 1 being an *indica* ecotype and NL44 being a NERICA line and moderate size of mapping population (127 RILs) (Reflinur et al., 2014). Segregation distortion was commonly reported in rice (Koide et al., 2012; Reflinur et al., 2014; Xu et al., 2014). The length of the maps constructed through SSR, and SNP markers was 1568.64 cM and 2977 cM, respectively. The map length obtained through SNP in the present study almost matched with the map length reported by Xiao et al. (2011a, b) (2025.2 cM), but greater than that reported by Cheng et al. (2012) (1591.1 cM), Seo et al. (2019) (1603 cM), Zhu et al. (2005) (1179.9 cM). An integrated linkage map utilizing both SSR and SNP markers was used for downstream QTL analysis.

Several of the major QTLs associated with major abiotic stress such as drought tolerance (Venuprasad et al., 2009; Sandhu et al., 2014) and salt tolerance (Bonilla et al., 2002) have been mapped using SSR markers. More than half of the QTL studies of tolerance to RSHS in rice have been carried out using SSR markers. Additionally, rice being a genomic resource rich crop, SNP based biparental QTL mapping has also been previously attempted to map traits including tolerance to various abiotic stresses such as heat (Yeo et al., 2012, 2015a, b; Shanmugavadivel et al., 2017; Chen et al., 2021), drought (Yadav et al., 2019) and salt (Tiwari et al., 2016).

5.9. QTLs governing tolerance to RSHS

Identification of QTLs is an important prerequisite for molecular marker assisted selection for which the linked markers serve as inputs. Given the complexity of abiotic stresses, including heat stress, marker assisted selection provides for efficient and precise selection for breeding stress resilient crop varieties. Since grain yield and spikelet fertility showed consistently greater contrast between parents and high variability among RILs, these traits were primarily considered for QTL mapping for tolerance to RSHS in NL44. A total of seven QTLs through individual environment analysis and eight QTLs through combined environment analysis were found under RSHS in the present study most of them being moderate effect QTLs with PVE between 5-10% except *qSTIGY2.1*, *qSTIGY8.1*, *qSTISF12.1* which registered low PVE of 3.2%. Majority of the QTLs reported for tolerance to RSHS showed a PVE of $\leq 15\%$, barring few exceptions, as in the present study recounting that multitude of genes with small to moderate effects is responsible for tolerance to RSHS in rice. Furthermore, the positive alleles of majority of these QTLs are

being contributed by tolerant parent except *qSTIGY1.1*, *qSTIGY1.2* and *qSTISF12.1*. This is not uncommon since similar instances have been previously reported such as in *qHTSF1.1* identified for spikelet fertility by Ye et al. (2012) the positive allele was contributed by IR64, the sensitive parent. Similarly, the yield increasing allele of *qtl12.1*, a major effect QTL for drought tolerance in rice was contributed by sensitive parent, Way Rarem (Bernier et al., 2007). It was reasoned that the epistatic interaction between the QTLs contributed by tolerant and sensitive parents might contribute to tolerance. Two major QTLs through individual environment analysis and four moderate effect QTLs through combined environment analysis were identified under control condition. Perhaps, an increase in the contrast between the parents coupled with increased range in variation among the RILs for the trait under study might result in the increased capture of phenotypic variance by the QTLs.

QTL analysis was also attempted with other yield contributing traits as well. Interestingly, in contrast with grain yield and spikelet fertility, the favourable alleles of all the other traits were contributed by PB 1. This reflects that PB 1 is superior for most of yield contributing traits except grain yield and spikelet fertility for which NL44 was significantly superior than PB 1 (irrespective of treatments). As mentioned earlier and as pointed out by Ye et al. (2012), tolerance is relative term and both sensitive and tolerant parents can contribute alleles with positive effects under stress. Another intriguing finding was that a major effect QTL (*qCPH8.1*) was identified on chromosome 8 which was unveiled under both in control and heat stress treatment through combined environment analysis and in control in individual environment analysis. What makes this even more remarkable is that the difference between parents for plant height is < 10 cm across environments. Similar major QTL for plant height (PVE of 71%) was also reported by Ye et al. (2012) on chromosome 1 from a cross between IR64 and N22. Rest of the QTLs for the remaining traits are moderate effects akin to grain yield and spikelet fertility.

Several advantages have been observed for QTL mapping through SNP makers over SSRs. SNP genotyping revealed greater polymorphism between the parents than through SSRs which means more markers are available for genotyping, thus enriching the linkage map. Thus, coverage of markers was significantly improved for all chromosomes, but particularly chromosomes 1, 5, 7 and 10 through SNP genotyping. Though QTLs were initially identified through SSR markers, their PVE values were inflated. However, through SNP based linkage map, these turned out to be either minor or false positives. Though PVE

values obtained through SNP based mapping were moderate, their LOD values are higher, enhancing QTL resolution. The intervals obtained for QTLs identified through SNP mapping are also significantly narrower. Thus, these QTLs have greater chance to serve as candidates of marker assisted selection.

5.10. Co-localization with previously reported QTLs

The physical location of markers linked to the QTLs identified (grain yield and spikelet fertility) were compared with the positions of previously reported QTLs for tolerance to RSHS in rice (except the report by Seo et al. (2019) due to lack of sufficient information). Nearly half of the QTLs co-localized with previous reports. The QTL, *qHTSF7.1* was flanked by *qAL10-7*, *qGN7.1* and *qHTSF7.1* within 1 Mb distance. QTL *qAL10-7* was reported by Tazib et al. (2015) for anther length, *qGN7.1* was a major QTL identified for spikelet number and *qHTSF7.1* for spikelet fertility by Ye et al. (2012). *qSTISF10.1* overlapped with *qHTB10-1* found for spikelet fertility by Zhu et al. (2017). *qSTIGY1.1* identified through combined environment analysis was around 2 Mb from a QTL hotspot with five QTLs reported by various authors for spikelet fertility (Figure 5.1). This hotspot includes *qtl_1.1* reported for both absolute spikelet fertility and relative spikelet fertility by Jagadish et al. (2010), *qHTB1* for spikelet fertility by Zhu et al. (2017), *qGN1.1* and *qHTSF1.1* governing spikelet number and spikelet fertility found from two different mapping populations by Ye et al. (2012). Another QTL, *qSTIGY1.2* co-localized with *qAL09-1* reported for anther length by Tazib et al. (2015). Finally, *qSTIGY8.1* co-localized with *qtl_8.2* found for absolute spikelet fertility by Jagadish et al. (2010). It's worthy to mention here that of these, the QTLs identified by Ye et al. (2012) were not found when tested in different background (Ye et al., 2015a). Furthermore, none of the remaining QTLs were validated by the respective authors. Thus, the remaining QTLs can be considered novel. Additionally, no QTL was found on chromosome 4 in the hotspot region which houses the *qHTSF4.1*, the fine-mapped and validated QTL for tolerance to RSHS from N22 and on chromosome 5, anywhere near the hotspot region further affirming the novelty of the donor and the QTLs identified in the present study.

5.10. Convergence of QTNs identified using GWAS and QTLs found through biparental mapping

The physical positions of QTLs identified through biparental mapping were compared with the physical positions of QTNs found in GWAS. It was found that QTL *qSTIGY1.1* governing STI for grain yield identified on chromosome 1 co-localized with

qHTGY1.1 and *qHTSF1.2* found for grain yield and spikelet fertility under stress, respectively through GWAS. Another QTL, *qSTIGY1.2* controlling STI for grain yield on chromosome 1 was found 3 Mb away from QTN, *qHTGY1.2* of grain yield under RSHS. On chromosome 5, QTL *qHTSF5.1* of spikelet fertility under stress was found to be 6 Mb away from QTNs, *qSTISF5.2*, *qSTISF5.1* and *qHTSF5.1* governing STI for spikelet fertility and spikelet fertility under RSHS. QTL for spikelet fertility, *qHTSF7.1* on chromosome 7 co-localized with the QTN governing spikelet fertility, *qHTSF7.1*. Among these QTL, *qHTSF5.1* did not colocalize with any of the previously reported QTL for tolerance to RSHS in rice.

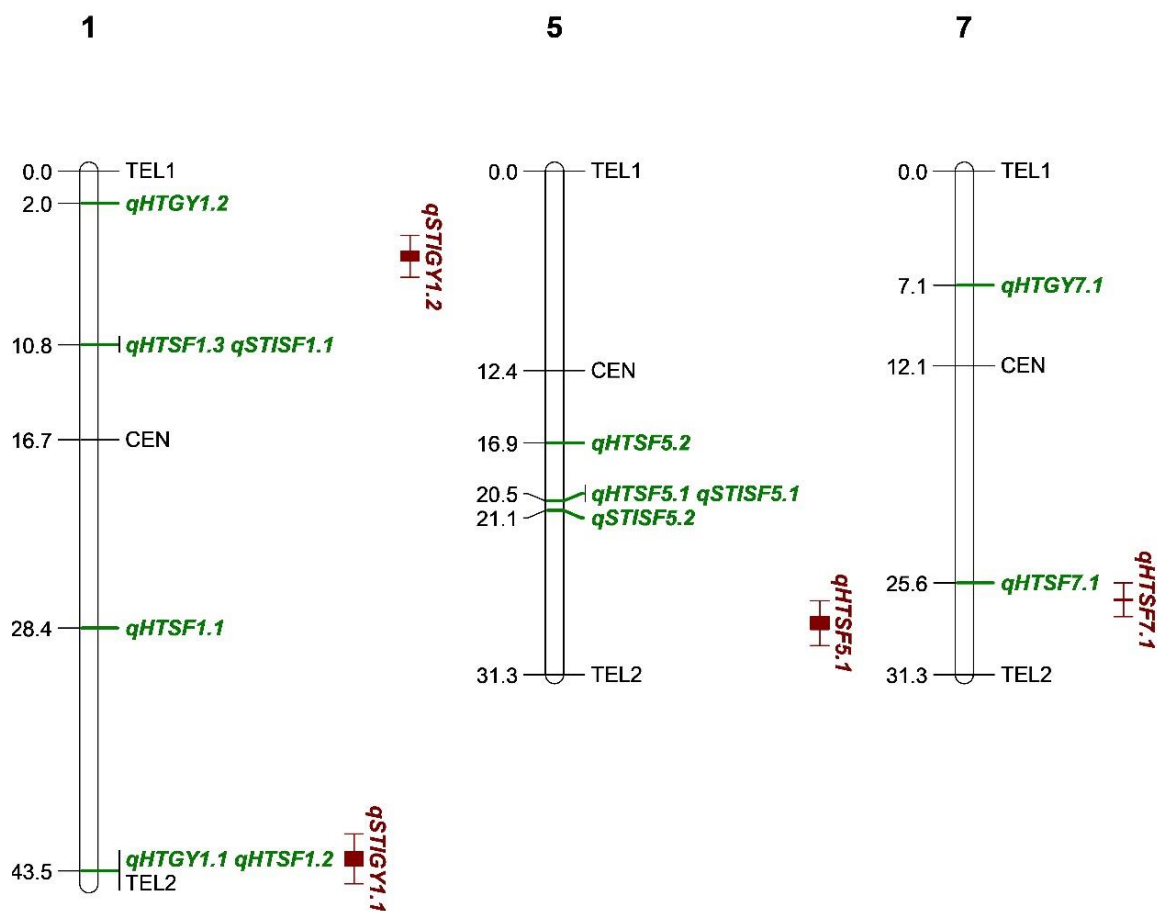


Figure 5.1. Co-localization of QTLs identified through biparental mapping (in brown) and QTNs found through GWAS (in green).

Chapter VI

SUMMARY AND CONCLUSION

Post the industrial era, the earth's mean surface temperature increased by 0.85°C and is expected to further raise by 5°C towards the end of this century (IPCC 2014). Historically several instances of heat waves have been documented across the world resulting in huge economic losses to farmers. Their frequency and intensity are on an increasing trend and is expected to further scale up in the near future. Rice, a principal staple cereal, is highly sensitive to heat stress. Seedling, reproductive and post reproductive stages of rice are the most sensitive stages for heat stress. Heat stress during seedling stage results in leaf discoloration and seedling death. Anthesis and booting are the reproductive stages which are highly vulnerable to heat stress (Satake and Yoshida, 1978). Severe spikelet fertility and yield decline are observed when supra-optimal temperatures coincide with these stages. This has been primarily attributed to pollen sterility, pollen desiccation, abnormal anther dehiscence, malfunctioning of tapetum which ultimately result in significant decrease in number of viable pollens reaching the stigma (Xu et al. 2021). Thus, tolerance at reproductive stage heat stress is highly significant since it directly decides the ultimate grain yield. Heat stress post-fertilization also results in deterioration of grain quality (Lyman et al. 2013). Breeding heat stress tolerant varieties is an urgent need of the hour to avert the risk of crop and quality loss due to these extreme weather events.

Screening of diverse germplasm for tolerance to heat stress in order to identify novel donors is the first step in this direction. The tolerant genotypes need to be characterized to map the genomic regions governing heat stress tolerance through appropriate mapping strategy. After further validation, these QTLs can be introgressed into popular varieties of the region sensitive to heat stress so that resilience to heat stress can be improved. Huge variability has already been reported in rice for tolerance to RSHS. Similarly, several QTLs governing tolerance to RSHS have been mapped, on almost on all chromosomes using various donors. However, only a couple of studies validated these QTL in different genetic backgrounds, which enables its use in trait improvement. Recently, a new reproductive stage heat stress tolerant donor, NL44, a NERICA line, was reported to show reproductive heat stress resilience almost at par with N22, a popular heat stress tolerant donor (Bahuguna et al., 2015). However, the genetic and molecular basis of its tolerance remained elusive. Thus, the current study was carried out with the aim of identifying genomic regions encoding tolerance to RSHS in NL44 along with a search for new donors with tolerance to

RSHS in the rice germplasm. Accordingly, the following objectives have been framed to achieve this:

1. To assess the phenotypic variation in the rice germplasm for heat stress tolerance at reproductive stage.
2. To map QTLs governing heat stress tolerance at reproductive stage in the NERICA rice cultivar, NL44.
3. To validate the molecular markers associated with QTLs governing heat stress tolerance in rice.

Characterization of variability for tolerance to RSHS was done with two sets, one with a set of 192 diverse rice germplasm, under natural field conditions in off-season of 2019 at Aduthurai, Tamil Nadu through two staggered sowing separated by one month. The first set sown in late December flowered in early March when the ambient temperature is around 33-35°C whereas the second set sown in late January came to flowering in early to late April when the temperatures rose to 38-40°C, hence, were designated as control and RSHS treatments, respectively. Significant reduction was observed in grain yield and spikelet fertility under heat stress with an average 26% and 19% decline relative to control, respectively. Additionally, stress tolerance index (STI) was also calculated for both the traits. Based on *per se* trait values and STI, Bhuban and PRR127 were found promising for grain yield, whilst DV85 and Improved Samba Mahsuri were found to be the best genotypes for spikelet fertility.

In the second experiment, a set of 35 Basmati genotypes were assessed for tolerance to RSHS with two staggered sowing like the above study during off-seasons, 2018-19 and 2019-20, respectively. In contrast to first set of rice germplasm, most of the Basmati genotypes showed severe reduction in grain yield and spikelet fertility. The mean reduction in grain yield was 54% and 39% in 2018-19 and 2019-20, respectively whereas the mean reduction in spikelet fertility was 46% and 28% in 2018-19 and 2019-20, respectively. Considering both *per se* trait values and STI, the genotypes, Basmati 370, Type 23, Basmati Jamuna were found to be moderately heat stress tolerant. However, this classification was only relative and not exclusive since these genotypes also suffered a considerable reduction in trait values.

The genotype data of 192 rice genotypes (Bollinedi et al., 2020) was utilized for GWAS through R package, GAPIT. PCA based structure analysis revealed the existence of three sub-populations as reported before primarily based on the extent of breeding

activity carried out on these genotypes. For GWAS, three models, MLM, FarmCPU and BLINK were used. No QTNs were found for grain yield and spikelet fertility under control. Under RSHS, seven and three QTNs were identified for grain yield through FarmCPU and BLINK, respectively of which *qHTGY8.1* was found through both the models. Similarly, for spikelet fertility, eight QTN were found using FarmCPU, and seven each through MLM and BLINK of which *qHTSF5.1* was shared by the three models. Furthermore, only two QTNs of grain yield and five QTNs of spikelet fertility co-localized with the previously reported QTLs for RSHST in rice making significant number of QTNs novel. Based on phenotype and number of favourable alleles accumulated, RIL 10, Selected Sabarmati, Sitwa Dhan, Bhadrakali, and Samba Mahsuri were identified for further detailed characterization.

To map QTLs for tolerance to RSHS in NL44, a RIL population was generated from the cross between NL44 and a popular Basmati variety, Pusa Basmati 1 (PB 1). Prior to actual QTL mapping, two experiments were conducted, one under controlled environment chamber, where the parents were validated along with N22, IR64 and PB1509 for tolerance to RSHS through various physiological parameters. As reported earlier, N22 and NL44 displayed cooler panicles and less membrane injury coupled with efficient ROS scavenging under RSHS making them tolerant, whereas IR64 showed moderate tolerance and the Basmati rice varieties, PB 1 and PB1509 were highly sensitive. Another pilot study was conducted during off-season, 2017-18 at Aduthurai, where the RILs and parents were exposed to high ambient temperatures under natural field conditions. A set of 54 QTLs with 116 linked SSR markers was analyzed for parental polymorphism out of which only 31 markers were found. A single marker analysis using the phenotypic (grain yield and spikelet fertility) and genotypic (19 SSR markers) data validated seven previously reported QTLs, albeit with < 5% PVE. Thus, it was confirmed that the genomic regions governing tolerance to RSHS in NL44 might be different from the previously reported regions. In addition, an evaluation of a separately generated F₁ from the original cross, PB1 × NL44 under heat stress showed that tolerance of NL44 to RSHS may be controlled by majority of genes with dominant gene action.

ANOVA of the data of RILs along with parents and checks, evaluated for two consecutive off-seasons, 2019 and 2020 at two locations, Aduthurai and Cuttack with two staggered sowings recorded on grain yield and its contributing traits including spikelet fertility revealed significant genotype (except panicle number in control), environment

(except days to 50% flowering) and genotype $\times$ environment (except panicle length and tiller number under heat stress) interactions. A consistent reduction was observed for grain yield and spikelet fertility under RSHS across environments, whereas the response of the genotypes varied across treatments and environments for remaining traits. This is expected since the genotypes were exposed to similar temperatures in both treatments till anthesis. The discrepancies of these traits might be due to other environmental variables. Grain yield and spikelet fertility showed near normal distributions in most of the environments. Under RSHS, lowest mean grain yield and spikelet fertility were observed at Cuttack, during 2018-19 (6.35g and 41.88%) followed by Cuttack, 2019-20 (11.31g and 52.31%), Aduthurai, 2019-20 (12.85g and 58.79%) and Aduthurai, 2018-19 (15.26g and 64.28%). Similarly, the contrast between the parents was clearer at Cuttack than at Aduthurai. However, GGE biplot analysis revealed that Aduthurai was the most discriminating location, both for grain yield and spikelet fertility. The RILs, RIL52, RIL49, RIL57, RIL119, RIL56 and RIL50 were found ideal for grain yield, whereas for spikelet fertility RIL126, RIL116, RIL123, RIL59, RIL87, RIL89, RIL51 and RIL84 were superior.

Characterization of the grain and cooking quality of the RILs and parents from two treatments of Aduthurai from off-season, 2018-19 and New Delhi, *Kharif*, 2019 showed that the grain and cooking quality was significantly affected by both the treatments of Aduthurai as compared to produce from New Delhi. This was particularly noticeable in HRR where 50% reduction was observed compared to favorable environment, i.e., New Delhi. Gel consistency and amylose contents also showed significant decline at Aduthurai compared to New Delhi. Grain chalkiness, the phenotypic marker for the effect of heat stress was highest from control treatment of Aduthurai followed by RSHS. This is understandable since main our objective was to expose the genotypes to high temperatures during anthesis and the location Aduthurai is not favourable for Basmati trait expression.

Initially, the genotypic data from 94 polymorphic markers from the RILs were used for constructing a linkage map with a span of 1568.64 cM using MAP function of QTL IciMapping software. This linkage map had large gaps as compared to the linkage map with a span of 2977 cM was constructed with 10924 SNPs markers from the SNP genotyping of data of the RILs with 80K SNP array, after accounting for monomorphism and segregation distortion.

For mapping tolerance to RSHS in NL44, grain yield and spikelet fertility and their respective STI values were primarily considered. Under control, only two QTLs were found

for only spikelet fertility through individual environment analysis, whereas two QTLs each were identified for grain yield and spikelet fertility through combined environment analysis. Under RSHS, seven QTLs were identified, one for grain yield, three for spikelet fertility, one for STI of grain yield and two for STI of spikelet fertility with positive alleles of most of the QTLs contributed by NL44. Most of them are moderate-effect QTLs with PVE varying between 3.2% (*qSTIGY2.1*) and 10.1% (*qSTISF10.1*). Through combined environment analysis, eight QTLs were revealed, one for grain yield, three for spikelet fertility, three for STI of grain yield and one for STI of spikelet fertility. As in individual environment analysis most of them were moderate effect QTLs with PVE varying between 1.41% (*qSTIGY8.1*) to 8.26% (*qHTSF7.1*). QTL mapping for other yield contributing traits identified QTLs only for plant height under control through individual environment analysis. However, through combined environment analysis, a total of 19 QTLs were identified with majority being small to moderate effect QTLs. Among these, *qCPH8.1* governing plant height showed remarkably high PVE, both through individual and combined environment analysis. Of the QTLs identified under heat stress for grain yield and spikelet fertility, only five QTLs, i.e., *qHTSF7.1*, *qSTISF10.1*, *qSTIGY1.1*, *qSTIGY1.2* and *qSTIGY8.1* validated previously reported QTLs making the majority rest novel. Furthermore, the physical locations of these QTLs were compared with the results of GWAS of which *qSTIGY1.1*, *qSTIGY1.2*, *qHTSF5.1* and *qHTSF7.1* coincided with QTNs identified through GWAS, thus validating them.

Thus, several novel QTLs were identified through present study governing tolerance to RSHS in NL44, some of which validated the MTAs identified through GWAS. Promising RILs have been identified which can serve as improved donors for introgression of these QTLs into elite varieties. These QTLs can be further fine-mapped in future studies to pinpoint candidate genes encoding tolerance to RSHS in NL44. New heat stress tolerant genotypes were also identified which needs further confirmation and detailed characterization in future studies.

Abstract

Rice, the important staple crop of India, is highly sensitive to heat stress, primarily at anthesis and gametogenesis stages. The frequency and intensity of heat wave episodes are increasing every year, which is causing huge financial losses to farmers. Evolving heat stress resilient rice varieties is the only economically viable option available for managing these supra optimal temperatures. Screening of germplasm for donors of heat stress tolerance, followed by mapping of genomic regions governing tolerance, their validation and introgression through marker assisted selection into popular varieties are important steps in this direction. Thus, the present study was aimed to explore the heat stress tolerance potential of rice germplasm available at Rice Genetics, Division of Genetics, ICAR-IARI along with the identification and validation of genomic regions governing tolerance to reproductive stage heat stress (RSHS) in a NERicA rice genotype, NL44, which was recently reported to show heat stress tolerance almost at par with N22, a well-known heat stress tolerant genotype. To assess the variability present in the rice germplasm for tolerance to RSHS, a set of 192 diverse rice genotypes were assessed for their tolerance to RSHS under natural field conditions using two staggered sowings (one month apart) at Aduthurai during off-season, 2018-19 and another set of 35 Basmati genotypes were also assessed under heat stress at the same location, for two off-seasons, 2018-19 and 2019-2020, respectively. Significant reduction was recorded for grain yield and spikelet fertility under RSHS in both these experiments. Based on *per se* performance and STI, Bhuban, PRR127, DV85 and Improved Samba Mahsuri were found promising from the 192 genotypes. Though Basmati 370, Type 23, Basmati Jamuna were found relatively better among the 35 Basmati genotypes, significant loss in yield was observed under RSHS in most of the Basmati genotypes evaluated reflecting the heat stress vulnerability of Basmati rice cultivars. GWAS with SNP genotype data generated (using 50K SNP array) for 192 genotypes identified seven and three QTNs for grain yield (under RSHS and its STI) through FarmCPU and BLINK, respectively, of which *qHTGY8.1* was found through both the models. Eight QTNs were found using FarmCPU and seven each through MLM and BLINK for spikelet fertility (under RSHS and its STI), of which *qHTSF5.1* was shared by the three models. Furthermore, barring two QTNs for grain yield and five QTNs for spikelet fertility, rest of them were found to be novel. Based on phenotype and number of favourable alleles of QTN, RIL 10, Selected Sabarmati, Sitwa Dhan, Bhadrakali, and Samba Mahsuri were identified as candidates for future characterization of RSHS. Significant reduction

was observed under heat stress for grain yield plant⁻¹ (GYPP) and spikelet fertility (SF) for sensitive parent PB1 and some RILs (out of 127) generated from PB1/NL44 assessed for two off-seasons (2018-19 and 2019-20) and two locations (Aduthurai and Cuttack), both showing near normal distributions. GGE biplot based multi environment analysis revealed the RILs, RIL49, RIL52, RIL56, RIL57, RIL59, RIL87, RIL116, RIL119, RIL123, and RIL126 were ideal. For QTL mapping of genomic regions governing tolerance to RSHS in NL44 genotyping of RILs was done using 94 SSR markers and later with 10,924 polymorphic SNP markers from a high-density SNP array with 60,026 markers and a linkage map of 1568.64 cM, and 2977 cM, was generated respectively. Through individual environment analysis, nine QTLs were identified through inclusive composite interval mapping, two under control, four under RSHS and three for stress indices. PVE values ranged from 5.1% (*qHTGY2.1* governing grain yield under heat stress) to 13.6% (*qCSF10.1* controlling spikelet fertility under control). Combined environment analysis revealed 12 QTLs, four under control, four under RSHS and four for stress indices. As in individual environment analysis, most of them are moderate effect QTLs with PVE varying between 1.41% (*qSTIGY8.1*) to 8.26% (*qHTSF7.1*). Similarly, 19 QTLs were also identified for other yield contributing traits, of which *qCPH8.1* governing plant height showed exceptionally high PVE both through individual and combined environment analysis. Of the QTLs identified under heat stress for grain yield and spikelet fertility, only five QTLs, i.e., *qHTSF7.1*, *qSTISF10.1*, *qSTIGY1.1*, *qSTIGY1.2* and *qSTIGY8.1* co-localized with previously reported QTLs making the remaining QTLs novel. Furthermore, the physical locations of these QTLs were compared with the results of GWAS of which *qSTIGY1.1*, *qSTIGY1.2*, *qHTSF5.1* and *qHTSF7.1* matched with QTNs identified through GWAS, thus validating them. These QTLs are valuable candidates for fine mapping and candidate gene identification in further studies. Additionally, a set of 20 F₆ families derived from PB1509/NL44 were also assessed for their tolerance to RSHS and genotyped using SSRs near the physical positions of putative QTLs. However, these SSR markers could not validate these QTLs warranting a systematic SNP based genotyping in future.

धान, भारत की एक महत्वपूर्ण प्रधान फसल है, जो मुख्य रूप से एंथेसिस एवं गैमेटोजेनेसिस चरणों में ताप तनाव के प्रति संवेदनशील है। ताप की लहरों की आवृत्ति और तीव्रता हर वर्ष बढ़ रही है जिससे किसानों को भारी वित्तीय नुकसान हो रहा है। इस उच्च तापमान के प्रबंधन के लिए ताप सहनशील किस्मों का विकास करना ही एकमात्र विकल्प है। इस दिशा में ताप सहनशील किस्मों की पहचान के लिये जर्मप्लाज्म की परीक्षण, सहनशीलता के उत्तरदायी जीनोमिक क्षेत्रों की पहचान, और उनका लोकप्रिय किस्मों में स्थानान्तरण करना सहायनीय कदम हैं। इसलिए वर्तमान अध्ययन का उद्देश्य, एक NERicA लाइन एनएल44 में ताप तनाव सहनशीलता को नियंत्रित करने वाले जीनोमिक क्षेत्रों की पहचान और मान्यकरण के साथ-साथ अनुवांशिकी संभाग आईसीएआर-आईएआरआई में उपलब्ध धान के जर्मप्लाज्म की ताप तनाव सहनशीलता का पता लगाना था। एनएल44 और एन22, जो एक प्रसिद्ध ताप तनाव सहनशील जीनोटाइप है, में लगभग बराबर सहनशीलता दिखाई दी। प्रजनन अवस्था पर धान के जर्मप्लाज्म में मौजूद ताप तनाव सहनशीलता का आकलन करने के लिए दो प्रयोग किए गए - एक प्रयोग में 192 धान जीनोटाइप के एक सेट का ताप तनाव सहनशीलता का मूल्यांकन स्थानीय क्षेत्र की स्थिति में किया गया, जिसमें 2018-19 के दौरान आडुतुरई में दो बुवाई (एक महीने के अंतराल में) का उपयोग किया गया। दूसरा प्रयोग में 35 बासमती जीनोटाइप के एक सेट का मूल्यांकन भी उसी स्थान पर ताप तनाव के तहत दो 2018-19 और 2019-20 में किया गया। ताप तनाव (दूसरी बुवाई) के तहत उपज और बाली की उर्वरता दोनों प्रयोगों में कमी पायी गयी। अपने प्रदर्शन एवं एसटीआई के आधार पर भूबन, पीआरआर127, डीवी85 और इम्पूव्ड सांबा मशुरी आशाजनक पाया गया। हालांकि बासमती 370, टाइप 23, बासमती जमुना को अपेक्षाकृत बेहतर पाया गया, मूल्यांकन किए गए अधिकतर बासमती जीनोटाइपस में ताप तनाव ट्रीटमेंट में कोई आर्थिक उपज नहीं देखी गई। इन 192 जीनोटाइप के लिए उत्पन्न एसएनपी जीनोटाइप डेटा (60के एसएनपी सरणी का उपयोग करके) का उपयोग कर धान में ताप तनाव सहनशीलता के लिए जीनोम वाइड एसोसिएशन मैपिंग किया गया। फार्मसीपीयू और ब्लिंक के माध्यम से अनाज की उपज (तनाव और इसके एसटीआई के तहत) के लिए सात और तीन क्यूटीएन की पहचान की गई, जिनमें से क्यूएचटीजीवाई8.1 दोनों मॉडलों के माध्यम से पाया गया। बाली उर्वरता (तनाव और इसके एसटीआई के तहत) के लिए आठ क्यूटीएन को फार्मसीपीयू के द्वारा, एमएलएम और ब्लिंक के माध्यम से सात-सात क्यूटीएन पाया गया जिनमें से क्यूएचटीएसएफ5.1 को तीन मॉडलों द्वारा साझा किया गया। इसके अलावा उपज के दो क्यूटीएन और बाली उर्वरता के पांच क्यूटीएन को छोड़कर बाकी क्यूटीएन नए पाए गए। जीनोटाइप और क्यूटीएन के सकारात्मक एलील की संख्या के आधार पर आरआईएल10, चयनित साबरमती, सितवा धन, भद्रकाली और सांभा मसूरी को भविष्य के विलक्षण वर्णन हेतु चुना गया। दो बे-मौसमों (2018-19 और 2019-20) और दो स्थान (अदुथुराई और कटक) में मूल्यांकन किए गए पीबी1/एनएल44 से उत्पन्न 127 आरआईएल में से कुछ आरआईएलस और संवेदनशील पेरेंट पीबी1 के लिए अनाज उपज और चोटी बाली उर्वरता में ताप तनाव के तहत सहायनीय कमी

देखी गई। दोनों लक्षण नॉर्मल डिस्ट्रिब्यूशन के करीब दिखे। जीजीई बाइप्लॉट आधारित बहु पर्यावरण विश्लेषण से आरआईएल 49, 52, 56, 57, 59, 87, 116, 119, 123, 126 आदर्श पाए गए। एनएल44 में ताप तनाव के प्रति सहिष्णुता को नियंत्रित करने वाले जीनोमिक क्षेत्रों की क्यूटीएल मैपिंग के लिए 94 एसएसआर चिह्नको और बाद में हाई डेन्सिटी (60,026) चिह्नोंके एसएनपी चिप के 10,924 पॉलीमॉर्फिक एसएनपी चिह्नोंसे से आरआईएल की जीनोटाइपिंग करके 1568.64 सीएम और 2977 सीएम के लिंकेज मैप का निर्माण किया गया। एकल पर्यावरण विश्लेषण (आईसीआईएम का उपयोग करके) के माध्यम से, नौ क्यूटीएल की पहचान की गई, जिनमें से दो नियंत्रण में, चार ताप तनाव के तहत और तीन तनाव सूचकांकों के लिए पाया गया। पीवीई मान 5.1% (क्यूहेचटीजीवै2.1 ताप तनाव के तहत अनाज की उपज को नियंत्रित करने वाले) से लेकर 13.6% (क्यूसीयसएफ10.1 नियंत्रण में बाली की उर्वरता को नियंत्रित करने) तक था। संयुक्त पर्यावरण विश्लेषण से 12 क्यूटीएल का पता चला, चार नियंत्रण में, चार ताप तनाव के तहत और चार तनाव सूचकों के लिए। एकल पर्यावरण विश्लेषण के तरह, उनमें से अधिकतर मध्यम प्रभाव वाले क्यूटीएल थे जिनका पीवीई 1.41% (क्यूहेचटीजीवै8.1) से 8.26% (क्यूहेचटीयसएफ7.1) के बीच पाया गया। इसी तरह, 19 क्यूटीएल को अन्य उपज योगदान लक्षणों के लिए भी पहचाना गया था, जिनमें से पौधे की ऊंचाई नियंत्रित करने वाली क्यूसीपीएच8.1 ने एकल और संयुक्त पर्यावरण विश्लेषण दोनों के माध्यम से उच्च पीवीई दिखाया। अनाज की उपज और चोटी बाली उर्वरता के लिए ताप तनाव के तहत पहचाने गए क्यूटीएल में से, केवल पांच क्यूटीएल, यानी, क्यूएचटीएसएफ7.1, क्यूएसटीआईएसएफ10.1, क्यूएसटीआईजीवै1.1, क्यूएसटीआईजीवै1.2 और क्यूएसटीआईजीवै8.1 पहले प्रस्थाप किए गए क्यूटीएल के स्थान से मिला जिससे शेष क्यूटीएल को नया माना जा सकता है। इसके अलावा, इन क्यूटीएल के भौतिक स्थानों की तुलना जीडब्ल्यूएस के परिणामों से की गई, जिनमें से क्यूएसटीजीवै1.1, क्यूएसटीआईजीवै1.2, क्यूएचटीएसएफ5.1 और क्यूएचटीएसएफ7.1 जीडब्ल्यूएस के माध्यम से पहचाने गए क्यूटीएल के साथ मिलान किया, जिससे उन्हें मान्य किया गया। ये क्यूटीएल आगे के अध्ययनों में फाइन मैपिंग और कैंडीडेट जीन पहचान के लिए मूल्यवान हैं। इसके अतिरिक्त, पीबी1509/एनएल44 से प्राप्त 20 एफ₆ परिवारों के एक सेट का भी ताप तनाव के प्रति उनकी सहनशीलता के लिए मूल्यांकन किया गया था और क्यूटीएल की भौतिक स्थिति के पास यसयसआरस् का उपयोग करके जीनोटाइपिंग किया गया। हालाँकि, ये यसयसआर मार्कर क्यूटीएल को मान्य नहीं कर सके, भविष्य में यसएनपी आधारित जीनोटाइपिंग की आवश्यकता है।

References

- Abayawickrama, A.S.M.T., Reinke, R.F., Fitzgerald, M.A., Harper, J.D. and Burrows, G.E., 2017. Influence of high daytime temperature during the grain filling stage on fissure formation in rice. *Journal of Cereal Science*, 74, pp.256-262.
- Aghamolki, M.T.K., Yusop, M.K., Oad, F.C., Zakikhani, H., Jaafar, H.Z., Kharidah, S. and Musa, M.H., 2014. Heat stress effects on yield parameters of selected rice cultivars at reproductive growth stages. *Journal of Food, Agriculture and Environment*, 12(2), pp.741-746.
- Ahmed, N., Tetlow, I.J., Nawaz, S., Iqbal, A., Mubin, M., Nawaz ul Rehman, M.S., Butt, A., Lightfoot, D.A. and Maekawa, M., 2015. Effect of high temperature on grain filling period, yield, amylose content and activity of starch biosynthesis enzymes in endosperm of basmati rice. *Journal of the Science of Food and Agriculture*, 95(11), pp.2237-2243.
- Alexieva, V., Sergiev, I., Mapelli, S. and Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell & Environment*, 24(12), pp.1337-1344.
- Ali, F., Waters, D.L., Ovenden, B., Bundock, P., Raymond, C.A. and Rose, T.J., 2019. Australian rice varieties vary in grain yield response to heat stress during reproductive and grain filling stages. *Journal of Agronomy and Crop Science*, 205(2), pp.179-187.
- Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R. and Mohanty, P., 2008. Heat stress: an overview of molecular responses in photosynthesis. *Photosynthesis Research*, 98(1), pp.541-550.
- Asaoka, M., Okuno, K. and Fuwa, H., 1985. Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). *Agricultural and Biological Chemistry*, 49(2), pp.373-379.
- Asaoka, M., Okuno, K., Hara, K., Oba, M. and Fuwa, H., 1989. Effects of environmental temperature at the early developmental stage of seeds on the characteristics of endosperm starches of rice (*Oryza sativa* L.). *Journal of the Japanese Society of Starch Science*, 36(1), pp.1-8.
- Asaoka, M., Okuno, K., Sugimoto, Y., Kawakami, J. and Fuwa, H., 1984. Effect of environmental temperature during development of rice plants on some properties of endosperm starch. *Starch*, 36(6), pp.189-193.
- Ayeneh, A., Van Ginkel, M., Reynolds, M.P. and Ammar, K., 2002. Comparison of leaf, spike, peduncle and canopy temperature depression in wheat under heat stress. *Field Crops Research*, 79(2-3), pp.173-184.

- Bahuguna, R.N., Jha, J., Pal, M., Shah, D., Lawas, L.M., Khetarpal, S. and Jagadish, K.S., 2015. Physiological and biochemical characterization of NERICA-L-44: a novel source of heat tolerance at the vegetative and reproductive stages in rice. *Physiologia Plantarum*, 154(4), pp.543-559.
- Baker, J.T., 2004. Yield responses of southern US rice cultivars to CO₂ and temperature. *Agricultural and Forest Meteorology*, 122(3-4), pp.129-137.
- Baker, J.T., Allen Jr, L.H. and Boote, K.J., 1992. Temperature effects on rice at elevated CO₂ concentration. *Journal of Experimental Botany*, 43(7), pp.959-964.
- Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239(1), pp.70-76.
- Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. and Atlin, G., 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Science*, 47(2), pp.507-516.
- Bheemanahalli, R., Sathishraj, R., Tack, J., Nalley, L.L., Muthurajan, R. and Jagadish, K.S., 2016. Temperature thresholds for spikelet sterility and associated warming impacts for sub-tropical rice. *Agricultural and Forest Meteorology*, 221, pp.122-130.
- Bollinedi, H., Yadav, A.K., Vinod, K.K., Gopala Krishnan, S., Bhowmick, P.K., Nagarajan, M., Neeraja, C.N., Ellur, R.K. and Singh, A.K., 2020. Genome-wide association study reveals novel marker-trait associations (MTAs) governing the localization of Fe and Zn in the rice grain. *Frontiers in Genetics*, 11, pp.213.
- Bonilla, P., Mackell, D., Deal, K. and Gregorio, G., 2002. RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philippine Agricultural Scientist (Philippines)*, 85, pp.68-76.
- Buu, B.C., Ha, P.T.T., Tam, B.P., Nhien, T.T., Van Hieu, N., Phuoc, N.T., Giang, L.H. and Lang, N.T., 2014. Quantitative trait loci associated with heat tolerance in rice (*Oryza sativa* L.). *Plant Breeding and Biotechnology*, 2(1), pp.14-24.
- Čajánek, M., Štroch, M., Lachetova, I., Kalina, J. and Spunda, V., 1998. Characterization of the photosystem II inactivation of heat-stressed barley leaves as monitored by the various parameters of chlorophyll a fluorescence and delayed fluorescence. *Journal of Photochemistry and Photobiology B: Biology*, 47(1), pp.39-45.
- Cao, L., Zhao, J., Zhan, X., Li, D., He, L. and Cheng, S., 2003. Mapping QTLs for heat tolerance and correlation between heat tolerance and photosynthetic rate in rice. *Chinese Journal of Rice Science*, 17, 223-227.

- Cao, L.Y., Zhu, J., Zhao, S.T., He, L.B. and Yan, Q.C., 2002. Mapping QTLs for heat tolerance in a DH population from *indica-japonica* cross rice (*Oryza sativa*). *Journal of Agricultural Biotechnology*, 10, pp.210-214. (In Chinese)
- Cao, Y.Y., Duan, H., Yang, L.N., Wang, Z.Q., Zhuo, S.C. and Yang, J.C., 2008. Effective heat stress during meiosis on grain yield of rice cultivars differing in heat tolerance and its physiological mechanism. *Acta Agronomica Sinica*, 34, pp.2134-2142.
- Cao, Z., Li, Y., Tang, H., Zeng, B., Tang, X., Long, Q., Wu, X., Cai, Y., Yuan, L. and Wan, J., 2020. Fine mapping of the *qHTB1-1*QTL, which confers heat tolerance at the booting stage, using an *Oryza rufipogon* Griff. introgression line. *Theoretical and Applied Genetics*, 133(4), pp.1161-1175.
- Cao, Z., Xie, H., Nie, Y., Mao, L., Li, Y. and Cai, Y., 2015. Mapping a QTL (*qHTH5*) for heat tolerance at the heading stage on rice chromosome 5 and its genetic effect analysis. *Chinese Journal of Rice Science*, 29(2), pp.119-125.
- Ceccarelli, S., Grando, S., Maatougui, M., Michael, M., Slash, M., Haghparast, R., Rahmanian, M., Taheri, A., Al-Yassin, A., Benbelkacem, A. and Labdi, M., 2010. Plant breeding and climate changes. *The Journal of Agricultural Science*, 148(6), pp.627-637.
- Cen, Y.P. and Sage, R.F., 2005. The regulation of Rubisco activity in response to variation in temperature and atmospheric CO₂ partial pressure in sweet potato. *Plant Physiology*, 139(2), pp.979-990.
- Chang-lan, Z., Ying-hui, X., Chun-ming, W., Ling, J., Hu-Qu, Z. and Jian-min, W., 2005. Mapping QTL for heat-tolerance at grain filling stage in rice. *Rice Science*, 12(1), pp.33.
- Chang-Lan, Z., Ying-Hui, X., Chun-Ming, W., Ling, J., Qu, Z.H. and Min, W.J. 2005. Mapping QTL for heat-tolerance at grain filling stage in rice. *Rice Science*, 12, pp.33–38.
- Chaturvedi, A.K., Bahuguna, R.N., Shah, D., Pal, M. and Jagadish, S.V., 2017. High temperature stress during flowering and grain filling offsets beneficial impact of elevated CO₂ on assimilate partitioning and sink-strength in rice. *Scientific Reports*, 7(1), pp.1-13.
- Chauhan, H., Khurana, N., Agarwal, P. and Khurana, P., 2011. Heat shock factors in rice (*Oryza sativa* L.): genome-wide expression analysis during reproductive development and abiotic stress. *Molecular Genetics and Genomics*, 286(2), pp.171-187.
- Cheabu, S., Moung-Ngam, P., Arikrit, S., Vanavichit, A. and Malumpong, C., 2018. Effects of heat stress at vegetative and reproductive stages on spikelet fertility. *Rice Science*, 25(4), pp.218-226.

- Cheabu, S., Panichawong, N., Rattanamettha, P., Wasuri, B., Kasemsap, P., Arikitt, S., Vanavichit, A. and Malumpong, C., 2019. Screening for spikelet fertility and validation of heat tolerance in a large rice mutant population. *Rice Science*, 26(4), pp.229-238.
- Chen, E., Huang, X., Tian, Z., Wing, R.A. and Han, B., 2019. The genomics of *Oryza* species provides insights into rice domestication and heterosis. *Annual Review of Plant Biology*, 70, pp.639-665.
- Chen, J.H., Chen, S.T., He, N.Y., Wang, Q.L., Zhao, Y., Gao, W. and Guo, F.Q., 2020. Nuclear-encoded synthesis of the D1 subunit of photosystem II increases photosynthetic efficiency and crop yield. *Nature Plants*, 6(5), pp.570-580.
- Chen, L., Wang, Q., Tang, M., Zhang, X., Pan, Y., Yang, X., Gao, G., Lv, R., Tao, W., Jiang, L. and Liang, T., 2021. QTL mapping and identification of candidate genes for heat tolerance at the flowering stage in rice. *Frontiers in Genetics*, 11, pp.621871.
- Chen, W., Gao, Y., Xie, W., Gong, L., Lu, K., Wang, W., Li, Y., Liu, X., Zhang, H., Dong, H. and Zhang, W., 2014a. Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. *Nature genetics*, 46(7), pp.714-721.
- Chen, X., Lin, S., Liu, Q., Huang, J., Zhang, W., Lin, J., Wang, Y., Ke, Y. and He, H., 2014b. Expression and interaction of small heat shock proteins (sHsps) in rice in response to heat stress. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1844(4), pp.818-828.
- Cheng, F., Zhong, L., Zhao, N., Liu, Y. and Zhang, G., 2005b. Temperature induced changes in the starch components and biosynthetic enzymes of two rice varieties. *Plant Growth Regulation*, 46(1), pp.87-95.
- Cheng, F.M., Ding, Y.S. and Zhu, B.Y., 2000. The formation of amylose content in rice grain and its relation with field temperature. *Acta Ecologica Sinica*, 20(4), pp.646-652.
- Cheng, F.M., Zhong, L.J., Wang, F. and Zhang, G.P., 2005a. Differences in cooking and eating properties between chalky and translucent parts in rice grains. *Food Chemistry*, 90(1-2), pp.39-46.
- Cheng, L., Wang, J., Uzokwe, V., Meng, L., Wang, Y., Sun, Y., Zhu, L., Xu, J. and Li, Z., 2012. Genetic analysis of cold tolerance at seedling stage and heat tolerance at anthesis in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, 11, pp.359-367.
- Cheng, L.-R., Wang, J.-M., Uzokwe, V., Meng, L.-J., Wang, Y., Sun, Y., Zhu, L.-H., Xu, J.-L. and Li, Z.-K., 2012. Genetic analysis of cold tolerance at seedling stage and

- heat tolerance at anthesis in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, 11(3), pp.359-367.
- Chiba, M. and Terao, T., 2014. Open-top chambers with solar-heated air introduction tunnels for the high-temperature treatment of paddy fields. *Plant Production Science*, 17(2), pp.152-165.
- Chidambaranathan, P., Balasubramaniasai, C., Behura, N., Purty, M., Samantaray, S., Subudhi, H., Ngangkham, U., Devanna, B.N., Katara, J.L., Kumar, A. and Behera, L., 2021. Effects of high temperature on spikelet sterility in rice (*Oryza sativa* L.): association between molecular markers and allelic phenotypic effect in field condition. *Genetic Resources and Crop Evolution*, 68(5), pp.1923-1935.
- Chuan, Y.A.N., Ding, Y.F., Liu, Z.H., Wang, Q.S., Li, G.H., Ying, H.E. and Wang, S.H., 2008. Temperature difference between the air and organs of rice plant and its relation to spikelet fertility. *Agricultural Sciences in China*, 7(6), pp.678-685.
- Chun, A., Lee, H.J., Hamaker, B.R. and Janaswamy, S., 2015. Effects of ripening temperature on starch structure and gelatinization, pasting, and cooking properties in rice (*Oryza sativa*). *Journal of Agricultural and Food Chemistry*, 63(12), pp.3085-3093.
- Coast, O., Murdoch, A.J., Ellis, R.H., Hay, F.R. and Jagadish, K.S., 2016. Resilience of rice (*Oryza* spp.) pollen germination and tube growth to temperature stress. *Plant, cell & environment*, 39(1), pp.26-37.
- Cobb, J.N., Biswas, P.S. and Platten, J.D., 2019. Back to the future: revisiting MAS as a tool for modern plant breeding. *Theoretical and Applied Genetics*, 132(3), pp.647-667.
- Cullis, B.R., Smith, A.B. and Coombes, N.E., 2006. On the design of early generation variety trials with correlated data. *Journal of Agricultural, Biological, and Environmental Statistics*, 11(4), pp.381-393.
- Das, S., Krishnan, P., Nayak, M. and Ramakrishnan, B., 2014. High temperature stress effects on pollens of rice (*Oryza sativa* L.) genotypes. *Environmental and Experimental Botany*, 101, pp.36-46.
- Daware, A., Malik, A., Srivastava, R., Das, D., Ellur, R.K., Singh, A.K., Tyagi, A.K. and Parida, S.K., 2022. Rice Pan-genome Array (RPGA): an efficient genotyping solution for pan-genome-based accelerated crop improvement in rice. *The Plant Journal*. <https://doi.org/10.1111/tpj.16028>
- de Brito, G.G., de Moraes, I.L., Moura, D.S., Fagundes, P.R.R., Campos, A.D., Andres, A., Parfitt, J., Panazzo, L. and Deuner, S., 2019. Non-invasive physiological approaches for plant phenotyping: rice responses to heat stress. *Journal of Agricultural Science*, 11, pp.453 – 464.

- Deng, Y., Tian, X., Wu, C., Matsui, T. and Xiao, B., 2010. Early signs of heat stress-induced abnormal development of anther in rice. *Chinese Journal of Eco-Agriculture*, 18(2), pp.377-383.
- Devate, N.B., Krishna, H., Sunilkumar, V.P., Manjunath, K.K., Mishra, C.N., Jain, N., Singh, G.P. and Singh, P.K., 2022. Identification of genomic regions of wheat associated with grain Fe and Zn content under drought and heat stress using genome-wide association study. *Frontiers in Genetics*, 13, pp.1034947.
- Endo, M., Tsuchiya, T., Hamada, K., Kawamura, S., Yano, K., Ohshima, M., Higashitani, A., Watanabe, M. and Kawagishi-Kobayashi, M., 2009. High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology*, 50(11), pp.1911-1922.
- Ezin, V., Ahanchede, W.W., Ayenan, M.A.T. and Ahanchede, A., 2022. Physiological and agronomical evaluation of elite rice varieties for adaptation to heat stress. *BMC Plant Biology*, 22(1), pp.1-14.
- Falconer, D.S. and Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*. 4th Edition, Addison Wesley Longman, Harlow.
- Fan, X., Li, Y., Zhu, Y., Wang, J., Zhao, J., Sun, X., Pan, Y., Bian, X., Zhang, C., Zhao, D. and Liu, Q., 2020. Characterization of physicochemical qualities and starch structures of two indica rice varieties tolerant to high temperature during grain filling. *Journal of Cereal Science*, 93, pp.102966.
- Fang, C., Dou, L., Liu, Y., Yu, J. and Tu, J., 2018. Heat stress-responsive transcriptome analysis in heat susceptible and tolerant rice by high-throughput sequencing. *Ecological Genetics and Genomics*, 6, pp.33-40.
- FAOSTAT., 2014. Food and Agriculture Organization of the United Nations. <http://faostat.fao.org/site/567/default.aspx#ancor>
- Feng, B., Zhang, C., Chen, T., Zhang, X., Tao, L. and Fu, G., 2018. Salicylic acid reverses pollen abortion of rice caused by heat stress. *BMC Plant Biology*, 18(1), pp.1-16.
- Feng, W., Fangmin, C. and Yi, L., 2006. Dynamic changes of plant hormones in developing grains at rice filling stage under different temperatures. *Acta Agronomica Sinica*, 32(1), 25-29.
- Fitzgerald, M.A. and Resurreccion, A.P., 2009. Maintaining the yield of edible rice in a warming world. *Functional Plant Biology*, 36(12), pp.1037-1045.
- Fu, G., Feng, B., Zhang, C., Yang, Y., Yang, X., Chen, T., Zhao, X., Zhang, X., Jin, Q. and Tao, L., 2016. Heat stress is more damaging to superior spikelets than inferiors of rice (*Oryza sativa* L.) due to their different organ temperatures. *Frontiers in Plant Science*, 7, p.1637.
- Fu, G., Zhang, C., Yang, X., Yang, Y., Chen, T., Zhao, X., Fu, W., Feng, B., Zhang, X., Tao, L. and Jin, Q., 2015. Action mechanism by which SA alleviates high

- temperature-induced inhibition to spikelet differentiation. *Chinese Journal of Rice Science*, 29(6), pp.637-647.
- Fukuoka, M., Yoshimoto, M. and Hasegawa, T., 2012. Varietal range in transpiration conductance of flowering rice panicle and its impact on panicle temperature. *Plant Production Science*, 15(4), pp.258-264.
- Funaba, M., Ishibashi, Y., Molla, A.H., Iwanami, K. and Iwaya-Inoue, M., 2006. Influence of low/high temperature on water status in developing and maturing rice grains. *Plant Production Science*, 9(4), pp.347-354.
- Glaubitz, U., Erban, A., Kopka, J., Hinch, D.K. and Zuther, E., 2015. High night temperature strongly impacts TCA cycle, amino acid and polyamine biosynthetic pathways in rice in a sensitivity-dependent manner. *Journal of Experimental Botany*, 66(20), pp.6385-6397.
- GoI (2021). *Agricultural statistics at a glance 2021*. Ministry of Agriculture and Farmers Welfare, Department of Agriculture and Farmers Welfare, Directorate of Economics and Statistics, pp.50.
- González-Schain, N., Dreni, L., Lawas, L.M., Galbiati, M., Colombo, L., Heuer, S., Jagadish, K.S. and Kater, M.M., 2016. Genome-wide transcriptome analysis during anthesis reveals new insights into the molecular basis of heat stress responses in tolerant and sensitive rice varieties. *Plant and Cell Physiology*, 57(1), pp.57-68.
- Gourdji, S.M., Sibley, A.M. and Lobell, D.B., 2013. Global crop exposure to critical high temperatures in the reproductive period: historical trends and future projections. *Environmental Research Letters*, 8(2), pp.024041.
- Guan-fu, F., Cai-xia, Z., Yong-jie, Y., Jie, X., Xue-qin, Y., Xiu-fu, Z., Qian-yu, J. and Long-xing, T., 2015. Male parent plays more important role in heat tolerance in three-line hybrid rice. *Rice Science*, 22(3), pp.116-122.
- Guo, J., Shi, W., Guo, J., Yue, L., Zhuang, L., Zhang, W., Tian, X., Xu, Y. and Zhou, M., 2020. Genome-Wide association studies on heat stress tolerance during grain development in wheat (*Triticum aestivum* L.). *Research Square* [Preprint]. doi:10.21203/rs.2.20460/v1
- Guo, J., Wu, J., Ji, Q., Wang, C., Luo, L., Yuan, Y., Wang, Y. and Wang, J., 2008. Genome-wide analysis of heat shock transcription factor families in rice and Arabidopsis. *Journal of Genetics and Genomics*, 35(2), pp.105-118.
- Hakata, M., Kuroda, M., Miyashita, T., Yamaguchi, T., Kojima, M., Sakakibara, H., Mitsui, T. and Yamakawa, H., 2012. Suppression of α -amylase genes improves quality of rice grain ripened under high temperature. *Plant Biotechnology Journal*, 10(9), pp.1110-1117.

- Hakata, M., Wada, H., Masumoto-Kubo, C., Tanaka, R., Sato, H. and Morita, S., 2017. Development of a new heat tolerance assay system for rice spikelet sterility. *Plant Methods*, 13(1), pp.1-8.
- Hasegawa, T., Ishimaru, T., Kondo, M., Kuwagata, T., Yoshimoto, M. and Fukuoka, M., 2011. Spikelet sterility of rice observed in the record hot summer of 2007 and the factors associated with its variation. *Journal of Agricultural Meteorology*, 67(4), pp.225-232.
- Hasegawa, T., Kuwagata, T., Nishimori, M., Ishigooka, Y., Murakami, M., Yoshimoto, M., Kondo, M., Ishimaru, T., Sawano, S., Masaki, Y. and Matsuzaki, H., 2009. Recent warming trends and rice growth and yield in Japan. In *MARCO Symposium on Crop Production under Heat Stress: Monitoring, Impact Assessment and Adaptation*. National Institute for Agro-Environmental Studies, Tsukuba, Japan, pp.44-51.
- Hermann, R.D. and Gabriel, G.V., 2013. Response of rice plants to heat stress during initiation of panicle primordia or grain-filling phases. *Journal of Stress Physiology & Biochemistry*, 9(3), pp.318-325.
- Hirabayashi, H., Sasaki, K., Kambe, T., Gannaban, R.B., Miras, M.A., Mendiolo, M.S., Simon, E.V., Lumanglas, P.D., Fujita, D., Takemoto-Kuno, Y. and Takeuchi, Y., 2015. *qEMF3*, a novel QTL for the early-morning flowering trait from wild rice, *Oryza officinalis*, to mitigate heat stress damage at flowering in rice, *O. sativa*. *Journal of Experimental Botany*, 66(5), pp.1227-1236.
- Hori, K., Kataoka, T., Miura, K., Yamaguchi, M., Saka, N., Nakahara, T., Sunohara, Y., Ebana, K. and Yano, M., 2012. Variation in heading date conceals quantitative trait loci for other traits of importance in breeding selection of rice. *Breeding science*, 62(3), pp.223-234.
- Hossain, M., 2021. As rising 'heat shocks' ruin rice crops, bangladesh faces hunger risk. Thomson Reuters Foundation News, Thomson Reuters Foundation. <https://news.trust.org/item/20210528065131-wnlhg/> (Accessed October, 2022).
- Hsuan, T.P., Jhuang, P.R., Wu, W.C. and Lur, H.S., 2019. Thermotolerance evaluation of Taiwan Japonica type rice cultivars at the seedling stage. *Botanical Studies*, 60(1), pp.1-18.
- Hu, Q., Wang, W., Lu, Q., Huang, J., Peng, S. and Cui, K., 2021. Abnormal anther development leads to lower spikelet fertility in rice (*Oryza sativa* L.) under high temperature during the panicle initiation stage. *BMC Plant Biology*, 21(1), pp.1-17.
- Hu, S., Zhang, Y., Zhu, D., Lin, X. and Xiang, J., 2012. Evaluation of heat resistance in hybrid rice. *Chinese Journal of Rice Science*, 26(6), pp.751-756. (in Chinese with English abstract).

- Hu, W., Hu, G. and Han, B., 2009. Genome-wide survey and expression profiling of heat shock proteins and heat shock factors revealed overlapped and stress specific response under abiotic stresses in rice. *Plant Science*, 176(4), pp.583-590.
- Huang, L., Sun, Y., Peng, S. and Wang, F., 2016. Genotypic differences of japonica rice responding to high temperature in China. *Agronomy Journal*, 108(2), pp.626-636.
- Iida, Y., Yokota, K., Kirihaara, T. and Suga, R., 2002. Comparison of the occurrence of kernel damage in rice plants grown in a heated greenhouse and in a paddy field of high temperature year. *Japanese Journal of Crop Science*, 71(2), pp.174-177.
- Im, E.S., Pal, J.S. and Eltahir, E.A., 2017. Deadly heat waves projected in the densely populated agricultural regions of South Asia. *Science Advances*, 3(8), pp.e1603322.
- Inouchi, N., Ando, H., Asaoka, M., Okuno, K. and Fuwa, H., 2000. The effect of environmental temperature on distribution of unit chains of rice amylopectin. *Starch*, 52(1), pp.8-12.
- IPCC, 2007. Summary for Policymakers. In: Solomon, S.; Qin, D.; Manning, M.; Chen, Z.; Marquis, M.; Averyt, K.B.; Tignor, M.; Miller, H.L. (eds) *Climate change 2007: The Physical Science Basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, pp.1–18.
- IPCC, Working Group I Contribution to the IPCC Fifth Assessment Report Climate Change 2013: The Physical Science Basis, Summary for Policymakers. 2013, www.Climatechange2013.org/images/uploads/WGIAR5-SPMAApproved27Sep2013.pdf.
- IRRI, 2013. Standard Evaluation System (SES) for Rice, 3rd ed. International Rice Research Institute, Manila, Philippines.
- Ishimaru, T., Hirabayashi, H., Ida, M., Takai, T., San-Oh, Y.A., Yoshinaga, S., Ando, I., Ogawa, T. and Kondo, M., 2010. A genetic resource for early-morning flowering trait of wild rice *Oryza officinalis* to mitigate high temperature-induced spikelet sterility at anthesis. *Annals of Botany*, 106(3), pp.515-520.
- Ishimaru, T., Horigane, A.K., Ida, M., Iwasawa, N., San-oh, Y.A., Nakazono, M., Nishizawa, N.K., Masumura, T., Kondo, M. and Yoshida, M., 2009. Formation of grain chalkiness and changes in water distribution in developing rice caryopses grown under high-temperature stress. *Journal of Cereal Science*, 50(2), pp.166-174.
- Ishimaru, T., Xaiyalath, S., Nallathambi, J., Sathishraj, R., Yoshimoto, M., Phoudalay, L., Samson, B., Hasegawa, T., Hayashi, K., Arumugam, G. and Muthurajan, R., 2016. Quantifying rice spikelet sterility in potential heat-vulnerable regions: Field surveys in Laos and southern India. *Field Crops Research*, 190, pp.3-9.

- Jagadish, K.S., Craufurd, P., Shi, W. and Oane, R., 2013. A phenotypic marker for quantifying heat stress impact during microsporogenesis in rice (*Oryza sativa* L.). *Functional Plant Biology*, 41(1), pp.48-55.
- Jagadish, S.K., 2020. Heat stress during flowering in cereals—effects and adaptation strategies. *New Phytologist*, 226(6), pp.1567-1572.
- Jagadish, S.K., Craufurd, P.Q. and Wheeler, T., 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of experimental botany*, 58(7), pp.1627-1635.
- Jagadish, S.V., Muthurajan, R., Rang, Z.W., Malo, R., Heuer, S., Bennett, J. and Craufurd, P.Q., 2011. Spikelet proteomic response to combined water deficit and heat stress in rice (*Oryza sativa* cv. N22). *Rice*, 4(1), pp.1-11.
- Jagadish, S.V.K., Cairns, J., Lafitte, R., Wheeler, T.R., Price, A.H. and Craufurd, P.Q., 2010b. Genetic analysis of heat tolerance at anthesis in rice. *Crop Science*, 50(5), pp.1633-1641.
- Jagadish, S.V.K., Craufurd, P.Q. and Wheeler, T.R., 2008. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Science*, 48(3), pp.1140-1146.
- Jagadish, S.V.K., Murty, M.V.R. and Quick, W.P., 2015. Rice responses to rising temperatures—challenges, perspectives and future directions. *Plant, cell & environment*, 38(9), pp.1686-1698.
- Jagadish, S.V.K., Muthurajan, R., Oane, R., Wheeler, T.R., Heuer, S., Bennett, J. and Craufurd, P., 2010a. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, 61(1), pp.143-156.
- Jagadish, S.V.K., Muthurajan, R., Rang, Z.W., Malo, R., Heuer, S., Bennett, J. and Craufurd, P.Q., 2011. Spikelet proteomic response to combined water deficit and heat stress in rice (*Oryza sativa* cv. N22). *Rice*, 4(1), pp.1-11.
- Jain, S.M., Brar, D.S. and Ahloowalia, B., 2010. *Molecular Techniques in Crop Improvement*, Springer, The Netherlands.
- Jennings, P.R., Coffman, W.R. and Kauffman, H.E., 1979. *Rice improvement*, IRRI, Los Banos, Phillippines.
- Jiang, H., Dian, W. and Wu, P., 2003. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. *Phytochemistry*, 63(1), pp.53-59.
- Julia, C. and Dingkuhn, M., 2012. Variation in time of day of anthesis in rice in different climatic environments. *European Journal of Agronomy*, 43, pp.166-174.

- Julia, C. and Dingkuhn, M., 2013. Predicting temperature induced sterility of rice spikelets requires simulation of crop-generated microclimate. *European Journal of Agronomy*, 49, pp.50-60.
- Jung, K.H., Gho, H.J., Nguyen, M.X., Kim, S.R. and An, G., 2013. Genome-wide expression analysis of HSP70 family genes in rice and identification of a cytosolic HSP70 gene highly induced under heat stress. *Functional & integrative genomics*, 13(3), pp.391-402.
- Karwa, S., Bahuguna, R.N., Chaturvedi, A.K., Maurya, S., Arya, S.S., Chinnusamy, V. and Pal, M., 2020. Phenotyping and characterization of heat stress tolerance at reproductive stage in rice (*Oryza sativa* L.). *Acta Physiologiae Plantarum*, 42(2), pp.1-16.
- Kilasi, N.L., Singh, J., Vallejos, C.E., Ye, C., Jagadish, S.V., Kusolwa, P. and Rathinasabapathi, B., 2018. Heat stress tolerance in rice (*Oryza sativa* L.): identification of quantitative trait loci and candidate genes for seedling growth under heat stress. *Frontiers in Plant Science*, 9, pp.1578.
- Kim, B., Jang, S.M., Chu, S.H., Bordiya, Y., Akter, M.B., Lee, J., Chin, J.H. and Koh, H.J., 2014. Analysis of segregation distortion and its relationship to hybrid barriers in rice. *Rice*, 7(1), pp.1-12.
- Kim, J., Shon, J., Lee, C.K., Yang, W., Yoon, Y., Yang, W.H., Kim, Y.G. and Lee, B.W., 2011. Relationship between grain filling duration and leaf senescence of temperate rice under high temperature. *Field Crops Research*, 122(3), pp.207-213.
- Kobata, T. and Uemuki, N., 2004a. High temperatures during the grain-filling period do not reduce the potential grain dry matter increase of rice. *Agronomy Journal*, 96(2), pp.406-414.
- Kobata, T., Uemuki, N., Inamura, T. and Kagata, H., 2004b. Shortage of assimilate supply to grain increases the proportion of milky white rice kernels under high temperatures. *Japanese Journal of Crop Science*, 73(3), pp.315-322.
- Kobayashi, K., Matsui, T., Murata, Y. and Yamamoto, M., 2011. Percentage of dehiscent thecae and length of dehiscence control pollination stability of rice cultivars at high temperatures. *Plant Production Science*, 14(2), pp.89-95.
- Koide, Y., Shinya, Y., Ikenaga, M., Sawamura, N., Matsubara, K., Onishi, K., Kanazawa, A. and Sano, Y., 2012. Complex genetic nature of sex-independent transmission ratio distortion in Asian rice species: the involvement of unlinked modifiers and sex-specific mechanisms. *Heredity*, 108(3), pp.242-247.
- Koike, S., Yamaguchi, T., Ohmori, S., Hayashi, T., Yatou, O. and Yoshida, H., 2015. Cleistogamy decreases the effect of high temperature stress at flowering in rice. *Plant Production Science*, 18(2), pp.111-117.

- Kosambi, D.D., 1944. The estimation of map distance from recombination values. *Annals of Eugenics*, 12, pp.172–175.
- Krishnan, R., Sanjay, J., Gnanaseelan, C., Mujumdar, M., Kulkarni, A. and Chakraborty, S., 2020. *Assessment of Climate Change over the Indian Region*. Springer Nature.
- Ku, S., Yoon, H., Suh, H.S. and Chung, Y.Y., 2003. Male-sterility of thermosensitive genic male-sterile rice is associated with premature programmed cell death of the tapetum. *Planta*, 217(4), pp.559-565.
- Kumar, N., Kumar, N., Shukla, A., Shankhdhar, S.C. and Shankhdhar, D., 2015b. Impact of terminal heat stress on pollen viability and yield attributes of rice (*Oryza sativa* L.). *Cereal Research Communications*, 43(4), pp.616-626.
- Kumar, V., Singh, A., Mithra, S.A., Krishnamurthy, S.L., Parida, S.K., Jain, S., Tiwari, K.K., Kumar, P., Rao, A.R., Sharma, S.K. and Khurana, J.P., 2015a. Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). *DNA research*, 22(2), pp.133-145.
- Kurek, I., Chang, T.K., Bertain, S.M., Madrigal, A., Liu, L., Lassner, M.W. and Zhu, G., 2007. Enhanced thermostability of Arabidopsis Rubisco activase improves photosynthesis and growth rates under moderate heat stress. *The Plant Cell*, 19(10), pp.3230-3241.
- Laborte, A., Nelson, A., Jagadish, S.V.K., Aunario, J., Sparks, A., Ye, C. and Redoña, E., 2012. Rice feels the heat. *Rice Today*, pp.30–31.
- Lafarge, T., Bueno, C., Frouin, J., Jacquin, L., Courtois, B., and Ahmadi, N. (2017). Genome-wide association analysis for heat tolerance at flowering detected a large set of genes involved in adaptation to thermal and other stresses. *PLoS One* 12, pp.e0171254.
- Lanceras, J.C., Huang, Z.L., Naivikul, O., Vanavichit, A., Ruanjaichon, V. and Tragoonrung, S., 2000. Mapping of genes for cooking and eating qualities in Thai jasmine rice (KDML105). *DNA Research*, 7(2), pp.93-101.
- Larkin, P.D. and Park, W.D., 1999. Transcript accumulation and utilization of alternate and non-consensus splice sites in rice granule-bound starch synthase are temperature-sensitive and controlled by a single-nucleotide polymorphism. *Plant Molecular Biology*, 40(4), pp.719-727.
- Larkindale, J. and Knight, M.R., 2002. Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiology*, 128(2), pp.682-695.
- Lawas, L.M., Bheemanahalli, R., Solis, C.A. and Jagadish, S.K., 2018. Sheathed panicle phenotype (cv. Sathi) maintains normal spikelet fertility and grain filling under prolonged heat stress in rice. *Crop Science*, 58(4), pp.1693-1705.

- Li C. Y., Peng C. H., Zhao Q. B., Xie P. and Chen W., 2004. Characteristic analysis of the abnormal high temperature in 2003s mid-summer in Wuhan City. *Journal of Central China Normal University (Natural Sciences)*, 38, pp.379–381.
- Li, H., Chen, Z., Hu, M., Wang, Z., Hua, H., Yin, C. and Zeng, H., 2011. Different effects of night versus day high temperature on rice quality and accumulation profiling of rice grain proteins during grain filling. *Plant Cell Reports*, 30(9), pp.1641-1659.
- Li, H., Ye, G. and Wang, J., 2007. A modified algorithm for the improvement of composite interval mapping. *Genetics*, 175(1), pp.361-374.
- Li, M.M., Li, X., Yu, L.Q., Wu, J.W., Li, H., Liu, J., Ma, X.D., Jo, S.M., Park, D.S., Song, Y. and Shin, D., 2018. Identification of QTLs associated with heat tolerance at the heading and flowering stage in rice (*Oryza sativa* L.). *Euphytica*, 214(4), pp.1-11.
- Li, X.M., Chao, D.Y., Wu, Y., Huang, X., Chen, K., Cui, L.G., Su, L., Ye, W.W., Chen, H., Chen, H.C. and Dong, N.Q., 2015. Natural alleles of a proteasome $\alpha 2$ subunit gene contribute to thermotolerance and adaptation of African rice. *Nature Genetics*, 47(7), pp.827-833.
- Lin, C.J., Li, C.Y., Lin, S.K., Yang, F.H., Huang, J.J., Liu, Y.H. and Lur, H.S., 2010. Influence of high temperature during grain filling on the accumulation of storage proteins and grain quality in rice (*Oryza sativa* L.). *Journal of Agricultural and Food Chemistry*, 58(19), pp.10545-10552.
- Lin, S.K., Chang, M.C., Tsai, Y.G. and Lur, H.S., 2005. Proteomic analysis of the expression of proteins related to rice quality during caryopsis development and the effect of high temperature on expression. *Proteomics*, 5(8), pp.2140-2156.
- Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J., Gore, M.A., Buckler, E.S. and Zhang, Z., 2012. GAPIT: genome association and prediction integrated tool. *Bioinformatics*, 28(18), pp.2397-2399.
- Li-rui, C., Uzokwe, V., Yun, W., Linghua, Z., 2012. Genetic analysis of cold tolerance at seedling stage and heat tolerance at anthesis in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, 11(3), pp.359-367.
- Lisle, A.J., Martin, M. and Fitzgerald, M.A., 2000. Chalky and translucent rice grains differ in starch composition and structure and cooking properties. *Cereal Chemistry*, 77(5), pp.627-632.
- Little, R.P., Hilder, G.B. and Dawson, E.H., 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. *Cereal Chemistry*, 35, pp.111–26.
- Liu, S. and Jiang, Y., 2010. Identification of differentially expressed genes under drought stress in perennial ryegrass. *Physiologia Plantarum*, 139(4), pp.375-387.
- Liu, X., Guo, T., Wan, X., Wang, H., Zhu, M., Li, A., Su, N., Shen, Y., Mao, B., Zhai, H. and Mao, L., 2010. Transcriptome analysis of grain-filling caryopses reveals

- involvement of multiple regulatory pathways in chalky grain formation in rice. *BMC Genomics*, 11(1), pp.1-15.
- Longmei, N., Gill, G.K., Zaidi, P.H., Kumar, R., Nair, S.K., Hindu, V., Vinayan, M.T. and Vikal, Y., 2021. Genome wide association mapping for heat tolerance in sub-tropical maize. *BMC Genomics*, 22(1), pp.1-14.
- Lyman, N.B., Jagadish, K.S., Nalley, L.L., Dixon, B.L. and Siebenmorgen, T., 2013. Neglecting rice milling yield and quality underestimates economic losses from high-temperature stress. *PloS One*, 8(8), pp.e72157.
- Mackill, D.J., 1995. Classifying japonica rice cultivars with RAPD markers. *Crop Science*, 35(3), pp.889-894.
- Mackill, D.J., Coffman, W.R. and Rutger, J.N., 1982. Pollen Shedding and Combining Ability for High Temperature Tolerance in Rice 1. *Crop Science*, 22(4), pp.730-733.
- Madan, P., Jagadish, S.V.K., Craufurd, P.Q., Fitzgerald, M., Lafarge, T. and Wheeler, T.R., 2012. Effect of elevated CO₂ and high temperature on seed-set and grain quality of rice. *Journal of experimental botany*, 63(10), pp.3843-3852.
- Mahajan, G., Sekhon, N.K., Singh, N., Kaur, R. and Sidhu, A.S., 2010. Yield and nitrogen-use efficiency of aromatic rice cultivars in response to nitrogen fertilizer. *Journal of New Seeds*, 11(4), pp.356-368.
- Malumpong, C., Cheabu, S., Mongkolsiriwatana, C., Detpittayanan, W. and Vanavichit, A., 2019. Spikelet fertility and heat shock transcription factor (Hsf) gene responses to heat stress in tolerant and susceptible rice (*Oryza sativa* L.) genotypes. *The Journal of Agricultural Science*, 157(4), pp.283-299.
- Maruyama, A., Weerakoon, W.M.W., Wakiyama, Y. and Ohba, K., 2013. Effects of increasing temperatures on spikelet fertility in different rice cultivars based on temperature gradient chamber experiments. *Journal of Agronomy and Crop Science*, 199(6), pp.416-423.
- Masutomi, Y., Arakawa, M., Minoda, T., Yonekura, T. and Shimada, T., 2015. Critical air temperature and sensitivity of the incidence of chalky rice kernels for the rice cultivar “Sai-no-kagayaki”. *Agricultural and Forest Meteorology*, 203, pp.11-16.
- Matsue, Y., 2012. *Rice palatability science from the viewpoint of crop production*. Tokyo: Yokendo.
- Matsui, T. and Omasa, K., 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: anther characteristics. *Annals of Botany*, 89(6), pp.683-687.
- Matsui, T., Kobayasi, K., Kagata, H. and Horie, T., 2005. Correlation between viability of pollination and length of basal dehiscence of the theca in rice under a hot-and-humid condition. *Plant Production Science*, 8(2), pp.109-114.

- Matsui, T., Kobayasi, K., Yoshimoto, M. and Hasegawa, T., 2007. Stability of rice pollination in the field under hot and dry conditions in the Riverina region of New South Wales, Australia. *Plant Production Science*, 10(1), pp.57-63.
- Matsui, T., Omasa, K. and Horie, T., 1997. High temperature-induced spikelet sterility of japonica rice at flowering in relation to air temperature, humidity and wind velocity conditions. *Japanese Journal of Crop Science*, 66(3), pp.449-455.
- Matsui, T., Omasa, K. and Horie, T., 1999a. Rapid swelling of pollen grains in response to floret opening unfolds anther locules in rice (*Oryza sativa* L.). *Plant Production Science*, 2(3), pp.196-199.
- Matsui, T., Omasa, K. and Horie, T., 1999b. Mechanism of anther dehiscence in rice (*Oryza sativa* L.). *Annals of Botany*, 84(4), pp.501-506.
- Matsui, T., Omasa, K. and Horie, T., 2000. High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Production Science*, 3(4), pp.430-434.
- Matsui, T., Omasa, K. and Horie, T., 2001. The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Production Science*, 4(2), pp.90-93.
- Matsushima, S., Ikewada, H., Maeda, A., Honma, S. and Niki, H., 1982. Studies on Rice Cultivation in the Tropics 1. Yielding and ripening responses of the rice plant to the extremely hot and dry climate in Sudan. *Japanese Journal of Tropical Agriculture*, 26(1), pp.19-25.
- McCouch, S.R., Cho, Y.G., Yano, M., Paul, E., Blinstrub, M., Morishima, H. and Kinoshita, T., 1997. Report on QTL nomenclature. *Rice Genetics Newsletter*, 14, pp.11-13.
- McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y. and Zhang, Q., 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA research*, 9(6), pp.199-207.
- McCouch, S.R., Zhao, K., Wright, M., Tung, C.W., Ebana, K., Thomson, M., Reynolds, A., Wang, D., DeClerck, G., Ali, M.L. and McClung, A., 2010. Development of genome-wide SNP assays for rice. *Breeding Science*, 60(5), pp.524-535.
- Meng, L., Li, H., Zhang, L. and Wang, J., 2015. QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal*, 3(3), pp.269-283.
- Merrick, L.F., Burke, A.B., Zhang, Z. and Carter, A.H., 2021. Comparison of single-trait and multi-trait genome-wide association models and inclusion of correlated traits in the dissection of the genetic architecture of a complex trait in a breeding program. *Frontiers in Plant Science*, 12, pp.772907.

- Mitsui, T., Yamakawa, H. and Kobata, T., 2016. Molecular physiological aspects of chalking mechanism in rice grains under high-temperature stress. *Plant Production Science*, 19(1), pp.22-29.
- Mohammad, S. and Avesi, G.M., 1985. Evaluation of rice germplasm for high temperature tolerance. *Pakistan Journal of Agricultural Research*, 6(3), pp.162-164.
- Mohammed, A.R. and Tarpley, L., 2010. Effects of high night temperature and spikelet position on yield-related parameters of rice (*Oryza sativa* L.) plants. *European journal of agronomy*, 33(2), pp.117-123.
- Monna, L., Miyao, A., Inoue, T., Fukuoka, S., Yamazaki, M., Zhong, H.S., Sasaki, T. and Minobe, Y., 1994. Determination of RAPD markers in rice and their conversion into sequence tagged sites (STSs) and STS-specific primers. *DNA Research*, 1(3), pp.139-148.
- Monna, L., Miyao, A., Zhong, H.S., Sasaki, T. and Minobe, Y., 1995. Screening of RAPD markers linked to the photoperiod-sensitivity gene in rice chromosome 6 using bulked segregant analysis. *DNA Research*, 2(3), pp.101-106.
- Morita, S., 2008. Prospect for developing measures to prevent high-temperature damage to rice grain ripening. *Japanese Journal of Crop Science*, 77, pp.1–12.
- Morita, S., Shiratsuchi, H., Takahashi, J. and Fujita, K., 2004. Effect of high temperature on grain ripening in rice plants: analysis of the effects of high night and high day temperatures applied to the panicle and other parts of the plant. *Japanese Journal of Crop Science*. 73, pp.77–83.
- Morita, S., Wada, H. and Matsue, Y., 2016. Countermeasures for heat damage in rice grain quality under climate change. *Plant Production Science*, 19(1), pp.1-11.
- Mu, Q., Zhang, W., Zhang, Y., Yan, H., Liu, K., Matsui, T., Tian, X. and Yang, P., 2017. iTRAQ-based quantitative proteomics analysis on rice anther responding to high temperature. *International journal of molecular sciences*, 18(9), pp.1811.
- Murray, M.G. and Thompson, W.F., 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8(19), 4321–4325.
- Nagahata, H. and Yamamoto, K., 2005. Evaluation of high temperature ripening ability using the temperature gradient chamber. *Breeding Research*, 7, 95-101.
- Nagata, K., Sasaki, R. and Ohdaira, Y., 2013. Cultivar differences in the grain cracking of rice under the high air temperature conditions during grain filling. *Japanese Journal of Crop Science*, 82(1), pp.42-48.
- Nagata, K., Takita, T., Yoshinaga, S., Terashima, K. and Fukuda, A., 2004. Effect of air temperature during the early grain-filling stage on grain fissuring in rice [*Oryza sativa*]. *Japanese Journal of Crop Science*, 73, 336–342.

- Nagato, K. and Ebata, M., 1965. Effects of high temperature during ripening period on the development and the quality of rice kernels. *Japanese Journal of Crop Science*, 34(1), pp.59-66.
- Nakagawa, H., Horie, T. and Matsui, T., 2003. Effects of climate change on rice production and adaptive technologies. In: Mew, T.W., Brar, D.S., Peng, S., Dawe, D. and Hardy, B., (Eds.) *Rice science: innovations and impact for livelihood. Proceedings of the International Rice Research Conference, Beijing, China, 16-19 September 2002*. International Rice Research Institute (IRRI). pp.635-658
- Nguyen, T., Shen, S., Cheng, M. and Chen, Q., 2022. Identification of QTLs for heat tolerance at the flowering stage using chromosome segment substitution lines in rice. *Genes*, 13, pp.2248.
- Nubankoh, P., Wanchana, S., Saensuk, C., Ruanjaichon, V., Cheabu, S., Vanavichit, A., Toojinda, T., Malumpong, C. and Arikrit, S., 2020. QTL-seq reveals genomic regions associated with spikelet fertility in response to a high temperature in rice (*Oryza sativa* L.). *Plant Cell Reports*, 39(1), pp.149-162.
- Ohdan, T., Sawada, T. and Nakamura, Y., 2010. Effects of temperature on starch branching enzyme properties of rice. *Journal of Applied Glycoscience*, 58(1), pp.19-26.
- Okada, M., Iizumi, T., Hayashi, Y. and Yokozawa, M., 2009. A climatological analysis on the recent declining trend of rice quality in Japan. *Journal of Agricultural Meteorology*, 65, 327-337.
- Okamoto, M., 1994. Studies on effect of chemical components on stickiness of cooked rice and their selection methods for breeding. *Bulletin of the Chugoku National Agricultural Experiment Station*, 14, pp.1-68.
- Osada, A., Sasiprapa, V., Rahong, M., Dhammanuvong, S. and Chakrabndhu, H., 1973. Abnormal occurrence of empty grains of indica rice plants in the dry, hot season in Thailand. *Japanese Journal of Crop Science*, 42(1), pp.103-109.
- Pan, Y., Luo, L., Deng, H., Zhang, G., Tang, W., Chen, L. and Xiao, Y., 2011. Quantitative trait loci associated with pollen fertility under high temperature stress at flowering stage in rice. *Chinese Journal of Rice Science*, 25(1), pp.99-102.
- Panda, B.B., Kariali, E., Panigrahi, R. and Mohapatra, P.K., 2009. High ethylene production slackens seed filling in compact panicked rice cultivar. *Plant Growth Regulation*, 58(2), pp.141-151.
- Pasuquin, E.M., Hasegawa, T., Eberbach, P., Reinke, R., Wade, L.J. and Lafarge, T., 2013. Responses of eighteen rice (*Oryza sativa* L.) cultivars to temperature tested using two types of growth chambers. *Plant Production Science*, 16(3), pp.217-225.
- Paul, P., Dhatt, B.K., Sandhu, J., Hussain, W., Irvin, L., Morota, G., Staswick, P. and Walia, H., 2020. Divergent phenotypic response of rice accessions to transient heat stress during early seed development. *Plant Direct*, 4(1), pp.e00196.

- Poli, Y., Basava, R.K., Panigrahy, M., Vinukonda, V.P., Dokula, N.R., Voleti, S.R., Desiraju, S. and Neelamraju, S., 2013. Characterization of a Nagina22 rice mutant for heat tolerance and mapping of yield traits. *Rice*, 6(1), pp.1-9.
- Pradhan, S.K., Barik, S.R., Sahoo, A., Mohapatra, S., Nayak, D.K., Mahender, A., Meher, J., Anandan, A. and Pandit, E., 2016. Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. *PloS One*, 11(8), pp.e0160027.
- Prasad, P.V.V., Boote, K.J., Allen Jr, L.H., Sheehy, J.E. and Thomas, J.M.G., 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, 95(2-3), pp.398-411.
- Prasanth, V.V., Babu, M.S., Basava, R.K., Tripura Venkata, V.G.N., Mangrauthia, S.K., Voleti, S.R. and Neelamraju, S., 2017a. Trait and marker associations in *Oryza nivara* and *O. rufipogon* derived rice lines under two different heat stress conditions. *Frontiers in Plant Science*, 8, pp.1819.
- Prasanth, V.V., Babu, M.S., Venkata, T.V.G.N., Kiran, T.V., Swamy, K.N., Babu, R.V., Mangrauthia, S.K., Subrahmanyam, D., Voleti, S.R. and Sarla, N., 2017b. Response of popular rice varieties to late sown high temperature conditions in field. *Indian Journal of Plant Physiology*, 22(2), pp.156-163.
- Qingquan, C., Sibin, Y. and Chunhai, L., 2008. Identification of QTLs for heat tolerance at flowering stage in rice. *Scientia Agricultura Sinica*, 41, pp.315–321.
- Qing-quan, C., Si-bin, Y., Chun-hai, LI. and Tong-min, M., 2008. Identification of QTLs for heat tolerance at flowering stage in rice. *Scientia Agricultura Sinica*, 41(2), 315-321.
- Raghunathachari, P., Khanna, V.K., Singh, U.S. and Singh, N.K., 2000. RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). *Current Science*, pp.994-998.
- Rahaman, M., Mamidi, S., and Rahman, M. (2018). Genome-wide association study of heat stress-tolerance traits in spring-type *Brassica napus* L. under controlled conditions. *Crop Journal*, 6, pp.115–125.
- Rang, Z.W., Jagadish, S.V.K., Zhou, Q.M., Craufurd, P.Q. and Heuer, S., 2011. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. *Environmental and Experimental Botany*, 70(1), pp.58-65.
- Restrepo-Diaz, H. and Garces-Varon, G., 2013. Response of rice plants to heat stress during initiation of panicle primordia or grain-filling phases. *Journal of Stress Physiology and Biochemistry*, 9(3), pp.318-325.
- Rieseberg, L.H., Archer, M.A. and Wayne, R.K., 1999. Transgressive segregation, adaptation and speciation. *Heredity*, 83(4), pp.363-372.

- Sailaja, B., Subrahmanyam, D., Neelamraju, S., Vishnukiran, T., Rao, Y.V., Vijayalakshmi, P., Voleti, S.R., Bhadana, V.P. and Mangrauthia, S.K., 2015. Integrated physiological, biochemical, and molecular analysis identifies important traits and mechanisms associated with differential response of rice genotypes to elevated temperature. *Frontiers in Plant Science*, 6, pp.1044.
- Sakata, T., Oshino, T., Miura, S., Tomabechi, M., Tsunaga, Y., Higashitani, N., Miyazawa, Y., Takahashi, H., Watanabe, M. and Higashitani, A., 2010. Auxins reverse plant male sterility caused by high temperatures. *Proceedings of the National Academy of Sciences, U.S.A.*, 107(19), pp.8569-8574.
- Salvucci, M.E. and Crafts-Brandner, S.J., 2004a. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum*, 120(2), pp.179-186.
- Salvucci, M.E. and Crafts-Brandner, S.J., 2004b. Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. *Plant physiology*, 134(4), pp.1460-1470.
- Sanchez, B., Rasmussen, A. and Porter, J.R., 2014. Temperatures and the growth and development of maize and rice: a review. *Global change biology*, 20(2), pp.408-417.
- Sandhu, N., Singh, A., Dixit, S., Sta Cruz, M.T., Maturan, P.C., Jain, R.K. and Kumar, A., 2014. Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. *BMC Genetics*, 15(1), pp.1-15.
- Satake, T. and Yoshida, S., 1978. High temperature-induced sterility in indica rices at flowering. *Japanese Journal of Crop Science*, 47(1), pp.6-17.
- Sathishraj, R., Bheemanahalli, R., Ramachandran, M., Dingkuhn, M., Muthurajan, R. and Krishna, J.S., 2016. Capturing heat stress induced variability in spikelet sterility using panicle, leaf and air temperature under field conditions. *Field Crops Research*, 190, pp.10-17.
- Sato K., 1979. High temperature damage to ripening in rice plant. *Japan Agricultural Research Quarterly*, 13(2), pp.90-95
- Sato, K. and Inaba, K., 1973. High temperature injury of ripening in rice plant: II. Ripening of rice grains when the panicle and straw were separately treated under different temperature. *Japanese Journal of Crop Science*, 42(2), pp.214-219.
- Sato, K. and Takahashi, M., 1971. The development of rice grains under controlled environment. I. The effects of temperature, its daily range and photoperiod during ripening on grain development. *Tohoku Journal of Agricultural Research*, 22, 57-67.
- Sato, K., 1979. High temperature damage to ripening in rice plant. *The Japan Agricultural Research Quarterly*, 13, pp.90-95.

- Scafaro, A.P., Haynes, P.A. and Atwell, B.J., 2010. Physiological and molecular changes in *Oryza meridionalis* Ng., a heat-tolerant species of wild rice. *Journal of Experimental Botany*, 61(1), pp.191-202.
- Schaarschmidt, S., Lawas, L.M.F., Glaubitz, U., Li, X., Erban, A., Kopka, J., Jagadish, S.K., Hinch, D.K. and Zuther, E., 2020. Season affects yield and metabolic profiles of rice (*Oryza sativa*) under high night temperature stress in the field. *International Journal of Molecular Sciences*, 21(9), p.3187.
- Seo, J., Lee, S.M., Han, J.H., Shin, N.H., Koh, H.J. and Chin, J.H., 2019. Identification of yield and yield-related quantitative trait loci for the field high temperature condition in backcross populations of rice (*Oryza sativa* L.). *Plant Breeding and Biotechnology*, 7(4), pp.415-426.
- Shah, F., Huang, J., Cui, K., Nie, L., Shah, T., Chen, C. and Wang, K., 2011. Impact of high-temperature stress on rice plant and its traits related to tolerance. *The Journal of Agricultural Science*, 149(5), pp.545-556.
- Shanmugavadivel, P.S., Mithra, S.V.A., Prakash, C., Ramkumar, M.K., Tiwari, R., Mohapatra, T. and Singh, N.K., 2017. High resolution mapping of QTLs for heat tolerance in rice using a 5K SNP array. *Rice*, 10, pp.28.
- Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell & Environment*, 28(3), pp.269-277.
- Sharma, D., Jaiswal, J.P., Gahtyari, N.C., Chauhan, A., Chhabra, R., Saripalli, G. and Singh, N.K., 2020. Population structure, marker-trait association and identification of candidate genes for terminal heat stress relevant traits in bread wheat (*Triticum aestivum* L. em Thell). *Plant Genetic Resources*, 18(3), pp.168-178.
- Sheehy, J.E., Mitchell, P.L., Beerling, D.J., Tsukaguchi, T. and Woodward, F.I., 1998. Temperature of rice spikelets: thermal damage and the concept of a thermal burden. *Agronomie*, 18(7), pp.449-460.
- Shi, P., Tang, L., Wang, L., Sun, T., Liu, L., Cao, W. and Zhu, Y., 2015a. Post-Heading heat stress in Rice of South China during 1981-2010. *PloS One*, 10(6), p.e0130642.
- Shi, P., Zhu, Y., Tang, L., Chen, J., Sun, T., Cao, W. and Tian, Y., 2016. Differential effects of temperature and duration of heat stress during anthesis and grain filling stages in rice. *Environmental and Experimental Botany*, 132, pp.28-41.
- Shi, W., Ishimaru, T., Gannaban, R.B., Oane, W. and Jagadish, S.V.K., 2015b. Popular rice (*Oryza sativa* L.) cultivars show contrasting responses to heat stress at gametogenesis and anthesis. *Crop Science*, 55(2), pp.589-596.
- Shi, W., Li, X., Schmidt, R.C., Struik, P.C., Yin, X. and Jagadish, S.K., 2018. Pollen germination and in vivo fertilization in response to high-temperature during

- flowering in hybrid and inbred rice. *Plant, Cell & Environment*, 41(6), pp.1287-1297.
- Shi, W., Muthurajan, R., Rahman, H., Selvam, J., Peng, S., Zou, Y. and Jagadish, K.S., 2013. Source–sink dynamics and proteomic reprogramming under elevated night temperature and their impact on rice yield and grain quality. *New Phytologist*, 197(3), pp.825-837.
- Shi, W., Yin, X., Struik, P.C., Solis, C., Xie, F., Schmidt, R.C., Huang, M., Zou, Y., Ye, C. and Jagadish, S.K., 2017. High day-and night-time temperatures affect grain growth dynamics in contrasting rice genotypes. *Journal of Experimental Botany*, 68(18), pp.5233-5245.
- Shimoda, S., 2012. Contribution of leaf nitrogen to photosynthetic gas exchange in contrasting rice (*Oryza sativa* L.) cultivars during the grain-filling period. *Photosynthetica*, 50(3), pp.387-394.
- Siddiq, E.A., Vemireddy, L.R. and Nagaraju, J., 2012. Basmati rices: genetics, breeding and trade. *Agricultural Research*, 1(1), pp.25-36.
- Sidhu, G.S., Khush, G.S. and Mew, T.W., 1978. Genetic analysis of bacterial blight resistance in seventy-four cultivars of rice, *Oryza sativa* L. *Theoretical and Applied Genetics*, 53(3), pp.105-111.
- Singh, N., Jayaswal, P.K., Panda, K., Mandal, P., Kumar, V., Singh, B., Mishra, S., Singh, Y., Singh, R., Rai, V. and Gupta, A., 2015. Single-copy gene based 50 K SNP chip for genetic studies and molecular breeding in rice. *Scientific reports*, 5(1), pp.1-9.
- Soda, N., Gupta, B.K., Anwar, K., Sharan, A., Singla-Pareek, S.L. and Pareek, A., 2018. Rice intermediate filament, OsIF, stabilizes photosynthetic machinery and yield under salinity and heat stress. *Scientific Reports*, 8(1), pp.1-13.
- Song, Z. P., Lu, B.R. and Chen, K.J., 2001. A study of pollen viability and longevity in *Oryza rufipogon*, *O. sativa* and their hybrids. *International Rice Research Notes*, 26, 31–32.
- Sreenivasulu, N., Butardo Jr, V.M., Misra, G., Cuevas, R.P., Anacleto, R. and Kavi Kishor, P.B., 2015. Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *Journal of Experimental Botany*, 66(7), pp.1737-1748.
- Su, C.C., Wan, J., Zhai, H.Q., Wang, C.M., Sun, L.H., Yasui, H. and Yoshimura, A., 2005. A new locus for resistance to brown planthopper identified in the indica rice variety DV85. *Plant breeding*, 124(1), pp.93-95.
- Subudhi, P.K. and Huang, N., 1999. RAPD Mapping in a Doubled Haploid Population of Rice (*Oryza Sativa* L.). *Hereditas*, 130(1), pp.41-49.
- Sukkeoa, S., Rerkasem, B. and Jamjoda, S., 2017. Heat tolerance in Thai rice varieties. *Science Asia*, 43, pp.61-69.

- Sun, Q., Miao, C., Hanel, M., Borthwick, A.G., Duan, Q., Ji, D. and Li, H., 2019. Global heat stress on health, wildfires, and agricultural crops under different levels of climate warming. *Environment international*, 128, pp.125-136.
- Suriyasak, C., Harano, K., Tanamachi, K., Matsuo, K., Tamada, A., Iwaya-Inoue, M. and Ishibashi, Y., 2017. Reactive oxygen species induced by heat stress during grain filling of rice (*Oryza sativa* L.) are involved in occurrence of grain chalkiness. *Journal of plant physiology*, 216, pp.52-57.
- Tadesse, W., Suleiman, S., Tahir, I., Sanchez-Garcia, M., Jighly, A., Hagra, A., Thabet, S. and Baum, M., 2019. Heat-tolerant QTLs associated with grain yield and its components in spring bread wheat under heat-stressed environments of Sudan and Egypt. *Crop Science*, 59(1), pp.199-211.
- Tafesse, E.G., Gali, K.K., Lachagari, V.R., Bueckert, R. and Warkentin, T.D., 2020. Genome-wide association mapping for heat stress responsive traits in field pea. *International Journal of Molecular Sciences*, 21(6), pp.2043.
- Takahashi, W., Ojima, T., Nomura, M. and Nabeshima, M., 2002. Development of a model for predicting cracked rice kernel of rice cultivar “Koshihikari”. *The Hokuriku Crop Science*, 37, 48–51.
- Takai, T., Lumanglas, P. and Simon, E.V., 2020. Genetic mechanism of heat stress tolerance at anthesis among three different rice varieties with different fertilities under heat stress. *Plant Production Science*, 23(4), pp.529-538.
- Takeoka, Y., Hiroi, K., Kitano, H. and Wada, T., 1991. Pistil hyperplasia in rice spikelets as affected by heat stress. *Sexual Plant Reproduction*, 4(1), pp.39-43.
- Tanaka, K., Onishi, R., Miyazaki, M., Ishibashi, Y., Yuasa, T. and Iwaya-Inoue, M., 2009. Changes in NMR relaxation of rice grains, kernel quality and physicochemical properties in response to a high temperature after flowering in heat-tolerant and heat-sensitive rice cultivars. *Plant Production Science*, 12(2), pp.185-192.
- Tanamachi, K., Miyazaki, M., Matsuo, K., Suriyasak, C., Tamada, A., Matsuyama, K., Iwaya-Inoue, M. and Ishibashi, Y., 2016. Differential responses to high temperature during maturation in heat-stress-tolerant cultivars of Japonica rice. *Plant Production Science*, 19(2), pp.300-308.
- Tang, R.S., Zheng, J.C., Jin, Z.Q., Zhang, D.D., Huang, Y.H. and Chen, L.G., 2008. Possible correlation between high temperature-induced floret sterility and endogenous levels of IAA, GAs and ABA in rice (*Oryza sativa* L.). *Plant Growth Regulation*, 54(1), pp.37-43.
- Tang, R.S., Zheng, J.C., Zhang, D.D., Jin, Z.Q., Chen, L.G., Huang, Y.H., Shi, C.L. and Ge, D.K., 2006. The effects of high temperatures on pollen vitality and seed setting of different rice varieties. *Jiangsu Journal of Agricultural Sciences* 22, pp.369-373.

- Tao, Z., Li, Y. and Kaifeng, J., 2008. QTL mapping for heat tolerance of the tassel period of rice. *Molecular Plant Breeding*, 6(5), pp.867-873.
- Tashiro, T. and Ebata, M., 1975. Studies on white-belly rice kernel: IV. Opaque rice endosperm viewed with a scanning electron microscope. *Japanese journal of crop science*, 44(2), pp.205-214.
- Tashiro, T.W.I.F. and Wardlaw, I.F., 1991. The effect of high temperature on kernel dimensions and the type and occurrence of kernel damage in rice. *Australian Journal of Agricultural Research*, 42(3), pp.485-496.
- Tazib, T., Kobayashi, Y., Koyama, H. and Matsui, T., 2015. QTL analyses for anther length and dehiscence at flowering as traits for the tolerance of extreme temperatures in rice (*Oryza sativa* L.). *Euphytica*, 203(3), pp.629-642.
- Teixeira, E.I., Fischer, G., Van Velthuizen, H., Walter, C. and Ewert, F., 2013. Global hot-spots of heat stress on agricultural crops due to climate change. *Agricultural and Forest Meteorology*, 170, pp.206-215.
- Tenorio, F.A., Ye, C., Redoña, E., Sierra, S., Laza, M. and Argayoso, M.A., 2013. Screening rice genetic resources for heat tolerance FA Tenorio¹, C. Ye¹, E. Redoña¹, S. Sierra¹, M. Laza¹ and MA Argayoso¹. *SABRAO Journal of Breeding and Genetics*, 45(3), pp.371-381.
- Terashima, K., Saito, Y., Sakai, N., Watanabe, T., Ogata, T. and Akita, S., 2001. Effects of high air temperature in summer of 1999 on ripening and grain quality of rice. *Japanese Journal of Crop Science*, 70(3), pp.449-458.
- Thuy, T.L., Lee, C.K., Jeong, J.H., Lee, H.S., Yang, S.Y., Im, Y.H. and Hwang, W.H., 2020. Impact of heat stress on pollen fertility rate at the flowering stage in korean rice (*Oryza sativa* L.) cultivars. *Korean Journal Crop Science*, 65(1), pp.22-29
- Tian, X., Luo, H., Zhou, H. and Wu, C., 2009. Research on heat stress of rice in China: progress and prospect. *Chinese Agricultural Science Bulletin*, 25(22), pp.166-168.
- Tiwari, S., Krishnamurthy, S.L., K., Kumar, V., Singh, B., Rao, A.R., Mithra SV, A., Rai, V., Singh, A.K. and Singh, N.K., 2016. Mapping QTLs for salt tolerance in rice (*Oryza sativa* L.) by bulked segregant analysis of recombinant inbred lines using 50K SNP chip. *PloS one*, 11(4), pp.e0153610.
- Tripathy, J.N., Zhang, J., Robin, S., Nguyen, T.T. and Nguyen, H.T., 2000. QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theoretical and Applied Genetics*, 100(8), pp.1197-1202.
- Tsukaguchi, T. and Iida, Y., 2008. Effects of assimilate supply and high temperature during grain-filling period on the occurrence of various types of chalky kernels in rice plants (*Oryza sativa* L.). *Plant Production Science*, 11(2), pp.203-210.

- Tsukaguchi, T., Ohashi, K., Sakai, H. and Hasegawa, T., 2011. Varietal difference in the occurrence of milky white kernels in response to assimilate supply in rice plants (*Oryza sativa* L.). *Plant production science*, 14(2), pp.111-117.
- Tsukaguchi, T., Yamamura, T., Inoue, H., Nakagawa, H., Murakami, K. and Kita, E., 2012. The response of the occurrence of milky white kernels with different cross-sectional patterns of chalkiness in the endosperm to grain-filling temperature and to assimilate supply in Koshihikari. *Japanese Journal of Crop Science*, 81(3), pp.267-274.
- Tung, C.W., Zhao, K., Wright, M.H., Ali, M.L., Jung, J., Kimball, J., Tyagi, W., Thomson, M.J., McNally, K., Leung, H. and Kim, H., 2010. Development of a research platform for dissecting phenotype–genotype associations in rice (*Oryza* spp.). *Rice*, 3(4), pp.205-217.
- Umemoto, T. and Terashima, K., 2002. Research note: Activity of granule-bound starch synthase is an important determinant of amylose content in rice endosperm. *Functional Plant Biology*, 29(9), pp.1121-1124.
- Usui, Y., Sakai, H., Tokida, T., Nakamura, H., Nakagawa, H. and Hasegawa, T., 2014. Heat-tolerant rice cultivars retain grain appearance quality under free-air CO₂ enrichment. *Rice*, 7(1), pp.1-9.
- Venuprasad, R., Dalid, C.O., Del Valle, M., Zhao, D., Espiritu, M., Sta Cruz, M.T., Amante, M., Kumar, A. and Atlin, G.N., 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics*, 120(1), pp.177-190.
- Vivitha, P., Raveendran, M. and Vijayalakshmi, D., 2017. Introgression of QTLs controlling spikelet fertility maintains membrane integrity and grain yield in improved white Ponni derived progenies exposed to heat stress. *Rice Science*, 24(1), pp.32-40.
- Wada, H., Hatakeyama, Y., Onda, Y., Nonami, H., Nakashima, T., Erra-Balsells, R., Morita, S., Hiraoka, K., Tanaka, F. and Nakano, H., 2019. Multiple strategies for heat adaptation to prevent chalkiness in the rice endosperm. *Journal of Experimental Botany*, 70(4), pp.1299-1311.
- Wada, Y., Oogaki, M. and Konishi, T., 2010. Effects of a high temperature before anthesis on rice growth and grain quality. *Japanese Journal of Crop Science*, 79, 460–467.
- Wahab, M.M.S., Akkareddy, S., Shanthi, P. and Latha, P., 2020. Identification of differentially expressed genes under heat stress conditions in rice (*Oryza sativa* L.). *Molecular Biology Reports*, 47, pp.1935-1948.

- Wakamatsu, K., Sasaki, O., Uezono, I. and Tanaka, A., 2007. Effects of high air temperature during the ripening period on the grain quality of rice in warm regions of Japan. *Japanese Journal of Crop Science* 76, 71–78.
- Wang, C., Su, C., Zhai, H. and Wan, J., 2005. Identification of QTLs underlying resistance to a virulent strain of *Xanthomonas oryzae* pv. *oryzae* in rice cultivar DV85. *Field Crops Research*, 91(2-3), pp.337-343.
- Wang, D., Qin, B., Li, X., Tang, D., Zhang, Y.E., Cheng, Z. and Xue, Y., 2016. Nucleolar DEAD-box RNA helicase TOGR1 regulates thermotolerant growth as a pre-rRNA chaperone in rice. *PLoS Genetics*, 12(2), pp.e1005844.
- Wang, D.R., Bunce, J.A., Tomecek, M.B., Gealy, D., McClung, A., McCouch, S.R. and Ziska, L.H., 2016b. Evidence for divergence of response in Indica, Japonica, and wild rice to high CO₂ × temperature interaction. *Global Change Biology*, 22(7), pp.2620-2632.
- Wang, Y., Impa, S.M., Sunkar, R. and Jagadish, S.K., 2021. The neglected other half-role of the pistil in plant heat stress responses. *Plant, Cell & Environment*, 44(7), pp.2200-2210.
- Wang, Y., Lin, S., Song, Q., Li, K., Tao, H., Huang, J., Chen, X., Que, S. and He, H., 2014. Genome-wide identification of heat shock proteins (Hsps) and Hsp interactors in rice: Hsp70s as a case study. *BMC genomics*, 15(1), pp.1-15.
- Wang, Y., Zhang, Y., Zhang, Q., Cui, Y., Xiang, J., Chen, H., Hu, G., Chen, Y., Wang, X., Zhu, D. and Zhang, Y., 2019. Comparative transcriptome analysis of panicle development under heat stress in two rice (*Oryza sativa* L.) cultivars differing in heat tolerance. *PeerJ*, 7, p.e7595.
- Wang, Z.Y., Second, G. and Tanksley, S.D., 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theoretical and Applied Genetics*, 83(5), pp.565-581.
- Weerakoon, W.M.W., Maruyama, A. and Ohba, K., 2008. Impact of humidity on temperature-induced grain sterility in rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science*, 194(2), pp.135-140.
- Welch, J.R., Vincent, J.R., Auffhammer, M., Moya, P.F., Dobermann, A. and Dawe, D., 2010. Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proceedings of the National Academy of Sciences USA*, 107(33), pp.14562-14567.
- Wise, R.R., Olson, A.J., Schrader, S.M. and Sharkey, T.D., 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant, Cell & Environment*, 27(6), pp.717-724.
- Wu, C., Cui, K., Li, Q., Li, L., Wang, W., Hu, Q., Ding, Y., Li, G., Fahad, S., Huang, J. and Nie, L., 2021. Estimating the yield stability of heat-tolerant rice genotypes

- under various heat conditions across reproductive stages: a 5-year case study. *Scientific Reports*, 11(1), pp.1-11.
- Wu, C., Cui, K., Wang, W., Li, Q., Fahad, S., Hu, Q., Huang, J., Nie, L. and Peng, S., 2016. Heat-induced phytohormone changes are associated with disrupted early reproductive development and reduced yield in rice. *Scientific reports*, 6(1), pp.1-14.
- Wu, J., Qin, Y. and Zhao, J., 2008. Pollen tube growth is affected by exogenous hormones and correlated with hormone changes in styles in *Torenia fournieri* L. *Plant Growth Regulation*, 55(2), pp.137-148.
- Wu, N., Yao, Y., Xiang, D., Du, H., Geng, Z., Yang, W., Li, X., Xie, T., Dong, F. and Xiong, L., 2022. A MITE variation-associated heat-inducible isoform of a heat-shock factor confers heat tolerance through regulation of JASMONATE ZIM-DOMAIN genes in rice. *New Phytologist*, 234(4), pp.1315-1331.
- Wu, Y.S. and Yang, C.Y., 2019. Ethylene-mediated signaling confers thermotolerance and regulates transcript levels of heat shock factors in rice seedlings under heat stress. *Botanical Studies*, 60(1), pp.1-12.
- Xiao, Y., Pan, Y., Luo, L., Zhang, G., Deng, H., Dai, L., Liu, X., Tang, W., Chen, L. and Wang, G.L., 2011a. Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.). *Euphytica*, 178(3), pp.331-338.
- Xiao, Y.H., Pan, Y., Luo, L.H., Deng, H.B., Zhang, G.L., Tang, W.B. and Chen, L.Y., 2011b. Quantitative trait loci associated with pollen fertility under high temperature stress at flowering stage in rice (*Oryza sativa* L.). *Rice Science*, 18, 1–7.
- Xie, X.J., Shen, S.H.H., Li, Y.X., Zhao, X.Y., Li, B.B. and Xu, D.F., 2011. Effect of photosynthetic characteristic and dry matter accumulation of rice under high temperature at heading stage. *African Journal of Agricultural Research*, 6, 1931-1940.
- Xiong, D., Ling, X., Huang, J. and Peng, S., 2017. Meta-analysis and dose-response analysis of high temperature effects on rice yield and quality. *Environmental and Experimental Botany*, 141, pp.1-9.
- Xu, P., Zhou, J., Li, J., Hu, F., Deng, X., Feng, S., Ren, G., Zhang, Z., Deng, W. and Tao, D., 2014. Mapping three new interspecific hybrid sterile loci between *Oryza sativa* and *O. glaberrima*. *Breeding science*, 63(5), pp.476-482.
- Xu, Y., Chu, C. and Yao, S., 2021. The impact of high-temperature stress on rice: challenges and solutions. *The Crop Journal*, 9(5), pp.963-976.
- Xu, Y., Zhang, L., Ou, S., Wang, R., Wang, Y., Chu, C. and Yao, S., 2020. Natural variations of SLG1 confer high-temperature tolerance in indica rice. *Nature Communications*, 11(1), pp.1-13.

- Yamada, I., 1964. *Studies on artificial germination and germination physiology of rice pollen*. Ph.D. Thesis, Kyoto University, Kyoto.
- Yamakawa, H. and Hakata, M., 2010. Atlas of rice grain filling-related metabolism under high temperature: joint analysis of metabolome and transcriptome demonstrated inhibition of starch accumulation and induction of amino acid accumulation. *Plant and Cell Physiology*, 51(5), pp.795-809.
- Yamakawa, H., Hirose, T., Kuroda, M. and Yamaguchi, T., 2007. Comprehensive expression profiling of rice grain filling-related genes under high temperature using DNA microarray. *Plant Physiology*, 144(1), pp.258-277.
- Yamane, Y., Kashino, Y., Koike, H. and Satoh, K., 1998. Effects of high temperatures on the photosynthetic systems in spinach: oxygen-evolving activities, fluorescence characteristics and the denaturation process. *Photosynthesis Research*, 57(1), pp.51-59.
- Yang, H.C., Huang, Z.Q., Jiang, Z.Y. and Wang, X.W., 2004. High temperature damage and its protective technologies of early and middle season rice in Anhui province. *Journal of Anhui Agricultural Sciences*, 32, pp.3-4.
- Ye, C., Argayoso, M.A., Redoña, E.D., Sierra, S.N., Laza, M.A., Dilla, C.J., Mo, Y., Thomson, M.J., Chin, J., Delaviña, C.B. and Diaz, G.Q., 2012. Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breeding*, 131(1), pp.33-41.
- Ye, C., Tenorio, F.A., Argayoso, M.A., Laza, M.A., Koh, H.J., Redoña, E.D., Jagadish, K.S. and Gregorio, G.B., 2015a. Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics*, 16(1), pp.1-10.
- Ye, C., Tenorio, F.A., Redoña, E.D., Morales-Cortezano, P.S., Cabrega, G.A., Jagadish, K.S. and Gregorio, G.B., 2015b. Fine-mapping and validating *qHTSF4.1* to increase spikelet fertility under heat stress at flowering in rice. *Theoretical and Applied Genetics*, 128(8), pp.1507-1517.
- Yonemaru, J.I. and Morita, S., 2012. Image analysis of grain shape to evaluate the effects of high temperatures on grain filling of rice, *Oryza sativa* L. *Field Crops Research*, 137, pp.268-271.
- Yoshida, S., Satake, T. and Mackill, D., 1981. High temperature stress. *IRRI Research Paper Series*, 67, pp.1-15.
- Yoshimoto, M., Fukuoka, M., Hasegawa, T., Matsui, T., Tian, X., Vijayalakshmi, C., Singh, M.P., MYINT, T.T., Weerakoon, W.M.W., Lafarge, T. and Lur, H.S., 2012. MINCERnet: a global research alliance to support the fight against heat stress in rice. *Journal of Agricultural Meteorology*, 68(2), pp.149-157.

- Yu, H., Xie, W., Li, J., Zhou, F. and Zhang, Q., 2014. A whole-genome SNP array (RICE 6 K) for genomic breeding in rice. *Plant Biotechnology Journal*, 12(1), pp.28-37.
- Yu, L.X. and Nguyen, H.T., 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 87(6), pp.668-672.
- Yun-Ying, C., Hua, D.U.A.N., Li-Nian, Y.A.N.G., Zhi-Qing, W.A.N.G., Li-Jun, L.I.U. and Jian-Chang, Y.A.N.G., 2009. Effect of high temperature during heading and early filling on grain yield and physiological characteristics in indica rice. *Acta Agronomica Sinica*, 35(3), pp.512-521.
- Zafar, S.A., Arif, M.H., Uzair, M., Rashid, U., Naeem, M.K., Rehman, O.U., Rehman, N., Zaid, I.U., Farooq, M.S., Zahra, N. and Saleem, B., 2022. Agronomic and physiological indices for reproductive stage heat stress tolerance in green super rice. *Agronomy*, 12(8), pp.1907.
- Zakaria, S., Matsuda, T., Tajima, S. and Nitta, Y., 2002. Effect of high temperature at ripening stage on the reserve accumulation in seed in some rice cultivars. *Plant Production Science*, 5(2), pp.160-168.
- Zhang, C., Li, G., Chen, T., Feng, B., Fu, W., Yan, J., Islam, M.R., Jin, Q., Tao, L. and Fu, G., 2018. Heat stress induces spikelet sterility in rice at anthesis through inhibition of pollen tube elongation interfering with auxin homeostasis in pollinated pistils. *Rice*, 11(1), pp.1-12.
- Zhang, C.X., Feng, B.H., Chen, T.T., Zhang, X.F., Tao, L.X. and Fu, G.F., 2017. Sugars, antioxidant enzymes and IAA mediate salicylic acid to prevent rice spikelet degeneration caused by heat stress. *Plant Growth Regulation*, 83(2), pp.313-323.
- Zhang, C.X., Fu, G.F., Yang, X.Q., Yang, Y.J., Zhao, X., Chen, T.T., Zhang, X.F., Jin, Q.Y. and Tao, L.X., 2016. Heat stress effects are stronger on spikelets than on flag leaves in rice due to differences in dissipation capacity. *Journal of Agronomy and Crop Science*, 202(5), pp.394-408.
- Zhang, G., Wang, S., You, J., Wang, Q., Ding, Y. and Ji, Z., 2006. Effect of higher temperature in different filling stages on rice qualities. *Acta Agronomica Sinica*, 32(2), p.283.
- Zhang, G.L., Chen, L.Y., Xiao, G.Y., Xiao, Y.H., Chen, X.B. and Zhang, S.T., 2009. Bulk segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers. *Agricultural Sciences in China*, 8(4), pp.482-487.
- Zhang, H., Duan, L., Dai, J.S., Zhang, C.Q., Li, J., Gu, M.H., Liu, Q.Q. and Zhu, Y., 2014. Major QTLs reduce the deleterious effects of high temperature on rice amylose content by increasing splicing efficiency of Wx pre-mRNA. *Theoretical and Applied Genetics*, 127(2), pp.273-282.

- Zhang, H., Xu, H., Feng, M. and Zhu, Y., 2018. Suppression of OsMADS7 in rice endosperm stabilizes amylose content under high temperature stress. *Plant biotechnology journal*, 16(1), pp.18-26.
- Zhang, T., Yang, L., Jiang, K.F., Huang, M., Sun, Q., Chen, W.F. and Zheng, J.K., 2008. QTL mapping for heat tolerance of the tassel period of rice. *Molecular Plant Breeding*, 6, 867–873.
- Zhao, L., Lei, J., Huang, Y., Zhu, S., Chen, H., Huang, R., Peng, Z., Tu, Q., Shen, X. and Yan, S., 2016. Mapping quantitative trait loci for heat tolerance at anthesis in rice using chromosomal segment substitution lines. *Breeding Science*, 66(3), pp.358-66.
- Zhao, X. and Fitzgerald, M., 2013. Climate change: implications for the yield of edible rice. *PloS One*, 8(6), pp.e66218.
- Zhao, Z., Jiang, L., Xiao, Y., Zhang, W., Zhai, H. and Wan, J., 2006. Identification of QTLs for heat tolerance at the booting stage in rice (*Oryza sativa* L.). *Acta Agronomica Sinica*, 32(5), pp.540.
- Zhong, L.J., Cheng, F.M., Wen, X., Sun, Z.X. and Zhang, G.P., 2005. The deterioration of eating and cooking quality caused by high temperature during grain filling in early-season indica rice cultivars. *Journal of Agronomy and Crop Science*, 191(3), pp.218-225.
- Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T. and Bryan, G.J., 1998. AFLP markers for the study of rice biodiversity. *Theoretical and Applied genetics*, 96(5), pp.602-611.
- Zhu, S., Huang, R., Wai, H.P., Xiong, H., Shen, X., He, H. and Yan, S., 2017. Mapping quantitative trait loci for heat tolerance at the booting stage using chromosomal segment substitution lines in rice. *Physiology and Molecular Biology of Plants*, 23(4), pp.817-825.

Annexures

Annexure I. BLUP values for grain yield and spikelet fertility under control and heat stress situations during off-seasons, 2018-19 and 2019-20.

Genotype	Grain yield plant ⁻¹				Spikelet fertility			
	Off-season, 2019		Off-season, 2020		Off-season, 2019		Off-season, 2020	
	Control	Heat stress	Control	Heat stress	Control	Heat stress	Control	Heat stress
Taraori Basmati	13.14	5.34	10.77	6.47	81.88	46.83	63.38	45.85
CSR 30	13.91	5.60	11.54	6.73	84.54	49.49	57.80	40.27
Basmati 370	22.00	10.67	19.64	11.80	88.51	53.45	74.94	57.41
Ranbir Basmati	17.19	7.99	14.83	9.12	90.54	55.49	72.42	54.90
Super Basmati	17.25	7.97	14.89	9.10	77.45	42.40	63.09	45.56
Type 3	16.93	7.46	14.57	8.58	71.71	36.65	55.93	38.40
Type 23	20.30	10.93	17.93	12.06	79.44	44.39	68.52	51.00
Basmati 386	16.59	7.79	14.22	8.92	86.85	51.80	73.97	56.45
Punjab Basmati 5	13.06	5.50	10.70	6.63	70.07	35.01	42.31	24.78
Kasturi Basmati	18.49	10.04	16.13	11.17	81.00	45.95	64.38	46.85
Palam Basmati	14.50	6.85	12.13	7.98	71.39	36.33	56.60	39.07
Sanwal Basmati	15.88	7.29	13.52	8.42	65.52	30.47	60.47	42.95
Basmati Kota	15.33	7.20	12.96	8.33	85.83	50.77	71.79	54.26
Basmati Jamuna	21.48	9.62	19.11	10.74	89.75	54.70	76.03	58.50
Seond Basmati	9.33	1.71	6.96	2.84	56.71	21.66	44.80	27.27
Chimbalate Basmati	20.31	10.85	17.94	11.98	87.37	52.31	77.24	59.71
Domsiah	18.75	9.63	16.39	10.76	85.06	50.00	69.73	52.20
Shahi Pasand	18.14	8.66	15.78	9.79	75.25	40.20	56.85	39.33
Hasan Serai	16.87	7.48	14.50	8.60	64.72	29.67	56.84	39.31
Haryana Basmati 1	18.55	9.94	16.18	11.07	90.59	55.54	77.53	60.01
Punjab Basmati 4	17.51	8.51	15.15	9.64	87.90	52.85	75.74	58.22
Punjab Basmati 2	14.94	6.31	12.58	7.44	78.55	43.49	63.12	45.59
Punjab Basmati 3	15.96	6.91	13.59	8.04	82.04	46.99	70.43	52.90
Vallabh Basmati 21	18.17	9.67	15.80	10.80	84.24	49.18	70.66	53.13
Vallabh Basmati 22	15.51	7.29	13.15	8.42	68.86	33.81	52.04	34.51
Vallabh Basmati 23	15.38	7.61	13.01	8.74	74.69	39.63	59.38	41.85
Pusa Basmati 6	15.96	6.90	13.60	8.03	72.45	37.40	60.05	42.52
Pusa Basmati 1121	14.54	6.23	12.18	7.36	65.72	30.67	44.03	26.51
Pusa Basmati 1509	17.76	7.82	15.39	8.95	61.42	26.37	52.14	34.61
Pusa Basmati 1728	19.11	8.62	16.75	9.75	73.04	37.99	62.38	44.85
Pusa Basmati 1637	18.59	9.20	16.22	10.33	68.09	33.03	62.07	44.55
Pusa Basmati 1718	11.71	3.11	9.34	4.24	50.52	15.46	33.44	15.92
Punjab Basmati 1	16.68	7.18	14.31	8.30	78.57	43.52	57.94	40.42
Haryana Basmati 2	15.62	6.30	13.26	7.42	65.55	30.50	50.68	33.16
Pusa Basmati 1	20.30	10.87	17.94	12.00	81.44	46.38	74.62	57.09

Annexure IIa. Daily maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures during off-season, 2017-18 at Aduthurai, Tamil Nadu.

Day	December		January		February		March		April		May		June	
	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$
1	29	24	33	20	32	20	34	23	38	27	40	26	37	27
2	26	21	33	22	32	17	35	22	38	27	41	28	38	28
3	29	22	32	23	31	17	36	21	38	27	40	26	39	28
4	30	23	31	22	32	18	36	21	38	27	38	25	37	26
5	30	23	30	20	31	19	35	20	38	26	39	28	38	27
6	30	23	30	18	32	22	35	20	37	26	38	27	39	27
7	31	23	30	19	34	24	34	22	38	26	40	27	39	27
8	31	22	31	18	32	23	35	22	37	26	39	25	37	27
9	32	21	28	21	34	23	34	21	37	27	37	24	35	28
10	32	23	27	21	34	23	34	23	37	26	36	23	38	27
11	32	23	28	22	32	22	34	23	38	27	38	26	38	29
12	32	24	31	23	33	23	35	24	38	27	38	27	37	27
13	31	24	30	22	32	22	32	25	38	27	38	26	36	28
14	31	23	32	21	33	22	34	25	38	27	37	25	36	27
15	29	23	31	21	33	23	36	25	37	27	38	28	39	27
16	31	22	30	21	33	23	37	25	38	26	37	28	39	25
17	30	22	31	21	34	20	30	26	38	27	37	28	38	24
18	30	20	31	19	33	22	36	24	39	27	38	27	38	27
19	29	21	30	18	32	19	35	24	38	28	37	27	38	27
20	31	23	30	18	32	20	36	22	40	27	39	27	37	27
21	27	23	31	18	33	21	38	23	40	28	37	28	36	27
22	29	21	30	19	34	20	39	25	41	27	38	25	37	27
23	30	22	30	20	33	21	40	26	40	27	38	26	38	27
24	30	21	31	20	34	21	38	27	41	27	37	24	39	27
25	29	21	32	21	33	22	36	24	41	27	35	24	38	27
26	30	22	32	21	34	22	35	23	40	28	34	24	38	26
27	30	21	31	20	35	23	39	24	38	28	36	26	38	27
28	29	21	31	21	35	23	39	27	39	27	36	26	38	28
29	30	21	31	20			39	26	38	27	36	26	39	26
30	31	21	32	21			40	26	41	27	36	26	39	25
31	32	21	32	21			39	26			38	27		

Annexure IIb. Daily maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures during off-season, 2018-19 at Aduthurai, Tamil Nadu.

Day	December		January		February		March		April		May		June	
	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$
1	31	23	30	20	32	21	37	25	40	25	39	29	41	28
2	30	21	30	19	32	21	35	25	39	26	41	29	39	29
3	31	23	30	18	34	22	36	24	38	26	42	28	39	27
4	29	23	30	18	33	22	36	24	38	25	41	29	40	28
5	32	24	31	20	32	24	38	25	39	26	43	28	40	26
6	31	23	30	20	34	23	40	25	39	26	42	29	39	26
7	32	23	31	19	34	22	40	26	39	26	42	29	40	28
8	31	23	32	21	34	23	39	27	40	27	38	24	40	27
9	31	23	31	20	34	24	38	26	40	28	39	21	40	28
10	31	22	31	19	34	25	39	27	40	28	40	26	38	28
11	31	22	31	20	34	24	37	25	40	27	40	29	40	28
12	31	23	31	21	33	23	37	23	40	27	42	29	40	29
13	31	23	31	20	33	23	36	22	40	27	42	29	40	28
14	32	21	32	20	34	22	37	23	40	26	41	29	40	29
15	32	23	31	19	35	24	36	21	40	25	41	28	40	28
16	31	22	32	20	36	24	36	23	41	27	41	28	41	29
17	32	20	31	20	38	25	36	23	40	28	41	27	40	29
18	32	22	31	21	37	26	37	24	40	28	40	25	40	29
19	29	23	31	20	36	26	37	25	40	28	41	28	40	29
20	31	21	30	19	35	25	37	25	40	27	41	27	40	28
21	32	22	30	19	35	24	38	24	36	27	41	28	38	28
22	28	24	32	18	35	22	38	25	40	28	41	29	38	27
23	29	23	32	20	35	21	39	26	37	27	40	28	38	28
24	29	23	33	21	36	20	39	27	39	27	40	30	40	28
25	31	22	33	22	36	21	38	26	39	26	41	29	41	28
26	31	21	34	23	37	23	38	24	40	28	42	29	39	28
27	32	21	33	23	38	25	38	23	40	28	42	29	40	29
28	31	22	32	23	37	26	39	24	40	28	42	29	40	28
29	30	23	32	23	37	26	39	23	39	29	41	27	40	28
30	31	22	32	22	37	22	40	24	41	28	41	26	37	28
31	30	21	32	22	37	22	41	24	41	25	39	25	37	28

Annexure IIc. Daily maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures during off-season, 2019-20 at Aduthurai, Tamil Nadu.

Day	December		January		February		March		April		May		June	
	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$
1	26	23	32	24	34	22	35	23	37	29	40	25	40	25
2	29	23	31	23	34	21	35	23	37	28	40	28	38	24
3	30	22	33	24	33	21	36	24	37	28	40	28	39	25
4	31	23	34	24	33	21	38	24	39	24	39	28	39	27
5	31	22	33	24	34	24	37	25	38	27	39	27	41	27
6	28	23	31	24	33	21	37	25	39	32	41	28	39	26
7	31	24	30	23	34	21	36	26	37	28	40	28	38	26
8	30	23	32	22	34	22	36	26	36	32	41	28	38	27
9	31	21	31	21	34	22	36	24	37	30	40	28	39	28
10	31	22	32	21	33	22	37	23	36	31	41	28	37	28
11	32	22	32	19	34	22	37	23	35	30	40	29	35	28
12	32	22	32	20	34	23	36	23	37	29	36	28	37	28
13	30	22	32	22	34	23	36	23	37	29	39	26	39	26
14	27	23	32	19	34	21	36	24	37	30	40	28	39	28
15	30	22	31	19	35	21	36	24	37	30	40	28	39	28
16	32	22	33	19	34	20	37	24	38	31	40	29	40	28
17	29	23	33	21	35	20	37	25	38	32	41	25	40	29
18	31	22	29	23	34	19	37	24	39	32	35	24	39	29
19	31	23	32	23	34	20	37	24	36	31	39	28	40	28
20	30	22	32	22	34	20	39	24	39	27	41	29	39	28
21	30	23	32	21	34	21	39	26	39	26	37	32	37	29
22	31	22	32	21	35	24	38	26	40	27	40	29	37	28
23	31	22	32	23	35	23	38	25	40	27	42	29	36	28
24	28	23	33	21	35	23	37	24	40	28	42	27	38	28
25	27	22	32	19	36	23	37	24	41	28	42	26	38	25
26	29	23	32	20	36	24	35	29	37	26	41	28	37	24
27	30	22	33	19	35	23	36	28	39	25	39	30	38	25
28	31	21	34	21	35	23	36	27	39	27	40	28	38	27
29	31	22	34	22	35	23	36	28	37	27	39	26	39	26
30	30	21	34	21	34	21	36	29	35	28	39	28	38	26
31	31	22	34	21	34	21	36	28	35	27	40	27	38	26

Annexure II.d. Daily maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures during off-season, 2018-19 at Cuttack, Odisha.

Day	December		January		February		March		April		May		June	
	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$
1	29	18	32	24	34	22	35	23	37	29	40	25	40	25
2	31	16	31	23	34	21	35	23	37	28	40	28	38	24
3	30	16	33	24	33	21	36	24	37	28	40	28	39	25
4	30	15	34	24	33	21	38	24	39	24	39	28	39	27
5	30	16	33	24	34	24	37	25	38	27	39	27	41	27
6	29	18	31	24	33	21	37	25	39	32	41	28	39	26
7	30	16	30	23	34	21	36	26	37	28	40	28	38	26
8	29	17	32	22	34	22	36	26	36	32	41	28	38	27
9	29	15	31	21	34	22	36	24	37	30	40	28	39	28
10	29	14	32	21	33	22	37	23	36	31	41	28	37	28
11	30	15	32	19	34	22	37	23	35	30	40	29	35	28
12	31	17	32	20	34	23	36	23	37	29	36	28	37	28
13	30	16	32	22	34	23	36	23	37	29	39	26	39	26
14	30	18	32	19	34	21	36	24	37	30	40	28	39	28
15	27	18	31	19	35	21	36	24	37	30	40	28	39	28
16	25	19	33	19	34	20	37	24	38	31	40	29	40	28
17	22	16	33	21	35	20	37	25	38	32	41	25	40	29
18	19	17	29	23	34	19	37	24	39	32	35	24	39	29
19	25	16	32	23	34	20	37	24	36	31	39	28	40	28
20	27	14	32	22	34	20	39	24	39	27	41	29	39	28
21	28	13	32	21	34	21	39	26	39	26	37	32	37	29
22	27	14	32	21	35	24	38	26	40	27	40	29	37	28
23	28	11	32	23	35	23	38	25	40	27	42	29	36	28
24	28	10	33	21	35	23	37	24	40	28	42	27	38	28
25	28	11	32	19	36	23	37	24	41	28	42	26	38	25
26	28	12	32	20	36	24	35	29	37	26	41	28	37	24
27	28	13	33	19	35	23	36	28	39	25	39	30	38	25
28	24	13	34	21	35	23	36	27	39	27	40	28	38	27
29	26	11	34	22	35	23	36	28	37	27	39	26	39	26
30	27	8	34	21			36	29	35	28	39	28	38	26
31	27	10	34	21			36	28			40	27		

Annexure IIe. Daily maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures during off-season, 2019-20 at Cuttack, Odisha.

Day	December		January		February		March		April		May		June	
	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$	$T_{\max}$	$T_{\min}$
1	30	18	27	13	28	17	32	18	39	24	36	22	35	25
2	28	18	26	16	27	18	34	20	39	26	35	21	36	24
3	30	16	22	17	27	18	34	19	39	25	35	27	36	24
4	29	18	21	16	29	16	32	22	33	25	34	27	36	25
5	29	16	25	15	21	18	32	20	35	26	36	26	36	27
6	29	13	25	15	27	17	29	20	38	25	32	23	38	28
7	30	16	27	12	23	18	32	22	39	24	35	25	34	26
8	30	18	28	13	21	16	32	21	36	24	37	26	35	26
9	30	19	29	16	23	15	34	22	37	21	38	24	36	24
10	31	18	26	18	27	15	34	23	39	24	38	23	36	24
11	31	17	25	14	28	13	34	23	40	25	35	21	32	26
12	30	18	26	11	30	13	34	23	40	25	38	26	31	26
13	29	19	28	11	32	13	35	23	42	26	36	26	34	26
14	30	18	29	13	34	14	35	22	41	26	38	26	29	25
15	31	18	29	16	35	16	32	20	40	26	38	26	31	27
16	30	19	30	16	32	17	35	23	39	26	39	27	31	25
17	29	18	30	16	32	20	36	23	37	27	39	27	33	29
18	27	18	30	17	34	16	36	22	37	23	39	26	32	27
19	27	15	31	15	34	14	34	19	37	22	32	24	36	26
20	27	14	29	18	33	19	34	18	38	26	25	20	34	26
21	26	15	28	18	33	18	34	20	34	24	35	21	31	26
22	27	15	30	16	33	17	33	21	34	22	35	28	36	27
23	28	17	31	18	33	16	34	21	37	25	35	27	35	28
24	28	15	30	20	28	18	35	21	37	23	35	28	36	27
25	29	14	28	16	21	17	36	23	30	22	34	28	36	27
26	25	17	29	15	26	16	37	23	34	24	34	28	37	27
27	24	17	29	13	30	17	38	24	34	23	36	28	37	28
28	25	12	31	16	30	18	38	25	34	25	34	28	35	25
29	26	10	33	21	31	18	38	24	37	22	33	19	33	24
30	25	11	31	22			37	22	35	21	34	21	36	25
31	26	10	28	20			37	23			36	26		

Annexure III. Details of SSR based linkage map generated using QTL IciMapping

Chromosome	Marker Name	Interval (cM)	Position (cM)	Recombination frequency	Interference
Chromosome1	RM493	0	0	0.5	0
Chromosome1	RM220	72.2593	72.2593	0.4474	-0.009
Chromosome1	RM11928	28.9363	101.1956	0.2609	3.8518
Chromosome1	RM8085	2.9159	104.1115	0.0291	0.6269
Chromosome1	RM113	52.697	156.8085	0.3917	0
Chromosome2	RM12970	0	0	0.5	0
Chromosome2	RM1407	57.3363	57.3363	0.4083	-0.2074
Chromosome2	RM3355	47.1767	104.5131	0.3684	-0.4695
Chromosome2	Hv2.68	12.4293	116.9424	0.1218	0.4193
Chromosome2	RM6	9.7607	126.7031	0.0964	-1.1821
Chromosome2	RM3789	29.5283	156.2314	0.2652	-0.3771
Chromosome2	RM208	7.2672	163.4986	0.0722	0.2805
Chromosome2	RM12705	40.667	204.1656	0.3357	0.268
Chromosome2	RM341	38.4334	242.599	0.3231	-1.0035
Chromosome2	RM262	7.4225	250.0215	0.0737	0.5299
Chromosome2	RM211	51.5356	301.557	0.3871	0
Chromosome3	RM203	0	0	0.5	0
Chromosome3	Hv3.86	18.6804	18.6804	0.1786	-0.2216
Chromosome3	RM5864	54.3056	72.9859	0.3977	0.3746
Chromosome3	RM564	58.3844	131.3703	0.4118	-0.439
Chromosome3	RM7	53.5017	184.872	0.3947	-0.9214
Chromosome3	RM218	7.3247	192.1967	0.0727	0.6566
Chromosome3	RGNMS1289	49.4291	241.6257	0.3784	0
Chromosome4	RM16294	0	0	0.5	0
Chromosome4	RM261	17.5726	17.5726	0.1688	1.1878
Chromosome4	RM401	9.8974	27.47	0.0977	-5.7383
Chromosome4	RM5687	8.0947	35.5647	0.0802	4.3642
Chromosome4	RM3317	2.7201	38.2847	0.0272	-40.9277
Chromosome4	Hv4.21	0.8773	39.162	0.0088	10.9299
Chromosome4	RM5749	14.1599	53.3219	0.1379	0.0194
Chromosome4	RM5586	0.893	54.2149	0.0089	-0.4231
Chromosome4	RM273	33.9531	88.1679	0.2955	-0.3046
Chromosome4	RM1388	5.1199	93.2878	0.051	-2.3199
Chromosome4	RM3288	8.3235	101.6113	0.0825	-1.449
Chromosome4	RM3687	11.2381	112.8494	0.1105	1.8258
Chromosome4	RM567	15.0749	127.9243	0.1463	0
Chromosome4	RM127	0	127.9243	0	0
Chromosome4	RM559	7.877	135.8013	0.0781	-0.157
Chromosome4	RM280	4.2555	140.0569	0.0425	0.8193
Chromosome4	RM1359	40.667	180.7238	0.3357	0
Chromosome5	RM1089	0	0	0.5	0
Chromosome5	RM164	35.3963	35.3963	0.3047	0.3581

Chromosome	Marker Name	Interval (cM)	Position (cM)	Recombination frequency	Interference
Chromosome5	RGNMS3250	57.2853	92.6816	0.4082	0.0869
Chromosome5	RM538	67.1395	159.8211	0.4362	0
Chromosome6	RM588	0	0	0.5	0
Chromosome6	RM19288	3.3705	3.3705	0.0337	-11.4857
Chromosome6	RM19311	2.3382	5.7086	0.0234	-34.9906
Chromosome6	RM589	1.3517	7.0603	0.0135	4.7957
Chromosome6	RM225	13.4396	20.4999	0.1312	0
Chromosome6	RM584	0	20.4999	0	0
Chromosome6	RM217	1.0311	21.531	0.0103	2.3932
Chromosome6	Hv6.40	28.817	50.348	0.26	-1.2249
Chromosome6	RM3	6.4074	56.7553	0.0637	1.675
Chromosome6	RM3628	17.547	74.3024	0.1686	-2.9696
Chromosome6	Hv6.56	8.6718	82.9741	0.0859	-10.1495
Chromosome6	RM528	1.4155	84.3896	0.0142	1.3974
Chromosome6	RGNMS2303	17.3287	101.7183	0.1667	5.3363
Chromosome6	RGNMS2220	10.4963	112.2146	0.1034	0
Chromosome7	RM1364	0	0	0.5	0
Chromosome7	RGN814	61.4184	61.4184	0.4211	0.1638
Chromosome7	RM3691	46.3096	107.728	0.3644	-0.153
Chromosome7	RM432	6.672	114.4	0.0663	-2.212
Chromosome7	RM6394	2.8067	117.2066	0.028	0
Chromosome8	RM310	0	0	0.5	0
Chromosome8	RM22804	19.4426	19.4426	0.1852	-0.3026
Chromosome8	RM339	1.3765	20.8191	0.0138	-5.2889
Chromosome8	RM23150	10.4592	31.2783	0.1031	1.6054
Chromosome8	RM23498	50.9221	82.2004	0.3846	2.0639
Chromosome8	RM1308	9.9104	92.1107	0.0978	0
Chromosome9	RM245	0	0	0.5	0
Chromosome9	RM201	12.7706	12.7706	0.125	2.3282
Chromosome9	RM242	11.6177	24.3883	0.1141	-0.147
Chromosome9	RM257	6.3445	30.7328	0.0631	-1.8431
Chromosome9	Hv9.36	1.7398	32.4727	0.0174	-9.285
Chromosome9	RM566	3.4033	35.876	0.034	0.81
Chromosome9	RM7364	23.8878	59.7638	0.2222	1.152
Chromosome9	RM6920	3.2757	63.0395	0.0327	0
Chromosome10	RM6673	0	0	0.5	0
Chromosome10	RM1859	34.2069	34.2069	0.2971	2.1443
Chromosome10	RM25245	15.8997	50.1066	0.1538	-0.1643
Chromosome10	RM7492	31.0428	81.1494	0.2759	0
Chromosome11	RM7283	0	0	0.5	0
Chromosome11	RM287	16.2825	16.2825	0.1573	-0.7545
Chromosome11	RM21	12.3505	28.633	0.1211	2.5456
Chromosome11	RM1341	1.7551	30.3881	0.0175	-9.1571

Chromosome	Marker Name	Interval (cM)	Position (cM)	Recombination frequency	Interference
Chromosome11	RM26973	4.6866	35.0746	0.0467	38.2321
Chromosome11	Hv11.60	0.431	35.5057	0.0043	-81.4286
Chromosome11	RM26985	3.1013	38.607	0.031	0
Chromosome12	RM1246	0	0	0.5	0
Chromosome12	RM511	8.1356	8.1356	0.0806	-0.4175
Chromosome12	RM1337	6.5591	14.6947	0.0652	0.8772
Chromosome12	RM3103	2.9738	17.6685	0.0297	-0.8698
Chromosome12	RM7619	3.2453	20.9138	0.0324	4.257
Chromosome12	RM247	2.8603	23.774	0.0286	0



ORIGINAL ARTICLE

A trait specific QTL survey identifies NL44, a NERICA cultivar as a novel source for reproductive stage heat stress tolerance in rice

K. T. Ravikiran¹ · S. Gopala Krishnan¹ · K. K. Vinod¹ · Gaurav Dhawan¹ · Priyanka Dwivedi¹ · Pankaj Kumar¹ · Vijay Prakash Bansal¹ · M. Nagarajan² · Prolay K. Bhowmick¹ · Ranjith K. Ellur¹ · Haritha Bollinedi¹ · Madan Pal³ · Amitha C. R. Mithra⁴ · A. K. Singh¹

Received: 7 June 2020 / Accepted: 9 November 2020 / Published online: 24 November 2020
© Indian Society for Plant Physiology 2020

Abstract Rice is sensitive to heat stress at gametogenesis and anthesis stages. For sustaining rice yields under the predicted threat of reproductive stage heat stress (RSHS), identification of tolerant donors as well as mapping of genes governing tolerance is crucial. Recently a NERICA (NEwRIce for AfriCA) rice genotype, NL44 has been reported tolerant to RSHS. The present study aims to survey a recombinant inbred line (RIL) population developed from the cross, Pusa Basmati 1 (PB1)/NL44 using markers

linked to 54 RSHS quantitative trait loci (QTLs) through phenotypic and genotypic characterization. When exposed to RSHS, the susceptible parent PB1 and several RILs showed significant reduction for spikelet fertility and grain yield plant⁻¹ relative to NL44. Both these traits and the estimated stress tolerance index (STI) showed a quantitative pattern of inheritance. Out of the 116 SSR markers surveyed, 31 markers were polymorphic between PB1 and NL44. No discernible associations could be found through a preliminary bulked segregant analysis with these markers. A subsequent single marker analysis revealed five minor QTLs, four for spikelet fertility under heat stress and

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s40502-020-00547-z>) contains supplementary material, which is available to authorized users.

✉ S. Gopala Krishnan
krish.icar@gmail.com

K. T. Ravikiran
rkravikiran@icar.org

K. K. Vinod
kkvinodh@gmail.com

Gaurav Dhawan
gauravbiochem2007@gmail.com

Priyanka Dwivedi
priyankam28@gmail.com

Pankaj Kumar
ky.pankaj@gmail.com

Vijay Prakash Bansal
bansal.vijay0@gmail.com

M. Nagarajan
drmnagarajan2000@yahoo.co.in

Prolay K. Bhowmick
prolaybhowmick@gmail.com

Ranjith K. Ellur
ranjithellur@gmail.com

Haritha Bollinedi
haritha.agric@gmail.com

Madan Pal
madanpal@yahoo.com

Amitha C. R. Mithra
amithamithra.nrcpb@gmail.com

A. K. Singh
aks_gene@yahoo.com

¹ Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

² Rice Breeding and Genetics Research Centre, ICAR-IARI, Aduthurai, Tamil Nadu 612 101, India

³ Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

⁴ ICAR- National Institute for Plant Biotechnology, New Delhi 110 012, India

two for STI-spikelet fertility, of which one QTL was mapped for both the traits. These QTLs, however, could explain a very low level of total phenotypic variation. Additionally, the cumulative additive effect of these QTLs could account only for a possible 30% of the contrast between PB1 and NL44. Thus, the study clearly establishes that NL44 has novel genomic regions for RSHS tolerance.

Keywords Heat stress · Grain yield plant⁻¹ · SSR · Recombinant inbred line (RIL) · Spikelet fertility

Introduction

Climate change poses a serious threat to agricultural productivity worldwide. Since early twentieth century, the earth's mean surface temperature has risen by 0.8 °C, of which about 0.6 °C increase is encountered since 1980 (IPCC 2007). Predictions for future imply that, relative to 1986–2005 period, global mean surface temperature is likely to increase by 0.3–4.8 °C by 2081–2100 period under different greenhouse gas emission scenarios (IPCC 2014). Due to climate change, impending heat waves beyond 35 °C have been predicted in South Asia, particularly in India, at the densely populated agricultural hot-spots of the Ganges and Indus river basins (Im et al. 2017). Since rice is sensitive to heat stress, it is shown to result in significant reduction in grain yield under such adverse occurrences (Jagadish et al. 2007, 2010). Tolerance threshold of rice to heat stress is reported to be around 33 °C (Yoshida et al. 1981; Jagadish et al. 2010). Estimates with various simulation models suggest that rice will record yield loss ranging between 2.5 and 10.0% for every 1 °C rise in earth's surface temperature (Baker et al. 1992; Peng et al. 2004). Being a tropical country, the adversities due to heat stress is expected to be more pronounced in India, than in other rice growing Asian countries (Zhao et al. 2016). Episodes of rice yield decline due to heat stress have already been documented in several countries including India, Pakistan, Bangladesh, China, Thailand, Japan, Australia and the U.S.A. (Osada et al. 1973; Matsushima et al. 1982; Yang et al. 2004; Hasegawa et al. 2009; Tian et al. 2009; Welch et al. 2010).

Response of rice to heat stress differs with the growth stages. Heat stress sensitivity is the highest during early reproductive stages coinciding with booting followed by the anthesis (Satake and Yoshida 1978; Yoshida et al. 1981). Susceptibility at these stages results in severe yield penalty because anthers and pollens suffer severe desiccation leading to poor fertilisation and ultimate yield loss, particularly at prolonged temperatures over 35 °C. Additionally, the injury to male reproductive organ is manifested as abnormal anther dehiscence, poor pollen

production, and low pollen germination rate (Osada et al. 1973; Matsushima et al. 1982; Matsui et al. 1997, b; Jagadish et al. 2007, 2008, 2010; Prasad et al. 2006).

Rice exhibits diverse adaptation to heat stress particularly rendering the classification as upland and lowland cultivars. Upland cultivars show better tolerance to heat stress especially during terminal stages than wetland cultivars. Heat stress tolerance in rice has been reported to be governed by a number of quantitative trait loci (QTLs) (Jagadish et al. 2010; Ye et al. 2012, 2015a, b; Shanmugavadivel et al. 2017; Li et al. 2018). QTLs for reproductive stage heat stress (RSHS) tolerance in rice have been reported on all the chromosomes (Arshad et al. 2017). Among the traits related to RSHS, spikelet fertility has been the most frequently analysed trait. Besides, floral traits such as anther length and anther dehiscence have also been targeted (Tazib et al. 2015). In order to map these QTLs, markers such as simple sequence repeats (SSRs), restriction fragment length polymorphism (RFLP) and single nucleotide polymorphism (SNP) have been utilized in several studies (Ye et al. 2012, 2015a, b; Shanmugavadivel et al. 2017). However, most of the QTLs reported, except those by Ye et al. (2015a), have not been fine mapped and validated. Among the donors for RSHS tolerance, Nagina 22 (N22) has been the most widely studied.

Development of resilient cultivars tolerant to heat stress is an important pre-emptive strategy for sustaining rice production under the projected rise in temperature. Considering the complexity of RSHS tolerance and the growing importance of this problem, it is essential that diverse donors be identified, and QTLs mapped. An attempt towards this has resulted in the identification of a heat tolerant rice genotype among the NERICA (NEwRice for AfriCA) cultivars, Nerica L 44 (NL44). A comparative study of this line *vis-a-vis* N22, an established RSHS tolerant cultivar, has identified NL44 to perform at par with N22 in terms of spikelet fertility and physiological resilience, making it a strong candidate for further studies (Bahuguna et al. 2015). However, the genomic regions imparting the high RSHS tolerance of NL44 has not yet been identified. To address this, a recombinant inbred line (RIL) population was developed at the Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi involving Pusa Basmati 1 as the RSHS sensitive parent and NL44 as the tolerant parent. Keeping in perspective of the above, as a first step enroute to QTL mapping, the present investigation was carried out to check for the presence of any previously reported RSHS tolerance QTLs in NL44, by investigating marker trait association in the RIL population.

Materials and methods

Plant material

A population of 127 RILs was developed through single seed descent method from the cross Pusa Basmati 1/NL44. The RSHS sensitive parent, Pusa Basmati 1 is the world's first high yielding semi-dwarf Basmati rice variety released by ICAR-IARI, New Delhi. Developed through convergent breeding strategy from the cross Pusa150/Karnal local (Siddiq et al. 2009), Pusa Basmati 1 has become highly popular among the farmers and the consumers, particularly for its extra-long grain type, grain and cooking quality and mild aroma. Albeit it's superior qualities, this variety is highly sensitive to various biotic and abiotic stress in general and RSHS in particular. As mentioned previously, NL44 shows significant RSHS tolerance exhibiting higher seedling survival rate, superior growth and greater reproductive resilience, even when exposed to 42.2 °C under field conditions (Bahuguna et al. 2015). Furthermore, NL44 maintains higher spikelet and pollen fertility, as that of the widely documented RSHS tolerant *aus* variety N22 (Yoshida et al. 1981; Prasad et al. 2006; Jagadish et al. 2008; Rang et al. 2011). The RILs were advanced to F₇ generation, following standard agronomic management.

Heat stress screening

The RILs along with the parents were evaluated under natural field conditions at IARI – Rice Breeding and Genetics Research Centre at Aduthurai in Tamil Nadu (11.0154° N, 79.4809° E). Two staggered sowings were made during the *Rabi* season of 2017–2018, with the first sowing timed to coincide the normal growing season and the second sowing to coincide with the heat stress during flowering. This ensured two contrasting environments, the first sown set did not experience any stress (unstressed) while the second set experienced the heat stress (stressed) beginning from anthesis. Experiments were laid out in an incomplete block design, with parents replicated seven times. Plots were of uniform size with 3 m length and 0.6 m width. A spacing of 20 cm between rows and 15 cm between plants was maintained. At flowering, five randomly selected plants per genotype were tagged and pollen fertility (%) was determined using I₂-KI staining method. For this, the primary tiller florets that were exposed to heat stress were sampled. The day prior to the anthesis, three spikelets per each tagged plant were fixed in Carnoy's fixative (three parts of 70% ethanol mixed with one part of glacial acetic acid). Three anthers from each spikelet were processed in I₂-KI stain. The stained pollens were observed under a Nikon Alphaphot-2 YS2 compound microscope

(Tokyo, Japan) using 100 × magnification. The pollen grains which imbibed stain and appeared dark blue were considered fertile, while the unstained ones were treated sterile. The proportion of fertile to total number of pollen grains per microscopic field was used to calculate pollen fertility and expressed in percentage. After physiological maturity, the tagged plants were also used to measure traits such as grain yield plant⁻¹ and spikelet fertility (%). For estimating spikelet fertility, the panicle from primary tiller was sampled. Spikelet fertility was calculated as the ratio of filled spikelets to total number of spikelets per panicle and expressed as percentage. The observations averaged over five plants were used for statistical analyses. Further, stress tolerant index (STI; Fernandez 1992) was calculated for grain yield plant⁻¹ and spikelet fertility to assess the relative performance of RILs under heat stress using the following formula,

$$\frac{(Y_s)(Y_p)}{Y_p^2}$$

where Y_s and Y_p are the average yield/spikelet fertility of genotypes under stressed and unstressed conditions, respectively, while Y_p represents mean yield/spikelet fertility of all genotypes under unstressed condition.

Molecular marker selection and bulked segregant analysis

We have used two approaches to narrow down to the putative QTLs possessed by the NL44 for RSHS tolerance. For this, an exhaustive survey of literature was conducted to inventory the RSHS QTLs reported in rice. Information on the linked SSR, SNP and RFLP markers were collated. Around 116 SSR markers were selected for polymorphism survey between the parents (NL44 and Pusa Basmati 1). N22 was used as a positive control. For molecular analysis, the total genomic DNA was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) method from young leaves (Doyle and Doyle 1990). The DNA concentration and quality were checked using a Nano spectrophotometer (Microdigital Co. Ltd., Korea). For PCR amplification, a total of 10 µl reaction mix was assembled with 50 ng of template DNA, 5 pmol each of both forward and reverse primers, 0.2 mM dNTPs, 1.5 mM of MgCl₂ and 0.5 U of Taq DNA polymerase (Genei Laboratories Pvt. Ltd., Bengaluru). The PCR program consisted of an initial denaturation for 5 min at 95 °C, followed by 35 cycles consisting of 30 s denaturation at 95 °C, 30 s annealing at 55 °C and 1 min extension at 72 °C. After 35 cycles, the final extension was rendered at 72 °C for 7 min and the products were cooled to 4 °C. The amplicons were resolved through electrophoresis in 3.5% agarose gel (Seakem LE Agarose, Lonza, USA) mixed with 0.1 mg/ml

of ethidium bromide. A 100 bp ladder DNA (GeneDireX, USA) was used as standard for comparison between amplicons by electrophoresis and visualisation under transillumination in a gel documentation system (BioRad, USA).

In the initial approach to run a bulked segregant analysis (BSA), only those markers which were polymorphic between the parents were utilized. Thirteen genotypes (10% of RILs, i.e., ~ 13) that were found to show extreme phenotypes for grain yield plant^{-1} , spikelet fertility and pollen fertility were selected to construct two DNA pools, one for tolerant and the other for sensitive. The genomic DNA of the selected RILs was normalized to a concentration of $50 \text{ ng } \mu\text{l}^{-1}$ using TE buffer and equal volume of DNA ($10 \mu\text{l}$) was pooled to construct the bulks. The polymorphic SSR markers were then surveyed over the bulks and the parents. In the second approach, all the polymorphic markers were analysed over the entire RIL population. A simple linear regression was conducted to test the linkage between the putative markers and the trait variables, in order to determine the phenotypic variance explained.

Results

The two sowing windows used in the experiment provided adequate exposure to different temperatures during the flowering period (Fig. 1). In the first sowing window, the maximum temperature ranged from 32 to 37 °C during active flowering and grain formation, with an average of

35.5 °C. While in the second window, the maximum ambient temperature exceeded the critical minimum temperature of 35 °C completely during the flowering period. The maximum temperature during this period ranged between 37.0 and 42.0 °C with an average temperature of 40.3 °C.

Phenotypic characterization of the RILs and parents

The analysis of variance (ANOVA) revealed significant difference between the two sowing windows for all the traits (Table 1), while the genotypes including the parents and the RILs showed insignificant differences barring grain yield. The genotype by sowing time interaction was also significant for spikelet fertility and grain yield. Among the parents, under normal planting condition when plants were unstressed, average grain yield plant^{-1} of NL44 was 22.7 g and that of PB1 was 23.2 g (Table 2). Corresponding spikelet fertility was 77.8% and 71.0% in NL44 and PB1 respectively. However, the pollen fertility did not vary among the parents under unstressed conditions. Whereas, under late planted situation, when the plants experienced RSHS, the sensitive parent PB1 recorded a grain yield of 7.5 g plant^{-1} as against 14.6 g plant^{-1} in NL44 (Fig. 2). The yield reduction in NL44 was 35.7%, while in PB1 it was 67.7%. Similarly, significant reduction of pollen and spikelet fertility were also observed in PB1, while in NL44 the spikelet fertility (~ 77.0%) and pollen fertility (> 93.0%) remained almost similar in both stressed and unstressed conditions. Relative reduction in spikelet

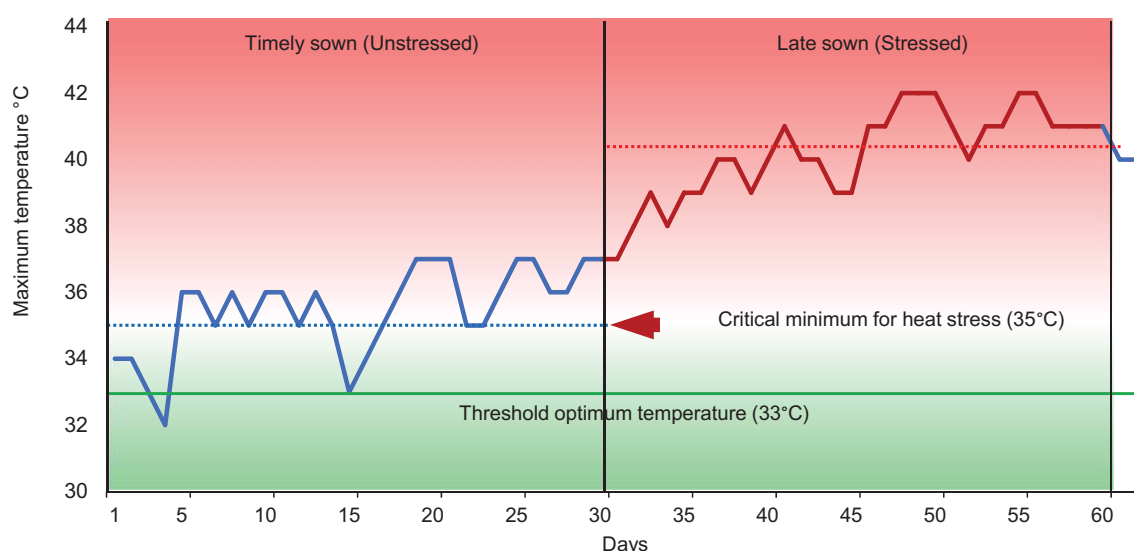


Fig. 1 Daily maximum temperatures experienced during the flowering period by the RILs sown timely and late, indicating the exposure to high temperature. The dotted lines indicate the average reproductive stage maximum temperatures for the two sowing windows. *The

30-day window indicated for timely sown (unstressed) and late sown (stressed) set of RILs coincides with 80–110 days after sowing

Table 1 Analysis of variance (ANOVA) of different traits between two staggered sowing

Source of variation	Df	Mean squares		
		Pollen fertility (%)	Spikelet fertility (%)	Grain yield plant ⁻¹ (g)
Sowing time	1	3217.5**	26,539.4**	5689.3**
Genotypes	256	49.7	120.0	3997.5**
Genotypes × sowing time	256	48.6	97.8**	1482.9**

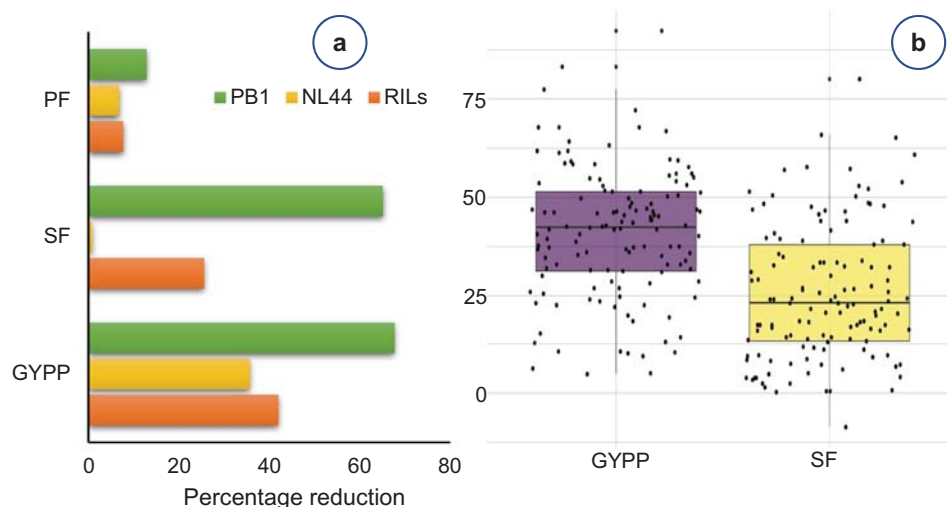
Df degrees of freedom

**Significant at 99% confidence level

Table 2 Summary statistics for grain yield, plant⁻¹, spikelet fertility (%) and pollen fertility (%) recorded in the 127 RILs and parents under timely sown (unstressed) and late sown (high temperature stressed) conditions

Statistic	Grain yield, plant ⁻¹ (g)		Spikelet fertility (%)		Pollen fertility (%)	
	Unstressed	Stressed	Unstressed	Stressed	Unstressed	Stressed
Range	9.6–38.2	2.1–22.7	64.0–90.8	16.6–82.2	97.0–100.0	40.4–99.9
Mean	22.4	13.0	79.1	58.9	98.2	90.7
SE	0.45	0.37	0.51	1.17	0.06	0.88
SD	5.08	4.17	5.74	13.23	0.74	9.89
Kurtosis	0.09	– 0.45	– 0.29	– 0.16	– 1.01	8.50
Skewness	– 0.15	0.09	– 0.36	– 0.52	– 0.38	– 2.74
NL44	22.7	14.6	77.8	77.2	100.0	93.3
PB1	23.2	7.5	71.0	24.8	99.0	86.3
CV (%)	22.7	32.0	7.3	22.5	0.73	10.9

SE standard error, SD standard deviation, CV (%) coefficient of variation in per cent

Fig. 2 Relative reduction in different traits **a** expressed in percentage among parents and RILs under reproductive stage temperature stress. Traits are pollen fertility (PF), spikelet fertility (SF) and grain yield plant⁻¹ (GYPP). **b** Box plots with jitters depicts the distribution of per cent reduction values of genotypes for GYPP and SF

fertility in PB1 was 65.1%, with a corresponding 12.8% reduction in pollen fertility.

Among the RILs, however, the mean grain yield plant⁻¹ ranged between 9.6 and 38.2 g plant⁻¹ with a mean of 22.4 g under unstressed conditions (Table 2). While under RSHS, the per plant grain yield among the RILs ranged from 2.1 to 22.7 g, with a mean of 13.0 g. With spikelet fertility, the RILs showed a range of 64.0–90.8% under unstressed conditions, and 16.6–82.2% under stress.

Corresponding averages in spikelet fertility among the RILs were 79.1% and 58.9%, respectively for stressed and unstressed treatments. Drawing a parallel to the parental performance, pollen fertility among the RILs was in a range of 97.0–100.0 with a mean of 98.2% under unstressed situation. Similar reduction was also observed in the RILs in comparison to the control. The per cent reduction under stress over non-stress showed that the grain yield reduction was relatively higher (mean reduction of 41.9%)

compared to per cent spikelet fertility (mean reduction of 25%) (Fig. 2). Furthermore, stress tolerance index (STI) was calculated for grain yield plant^{-1} and spikelet fertility as it is more indicative of relative tolerance of RILs (Table 3). The contrast between parents was more vivid in terms of STI calculated for spikelet fertility than for the grain yield *per se*.

The RILs exhibited normal distribution for grain yield and spikelet fertility. Whereas, pollen fertility showed a skewed distribution towards fertility (Fig. 3). The frequency distributions for these traits are indicative of their quantitative nature of inheritance. Grain yield plant^{-1} showed a near normal distribution followed by spikelet fertility under both stressed and unstressed conditions. Also, there were RILs with extreme values than the parents both under stressed and unstressed conditions, indicating transgressive segregation of heat stress response. The STI calculated from grain yield and spikelet fertility also showed near normal distributions.

Significant correlation was observed between grain yield plant^{-1} and spikelet fertility under heat stress conditions (Fig. 4). Pollen fertility showed very weak correlation with either grain yield per plant^{-1} , spikelet fertility and also with the STI computed from these traits. This is further evident from significant regression coefficient of spikelet fertility over grain yield (Supplementary Fig. 1). Despite the exposure to high temperature stress, pollen fertility among the RILs as well as the parents was relatively less affected. Contrary to the expectations, at higher temperature majority of the genotypes recorded higher pollen fertility, although their spikelet fertility remained lower (Supplementary Fig. 2). This loss of apparent correlation between pollen and spikelet fertilities could also be observed as high skewness of pollen fertility (– 2.74).

Quantitative trait loci (QTLs) for reproductive stage heat stress tolerance

Already reported 109 QTLs associated with RSHS tolerance and related traits are compiled in Fig. 5. A detailed list of these QTLs is summarized in Supplementary Table 1. Apparently, all the chromosomes are reported to hold QTLs associated to RSHS response. The number of QTLs reported was highest on chromosome 4 (18 QTLs)

followed by chromosome 1 (15 QTLs) and chromosome 3 (13 QTLs). Remaining chromosomes has number of QTLs ranging from two (Chromosome 12) to eleven (chromosome 5). Distinct ‘QTL hot spots’ could be delineated on chromosome 1 within the genomic region spanning from 38 to 40 Mb. There was a QTL hotspot found on chromosome 2 between 20 and 25 Mb region, that harboured five QTLs. On chromosome 4, there were two hotspots, one within 26–33 Mb region having eight QTLs, and another between 15 and 20 Mb with seven QTLs. Hotspot on chromosome 5 was within a shorter span between 0.1 and 3.1 Mb region, but possessed seven reported QTLs. On chromosome 8, four QTLs were located within a 1 Mb span, beginning from 24 Mb position. Further, on chromosome 10, there were four QTLs located within 18.7–20.8 Mb region.

Majority of the earlier mapping studies for RSHS tolerance used SSR markers, while a few recent studies used SNP markers (Ye et al. 2012, 2015a, b; Shanmugavadivel et al. 2017). Also, there are few initial studies that used RFLP markers. Among the donors investigated, most common donors were 966 and N22, followed by Azucena, Habataki and Kasalath. Different types of mapping populations such as F_2 , RILs, backcross inbred lines (BILs), doubled haploids (DH) and chromosome segment substitution lines (CSSLs) were used in different studies. The size of these populations ranged from 37 CSSLs (Zhao et al. 2016) to 310 BC_2F_2 lines (Buu et al. 2014). Many of the studies were conducted under controlled glass house conditions and the temperatures were maintained above 35 °C for heat stress treatment, while ~ 31 °C was used as unstressed treatment. However, in some of the studies, genotypes were exposed to heat stress under natural field conditions by adjusting their sowing dates to coincide the stress onset as done in the present study (Buu et al. 2014; Tazib et al. 2015; Zhao et al. 2016; Zhu et al. 2017; Seo et al. 2019). Another important feature of the earlier studies was the use of spikelet fertility as the single most important trait for mapping RSHS tolerance. Interestingly, grain yield was the least explored trait. Additionally, there was also a wide variation in the proportion of phenotypic variation explained (PVE) for the target trait reported with the QTLs. Reported PVEs ranged from 6 to 36% (Buu et al. 2014). Based on these studies, we have compiled a set of

Table 3 Summary of heat stress indices measured for grain yield, plant^{-1} and spikelet fertility among the RILs from PB1/NL44 cross

Indices	RILs					NL44	PB1
	Minimum	Maximum	Mean	Range	SE		
STI _(sf)	0.22	1.12	0.74	0.90	0.01	0.96	0.28
STI _(gy)	0.10	1.57	0.59	1.46	0.02	0.66	0.35

STI_(sf), stress tolerance index for spikelet fertility; STI_(gy), stress tolerance index for grain yield, plant^{-1} ; SE, standard error

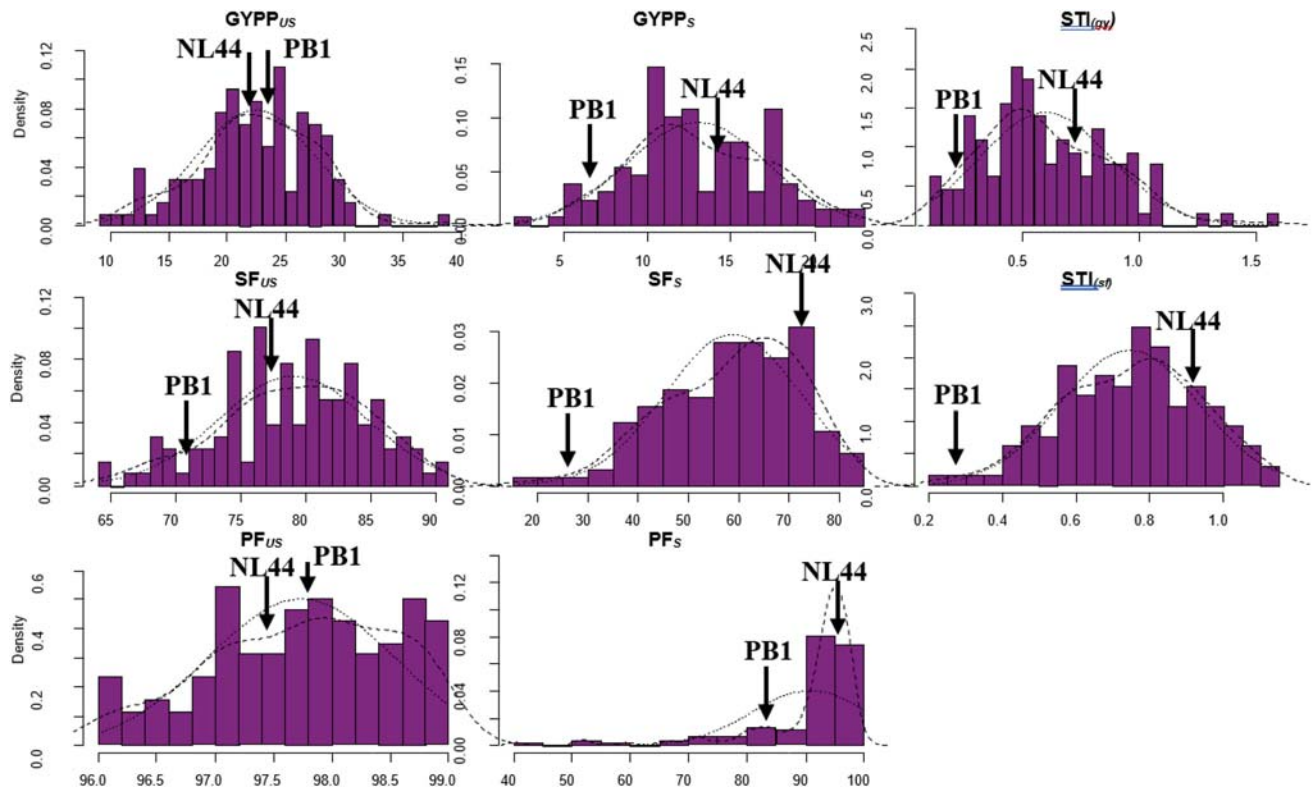


Fig. 3 Frequency distributions of grain yield plant⁻¹ (GYPP), spikelet fertility % (SF) and pollen fertility % (PF) under unstressed (US) and stressed (S) conditions with reproductive stage heat stress show near

normal distribution. The stress tolerance indices calculated for grain yield per plant $STI_{(gy)}$ and spikelet fertility % $STI_{(sf)}$ also show normality of distributions

116 QTL linked SSR markers for further analysis in the present study (Supplementary Fig. 3).

Parental polymorphism

Out of a total of 109 QTLs investigated, 54 QTLs were shortlisted based on the use of SSR markers and 116 linked SSR markers have been used that were distributed across all chromosomes (Supplementary Fig. 3). Genome wide survey of the two contrasting parents for RSHS response, PB1 (sensitive) and NL44 (tolerant) using the selected SSR markers had resulted in identification of 31 polymorphic markers (Table 4), indicating a putative 26.7% diversity between them. Of the polymorphic markers, the highest number was found on chromosome 4 (7 markers) followed by chromosome 3 (5 markers) whilst the least number of markers were from chromosome 7, 9 and 12 (1 marker).

Bulked segregant analysis

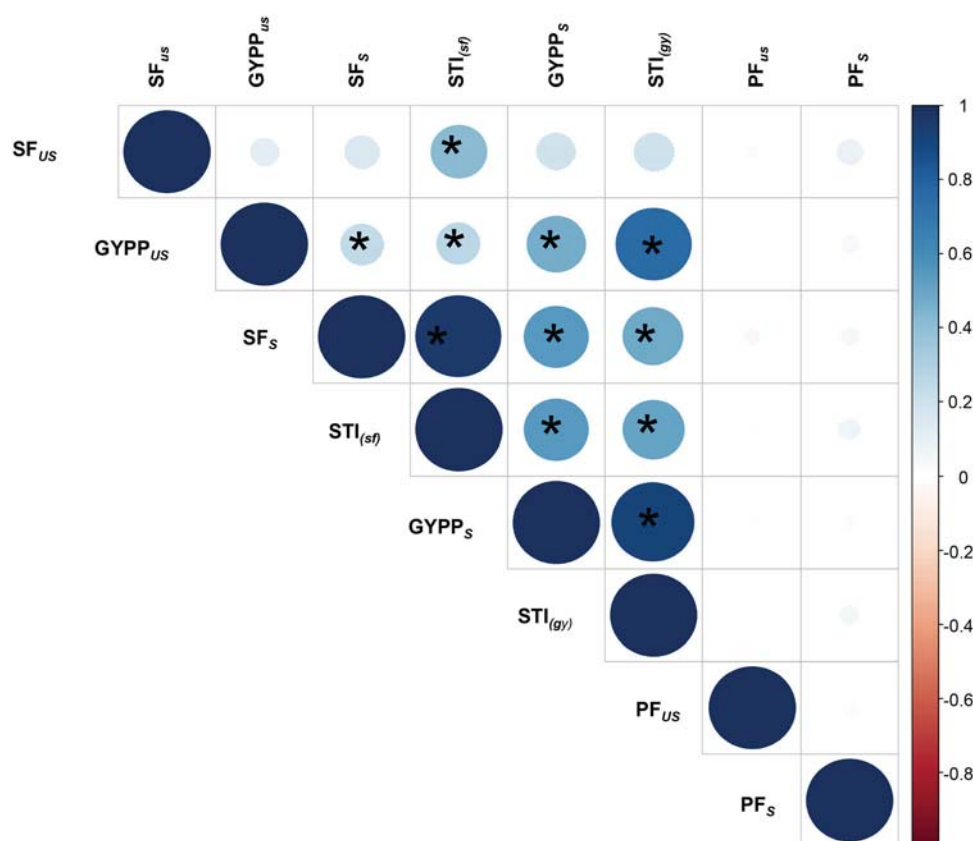
Thirteen genotypes that represent about 10% of the RILs, were drawn from extremes of phenotypic distribution based on grain yield plant⁻¹ (Supplementary Fig. 4). The lines were also compared for the effect on spikelet fertility. The criteria for selection was the highest and lowest per cent of

reduction for the target trait under heat stress over control. The lines with higher reduction were used to constitute the sensitive bulks, while those with lowest percent of reduction was used for constituting the tolerant bulk. The marker-based survey of the bulks along with the parents, however, could not resolve the phenotypic difference between the bulks.

Single marker analysis

Since no putative linkage between the marker and the traits could be established through BSA, we could assume that none of the already reported markers are adequate enough to associate to the RSHS tolerance in NL44. However, to resolve any weaker association, all the 31 polymorphic markers were run on the entire RIL population. Based on the segregation pattern, a subset of 19 markers that showed stark differences between the parents were scored. The subsequent single markers analysis revealed few significant associations (Table 5), particularly with spikelet fertility. The PVE, however, ranged between 3.3 and 6.9. The highest PVE was obtained for RM6394 for STI calculated for spikelet fertility (STI_{sf}), while the lowest was with RM7283 for spikelet fertility under stress (SF_s). Only one marker RM401 showed significant association both for

Fig. 4 Correlation plot between grain yield plant⁻¹ (GYPP), spikelet fertility % (SF) and pollen fertility % (PF) under unstressed (US) and stressed (S) conditions with reproductive stage heat stress as well as with the estimated stress tolerance indices for grain yield plant⁻¹ STI_(gy) and spikelet fertility % STI_(sf). The strength of the association is depicted by the colour code given in the legend and the significant (p value < 0.05) associations were indicated by asterisk



spikelet fertility under heat stress (SF_S) and STI for spikelet fertility (STI_(sf)). No significant associations were obtained for grain yield plant⁻¹ under stress and its respective STI. This warrants a detailed QTL mapping study involving genome wide markers to identify the genomics regions governing reproductive stage heat tolerance in NL44 using the RIL population.

Discussion

As per the IPCC fifth assessment report, heat stress coinciding with the reproductive stages of various crops is a serious threat to global food security (IPCC 2014). Frequent occurrence of heat stress could deter the crop yield potential in major food crops in general and rice in particular for two obvious reasons (1) it is the most important crop that is grown exclusively under flooded environments, and extensively in the most populated parts of the world (Redfern et al. 2012), and (2) is highly sensitive to heat stress at reproductive stages (Satake and Yoshida 1978; Yoshida et al. 1981). Although estimates of yield loss due to RSHS in rice around the world indicates an incoherent pattern, yield reduction up to 60% or higher can be expected depending upon the rice ecology and the duration and intensity of high temperature exposure. Rice breeding

programmes are to be devised to develop varieties tolerant to heat stress in order to be better equipped for the predicted temperature spikes.

Reduction of spikelet fertility and the consequent grain yield are the important ramifications of heat stress encountered in rice. In agreement to this fact, spikelet fertility and grain yield plant⁻¹ were significantly affected under heat stress at reproductive stage in the present study, particularly among the sensitive parent PB1 and sensitive set of RILs. This was ascertained by the clear contrast observed between parents, NL44 and PB1. The estimated advantage of the tolerant parent, NL44 over PB1 was two times in terms of yield, and three times in terms of spikelet fertility. This establishes the superiority of NL44 as a RSHS tolerant cultivar, a candidate donor for future rice improvement programmes. Transgressive segregants for spikelet fertility, particularly exceeding tolerant parent in the mapping population was also observed by Xiao et al. (2011a), Cheng et al. (2012), Ye et al. (2012), Li et al. (2018), and Cao et al. (2020). The contrast observed between the parents for spikelet fertility was noteworthy in the present study, because this supersedes earlier reported contrasts such as those between Azucena and Bala (Jagadish et al. 2010), Xiushui 09 and IR2061 (Cheng et al. 2012), IAPAR-9 and Liaoyan 241 (Li et al. 2018), and between IR64 and Koshihikari (Seo et al. 2019). However,

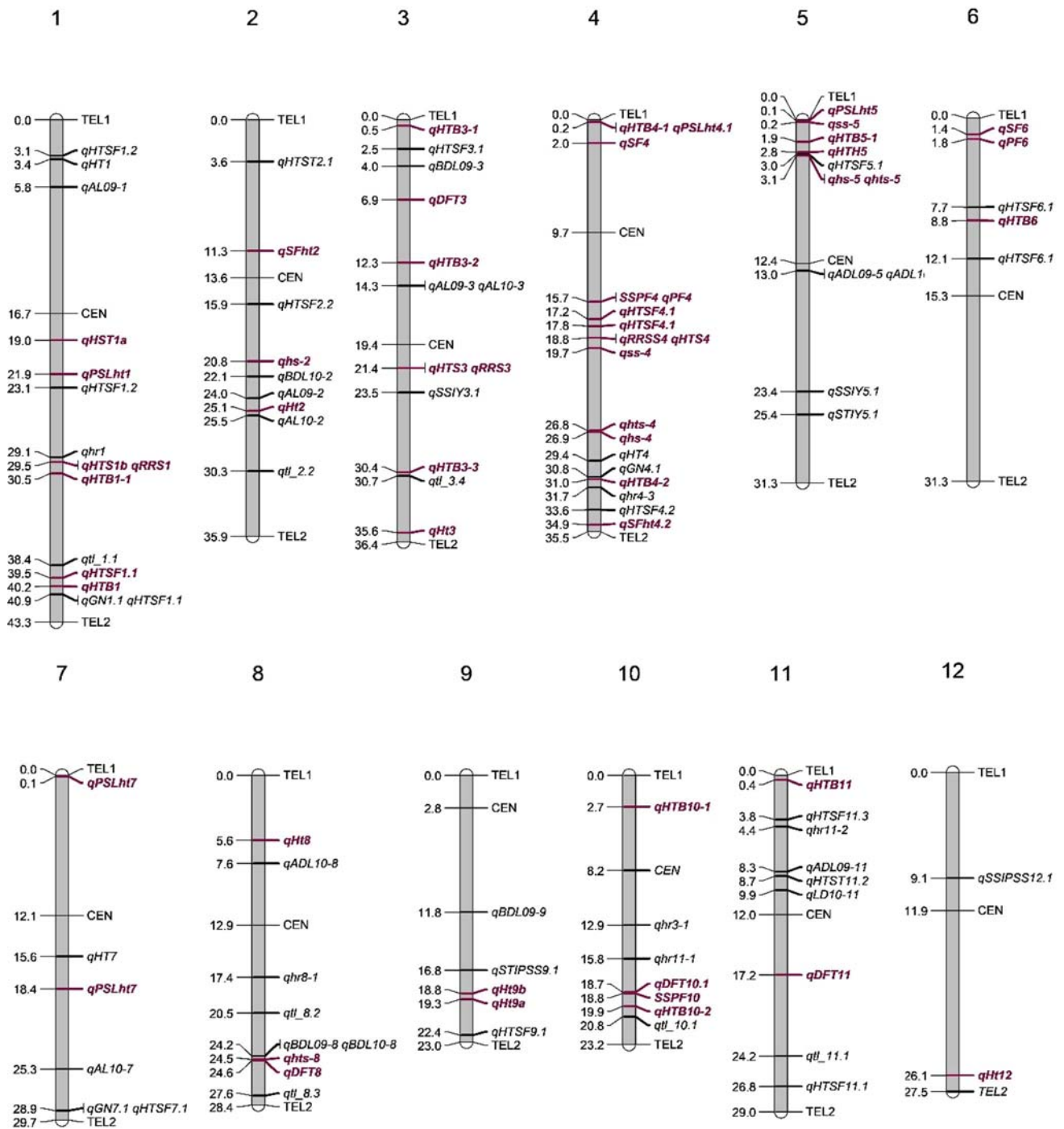


Fig. 5 The physical positions (in Mb) of various QTLs previously reported for reproductive stage heat stress tolerance in rice. The QTLs analysed in the present study using SSR markers are highlighted in

purple colour. TEL1 and 2 indicates telomeres and CEN represents centromere. The additional details of the QTLs are provided in supplementary table S1

superior contrasts than that in this study was also reported between cultivars such as 996 and 4628 (Xiao et al. 2011a), Sasanishiki and Habataki (Zhao et al. 2016; Zhu et al. 2017), N22 and IR64 (Ye et al. 2012) and between R53 and HHT4 (Cao et al. 2020). Moreover, the mean spikelet fertility of 77% under stressed conditions exhibited by

NL44 was higher than that was observed by Bahuguna et al. (2015) under similar temperature regime (38 °C) in the same cultivar. Also, the higher spikelet fertility among the RILs than that was reported in many of the previous studies indicated that NL44 can act as a potential donor for this trait. These observations were further corroborated by

Table 4 Summary of parent polymorphism survey carried out using reported QTL linked SSR markers reproductive stage heat stress tolerance

Chromosome no.	Number of QTL linked markers	Number of polymorphic markers ^a
1	11	3 (27%)
2	7	2 (29%)
3	22	5 (23%)
4	24	7 (29%)
5	10	3 (30%)
6	9	2 (22%)
7	4	1 (25%)
8	9	2 (22%)
9	4	1 (25%)
10	8	2 (25%)
11	6	2 (33%)
12	2	1 (50%)
Total	116	31 (26.7%)

^aThe percentage of polymorphic markers are given in parenthesis**Table 5** Markers showing significant association with the respective traits revealed through single marker analysis

Marker	SF _S			STI _(sf)			Linked QTL	
	PVE	Probability	Additive effect	PVE	Probability	Additive effect	QTL ID	Reported PVE
RM3355	5.7	0.016	– 3.03				<i>qhs2</i>	6.4
RM401	3.8	0.037	2.69	3.8	0.0261	0.02	<i>qSF4</i>	10.3
RM5687	4.8	0.026	3.05				<i>SSPF4, qPF4</i>	21.3
RM6394				6.9	0.0027	0.03	<i>qPSLht7</i>	–
RM6673	3.6	0.037	2.59				<i>qHTB10-2</i>	–
RM7283	3.3	0.047	2.49				<i>qHTB11</i>	–

SF_S, spikelet fertility under stress; STI_(sf), stress tolerance index for spikelet fertility; PVE, percent variation explained (%)

the estimated STIs for spikelet fertility and grain yield. These indices were previously utilized by Shanmugavadivel et al. (2017) for mapping reproductive stage heat stress tolerance.

One of the most interesting observations in this study was the stability of pollen fertility in NL44 and PB1 under RSHS. A similar pattern was also observed among the RILs. This implies that pollen fertility *per se* was not the factor that led to spikelet sterility and the consequent yield reduction in PB1 under RSHS. There are several factors that define successful fertilization and grain development in rice, that are attributable to high temperature tolerance/sensitivity. Such factors include anther dehiscence, pollen desiccation, poor pollen germination, drying up of stigma as well as embryo abortion. Some other impairment on the male reproductive system could also not be ruled out (Jagadish et al. 2010, 2015). The I₂-KI staining method, that is routinely used to estimate pollen fertility, captures the starch accumulation in the pollen grains which is essential for the germination on the stigma. Nevertheless, other

pollen traits such as pollen coat, composed of other biochemical compounds such as lipids and proteins, also play a vital role in pollen viability. Basing pollen viability solely on starch-based iodine staining may therefore be a false positive for fertility and may lead to misleading conclusions (De Storme and Geelen 2014). Therefore, rather than pollen fertility, spikelet fertility must be considered as the most reliable trait for determining RSHS response. Besides, spikelet fertility has been found to have an important bearing on grain yield under heat stress and hence qualifies as an important yardstick to judge genotypes for their heat stress tolerance (Prasad et al. 2006). Furthermore, we found that the spikelet fertility, grain yield and the derived stress tolerance indices followed normal distribution, qualifying these traits for mapping because of their true quantitative nature of inheritance, as reported earlier (Jagadish et al. 2010; Cheng et al. 2012; Ye et al. 2012, 2015a; Zhao et al. 2016; Shanmugavadivel et al. 2017; Zhu et al. 2017) meriting a QTL mapping study to identify the concerned genomic regions in further studies.

As previously mentioned, there has been studies that have mapped QTLs for RSHS tolerance in rice. Therefore, this warrants a pre-survey of the reported QTLs on any identified donor source before declaring it to be novel. BSA has been exemplified as a simple and rapid method to identify markers putatively linked to the target genomic regions when parents are highly contrasting for the trait of interest (Michelmore et al. 1991). This approach not only saves time but also saves resources that would otherwise go into mapping exercise. Propelled by next-generation sequencing (NGS) technologies, it has emerged as a robust alternative and method of choice for mapping studies in various crops (Takagi et al. 2013a, b; Zou et al. 2016). Since the parents used in this study, showed adequate contrast for RSHS response, we have employed BSA as the prefatory approach towards excluding the already known QTLs, if any. However, the polymorphic markers that defined 27% diversity between the parental genomes failed to strike any relation with the markers and the traits, with which the bulks were constituted such as grain yield reduction and spikelet fertility. It may be argued that the number of SSR markers used were limited, and the complexity of the trait itself (reproductive stage heat tolerance) might have been the reason for the lack of any putative association in BSA. This indicated that there may not be a specific set of markers or genomic regions among the ones targeted in the present study, that defined the RSHS response among the parents, hence the bulks failed to expose them.

In order to identify any QTLs that are involved in the stress response, we have run the polymorphic markers on all the RILs, leading to identification of six minor QTLs. The corresponding linked markers were distributed on chromosomes 2, 4, 7, 10 and 11 and were reported associated to QTLs, viz., *qhs2*, *qSF4*, *SSPF4*, *qPF4*, *qPSLht7*, *qHTB10-2* and *qHTB11*, respectively. However, the cumulative effect of these minor QTLs when exposed to RSHS also could not explain the contrast between NL44 and PB1, as well as between the bulks. For instance, the cumulative additive effect of these minor QTLs on NL44 was 7.86% for spikelet fertility, while the contrast between NL44 and PB1 was 52.4%. Therefore, the minor QTLs that were already identified from other genetic backgrounds could explain only 30% of the contrast between NL44 and PB1, leading us to conclude that NL44 must be possessing additional regions that may contribute to its high RSHS tolerance. Divulgence of these novel QTL regions requires exploration of genome wide variation between PB1 and NL44.

Among the numerous QTLs reported for RSHS tolerance, two major QTLs, *qHTSF1.1* and *qHTSF4.1* located respectively on chromosome 1 and 4, were reported from N22 for spikelet fertility (Ye et al. 2012). Notably in this

study, the markers RM431 and RM5757 linked to *qHTSF1.1* and *qHTSF4.1* respectively (Ye et al. 2012, 2015a, b; Vivitha et al. 2017), were monomorphic between NL44 and PB1. Both the genotypes possessed the alternate allele for these markers in contrast with N22, emphasizing that heat stress tolerance in NL44 could be different from that of N22. However, we could identify six minor QTLs, associated with markers related to seven putative QTLs already reported. Among these, the marker RM3355 was reported to be associated with spikelet fertility (*qhs2*) under high temperature (Zhao et al. 2006). Another marker, RM401 linked to *qSF4* located on chromosome 4 at physical location of 2.03 Mb (Cheng et al. 2012) was found associated to both spikelet fertility as well as STI in this study. This QTL located away from one of the robust QTLs, *qHTSF4.1* (17.69 Mb), could not be therefore implicated as associated to *qHTSF4.1*. Similarly, RM5687 that was reported associated with seed set percentage, *SSPC4* (Xiao et al. 2011a) and also to the QTL, *qPF4* for pollen fertility (Xiao et al. 2011b), was also located on chromosome 4. A marker on chromosome 7, RM6394 that was reportedly linked to pollen shedding level under heat stress (*qPSLht7*) (Zhao et al. 2016) was identified for spikelet fertility in our study. Further, another linked marker to spikelet fertility RM6673, was earlier reported to be associated with two QTLs (*qHTB10-2* and *qDFT10.1*) from two separate studies (Zhu et al. 2017; Zhao et al. 2016). Finally, the marker RM7283, that was associated with *qHTB11* as reported for spikelet fertility by Zhu et al. (2017) was also found linked to the same trait among the NL44 RILs. Thus, we could demonstrate that the major genomic regions conferring the RSHS tolerance in NL44 may be different, to a larger extent, from those reported in previous studies, and would act in tandem with few minor QTLs to display the tolerance.

Conclusion

In conclusion, the present study established that NL44 may be a plausible novel donor for RSHS tolerance in rice. Among the traits studied, the spikelet fertility has emerged as the most ideal trait for assessing the RSHS response. The SSR markers linked to the reported QTLs were found mostly ineffective in discerning the tolerance conferred by NL44, except for six minor ones. Since the percentage of total variation accounted for by these minor QTLs was low, and the cumulative additive effect of these QTLs was inadequate to explain the contrast between NL44 and PB1, we conclude that NL44 harbour novel genomic regions conferring RSHS tolerance. The total tolerance response of NL44 could be driven by the complementary effects of these minor QTLs with yet to be mapped genomic regions.

Identification of these novel regions require extensive genome wide studies, so that NL44 can be used as a potential donor for breeding RSHS tolerant climate resilient cultivars for the future.

Acknowledgements RKT is thankful to Director, ICAR-CSSRI, Karnal and ICAR-IARI, New Delhi for facilitating his Ph.D. and National Innovations on Climate Resilient Agriculture (NICRA) for funding the research study.

Authors contributions GKS and AKS conceived the idea and framed the research plan. RKT carried out the research work and prepared the manuscript. NM and PKB assisted in the execution of field trials. MACR gave inputs for the molecular marker analysis. DG, DP, PK and VP assisted in molecular work. VKK and ERK assisted in data analysis. MP shared donor seed material. GKS and HB improved the manuscript. AKS provided overall guidance in each of these activities.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

- Arshad, M. S., Farooq, M., Asch, F., Jagadish, S. V. K., Prasad, P. V. V., & Siddique, K. H. M. (2017). Thermal stress impacts reproductive development and grain yield in rice. *Plant Physiology and Biochemistry*, 115, 57–72.
- Bahuguna, R. N., Jha, J., Pal, M., Shah, D., Lawas, L. M., Khetarpal, S., et al. (2015). Physiological and biochemical characterization of NERICA-L-44: A novel source of heat tolerance at the vegetative and reproductive stages in rice. *Physiologia Plantarum*, 154(4), 543–559. <https://doi.org/10.1111/ppl.12299>.
- Baker, J. T., Allen, L. H., Jr., & Boote, K. J. (1992). Temperature effects on rice at elevated CO₂ concentration. *Journal of Experimental Botany*, 43(7), 959–964. <https://doi.org/10.1093/jxb/43.7.959>.
- Buu, B. C., Ha, P. T. T., Tam, B. P., Nhien, T. T., Van Hieu, N., Phuoc, N. T., et al. (2014). Quantitative trait loci associated with heat tolerance in rice (*Oryza sativa* L.). *Plant Breeding and Biotechnology*, 2(1), 14–24. <https://doi.org/10.9787/pbb.2014.2.1.014>.
- Cao, Z., Li, Y., Tang, H., Zeng, B., Tang, X., Long, Q., et al. (2020). Fine mapping of the *qHTB1-1* QTL, which confers heat tolerance at the booting stage, using an *Oryza rufipogon* Griff. introgression line. *Theoretical and Applied Genetics*. <https://doi.org/10.1007/s00122-020-03539-7>.
- Cheng, L. R., Wang, J. M., Uzokwe, V., Meng, L. J., Yun, W. A. N. G., Yong, S. U. N., et al. (2012). Genetic analysis of cold tolerance at seedling stage and heat tolerance at anthesis in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, 11(3), 359–367.
- De Storme, N., & Geelen, D. (2014). The impact of environmental stress on male reproductive development in plants: Biological processes and molecular mechanisms. *Plant, Cell and Environment*, 37(1), 1–18. <https://doi.org/10.1111/pce.12142>.
- Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.
- Fernandez, G. C. (1992). Effective selection criteria for assessing plant stress tolerance. In *Proceeding of the international symposium on adaptation of vegetables and other food crops in temperature and water stress*, Shanhua, Taiwan (pp. 257–270).
- Hasegawa, T., Kuwagata, T., Nishimori, M., Ishigooka, Y., Murakami, M., Yoshimoto, M., et al. (2009). Recent warming trends and rice growth and yield in Japan. In *Proceeding of the MARCO symposium* (pp. 44–51).
- Im, E. S., Pal, J. S., & Eltahir, E. A. B. (2017). Deadly heat waves projected in the densely populated agricultural regions of South Asia. *Science*, 3, e1603322.
- IPCC. (2007). Summary for policymakers. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, & H. L. Miller (Eds.), *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change* (pp. 1–18). Cambridge: Cambridge University Press.
- IPCC. (2014). Summary for policymakers. In T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, & P. M. Midgley (Eds.), *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change* (p. 1535). Cambridge: Cambridge University Press.
- Jagadish, S. V. K., Craufurd, P. Q., & Wheeler, T. R. (2007). High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, 58, 1627–1635. <https://doi.org/10.1093/jxb/erm003>.
- Jagadish, S. V. K., Craufurd, P. Q., & Wheeler, T. R. (2008). Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Science*, 48, 1140–1146.
- Jagadish, S. V. K., Murty, M. V. R., & Quick, W. P. (2015). Rice responses to rising temperatures—Challenges, perspectives and future directions. *Plant, Cell and Environment*, 38, 1686–1698. <https://doi.org/10.1111/pce.12430>.
- Jagadish, S. V. K., Muthurajan, R., Oane, R., Wheeler, T. R., Heuer, S., Bennett, J., et al. (2010). Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, 61(1), 143–156. <https://doi.org/10.1093/jxb/erp289>.
- Li, M. M., Li, X., Yu, L. Q., Wu, J. W., Li, H., Liu, J., et al. (2018). Identification of QTLs associated with heat tolerance at the heading and flowering stage in rice (*Oryza sativa* L.). *Euphytica*, 214(4), 70. <https://doi.org/10.1007/s10681-018-2136-0>.
- Matsui, T., Namuco, O. S., Ziska, L. H., & Horie, T. (1997a). Effect of high temperature and CO₂ concentration on spikelet sterility in indica rice. *Field Crops Research*, 51, 213–219. [https://doi.org/10.1016/S0378-4290\(96\)03451-X](https://doi.org/10.1016/S0378-4290(96)03451-X).
- Matsui, T., Omasa, K., & Horie, T. (1997b). High temperature induced spikelet sterility of japonica rice at flowering in relation to air humidity and wind velocity conditions. *Japanese Journal of Crop Science*, 66, 449–455. <https://doi.org/10.1626/jcs.66.449>.
- Matsushima, S., Ikewada, H., Maeda, A., Honda, S., & Niki, H. (1982). Studies on rice cultivation in the tropics. I. Yielding and ripening responses of the rice plant to the extremely hot and dry climate in Sudan. *Japanese Journal of Tropical Agriculture*, 26, 19–25.
- Michelmore, R. W., Paran, I., & Kesseli, R. V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of National Academy of Sciences USA*, 88(21), 9828–9832. <https://doi.org/10.1073/pnas.88.21.9828>.
- Osada, A., Sasiprapa, V., Rahong, M., Dhammanuvong, S., & Chakrabndhu, H. (1973). Abnormal occurrence of empty grains of indica rice plants in the dry, hot season in Thailand. *Japanese*

- Journal of Crop Science*, 42(1), 103–109. <https://doi.org/10.1626/jcs.42.103>.
- Peng, S., Huang, J., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X., et al. (2004). Rice yields decline with higher night temperature from global warming. *Proceedings of the National Academy of Sciences USA*, 101(27), 9971–9975. <https://doi.org/10.1073/pnas.0403720101>.
- Prasad, P., Boote, K., Allen, L., Sheehy, J., & Thomas, J. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, 95, 398–411. <https://doi.org/10.1016/j.fcr.2005.04.008>.
- Rang, Z. W., Jagadish, S. V. K., Zhou, Q. M., Craufurd, P. Q., & Heuer, S. (2011). Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. *Environment and Experimental Botany*, 70, 58–65.
- Redfern, S. K., Azzu, N., & Binamira, J. S. (2012). Rice in Southeast Asia: Facing risks and vulnerabilities to respond to climate change. *Build Resilience Adapt Climate Change Agri Sector*, 23, 295.
- Satake, T., & Yoshida, S. (1978). High temperature induced sterility in indica rice at flowering. *Japanese Journal of Crop Science*, 47(1), 6–17. <https://doi.org/10.1626/jcs.47.6>.
- Seo, J., Lee, S. M., Han, J. H., Shin, N. H., Koh, H. J., & Chin, J. H. (2019). Identification of yield and yield-related quantitative trait loci for the field high temperature condition in backcross populations of rice (*Oryza sativa* L.). *Plant Breeding and Biotechnology*, 7(4), 415–426.
- Shanmugavadeivel, P. S., Mithra, S. V. A., Prakash, C., Ramkumar, M. K., Tiwari, R., Mohapatra, T., et al. (2017). High resolution mapping of QTLs for heat tolerance in rice using a 5K SNP array. *Rice*, 10, 28. <https://doi.org/10.1186/s12284-017-0167-0>.
- Siddiq, E. A., Singh, V. P., Zaman, F. U., Sadananda, A. R., Abraham, M. J., Prasad, A. S. H., et al. (2009). Development of high yielding basmati quality rice varieties: A success story. *Indian Farming*, 59(1), 13–17.
- Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., Mitsuoka, C., et al. (2013a). QTL-seq: Rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant Journal*, 74(1), 174–183. <https://doi.org/10.1111/tpj.12105>.
- Takagi, H., Uemura, A., Yaegashi, H., Tamiru, M., Abe, A., Mitsuoka, C., et al. (2013b). MutMap-Gap: Whole-genome resequencing of mutant F₂ progeny bulk combined with de novo assembly of gap regions identifies the rice blast resistance gene *Pii*. *New Phytologist*, 200(1), 276–283. <https://doi.org/10.1111/nph.12369>.
- Tazib, T., Kobayashi, Y., Koyama, H., & Matsui, T. (2015). QTL analyses for anther length and dehiscence at flowering as traits for the tolerance of extreme temperatures in rice (*Oryza sativa* L.). *Euphytica*, 203(3), 629–642. <https://doi.org/10.1007/s10681-014-1291-1>.
- Tian, X., Luo, H., Zhou, H., & Wu, C. (2009). Research on heat stress of rice in China: Progress and prospect. *Chinese Agricultural Science Bulletin*, 25(22), 166–168.
- Vivitha, P., Raveendran, M., & Vijayalakshmi, D. (2017). Introgression of QTLs controlling spikelet fertility maintains membrane integrity and grain yield in improved white ponni derived progenies exposed to heat stress. *Rice Science*, 24(1), 32–40. <https://doi.org/10.1016/j.rsci.2016.05.006>.
- Welch, J. R., Vincent, J. R., Auffhammer, M., Moya, P. F., Dobermann, A., & Dawe, D. (2010). Rice yields in tropical/sub-tropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proceedings of the National Academy of Sciences USA*, 107(33), 14562–14567. <https://doi.org/10.1073/pnas.1001222107>.
- Xiao, Y. H., Pan, Y., Luo, L. H., Deng, H. B., Zhang, G. L., Tang, W. B., et al. (2011a). Quantitative trait loci associated with pollen fertility under high temperature stress at flowering stage in rice (*Oryza sativa* L.). *Rice Science*, 18, 1–7.
- Xiao, Y. H., Pan, Y., Luo, L., Zhang, G., Deng, H., Dai, L., et al. (2011b). Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.). *Euphytica*, 178, 331–338.
- Yang, H. C., Huang, Z. Q., Jiang, Z. Y., & Wang, X. W. (2004). High temperature damage and its protective technologies of early and middle season rice in Anhui province. *Journal of Anhui Agricultural Sciences*, 32(1), 3–4.
- Ye, C., Argayoso, M. A., Redoña, E. D., Sierra, S. N., Laza, M. A., Dilla, C. J., et al. (2012). Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breeding*, 131(1), 33–41. <https://doi.org/10.1111/j.1439-0523.2011.01924.x455>.
- Ye, C., Tenorio, F. A., Argayoso, M. A., Laza, M. A., Koh, H. J., Redoña, E. D., et al. (2015a). Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics*, 16(1), 41. <https://doi.org/10.1186/s12863-015-0199-7>.
- Ye, C., Tenorio, F. A., Redoña, E. D., Morales-Cortezano, P. S., Cabrega, G. A., Jagadish, S. V. K., et al. (2015b). Fine-mapping and validating *qHTSF4.1* to increase spikelet fertility under heat stress at flowering in rice. *Theoretical and Applied Genetics*, 128(8), 1507–1517. <https://doi.org/10.1007/s00122-015-2526-9>.
- Yoshida, S., Satake, T., & Mackill, D. (1981). High temperature stress. *IRRI Research Paper Series*, 67, 1–15.
- Zhao, L., Lei, J., Huang, Y., Zhu, S., Chen, H., Huang, R., et al. (2016). Mapping quantitative trait loci for heat tolerance at anthesis in rice using chromosomal segment substitution lines. *Breeding Science*. <https://doi.org/10.1270/jsbbs.15084>.
- Zhao, Z.-G., Jiang, L., Xiao, Y.-H., Zhang, W.-W., Zhai, H.-Q., & Wan, J.-M. (2006). Identification of QTLs for heat tolerance at the booting stage in rice (*Oryza saliva* L.). *Acta Agronomica Sinica*, 32, 640–644.
- Zhu, S., Huang, R., Wai, H. P., Xiong, H., Shen, X., He, H., et al. (2017). Mapping quantitative trait loci for heat tolerance at the booting stage using chromosomal segment substitution lines in rice. *Physiology and Molecular Biology of Plants*, 23(4), 817–825. <https://doi.org/10.1007/s12298-017-0465-4>.
- Zou, C., Wang, P., & Xu, Y. (2016). Bulk sample analysis in genetics, genomics and crop improvement. *Plant Biotechnology Journal*, 14(10), 1941–1955.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Genome-Wide Association Mapping Reveals Novel Putative Gene Candidates Governing Reproductive Stage Heat Stress Tolerance in Rice

K. T. Ravikiran^{1†}, S. Gopala Krishnan^{1*}, K. P. Abhijith¹, H. Bollinedi¹, M. Nagarajan², K. K. Vinod¹, P. K. Bhowmick¹, Madan Pal³, R. K. Ellur¹ and A. K. Singh¹

¹Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India, ²Rice Breeding and Genetics Research Centre, ICAR-IARI, Aduthurai, India, ³Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

OPEN ACCESS

Edited by:

Deepmala Sehgal,
International Maize and Wheat
Improvement Center, Mexico

Reviewed by:

Longbiao Guo,
China National Rice Research Institute
(CAAS), China
Vandana Jaiswal,
Institute of Himalayan Bioresource
Technology (CSIR), India

*Correspondence:

S. Gopala Krishnan
gopal_icar@yahoo.co.in

†Present address:

K. T. Ravikiran,
ICAR-Central Soil Salinity Research
Institute, Regional Research Station,
Lucknow, India

Specialty section:

This article was submitted to
Plant Genomics,
a section of the journal
Frontiers in Genetics

Received: 15 February 2022

Accepted: 25 March 2022

Published: 10 May 2022

Citation:

Ravikiran KT, Gopala Krishnan S,
Abhijith KP, Bollinedi H, Nagarajan M,
Vinod KK, Bhowmick PK, Pal M,
Ellur RK and Singh AK (2022) Genome-
Wide Association Mapping Reveals
Novel Putative Gene Candidates
Governing Reproductive Stage Heat
Stress Tolerance in Rice.
Front. Genet. 13:876522.
doi: 10.3389/fgene.2022.876522

Temperature rise predicted for the future will severely affect rice productivity because the crop is highly sensitive to heat stress at the reproductive stage. Breeding tolerant varieties is an economically viable option to combat heat stress, for which the knowledge of target genomic regions associated with the reproductive stage heat stress tolerance (RSHT) is essential. A set of 192 rice genotypes of diverse origins were evaluated under natural field conditions through staggered sowings for RSHT using two surrogate traits, spikelet fertility and grain yield, which showed significant reduction under heat stress. These genotypes were genotyped using a 50 k SNP array, and the association analysis identified 10 quantitative trait nucleotides (QTNs) for grain yield, of which one QTN (*qHTGY8.1*) was consistent across the different models used. Only two out of 10 MTAs coincided with the previously reported QTLs, making the remaining eight novel. A total of 22 QTNs were observed for spikelet fertility, among which *qHTSF5.1* was consistently found across three models. Of the QTNs identified, seven coincided with previous reports, while the remaining QTNs were new. The genes near the QTNs were found associated with the protein–protein interaction, protein ubiquitination, stress signal transduction, and so forth, qualifying them to be putative for RSHT. An *in silico* expression analysis revealed the predominant expression of genes identified for spikelet fertility in reproductive organs. Further validation of the biological relevance of QTNs in conferring heat stress tolerance will enable their utilization in improving the reproductive stage heat stress tolerance in rice.

Keywords: rice, GWAS, marker-trait association, quantitative trait nucleotides, reproductive stage heat stress tolerance

INTRODUCTION

Climate change and global warming are seriously affecting agricultural productivity. The mean surface temperature of the earth today is 0.8°C higher than that in the pre-industrial era and is projected to increase up to 4.8°C by the end of this century (IPCC, 2014). In India also, there has been a concurrent increase of 0.63°C since 1986, triggering intermittent heatwaves, and this is predicted to increase to 4.7°C by the end of 2100. The heatwave episodes are expected to intensify particularly in the Indo-Gangetic plains of India, where rice–wheat is the most prevalent cropping system (Im et al., 2017; Krishnan et al., 2020). Rice is the major staple food crop of India with an area of 43.79 million

hectares (mha) and a production of 116.42 million tons (mt) during 2018–19 (GoI, 2019). It is highly sensitive to heat stress at the reproductive stage with the optimum temperature ranging from 22 to 28°C (Prasad et al., 2006). Temperatures beyond the tolerant threshold (35°C) at anthesis and booting will adversely affect rice yields (Satake and Yoshida, 1978; Yoshida et al., 1981). Simulation models have predicted that for every 1°C rise in ambient temperature during the sensitive stages, the rice yield will suffer by 2.5 up to 10% (Baker et al., 1992; Peng et al., 2004). The temperatures of many tropical rice-growing countries have already reached ~33°C, and any further increase will have severe taxing on the grain yield and quality (Wassmann et al., 2009). For instance, a heatwave (~36°C) for 2 consecutive days in early April 2021 coupled with low rainfall and humidity has devastated the 68,000 acres of a spring-grown rice crop of Bangladesh with an estimated economic loss of \$39 million (Hossain, 2021).

Heat stress is one of the complex abiotic stresses, to which plants respond through an intricate network of signal transduction pathways (González-Schain et al., 2016; Wang et al., 2019). In rice, heat stress at the reproductive stage affects pollen viability and spikelet fertility, thereby reducing the grain yield. Pollen viability is reduced primarily due to pollen desiccation and denaturation of proteins. Spikelet sterility is attributed to poor anther dehiscence and reduced pollen production, reducing the number of viable pollens reaching the stigma (Matsui et al., 2000, 2001; Prasad et al., 2006). Additionally, tight closure of anther locules by cell layers could also hinder anther dehiscence (Matsui and Omasa, 2002). This is further exacerbated by impaired stigma receptivity due to its exertion out of the spikelet into hot ambience (Wu and Yang, 2019). However, the female reproductive organ is comparatively more resilient to heat stress compared to the male counterpart. Post-fertilization, grain filling, and maturation are equally sensitive to heat stress. High temperature reduces the grain filling period but hastens the grain maturation rate, leading to the impairment of grain filling and resulting in poorly filled chalky grains. These grains break easily on hulling and milling, affecting the head rice recovery in rice (Lyman et al., 2013).

Breeding rice for heat stress tolerance is one of the viable options for mitigating the ill effects of heat stress in rice. Genetic variability for tolerance to reproductive stage heat stress (RSHT) in rice has been well documented (Tenorio et al., 2013; Bheemanahalli et al., 2016; Pradhan et al., 2016; Cheabu et al., 2019; de Brito et al., 2019; Ravikiran et al., 2020). Various mechanisms conferring reproductive stage heat stress tolerance (RSHT) in rice have been reported, which include 1) escape through early morning flowering (Ishimaru et al., 2010; Julia and Dingkuhn, 2013; Hirabayashi et al., 2014), 2) avoidance through mainly evaporative cooling (Julia and Dingkuhn, 2013), and 3) tolerance (Jagadish et al., 2010). True tolerance is primarily adjudged through spikelet fertility, grain yield, and stress tolerance indices. Both spikelet fertility and grain yield under RSHT are quantitative traits, and a large number of QTLs governing RSHT have been documented (Ravikiran et al., 2020). Two major QTLs, *qHTSF1.1* and *qHTSF4.1*, for spikelet fertility were reported on chromosomes 1 and 4 from an upland *aus* cultivar, Nagina 22 (Ye et al., 2012). *qHTSF4.1* has been fine-mapped to around 1.2 Mb region using BC₅F₂ population, and its effect was

validated in a set of 24 rice varieties and different genetic backgrounds (Ye et al., 2015a; b). However, in the majority of the studies for mapping RSHT, the phenotypic variance accounted for by the QTLs identified is very low. Furthermore, most of them are neither validated nor finely mapped and cannot be reliably utilized for marker assisted selection for RSHT in rice. Several putative candidate genes associated with heat stress tolerance such as *TOGRI* (*Thermotolerant Growth Required 1*) (Wang et al., 2016), *SLG1* (*Slender guy 1*) (Xu et al., 2020), and *psbA* (Chen et al., 2020) in addition to *OgTTI1* (*Thermo Tolerance 1*) (Li et al., 2015) encoding the α_2 subunit of 26s proteasome, heat shock proteins and heat shock transcription factors, have been proposed in rice.

A biparental mapping population generally limits the number of genes that can be detected as its genetic variation is restricted between the contrasting parents, both phenotypic and genotypic. This would essentially lead to a large number of key genes/alleles contributing to the variability of a particular trait going unaccounted. Furthermore, the number of false positives in linkage mapping is higher due to the existence of extensive genomic regions under disequilibrium. Genome-wide association studies (GWAS) provide a valuable alternative to linkage mapping since it is based on historic recombination, which considerably breaks the linkage disequilibrium (LD) blocks and also enhances the resolution of QTL detected. It is a particularly useful tool to dissect complex traits such as heat stress tolerance. GWAS is popular in rice as it is endowed with vast genetic variability conserved in gene banks and access to rich genomic resources. Recently, pan-genome data of 67 diverse rice accessions with 16.5 million SNPs, 5.5 million indels, and 0.9 million structural variants have been made available (Zhao et al., 2018). Several SNP arrays with varying densities already exist in rice enabling high-throughput genotyping, which include 44 K (Zhao et al., 2011), 6 K (Yu et al., 2014), 50 K (Singh et al., 2015), 700 K (McCouch et al., 2016), and 7 K (Thomson et al., 2017). GWAS is routinely utilized for mapping biotic (Li et al., 2019; Hada et al., 2020), abiotic (Rohilla et al., 2020; Yuan et al., 2020), and grain quality (Misra et al., 2019; Tang et al., 2019)-related attributes in rice. Even though vast genetic variation was reported for RSHT in rice, it has not been properly utilized for identifying MTAs through a GWAS. There is only one systematic GWAS effort which compares three strategies of association mapping (Lafarge et al., 2017). Hence, in the present study, GWAS for RSHT was carried out through the phenotypic characterization of a set of 192 rice accessions for RSHT in terms of spikelet fertility and grain yield and by genotyping through 50K SNP markers, which lead to the identification of significant marker trait associations (MTAs) through three models. The SNPs associated with the significant MTAs are identified as quantitative trait nucleotides (QTNs). Furthermore, the candidate genes governing RSHT in the vicinity of these MTAs were also identified and discussed.

MATERIALS AND METHODS

Phenotypic Characterization of Germplasm for Reproductive Stage Heat Stress

The present study was conducted on a set of 192 diverse rice genotypes assembled from a germplasm collection maintained at

the Division of Genetics, ICAR–Indian Agricultural Research Institute (ICAR-IARI). The experiment was conducted at the IARI–Rice Breeding and Genetics Research Center, Aduthurai, Tamil Nadu (11° 00'N; 79° 28'E, 19.5 m) during the late *Rabi* (December–April) season of 2018–19. The crop was raised under irrigated transplanted conditions. The genotypes were laid out in an augmented randomized complete block design with four blocks – 48 genotypes along with five checks were randomly allocated to 53 plots per block. In each plot, genotypes were planted in three rows with a spacing of 20 × 15 cm. Two staggered sowings with a gap of 30 days were completed to adjust the flowering time of the germplasm to the targeted seasonal temperatures. The first sowing was completed in the second fortnight of December, which served as the unstressed control since the peak anthesis of the genotypes coincided with optimum (max) temperatures, 33–35°C. The second staggering was taken up in the second fortnight of January, which served as reproductive heat stress treatment wherein peak anthesis of genotypes occurred at higher ambient temperatures, that is, 39–41°C. There were no differences in other agronomic practices in both staggered experiments, and all necessary care was taken to raise a healthy rice crop.

Observations Recorded and Data Analysis

The genotypes from two staggered sowings were closely monitored particularly for their flowering and anthesis to make sure that there are no escapes due to variation in flowering duration in these genotypes. At physiological maturity, five randomly selected plants from the middle row of every plot were harvested separately. One panicle from the main tiller of each genotype was sampled for spikelet fertility. The plants were then threshed separately and weighed to record data on a single plant yield. For spikelet fertility, the panicles were threshed individually, and the filled and unfilled grains were counted manually. The proportion of filled grains among the total number of grains per panicle was expressed as spikelet fertility percent. Additionally, the stress tolerance index (STI; Fernandez, 1992) was calculated for both grain yield per plant and spikelet fertility using the following formula:

$$\frac{(Y_s)(Y_p)}{\bar{Y}p^2},$$

where Y_s and Y_p are the average yield/spikelet fertility of genotypes under stressed and unstressed conditions, respectively, while Y_p represents the mean yield/spikelet fertility of all genotypes under unstressed conditions. Statistical analysis of the phenotypic data was conducted using R statistical software by utilizing appropriate packages. The adjusted means from augmented RCB analysis were generated using the *agricolae* package run on the R studio (RStudio Team, 2016). The package *ggplot2* was utilized to draw frequency curves for different traits.

SNP Genotyping and Filtering

The SNP genotyping of the germplasm set carried out in an earlier study (Bollinedi et al., 2020) was utilized for this study as well.

Briefly, 2-week-old seedlings from the nursery were sampled and processed in liquid nitrogen. DNA was extracted using the cetyl trimethyl ammonium bromide method (Murray and Thompson, 1980). DNA quality was first assessed on 0.8% agarose gel, which was further confirmed using a nanospectrophotometer (NanoDrop™ 2000/2000c, Thermo Fisher Scientific, DE, United States). DNA samples were then sent for SNP genotyping using 50k Affymetrix GeneChip (Thermo Fisher Scientific, United States). The technical details of this custom-made gene chip were explained in Singh et al. (2015). The array houses 50,051 SNPs selected from 18,980 genes covering 12 rice chromosomes with an interval of 1 kb between two adjacent SNPs. The genotyping data of 50,051 SNPs were first filtered for rare alleles with a minor allele frequency cutoff of 5%, and then for missing values, markers with >20% missing reads were dropped. The final number of markers utilized for downstream analysis was reduced to 32,712 SNPs.

Population Structure, Linkage Disequilibrium, and Association Analysis

The population structure of the germplasm and LD decay was worked out as explained in Bollinedi et al. (2020). However, principal component analysis (PCA), inbuilt in the R platform for association analysis, genome association, and prediction integrated tool (GAPIT), was conducted to cross-check the number of subpopulations reported (Lipka et al., 2012). The scree plot generated from PCA was utilized to decide the number of components explaining the optimum population structure and thereby the number of subpopulations. Linkage disequilibrium was estimated based on squared allele frequency correlations (r^2) with significant p values (<0.05) for each pair of loci. LD decay was depicted using bins of 200 kb, and the average r^2 value was plotted against the physical distance. The distance at which the r^2 value plummeted to half of its average maximum value was considered as the rate of LD decay. The association analysis was conducted in GAPIT by executing three different models—mixed linear model (MLM), fixed and random model circulating probability unification (FarmCPU), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK). Phenotypic data generated under both normal and stressed conditions were used for association analysis. The significant threshold for marker trait associations (MTAs) was fixed at $-\log_{10} p > 5.8$ (Bonferroni threshold) to avoid type 1 errors (false positives). However, to prevent type 2 errors (false negatives), the threshold was relaxed to $-\log_{10} p > 5.0$, wherever appropriate (Melandri et al., 2020). For every significant MTA, quantitative trait nucleotide (QTN) was identified.

Co-Localized QTLs, Candidate Genes, and Their *In Silico* Expression Analysis

The physical positions of the MTAs in the rice genome were further analyzed for the presence of any reported QTLs for reproductive stage heat stress tolerance, and MTAs which did not co-localize with QTLs mapped in earlier studies were

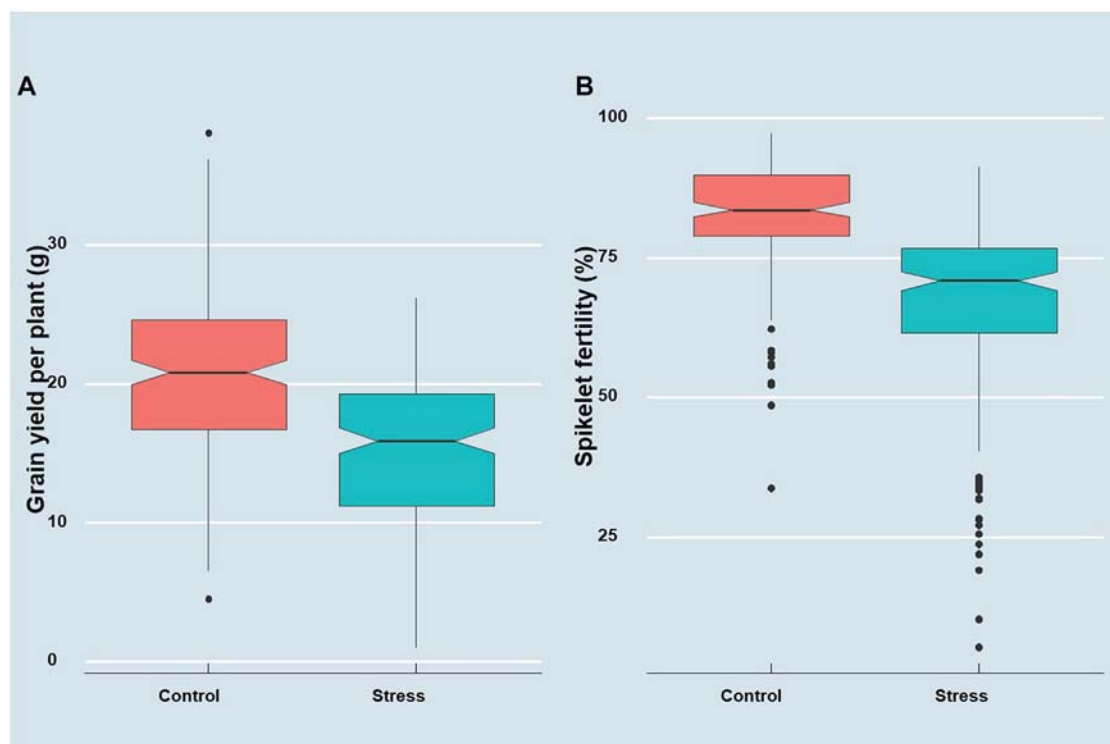


FIGURE 1 | Boxplots depicting the distribution of **(A)** grain yield plant⁻¹ (g) and **(B)** spikelet fertility (%) under control and heat stress conditions.

considered novel. The candidate genes in and around these MTAs were identified using the genome browser of the Rice Genome Annotation Project (<http://rice.uga.edu/cgi-bin/gbrowse/rice>). The tissue-specific expression of the putative candidates identified was analyzed using the datasets available on the RiceXPro website (<https://ricexpro.dna.affrc.go.jp>). Furthermore, the candidate genes were compared with the results of previous transcriptomic studies and the expression dynamics of common genes.

RESULTS

Phenotypic Characterization of the Germplasm for Reproductive Stage Heat Stress Tolerance

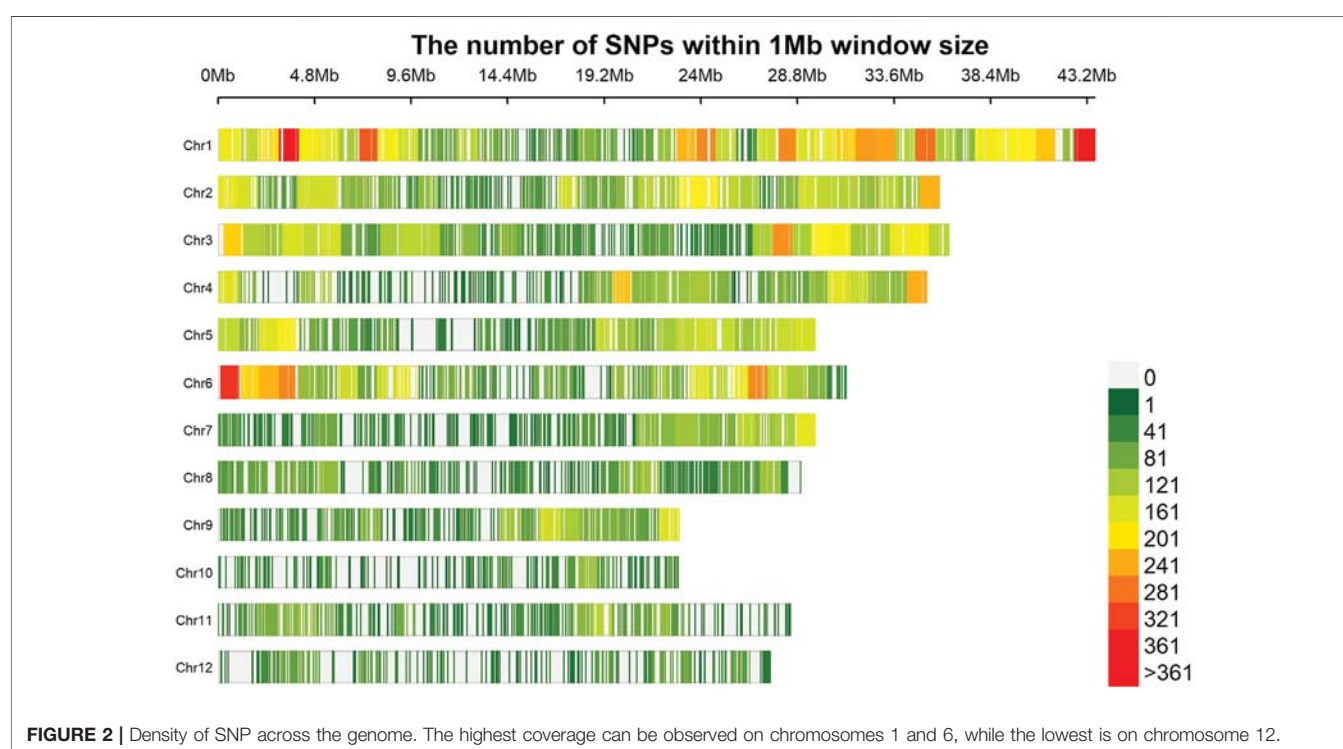
A preliminary augmented ANOVA revealed significant test genotype effects, check effects, and checks versus test entry effects for both grain yield and spikelet fertility, particularly under heat stress (**Supplementary Table S1**). A significant difference was observed in the diurnal mean temperatures during the peak anthesis stage of the germplasm between the two staggered sowing windows. The temperature range during anthesis for the first staggered sown set was between 33 and 35°C, whereas it was 38–40°C for the late sown set (**Supplementary Figure S1** and **Figure 1** of Ravikiran et al., 2020). As a result, the performance of the genotype showed a

significant reduction in the late sown set (**Figure 1**). A mean reduction of 26% was observed for grain yield, while it was 19% for spikelet fertility in the late sown set exposed to high-temperature stress at the reproductive stage. The grain yield and spikelet fertility ranged from 4.47 to 38.13 g and from 33.85 to 97.52%, respectively, under the timely sown unstressed situation. However, the grain yield under heat stress ranged from 1.04 to 26.23 g, while the spikelet fertility ranged from 5.30 to as high as 91.46% (**Table 1**). According to IRRI Standard Evaluation System (SES; IRRI, 2002), 27 genotypes are highly sterile with SF of <50%, 96 genotypes were partially sterile with SF ranging between 50 and 74%, 65 genotypes were fertile with SF varying between 75 and 89%, and the remaining four genotypes were highly fertile with spikelet fertility of ≥90% under heat stress conditions. For grain yield under heat stress, the genotypes Bhubana (26.24 g), Indravati (25.56 g), PRR127 (25.42 g), and PRR122 (24.93 g) were found to be superior, while for spikelet fertility, DV85 (91.46%), BJ1 (90.18%), and NDR359 (90.35%) were found to be the best. Grain yield under heat stress and spikelet fertility under the unstressed control showed high broad sense heritability. Both grain yield and spikelet fertility under heat stress followed near normal distributions (Shapiro–Wilk's p -value > 0.05). Furthermore, the range of stress tolerance index (STI) calculated for grain yield (STIGY) (0.01–2.14 with a mean of 0.81) was higher than that of spikelet fertility (STISF) (0.03–1.27 with a mean of 0.82).

TABLE 1 | Summary statistics of grain yield plant⁻¹ and spikelet fertility under control and reproductive stage heat stress conditions.

Statistics	Grain Yield plant ⁻¹ (g)		Spikelet Fertility (%)		STI _{sf}	STI _{gy}
	Control	Heat Stress	Control	Heat Stress		
Mean	20.41	15.14	82.49	66.82	0.82	0.81
Min	4.47	1.04	33.85	5.30	0.03	0.01
Max	38.13	26.23	97.52	91.46	1.27	2.14
S.D.	6.60	5.77	9.87	16.85	0.25	0.47
S.E.	0.47	0.42	0.72	1.22	0.01	0.03
C.V. (%)	24.05	16.28	6.30	23.09	30.54	58.25
PCV	31.93	37.62	11.72	24.75		
GCV	20.89	33.94	9.89	16.54		
h ² (broad sense)	42.83	81.36	71.19	65.91		
GA	5.78	9.58	14.21	10.08		

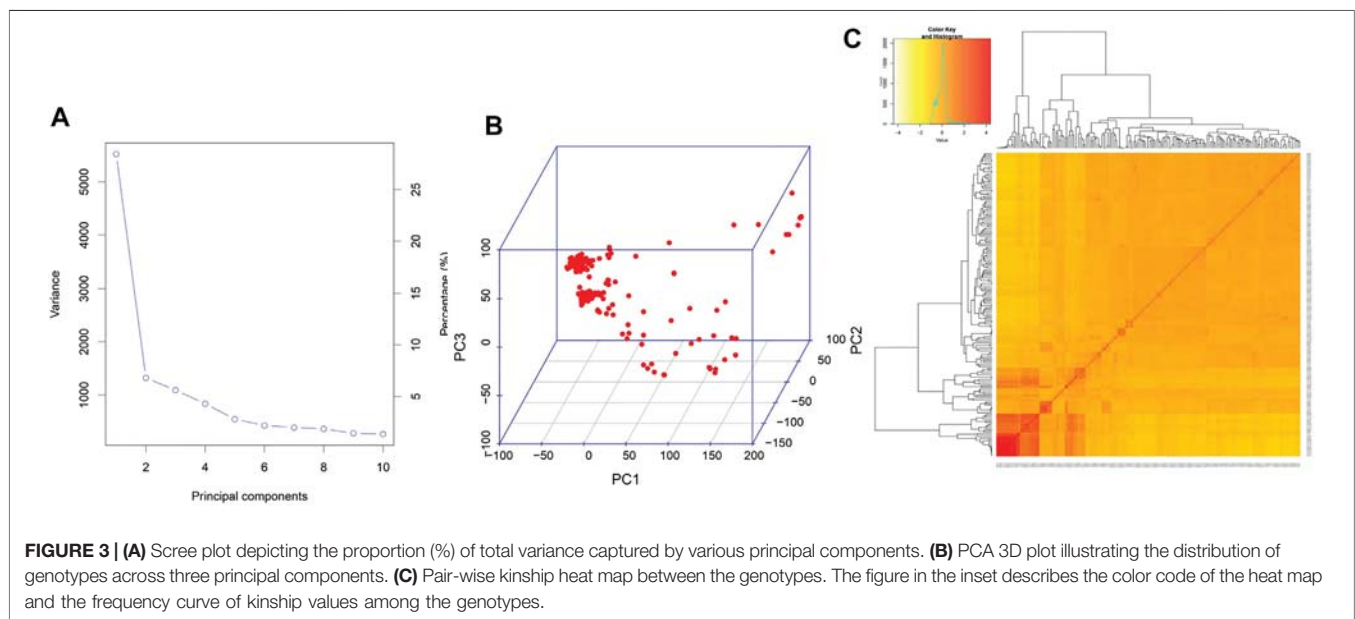
S.D., standard deviation; S.E., standard error of the mean; C.V., coefficient of variation; PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; h², heritability; GA, genetic advance; STI_{sf}, stress tolerance index calculated for spikelet fertility; STI_{gy}, stress tolerance index calculated for grain yield per plant.

**FIGURE 2** | Density of SNP across the genome. The highest coverage can be observed on chromosomes 1 and 6, while the lowest is on chromosome 12.

Population Structure and Linkage Disequilibrium

The marker density plot showed the coverage of markers with an average distance of around 30 kb with almost 90% of the markers spaced within a 5 kb distance (**Figure 2**). In addition, the majority of the genotypes (>150) and markers (>25,000) showed the least heterozygosity, reflecting the true breeding nature of genotypes. The highest r^2 (>0.8) was obtained with a genomic span of <5 kb, followed by a sudden dip (50%) at around 200 kb. Although there were many peaks and valleys, beyond this, there was a gradual general decline in LD which reached less than 0.1 at around 400 kb. Considering this, the marker coverage obtained in the present study is adequate and hence can be utilized for association analysis. Two covariates, population structure and kinship, were

employed to cull out false positives. As described earlier (Bollinedi et al., 2020), the population structure analysis using STRUCTURE based on the graph drawn between ΔK and K values showed the existence of three subpopulations, denoted as POP1, POP2, and POP3. This was further predicted by principal component analysis conducted in the present study. The scree plot showed that a significant portion of variance was captured by PC1 itself (29%), followed by PC2 (7%) and PC3 (6%) (**Figure 3A**). Beyond PC3, the variation contributed by individual PCs was meager (<5%) and can be safely ignored. Hence, a PCA 3D plot between PC1, PC2, and PC3 was considered for interpreting the subpopulation composition of the association panel (**Figure 3B**). One of the subpopulations possessed the maximum number of genotypes (139 genotypes),



followed by the second subpopulation with 35 accessions and the third with 15 genotypes. The first subpopulation with the maximum membership is composed of most of the popular rice varieties of the country such as MTU1001, ADT 39, MAS946-1, Improved Sabarmati, and some advanced breeding lines. The second subpopulation is made of unique temperate rice landraces of Jammu and Kashmir. Furthermore, the heatmap of a kinship value revealed that the maximum number of kinship values populated around 0 to 0.5, indicating a very weak relatedness or maximum genetic diversity in the association panel utilized in the present study (Figure 3C).

Genome-Wide Association Analysis

Genome-wide association analysis was performed by executing three different models, namely, MLM, FarmCPU, and BLINK. The significant MTAs obtained from heat-stressed conditions are summarized in Table 2 along with the corresponding Manhattan plots (Figure 4). However, no significant MTAs could be detected under normal unstressed conditions. The locations of the QTNs identified are depicted in Figure 5, and common QTNs across the models are shown in Figure 6. For grain yield, MLM failed to detect any significant association, while seven QTNs were detected through FarmCPU and three through BLINK. Only one MTA, *qHTGY8.1*, was common between these two models (Figure 6A). This MTA registered the highest probability through BLINK, while it was relatively lower through FarmCPU, but high R^2 values through both models. The MTA *qHTGY10.1* showed the highest probability under FarmCPU. MTAs with higher probability values, *qHTGY10.1* and *qHTGY11.1*, were also reported earlier but for other traits. For spikelet fertility, the highest number of MTAs was detected using the FarmCPU model (8), followed by MLM (7) and BLINK (7). Among these, one MTA was identified consistently across three models, *qHTSF5.1* (Figure 6B), which also displayed the highest R^2 value (0.10). Furthermore, R^2 values of MTAs identified through MLM are slightly higher than those identified through the other two models.

This reflects that MLM lays more emphasis on major QTLs and may miss some minor QTLs which play an equally important role in trait expression. The majority of these MTAs are novel in terms of RSHT except for five MTAs, which coincided with the positions of previously reported MTAs. The MTAs identified for STI are almost the same as that of original trait values, reflecting a high correlation between the two. Particularly under MLM, a group of MTAs clustered in the region 20.5 Mb. This region showed a significant hit with FarmCPU and BLINK as well.

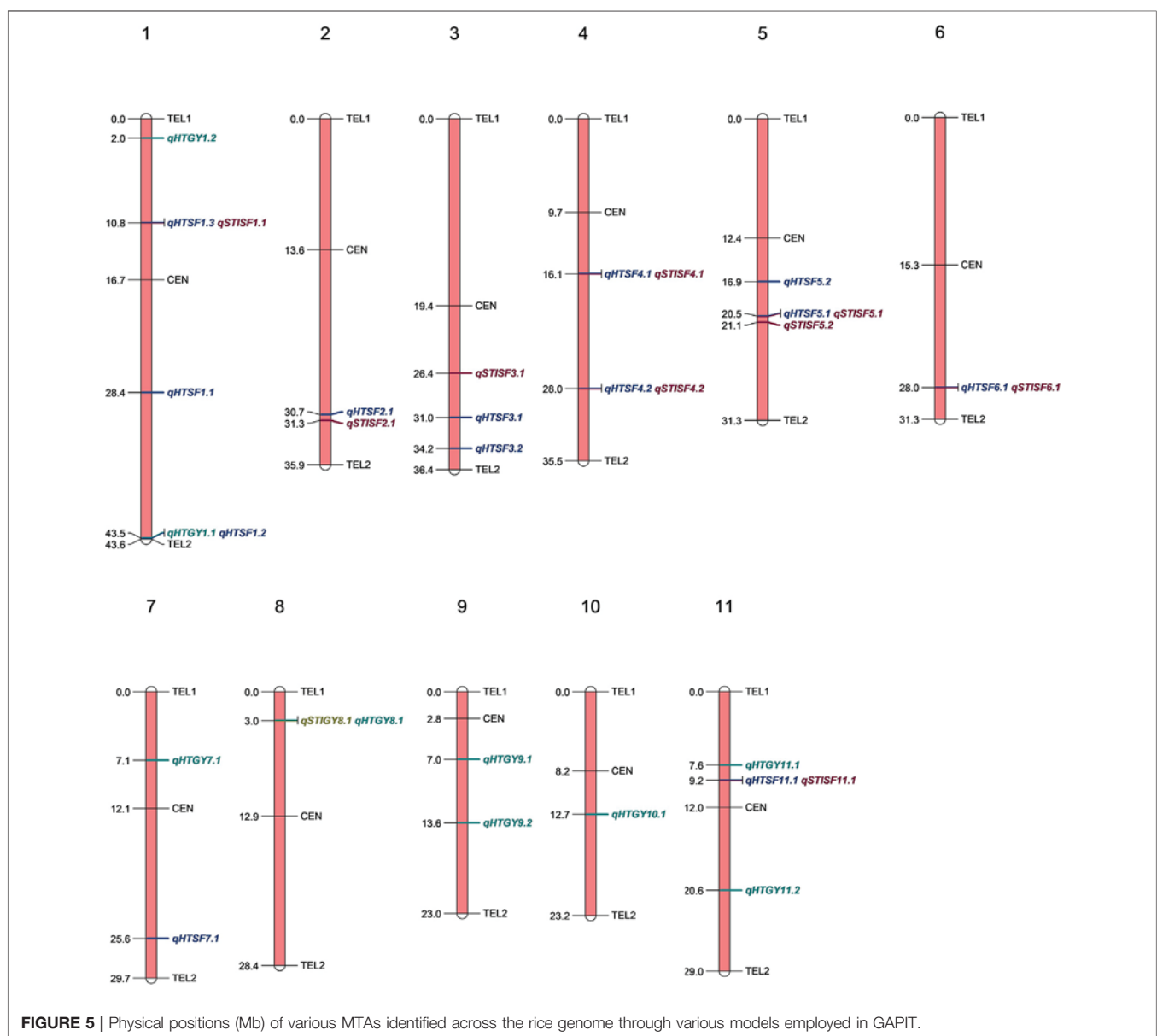
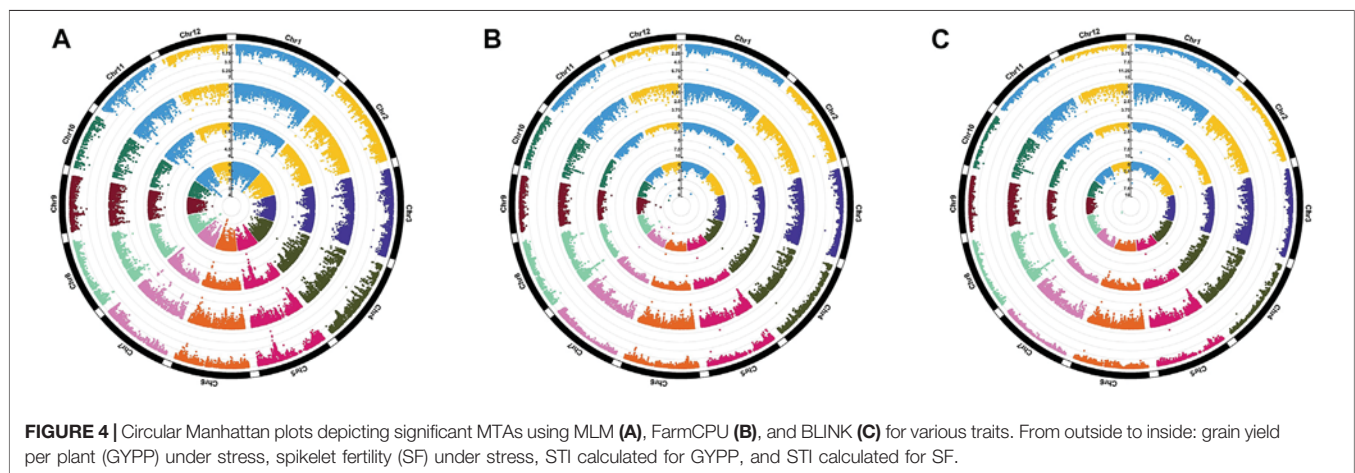
Allelic Effects of Major Quantitative Trait Nucleotides Identified

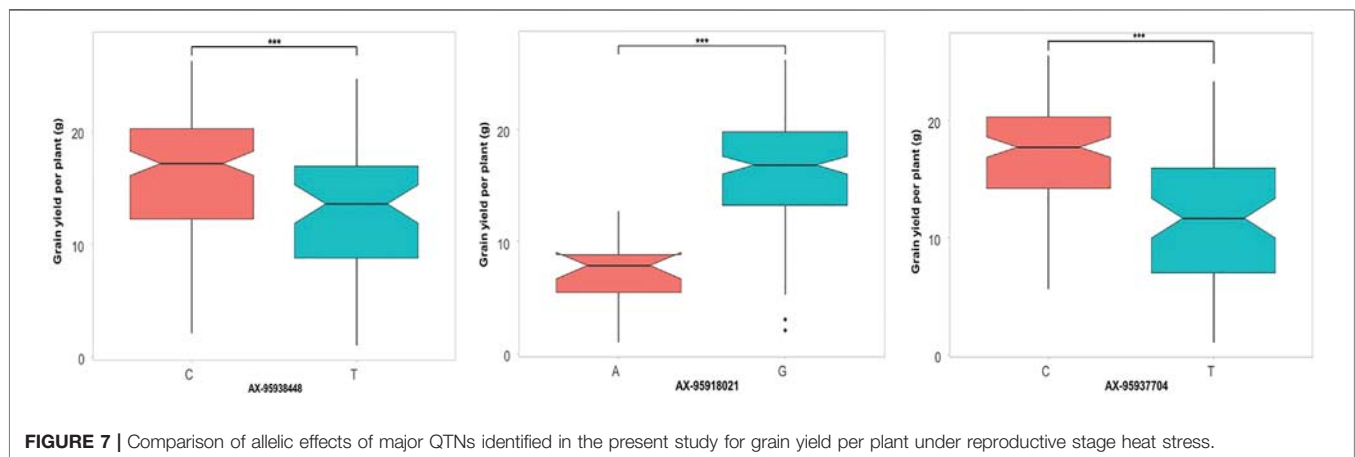
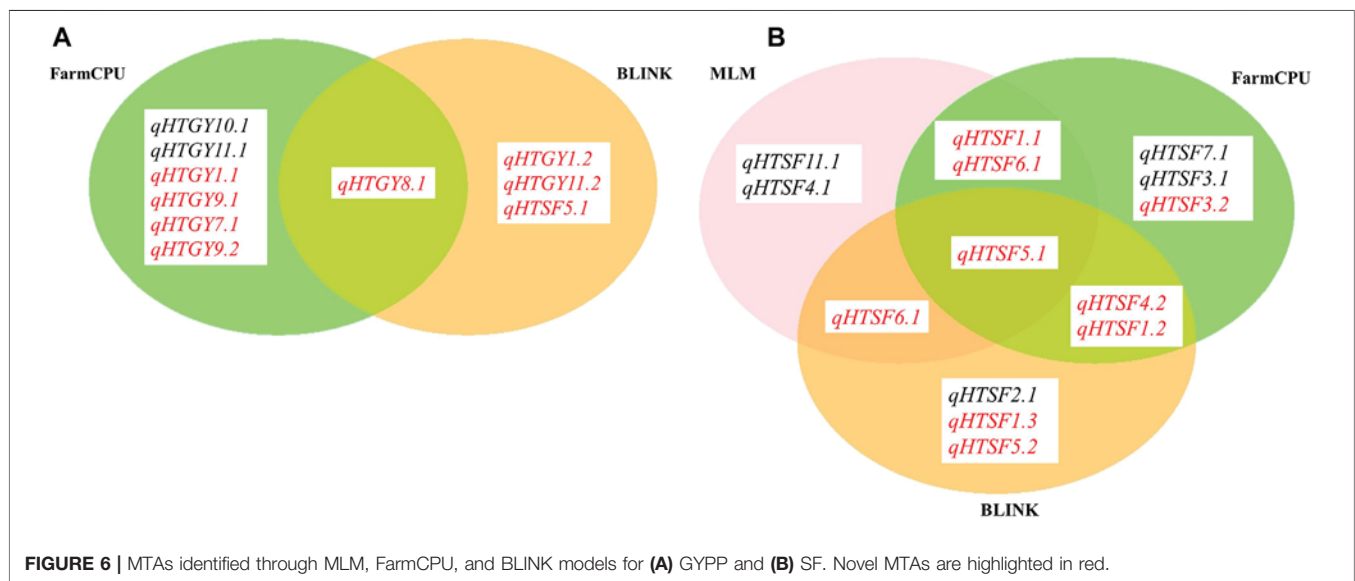
Additionally, the allelic effects of a subset of QTNs identified through GWAS showing significant effects on the respective trait were inspected. For grain yield (Figure 7), three QTNs were selected, namely, *qHTGY10.1*, *qHTGY1.1*, and *qHTGY8.1*, with the linked SNP AX-95938448, AX-95918021, and AX-95937704, respectively. Among these three markers, the greatest significant difference for the grain yield per plant between the genotypes carrying alternate alleles was found for the marker AX-95918021, followed by AX-95937704 and AX-95938448. The genotypes carrying the 'A' allele for AX-95918021 showed a mean grain yield of 8 g, while those carrying its alternate allele, 'G', had a mean grain yield of 18 g. Similarly, genotypes with the 'C' allele of AX-95937704 exhibited a grain yield of 12 g, while those carrying the 'T' allele showed a grain yield of 18 g. Similarly, for spikelet fertility, seven major QTNs were selected, among which AX-95940947 with its linked QTN *qHTSF1.3* showed a highly significant difference in spikelet fertility values for the two alternate alleles closely followed by AX-95918542 linked to QTN *qHTSF1.1* (Figure 8). The 'A' allele of AX-95940947 showed an allelic effect in terms of spikelet fertility (%) of 47, while the 'T' allele showed an effect of 75. The 'A' allele of AX-95918542 exhibited an effect of 75%

TABLE 2 | Significant MTAs identified for grain yield plant⁻¹ (GYPP) and spikelet fertility (SF) under heat stress and their respective stress tolerance indices.

Trait	SNP	Model	Fav Allele	Chr	Position	Probability	R ²	MTA	Previously Reported QTLs ^a
GYPP	AX-95938448	FarmCPU	C	10	12,691,143	9.11E-08	0.02	qHTGY10.1	qhr3-1 Cao et al. (2003)
	AX-95938539		T	11	7,598,615	2.03E-07	0.05	qHTGY11.1	qADL09-11 Tazib et al. (2015)
	AX-95918021		G	1	43,529,356	2.74E-07	0.03	qHTGY1.1	-
	AX-95958896		C	9	6,976,331	4.10E-06	0.02	qHTGY9.1	-
	AX-95957982		G	7	7,144,612	2.51E-05	0.02	qHTGY7.1	-
	AX-95938211		A	9	13,559,420	4.95E-05	0.02	qHTGY9.2	-
	AX-95937704		C	8	3,014,640	8.57E-05	0.05	qHTGY8.1	-
	AX-95937704		C	8	3,014,640	2.43E-10	0.05	qHTGY8.1	-
	AX-95916113		C	1	2,051,081	8.53E-07	0.04	qHTGY1.2	-
	AX-95932,408		C	11	20,682,446	2.31E-05	0.03	qHTGY11.2	-
SF	AX-95926541	MLM	C	5	20,542,291	1.62E-06	0.10	qHTSF5.1	qHTSF11.2 Ye et al. (2015a)
	AX-95961044		G	11	9,168,125	1.09E-05	0.08	qHTSF11.1	qLD10-11 Tazib et al. (2015)
	AX-95926170		G	5	20,547,603	1.70E-05	0.08	qHTSF5.1	-
	AX-95927077		T	5	20,540,979	1.70E-05	0.08	qHTSF5.1	-
	AX-95962,696		C	4	16,049,320	1.73E-05	0.08	qHTSF4.1	SSPF4 Xiao et al. (2011a)
	AX-95918542		A	1	28,440,408	4.95E-05	0.07	qHTSF1.1	qPF4 Xiao et al. (2011b)
	AX-95956527		T	6	28,019,302	5.42E-05	0.07	qHTSF6.1	-
	AX-95956527		T	6	28,019,302	2.53E-10	0.07	qHTSF6.1	-
	AX-95926541		C	5	20,542,291	3.57E-09	0.10	qHTSF5.1	-
	AX-95930097		G	7	25,603,077	6.96E-08	0.03	qHTSF7.1	qAL10-7 Tazib et al. (2015)
STIGY	AX-95924814	FarmCPU	C	4	28,011,513	9.78E-08	0.04	qHTSF4.2	-
	AX-95944879		T	1	28,469,004	6.78E-06	0.06	qHTSF1.1	-
	AX-95923670		G	3	31,000,572	1.45E-05	0.03	qHTSF3.1	qtl_3.4 Jagadish et al. (2010)
	AX-95918181		G	1	43,540,685	2.80E-05	0.04	qHTSF1.2	-
	AX-95949687		A	3	34,204,030	5.32E-05	0.04	qHTSF3.2	-
	AX-95926541		C	5	20,542,291	2.15E-10	0.10	qHTSF5.1	-
	AX-95924814		C	4	28,011,513	7.73E-10	0.04	qHTSF4.2	-
	AX-95921100		T	2	30,694,562	2.67E-09	0.03	qHTSF2.1	qtl_2.2 Jagadish et al. (2010)
	AX-95940947		T	1	10,799,807	5.58E-09	0.05	qHTSF1.3	-
	AX-95918181		G	1	43,540,685	3.68E-07	0.04	qHTSF1.2	-
STISF	AX-95936404	FarmCPU	C	5	16,852,857	4.32E-05	0.05	qHTSF5.2	-
	AX-95956527		T	6	28,019,302	6.21E-05	0.07	qHTSF6.1	-
	AX-95930775		A	8	3,009,287	8.98E-05	0.03	qSTIGY8.1	-
	AX-95930775		A	8	3,009,287	8.98E-05	0.03	qSTIGY8.1	-
	AX-95926541		C	5	20,542,291	5.10E-07	0.11	qSTISF5.1	-
	AX-95927077		T	5	20,540,979	5.11E-06	0.09	qSTISF5.1	-
	AX-95926170		G	5	20,547,603	5.11E-06	0.09	qSTISF5.1	-
	AX-95952,837		A	5	20,642,828	8.34E-06	0.09	qSTISF5.1	qHTSF11.2 Ye et al. (2015a)
	AX-95961044		G	11	9,168,125	1.53E-05	0.08	qSTISF11.1	qLD10-11 Tazib et al. (2015)
	AX-95927241	FarmCPU	A	5	21,126,325	4.49E-05	0.07	qSTISF5.2	-
	AX-95962,696		C	4	16,049,320	4.54E-05	0.07	qSTISF4.1	SSPF4 Xiao et al. (2011a)
	AX-95926541		C	5	20,542,291	3.77E-09	0.11	qSTISF5.1	qPF4 Xiao et al. (2011b)
	AX-95924814		C	4	28,011,513	1.48E-07	0.03	qSTISF4.2	-
	AX-95940947		G	1	10,799,807	1.17E-06	0.04	qSTISF1.1	-
	AX-95919771		T	2	31,278,025	6.76E-06	0.03	qSTISF2.1	-
	AX-95926541		C	5	20,542,291	8.87E-15	0.11	qSTISF5.1	-
	AX-95924814		C	4	28,011,513	1.50E-05	0.03	qSTISF4.2	-
	AX-95956527		T	6	28,019,302	3.67E-05	0.06	qSTISF6.1	-
	AX-95919771		T	2	31,278,025	5.25E-05	0.03	qSTISF2.1	-

^aQTLs, reported exclusively for reproductive stage heat stress tolerance in rice; Chr, chromosome; GYPP, grain yield per plant; SF, spikelet fertility; STISF, stress tolerance index calculated for spikelet fertility; STIGY, stress tolerance index calculated for grain yield per plant; MTAs which are common across two to three models are highlighted in bold for various traits.





spikelet fertility, and the ‘T’ allele showed an effect of 50%. Interestingly, both these QTNs are located on chromosome 1 but 18 Mb apart. The remaining QTNs also showed statically significant ($p < 0.001$) differences between the trait values conferred by their respective alleles, except AX-95956527, with its linked QTN, *qHTSF6.1*, significant at only $p < 0.05$. This further attests to the robustness of major QTNs identified in the present study.

Identification of Putative Candidate Genes for RSHT and Their In Silico Expression Analysis

A total of 11 candidate genes were identified near 10 SNPs associated with grain yield under heat stress distributed on chromosomes 1 (2), 7 (1), 8 (3), 9 (2), 10 (1), and 11 (2) (Table 3). Of these genes, five of them are kinases—diacylglycerol kinase, Ser/Thr protein kinase, and receptor-like kinase involved in stress signaling cascades. Others include glucosylceramidase, zinc ion binding protein,

DUF1336 domain-containing protein, cytochrome P450, and lipase with putative roles in plant defense responses. Similarly, 17 candidate genes were found in the vicinity of 10 SNPs associated with spikelet fertility under heat stress scattered across chromosomes 1 (2), 2 (3), 3 (5), 4 (1), 5 (3), 6 (1), 7 (1), and 11 (1). The majority of these genes are involved in protein processing and protein-protein interactions such as tetratricopeptide repeat domain-containing protein, an E3 ubiquitin ligase, BTB/POZ domain-containing protein, U-box domain-containing protein, ankyrin repeat-containing protein, and RING zinc finger protein. Others include genes involved in abiotic stress responses and other essential pathways of plant development. The survey of expression database (RiceXPro) revealed that the majority of the putative candidate genes identified for grain yield showed predominant vegetative stage-specific expression, whereas those identified for spikelet fertility expressed primarily in the reproductive organs (Supplementary Figures S3A,B). Additionally, the putative candidate genes identified in the present study

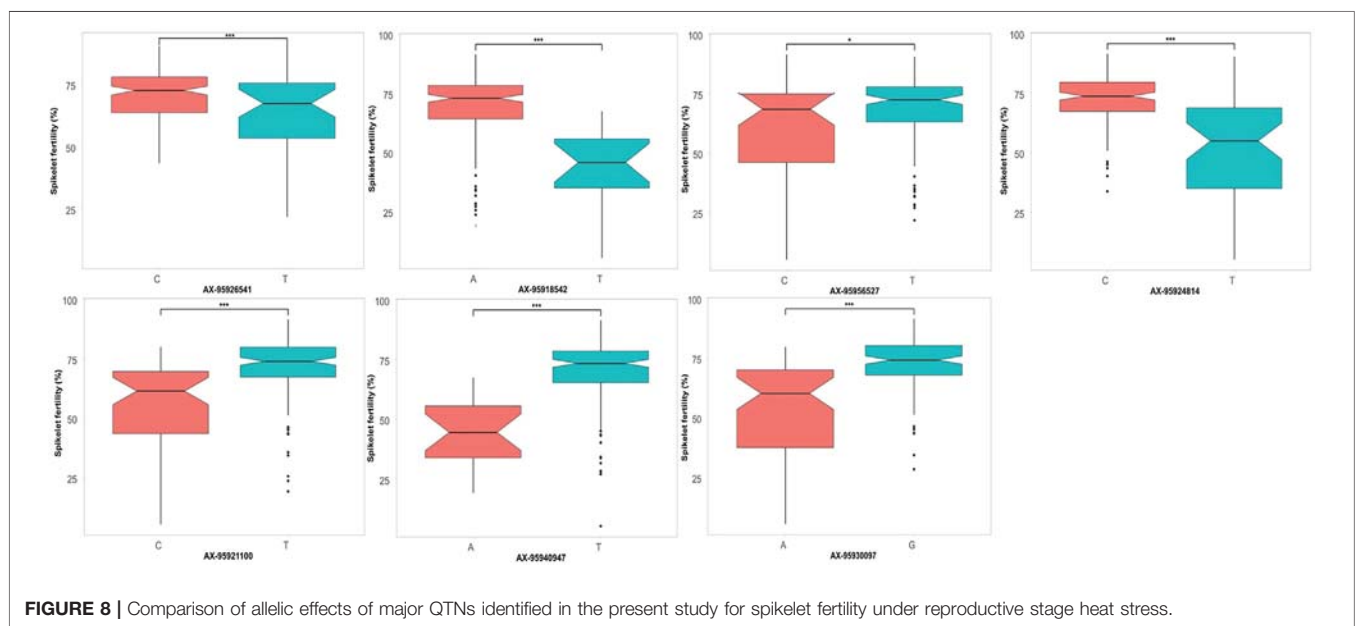


FIGURE 8 | Comparison of allelic effects of major QTNs identified in the present study for spikelet fertility under reproductive stage heat stress.

were compared with the differentially expressed genes (DEGs) identified in previous transcriptomic studies under heat stress in rice. Six genes were found common with the DEGs reported by Liu et al. (2020) in the thermo-tolerant genotype, SDWG005 (Table 4). Of these, three genes were upregulated, while three genes were downregulated under heat stress. There were four putative candidate genes that matched with the DEGs observed by Cai et al. (2020), of which two genes were upregulated and the other two were downregulated.

Selection of RSHT Genotypes With Superior Allelic Combination

The promising genotypes with the *per se* trait values of grain yield and spikelet fertility under stress and STI were shortlisted, and the number of positive alleles of 29 putative MTAs in these tolerant genotypes was investigated. A total of 18 genotypes were identified (Table 5). The genotype RIL 10 accumulated positive alleles for all the 29 SNPs coupled with superior grain yield and spikelet fertility under stress. This was followed closely by Selected Sabarmati, Sitwa Dhan, Bhadrakali, Samba Mashuri, Hema, and Haldamuri, which showed positive alleles for 28 SNPs. Similarly, UPRI 2003-45, B6144-MR-6-0-0, and Pant dhan 18 had positive alleles for 27 SNPs, while RNRM 7, Gouri, Indravati, and ADT 39 had positive alleles for 26 SNPs. The genotypes IR 77384-12-35-3-6-7-2-B and JR 75 showed positive alleles for 25 SNPs, while PRR117 showed positive alleles for 24 SNPs.

DISCUSSION

Crop yields and productivity are predicted to suffer acutely in the coming years due to climate change and global warming.

Breeding heat-tolerant crop varieties is essential to address crop losses due to heat stress in rice. The present study aimed to map MTAs governing reproductive stage heat stress tolerance in rice using a diverse set of 192 rice germplasm lines. The two sowing windows utilized could adequately distinguish sensitive and tolerant genotypes, providing a unique situation in which two staggered sowings differed only for the heat stress at the reproductive stage. The screening was carried out during the late *rabi* season in Tamil Nadu, where winter is normally felt inconspicuous from normal weather conditions. The heat stress normally occurs at Aduthurai during the end of the *rabi* season, marking the beginning of summer. The weather change occurs within a span of 30 days, which is a significant shift from the normal temperature to a high temperature. We had two overlapping conditions: 1) one under a normal sowing which provided the most ideal normal weather conditions all throughout the crop period including the reproductive stage and 2) a late sowing that provided a heat stress only at the terminal stage. The highest temperature reached during the flowering of the second sowing was $\sim 42^{\circ}\text{C}$. The overlap of both the sowings was almost 75%, meaning that both the normal and stressed conditions experienced the same environment most of the time, except for a 30-day window. In the first sowing, the window was during the initial stage, whereas in the second sowing, the window coincided with the reproductive stage. Therefore, both the sowings shared a common environmental influence most of the time. Moreover, weather-wise, the initial 30-day window for the first sowing was not different from that of the second sowing. This provides a unique situation, in which the environmental difference is maximized only during the reproductive stage between both the sowings, and hence, the data generated during this stage could be reliable for studying the RSHT among the genotypes. If found unique and reliable, single season data can be utilized for

TABLE 3 | Candidate genes in the QTN regions identified through three GWAS models for grain yield per plant, spikelet fertility, and their respective stress tolerance index.

Trait or gene ID	Closest SNP	Chr	Start	End	Gene Annotation	Putative Gene Function
Grain yield plant⁻¹						
LOC_Os10g24690	AX-95938448	10	12,690,416	12,689,328	Expressed protein	-
LOC_Os11g13810	AX-95938539	11	7,595,274	7,606,870	Non-lysosomal glucosylceramidase, putative, expressed	Catabolism of sphingolipids
LOC_Os09g12250	AX-95959896	9	6,975,494	6,978,083	Expressed protein	-
LOC_Os07g12520	AX-95957982	7	7,145,539	7,149,827	Zinc ion binding protein, putative, expressed	Reproductive development
LOC_Os09g22450	AX-95938211	9	13,559,548	13,563,071	Lipase, putative, expressed	Disease resistance
LOC_Os08g05640	AX-95937704	8	3,013,718	3,016,330	DUF1336 domain-containing protein	Disease resistance
LOC_Os08g05620	AX-95937704	8	3,007,241	3,009,195	Cytochrome P450, putative, expressed	Regulation of non-enzymatic antioxidant synthesis
LOC_Os08g05650	AX-95937704	8	3,017,356	3,022,047	Diacylglycerol kinase, putative, expressed	Plant stress response
LOC_Os01g04570	AX-95916113	1	2,048,717	2,052,521	Ser/Thr protein kinase, putative, expressed	Heat shock response
LOC_Os01g04580	AX-95937704	1	2,053,583	2,057,638	Ser/Thr protein kinase, putative, expressed	
LOC_Os11g35120	AX-95932,408	11	20,589,041	20,591,080	OsWAK116 - OsWAK receptor-like cytoplasmic kinase OsWAK-RLCK, expressed	Signal transduction
Spikelet fertility						
LOC_Os11g16540	AX-95961044	11	9,156,589	9,173,338	Tetratricopeptide repeat domain-containing protein, expressed	Protein–protein interactions
LOC_Os01g49470	AX-95918700	1	28,447,887	28,458,977	E3 ubiquitin ligase, putative, expressed	Proteolysis
LOC_Os06g46240	AX-95956527	6	28,007,285	28,017,490	BTB/POZ domain-containing protein, putative, expressed	Transcriptional regulation and protein degradation
LOC_Os07g42750	AX-95930097	7	25,601,642	25,606,513	DDT domain-containing protein, putative, expressed	Chromatin remodeling
LOC_Os04g47170	AX-95924814	4	28,013,719	28,017,003	ATROGEF7/ROPGEF7, putative, expressed	Secondary cell wall formation
LOC_Os01g49490	AX-95944879	1	28,467,690	28,471,172	Expressed protein	-
LOC_Os03g54084	AX-95923670	3	31,004,724	31,009,782	Phytochrome C, putative, expressed	Photoperiodic response
LOC_Os03g54091		3	31,009,978	31,013,940	OsTOP6A1-Topoisomerase 6 subunit A homolog 1, expressed	Meiotic recombination
LOC_Os03g60140	AX-95949687	3	34,198,836	34,201,827	U-box domain-containing protein, putative, expressed	Protein degradation
LOC_Os03g60130		3	34,194,882	34,197,992	Transcription elongation factor protein, putative, expressed	Regulation of flower induction
LOC_Os03g60150		3	34,202,415	34,206,308	Protein kinase domain-containing protein, expressed	Kinase activity
LOC_Os02g50270	AX-95921100	2	30,695,324	30,698,401	Ankyrin repeat-containing protein, putative, expressed	Protein–protein interactions and protein chaperoning
LOC_Os02g50280		2	30,698,990	30,701,615	Pentatricopeptide, putative, expressed	Abiotic stress responses
LOC_Os02g50290		2	30,701,962	30,703,636	RING zinc finger protein, putative, expressed	Protein ubiquitination
LOC_Os05g28730	AX-95936404	5	16,850,590	16,853,524	Zinc finger, C3HC4 type domain-containing protein, expressed	Signal transduction
LOC_Os05g28720		5	16,845,905	16,848,027	PPR repeat-containing protein, expressed	Abiotic stress responses
LOC_Os05g28740		5	16,859,683	16,860,977	Universal stress protein domain-containing protein, putative, expressed	Several abiotic stresses
LOC_Os03g46640	AX-95949747	3	26,396,243	26,397,459	Deoxyuridine 5-triphosphate nucleotidohydrolase, putative, expressed	Floral organ development
LOC_Os03g46650		3	26,398,523	26,406,544	WD domain, G-beta repeat domain-containing protein, expressed	Signal transduction

Chr, chromosome.

the GWAS study in rice (Melandri et al., 2020). Field-based phenotyping for RSHT was adopted in other studies (Bheemanahalli et al., 2016; Huang et al., 2016; Pradhan et al., 2016; Sukkeo et al., 2017; Cheabu et al., 2019). A similar sowing date (January last week) to expose the genotypes to heat stress was also chosen by Chidambaranathan et al. (2021) and Pradhan et al. (2016). Wide variation was observed for RSHT in terms of both grain yield and spikelet fertility. A similar variability at this scale (>150 genotypes) for RSHT was also reported in previous studies (Tenorio et al., 2013; Bheemanahalli et al., 2016; Pradhan et al., 2016; Cheabu et al., 2019; de Brito et al., 2019). From a set of 182 *indica*, *japonica*, and *indica/japonica* genotypes assessed for

RSHT, two genotypes, LTB 14301 and BRS Pampa, were found tolerant (de Brito et al., 2019). Out of 198 genotypes exposed to heat stress, 15 genotypes showed spikelet sterility of <15% (Chidambaranathan et al., 2021). Similarly, from 169 rice accessions evaluated under very high temperatures (40–45°C), four genotypes, namely, AUS17, M9962, SONALEE, and AUS16, were found to be tolerant (with a seed-setting rate of >75%) along with N22. In another study, a total of 240 genotypes were assessed for RSHT at Cuttack, of which 59 genotypes including N22 showed spikelet fertility >60% (Pradhan et al., 2016). From a total of 511 rice genotypes evaluated in an open field, 200 genotypes showing high spikelet fertility (>60%) were further

TABLE 4 | Differential expression of some putative candidate genes identified in the present study from published datasets.

Gene ID	Log ₂ FC ^a (6 h)	Log ₂ FC ^a (12 h)	Log ₂ FC ^b (36°C)	Log ₂ FC ^b (38°C)	Log ₂ FC ^c (36°C)	Log ₂ FC ^c (38°C)
LOC_Os08g05620	-1.51	-1.40	-	-	-	-
LOC_Os01g04580	-4.87	-2.35	-	-	-	-
LOC_Os05g28740	-	-2.12	-	-	-	-
LOC_Os07g42750	2.45	1.65	-	-	-	-
LOC_Os01g04570	1.68	1.48	-	-	-	-
LOC_Os03g54091	1.43	1.12	-	-	-	-
LOC_Os10g24690	-	-	-	-	-1.89	-3.02
LOC_Os05g28730	-	-	1.67	2.18	2.08	2.45
LOC_Os08g05620	-	-	1.66	2.20	-0.17	0.28
LOC_Os03g46640	-	-	-1.36	-2.10	-0.69	-1.02

^aLogarithm of fold change values in thermo-tolerant genotype SDWG005 exposed to 6 and 12 h of heat stress during anthesis (Liu et al., 2020).

^bLogarithm of fold change values in thermo-tolerant genotype SDWG005 exposed to heat stress (36 and 38°C) during the meiosis stage (Cai et al., 2020).

^cLogarithm of fold change values in thermo-sensitive genotype MH101 exposed to heat stress (36 and 38°C) during the meiosis stage (Cai et al., 2020).

TABLE 5 | List of best-performing genotypes in terms of grain yield, spikelet fertility, and their respective tolerance indices and number of positive alleles of 29 MTAs identified for RSHT.

S. No	Genotype	GY_C	GY_H	STI_GY	SF_C	SF_HT	STI_SF	NPA
1	RIL 10	29.87	23.5	1.68	90.59	86.23	1.14	29
2	Selected Sabarmati	21.98	20.38	1.07	86.8	86.79	1.1	28
3	Sitwa Dhan	26.15	24.66	1.54	77.49	75.02	0.85	28
4	Bhadrakali	25.5	21.54	1.31	90.73	89.81	1.19	28
5	Samba Mahsuri	24.23	22.32	1.29	88.64	78.24	1.02	28
6	Hema	24.88	22.22	1.32	86.79	86.01	1.09	28
7	Haldimuri	25.14	23.38	1.4	97.52	88.35	1.26	28
8	UPRI 2003-45	23.32	21.5	1.2	90.86	83.32	1.11	27
9	B6144-MR-6-0-0	16.81	15.33	0.62	83.59	82.6	1.01	27
10	Pant dhan 18	19.98	19.27	0.92	91.15	88.72	1.19	27
11	RNRM 7	24.62	20.25	1.19	90.63	84.11	1.12	26
12	Gouri	27.44	24.9	1.63	78.06	76.81	0.88	26
13	Indravati	27.88	25.56	1.7	83.64	82.81	1.02	26
14	ADT 39	29.21	23.57	1.64	89.85	79.38	1.05	26
15	IR 77384-12-35-3-6-7-2-B	17.23	15.2	0.81	87.58	83.88	1.08	25
16	JR 75	23.58	19.88	1.12	93.18	87.06	1.19	25
17	PRR 117	16.74	14.53	0.58	87.13	85.58	1.09	24
18	IR 70	25.04	22.48	1.34	81.31	80.29	0.96	24

GY_C, grain yield under control; GY_H, grain yield under heat stress; STI_GY, stress tolerance index for grain yield; SF_C, spikelet fertility under control; SF_H, spikelet fertility under heat stress; STI_SF, stress tolerance index for spikelet fertility; NPA, number of positive alleles.

assessed in a controlled environment (38°C), of which 28 genotypes with high fertility were finally selected as donors of RSHT (Tenorio et al., 2013). There were certain genotypes, such as Bhuban, PRR127, DV85, and Improved Samba Mashuri, which could maintain exceptionally higher yields and spikelet fertility under high temperature and can be valuable donors for RSHT after further validation. Grain yield suffered more than spikelet fertility, implying the role of other traits in addition to spikelet fertility which contributes to grain yield under heat stress. Both spikelet fertility and grain yield showed quantitative inheritance qualifying for a GWAS. Considering both phenotypes and genotypes (positive alleles of significant MTAs), RIL 10 was found to be the best performer of all the genotypes and can be a potential heat-tolerant donor after validation in further studies.

In the present study, a total of 32,712 SNPs were utilized for GWAS, which is greater than previous reports. PCA-based population structure analysis showed the presence of three subpopulations as in the previous report (Bollinedi et al.,

2020). Assessing the population structure is indispensable, particularly in rice, since it is a self-pollinated crop with clearly differentiated ecotypes—*indica*, *temperate japonica*, *tropical japonica*, *aus/boro*, and *Basmati/Sadri* (Chen et al., 2019). The classification of genotypes through structure analysis in the present study is mainly based on the degree of their domestication and improvement through breeding to which they had been exposed with popular varieties subsumed in one group, with the landraces in the other. Another important parameter to be considered for GWAS is familial relatedness. The kinship values between different genotype pairs clustered around near-zero values, reflecting optimum genetic diversity between the genotypes. Several models are available to conduct the GWAS study. Broadly, they are classified into two categories—single locus models and multi-locus models. Single locus methods test MTAs one SNP at a time akin to single-marker analysis or simple interval mapping of the QTL study. These include the general linear model (GLM) (fits only population

structure as a covariate in the model), mixed linear model (MLM) (Zhang Y-M. et al., 2005; Yu et al., 2006) (uses both ancestry coefficient and kinship as covariates), and its improvements and modifications. To detect more MTAs with the least type I errors, multi-locus models have been proposed, which control background noise generated by other loci (termed pseudo-QTNs) which are in LD with the locus being tested. This is similar to the CIM and MIM strategies of QTL mapping. The multi-locus methods include MLMM (Segura et al., 2012), FarmCPU (Liu et al., 2016), and BLINK (Huang et al., 2019). Several other models were proposed after these recently (Zhang et al., 2020). Among them, in the present study, we have utilized these three models inbuilt in the GAPIT package for association analysis—MLM, FarmCPU, and BLINK (Lipka et al., 2012). In a GWAS study, it is important to keep the critical value very stringent to identify true MTAs by eliminating any false positives. Although the Bonferroni threshold is commonly used, it is likely to exclude some of the real positives if the probability of such MTAs lies too close to the threshold. Therefore, it is common to relax the threshold to bring such MTAs into selection. In this study too, the cutoff was relaxed to 5.0, but only those MTAs consistently appearing under multiple models were considered.

Although GWAS had been frequently adopted for mapping in rice, there were only two previous reports of GWAS for RSHT in rice. A set of 20 previously reported SSR markers was validated in one study using 62 rice genotypes (Pradhan et al., 2016). In another study, GBS-based genotyping of 167 rice accessions was performed to identify MTAs for 20 traits (Lafarge et al., 2017) using a set of 13,160, including 6667 SNPs and 6593 DArT markers. The authors compared three strategies of arriving at MTAs, single-marker-based regression, haplotype-based GWAS, and Bayesian Lasso-based analysis, which were utilized for identifying the MTAs, as in the present study. GWAS was also utilized for identifying genes for RSHT in other plant species such as *Arabidopsis* (Bac-Molenaar et al., 2015), *Brassica napus* (Rahaman et al., 2018), wheat (Tadesse et al., 2019; Guo et al., 2020; Sharma et al., 2020), and field pea (Tafesse et al., 2020).

Both *per se* trait values and the stress tolerance index extracted from them were utilized to establish marker-trait associations. GWAS for stress tolerance indices have been carried out in wheat (Gahlaut et al., 2019), cotton (Baytar et al., 2018), and *Brassica* (Khanzada et al., 2020). Several MTAs were identified in the present study for grain yield and spikelet fertility. The number of MTAs identified through FarmCPU and BLINK was higher than MLM, indicating that multi-locus models are effective in identifying reliable MTAs. False negatives were found to be more in the MLM model, implying its medium power of QTL detection. MLM weakens the associations in an effort to control the inflation of *p*-values via the population structure, thus missing out many minor effect MTAs. These minor effect MTAs are particularly relevant for complex traits such as heat stress tolerance. MTAs identified through the remaining two models cannot be deemed false positives given the appreciable phenotypic variances (R^2 values) observed for these MTAs. Hence, either FarmCPU or BLINK can be employed for GWAS (Merrick et al., 2022). Only nine previously reported QTLs were identified through the present study, indicating

that most of the MTAs were novel. Two QTNs for grain yield per plant, five QTNs for spikelet fertility, and two QTNs for STISF were found to co-localize with previously reported QTLs. QTN for grain yield per plant identified through FarmCPU *qHTGY10.1* was co-localized with *qhr3-1* reported for heat tolerance by Cao et al. (2003) and *qHTGY11.1* overlapped with *qADL09-11* identified by Tazib et al. (2015) for anther dehiscence length. The QTN, *qHTST11.1* (or *qSTISF11.1* for STISF) for spikelet fertility identified in the present study through MLM coincided with two previously reported QTLs, *qHTSF11.2* (Ye et al., 2015a) for spikelet fertility and *qLD10-11* (Tazib et al., 2015) for longitudinal dehiscence of anthers. Another QTN, *qHTSF4.1* (and *qSTISF4.1* for STISF), for spikelet fertility on chromosome 4 coincided with *SSPF4* (Xiao et al., 2011a) and *qPF4* (Xiao et al., 2011b) mapped for seed set percentage and pollen fertility under heat stress, respectively. Furthermore, *qHTSF7.1* identified through FarmCPU for spikelet fertility co-localized with *qAL10-7* reported for anther length (Tazib et al., 2015). The MTA *qHTST2.1*, identified through BLINK, coincided with *qtl_2.2* reported for absolute spikelet fertility (Jagadish et al., 2010).

The regions identified as significant MTAs were analyzed using the genome browser on the Rice Genome Annotation Project website in order to identify putative candidate genes. The majority of those genes identified for grain yield per plant are involved in stress signaling, such as the glucosylceramidase (GCD) gene, zinc ion binding protein, DUF1336 domain-containing protein, cytochrome P450, diacylglycerol kinase, Ser/Thr protein kinase, OsWAK116–OsWAK receptor-like cytoplasmic kinase OsWAK-RLCK, and so forth. A DUF1336 domain-containing protein was identified on chromosome 8. Several transcriptomic studies reported differential expression of DUF domain-containing proteins under heat stress at the anthesis stage in rice, indicating their possible role in heat stress responses (Endo et al., 2009; Zhang et al., 2012; González-Schain et al., 2016). The cytochrome P450 proteins (like the one found on chromosome 8) have varied roles in the biotic and abiotic stress responses of plants, particularly in the synthesis of secondary metabolites (Jun et al., 2015; Pandian et al., 2020). Their relevance under heat stress has already been demonstrated in rice (Endo et al., 2009; González-Schain et al., 2016; Wang et al., 2019), mustard (Rahaman et al., 2018), *Panicum virgatum* (Li et al., 2013), *Rhazya stricta* (Obaid et al., 2016), and *Panicum maximum* (Wedow et al., 2019). Four kinase-encoding genes were found—one on chromosome 8, two on chromosome 1, and one on chromosome 11. Protein kinases are central to abiotic stress signal transduction pathways. Among these, OsWAK116, receptor-like wall-associated kinase proteins can be presumed to be a transducer of heat stress signal between the cytoplasm and cell wall (Zhang S. et al., 2005).

Several genes identified for spikelet fertility are involved in protein chaperoning pathways central to plant heat stress responses. The proteins containing the tetratricopeptide repeat (TRP) motif (like the one found on chromosome 11) are, in general, involved in protein–protein interactions which unite into multi-protein complexes to assist plants in warding off external stresses (Paeng et al., 2020). An E3 ubiquitin ligase identified on chromosome 1 might be involved in the degradation of denatured proteins due to heat stress. Ubiquitin ligases are frequently implicated under heat stress in rice (Mittal et al., 2012; Zhang

et al., 2012; González-Schain et al., 2016). Broad-complex Tramtrack and the Bric-a-brac (BTB) domain/POX virus and Zinc finger (POZ) (BTB/POX) domain-containing proteins (chromosome 6) are implicated in transcriptional regulation and protein degradation (He et al., 2019). These BPM proteins are reported to negatively regulate the degradation of DREB2A and contribute to plant thermo-tolerance (Morimoto et al., 2017). The U-box domain-containing proteins (chromosome 3), called PUBs (plant U-box proteins), are a part of the ubiquitin–proteasome system (UPS) involved in the targeted ubiquitination and degradation of proteins when exposed to various environmental stresses (Hur et al., 2012; Byun et al., 2017; Ryu et al., 2019).

Ankyrin (ANK) repeat-containing proteins (chromosome 2) are involved in various protein–protein interactions (Michaely and Bennett, 1992; Li et al., 2006) and protein chaperoning (Shen et al., 2010) with putative roles in pollen germination and pollen tube growth in lily (Huang et al., 2006) and rice (Huang et al., 2009). These ANK repeat proteins also showed differential expression under heat stress at the anthesis stage in rice, in line with the present finding (González-Schain et al., 2016). RING finger proteins (chromosome 2) are a family of zinc finger proteins, with the majority being U3 ubiquitin ligases (Ciechanover 1998), which stem from the RING domain. There are reports of association of RING finger proteins in heat stress responses in rice. For instance, both *Oryza sativa* heat- and cold-induced 1 (*OsHCl1*) and *Oryza sativa* heat-induced RING finger protein 1 (*OsHIRP1*) act as E3 ligases and positively regulate heat stress responses (Lim et al., 2013; Kim et al., 2019). The rice *OsRZFP34* gene and *HEAT TOLERANCE AT SEEDLING STAGE* (*OsHTAS*) gene (an E3 ligase) improve high-temperature tolerance (Hsu et al., 2014; Liu et al., 2016). Corroborating this, the candidate gene of thermo-tolerance 1 (TT1) QTL identified from *O. glaberrima* encodes the $\alpha 2$ subunit of the 26S proteasome (Li et al., 2015). Several other genes such as DDT domain-containing protein, ATROPGEF7/ROPGEF7, phytochrome C, OsTOP6A1–Topoisomerase 6 subunit A homolog 1, transcription elongation factor protein, C3HC4-type domain-containing protein, PPR repeat-containing protein, universal stress protein domain-containing protein, deoxyuridine 5-triphosphate nucleotidohydrolase and WD domain, and G-beta repeat domain-containing protein were also found in the regions associated with spikelet fertility with putative functions in plant abiotic stress responses. An *in silico* expression analysis of these genes revealed an interesting pattern. The majority of the genes identified for spikelet fertility showed upregulation in reproductive and grain tissues, while the genes identified for grain yield showed higher expression levels in vegetative organs. Furthermore, nearly 10 genes matched with two heat stress-related DEGs identified in the previous transcriptomics studies (Cai et al., 2020; Liu et al., 2020). However, the logarithm of foldchange (Log_2FC) values of the genes are <2.5 except for LOC_Os01g04580, a Ser/Thr protein kinase which was significantly downregulated at 6 h of heat stress exposure. This is understandable since the genotypes (SDWG005 and MH101) used in these two reports were completely different from those in the current study.

Thus, in the present study, significant reductions in grain yield and spikelet fertility were observed among the rice genotypes characterized for RSHT. GWAS identified many

novel MTAs, explaining high phenotypic variance for both these traits. There was a clear difference between the effects of alternate alleles, indicating their significance in governing RSHT in rice. The majority of the candidate genes identified around these MTAs were either directly or indirectly involved in heat stress and other abiotic stress responses, which are valuable candidates for marker-assisted selection for the improvement of heat stress tolerance at a reproductive stage after further validation in future. Some uncharacterized genes were also observed for both grain yield and spikelet fertility, whose function needs to be elucidated in future studies.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The name of the repository and link to the data can be found below: ICAR; <https://krishi.icar.gov.in/jspui/handle/123456789/31947>.

AUTHOR CONTRIBUTIONS

SG and AS conceived the idea and formulated the research plan. KR carried out the research work and prepared the manuscript. MN and PB assisted in the execution of field trials. KV, RE, and KA assisted in data analysis. BH shared seed materials and generated the genotypic data of the germplasm. SG, KV, and MP improved the manuscript. AS provided overall guidance in each of these activities.

FUNDING

This research was funded by the ICAR-funded network project on the National Innovations on Climate Resilient Agriculture (NICRA) and the National Agricultural Higher Education Project (NAHEP) – Center for Advanced Agricultural Science and Technology (CAAST) project on “Genomics Assisted Breeding for Crop Improvement” of the World Bank and the Indian Council of Agricultural Research, New Delhi.

ACKNOWLEDGMENTS

The study is part of the PhD research of the first author. RKT is thankful to the Director, ICAR-CSSRI, Karnal, and ICAR-IARI, New Delhi, for facilitating his PhD.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2022.876522/full#supplementary-material>

REFERENCES

- Bac-Molenaar, J. A., Fradin, E. F., Becker, F. F. M., Rienstra, J. A., van der Schoot, J., Vreugdenhil, D., et al. (2015). Genome-Wide Association Mapping of Fertility Reduction upon Heat Stress Reveals Developmental Stage-Specific QTLs in *Arabidopsis thaliana*. *Plant Cell* 27, 1857–1874. doi:10.1105/tpc.15.00248
- Baker, J. T., Allen, L. H., Jr, and Boote, K. J. (1992). Temperature Effects on Rice at Elevated CO₂ Concentration. *J. Exp. Bot.* 43, 959–964. doi:10.1093/jxb/43.7.959
- Baytar, A. A., Peynircioglu, C., Sezener, V., Basal, H., Frary, A., Frary, A., et al. (2018). Genome-Wide Association Mapping of Yield Components and Drought Tolerance-Related Traits in Cotton. *Mol. Breed.* 38, 1–16. doi:10.1007/s11032-018-0831-0
- Bheemanahalli, R., Sathishraj, R., Tack, J., Nalley, L. L., Muthurajan, R., and Jagadish, K. S. V. (2016). Temperature Thresholds for Spikelet Sterility and Associated Warming Impacts for Sub-Tropical Rice. *Agric. For. Meteorol.* 221, 122–130. doi:10.1016/j.agrformet.2016.02.003
- Bollinedi, H., Yadav, A. K., Vinod, K. K., Gopala Krishnan, S., Bhowmick, P. K., Nagarajan, M., et al. (2020). Genome-Wide Association Study Reveals Novel Marker-Trait Associations (MTAs) Governing the Localization of Fe and Zn in the Rice Grain. *Front. Genet.* 11, 213. doi:10.3389/fgene.2020.00213
- Byun, M. Y., Cui, L. H., Oh, T. K., Jung, Y.-J., Lee, A., Park, K. Y., et al. (2017). Homologous U-Box E3 Ubiquitin Ligases OsPUB2 and OsPUB3 Are Involved in the Positive Regulation of Low Temperature Stress Response in Rice (*Oryza sativa* L.). *Front. Plant Sci.* 8, 16. doi:10.3389/fpls.2017.00016
- Cai, Z., He, F., Feng, X., Liang, T., Wang, H., Ding, S., et al. (2020). Transcriptomic Analysis Reveals Important Roles of Lignin and Flavonoid Biosynthetic Pathways in Rice Thermotolerance During Reproductive Stage. *Front. Genet.* 11, 562937. doi:10.3389/fgene.2020.562937
- Cao, L., Zhao, J., Zhan, X., Li, D., He, L., and Cheng, S. (2003). Mapping QTLs for Heat Tolerance and Correlation Between Heat Tolerance and Photosynthetic Rate in Rice. *Chin. J. Rice Sci.* 17, 223–227.
- Cheabu, S., Panichawong, N., Rattanamettha, P., Wasuri, B., Kasemsap, P., Arikrit, S., et al. (2019). Screening for Spikelet Fertility and Validation of Heat Tolerance in a Large Rice Mutant Population. *Rice Sci.* 26, 229–238. doi:10.1016/j.rsci.2018.08.008
- Chen, E., Huang, X., Tian, Z., Wing, R. A., and Han, B. (2019). The Genomics of *Oryza* Species Provides Insights into Rice Domestication and Heterosis. *Annu. Rev. Plant Biol.* 70, 639–665. doi:10.1146/annurev-arplant-050718-100320
- Chen, J.-H., Chen, S.-T., He, N.-Y., Wang, Q.-L., Zhao, Y., Gao, W., et al. (2020). Nuclear-Encoded Synthesis of the D1 Subunit of Photosystem II Increases Photosynthetic Efficiency and Crop Yield. *Nat. Plants* 6, 570–580. doi:10.1038/s41477-020-0629-z
- Chidambaramanathan, P., Balasubramaniasai, C., Behura, N., Purty, M., Samantaray, S., Subudhi, H., et al. (2021). Effects of High Temperature on Spikelet Sterility in Rice (*Oryza sativa* L.): Association Between Molecular Markers and Allelic Phenotypic Effect in Field Condition. *Genet. Resour. Crop Evol.* 68, 1923–1935. doi:10.1007/s10722-021-01106-7
- Ciechanover, A. (1998). The Ubiquitin-Proteasome Pathway: On Protein Death and Cell Life. *EMBO J.* 17, 7151–7160. doi:10.1093/emboj/17.24.7151
- de Brito, G. G., Moraes, Í. L. d., Moura, D. S., Fagundes, P. R. R., Campos, A. D., Andres, A., et al. (2019). Non-Invasive Physiological Approaches for Plant Phenotyping: Rice Responses to Heat Stress. *Jas* 11, 453–464. doi:10.5539/jas.v11n3p453
- Endo, M., Tsuchiya, T., Hamada, K., Kawamura, S., Yano, K., Ohshima, M., et al. (2009). High Temperatures Cause Male Sterility in Rice Plants with Transcriptional Alterations During Pollen Development. *Plant Cell Physiol.* 50, 1911–1922. doi:10.1093/pcp/pcp135
- Fernandez, G. C. (1992). “Effective Selection Criteria for Assessing Plant Stress Tolerance,” in Proceeding of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress, Shanhua, Taiwan, 257–270.
- Gahlaut, V., Jaiswal, V., Singh, S., Balyan, H. S., and Gupta, P. K. (2019). Multi-Locus Genome Wide Association Mapping for Yield and its Contributing Traits in Hexaploid Wheat Under Different Water Regimes. *Sci. Rep.* 9, 1–15. doi:10.1038/s41598-019-55520-0
- GoI (2019). *Agricultural Statistics at a Glance*. India: Directorate of Economics & Statistics.
- González-Schain, N., Dreni, L., Lawas, L. M. F., Galbiati, M., Colombo, L., Heuer, S., et al. (2016). Genome-Wide Transcriptome Analysis During Anthesis Reveals New Insights into the Molecular Basis of Heat Stress Responses in Tolerant and Sensitive Rice Varieties. *Plant Cell Physiol.* 57, 57–68. doi:10.1093/pcp/pcv174
- Guo, J., Shi, W., Guo, J., Yue, L., Zhuang, L., Zhang, W., et al. (2020). Genome-Wide Association Studies on Heat Stress Tolerance During Grain Development in Wheat (*Triticum aestivum* L.). Research Square [Preprint]. doi:10.21203/rs.2.20460/v1
- Hada, A., Dutta, T. K., Singh, N., Singh, B., Rai, V., Singh, N. K., et al. (2020). A Genome-Wide Association Study in Indian Wild Rice Accessions for Resistance to the Root-Knot Nematode *Meloidogyne graminicola*. *PLoS ONE* 15, e0239085. doi:10.1371/journal.pone.0239085
- He, Y.-M., Liu, K.-K., Zhang, H.-X., Cheng, G.-X., Ali, M., Ul Haq, S., et al. (2019). Contribution of CaBPM4, a BTB Domain-Containing Gene, to the Response of Pepper to *Phytophthora capsici* Infection and Abiotic Stresses. *Agronomy* 9, 417. doi:10.3390/agronomy9080417
- Hirabayashi, H., Sasaki, K., Kambe, T., Gannaban, R. B., Miras, M. A., Mendioro, M. S., et al. (2014). *qEMF3*, a Novel QTL for the Early-Morning Flowering Trait from Wild Rice, *Oryza officinalis*, to Mitigate Heat Stress Damage at Flowering in Rice, *O. Sativa* L. *J. Exp. Bot.* 66, 1227–1236. doi:10.1093/jxb/eru474
- Hossain, M. (2021). *As Rising 'heat Shocks' Ruin rice Crops, Bangladesh Faces Hunger Risk*. Thomson Reuters Foundation News, Thomson Reuters Foundation. Available at: <https://news.trust.org/item/20210528065131-wnlhg/> (Accessed May, 2021).
- Hsu, K.-H., Liu, C.-C., Wu, S.-J., Kuo, Y.-Y., Lu, C.-A., Wu, C.-R., et al. (2014). Expression of a Gene Encoding a rice RING Zinc-finger Protein, OsRZFP34, Enhances Stomata Opening. *Plant Mol. Biol.* 86, 125–137. doi:10.1007/s11103-014-0217-6
- Huang, J., Chen, F., Del Casino, C., Autino, A., Shen, M., Yuan, S., et al. (2006). An Ankyrin Repeat-Containing Protein, Characterized as a Ubiquitin Ligase, Is Closely Associated with Membrane-Enclosed Organelles and Required for Pollen Germination and Pollen Tube Growth in Lily. *Plant Physiol.* 140, 1374–1383. doi:10.1104/pp.105.074922
- Huang, J., Zhao, X., Yu, H., Ouyang, Y., Wang, L., and Zhang, Q. (2009). The Ankyrin Repeat Gene Family in Rice: Genome-Wide Identification, Classification and Expression Profiling. *Plant Mol. Biol.* 71, 207–226. doi:10.1007/s11103-009-9518-6
- Huang, L., Sun, Y., Peng, S., and Wang, F. (2016). Genotypic Differences of Japonica Rice Responding to High Temperature in China. *Agron.j.* 108, 626–636. doi:10.2134/agronj2015.0507
- Huang, M., Liu, X., Zhou, Y., Summers, R. M., and Zhang, Z. (2019). BLINK: A Package for the Next Level of Genome-Wide Association Studies with Both Individuals and Markers in the Millions. *Gigascience* 8, giy154. doi:10.1093/gigascience/giy154
- Hur, Y.-J., Yi, Y. B., Lee, J. H., Chung, Y. S., Jung, H. W., Yun, D. J., et al. (2012). Molecular Cloning and Characterization of OsUPS, a U-Box Containing E3 Ligase Gene that Respond to Phosphate Starvation in rice (*Oryza sativa*). *Mol. Biol. Rep.* 39, 5883–5888. doi:10.1007/s11033-011-1399-5
- Im, E.-S., Pal, J. S., and Eltahir, E. A. B. (2017). Deadly Heat Waves Projected in the Densely Populated Agricultural Regions of South Asia. *Sci. Adv.* 3, e1603322. doi:10.1126/sciadv.1603322
- IPCC (2014). “Summary for Policymakers,” in *Climate Change 2013: The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Editors T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, and Allen (Cambridge, United Kingdom and New York, NY: Cambridge University Press).
- IRRI (2002). *Standard Evaluation System for rice (SES)*. Los Banos, Philippines: International Rice Research Institute.
- Ishimaru, T., Hirabayashi, H., Ida, M., Takai, T., San-Oh, Y. A., Yoshinaga, S., et al. (2010). A Genetic Resource for Early-Morning Flowering Trait of Wild Rice *Oryza officinalis* to Mitigate High Temperature-Induced Spikelet Sterility at Anthesis. *Ann. Bot.* 106, 515–520. doi:10.1093/aob/mcq124
- Jagadish, S. V. K., Cairns, J., Lafitte, R., Wheeler, T. R., Price, A. H., and Craufurd, P. Q. (2010). Genetic Analysis of Heat Tolerance at Anthesis in Rice. *Crop Sci.* 50, 1633–1641. doi:10.2135/cropsci2009.09.0516

- Julia, C., and Dingkuhn, M. (2013). Predicting Temperature Induced Sterility of Rice Spikelets Requires Simulation of Crop-Generated Microclimate. *Eur. J. Agron.* 49, 50–60. doi:10.1016/j.eja.2013.03.006
- Jun, X. U., Wang, X. Y., and Guo, W. Z. (2015). The Cytochrome P450 Superfamily: Key Players in Plant Development and Defense. *J. Integr. Agric.* 14, 1673–1686. doi:10.1016/S2095-3119(14)60980-1
- Khanzada, H., Wassan, G. M., He, H., Mason, A. S., Keerio, A. A., Khanzada, S., et al. (2020). Differentially Evolved Drought Stress Indices Determine the Genetic Variation of Brassica Napus at Seedling Traits by Genome-Wide Association Mapping. *J. Adv. Res.* 24, 447–461. doi:10.1016/j.jare.2020.05.019
- Kim, J. H., Lim, S. D., and Jang, C. S. (2019). *Oryza sativa* Heat-Induced RING Finger Protein 1 (*OsHIRP1*) Positively Regulates Plant Response to Heat Stress. *Plant Mol. Biol.* 99, 545–559. doi:10.1007/s11103-019-00835-9
- Krishnan, R., Sanjay, J., Gnanaseelan, C., Mujumdar, M., Kulkarni, A., and Chakraborty, S. (2020). *Assessment of Climate Change Over the Indian Region*. Singapore: SpringerOpen. doi:10.1007/978-981-15-4327-2
- Lafarge, T., Bueno, C., Frouin, J., Jacquin, L., Courtois, B., and Ahmadi, N. (2017). Genome-Wide Association Analysis for Heat Tolerance at Flowering Detected a Large Set of Genes Involved in Adaptation to thermal and Other Stresses. *PLoS ONE* 12, e0171254. doi:10.1371/journal.pone.0171254
- Li, J., Mahajan, A., and Tsai, M.-D. (2006). Ankyrin Repeat: A Unique Motif Mediating Protein–Protein Interactions. *Biochemistry* 45, 15168–15178. doi:10.1021/bi062188q
- Li, Y.-F., Wang, Y., Tang, Y., Kakani, V. G., and Mahalingam, R. (2013). Transcriptome Analysis of Heat Stress Response in Switchgrass (*Panicum virgatum* L.). *BMC Plant Biol.* 13, 153. doi:10.1186/1471-2229-13-153
- Li, X.-M., Chao, D.-Y., Wu, Y., Huang, X., Chen, K., Cui, L.-G., et al. (2015). Natural Alleles of a Proteasome $\alpha 2$ Subunit Gene Contribute to Thermotolerance and Adaptation of African rice. *Nat. Genet.* 47, 827–833. doi:10.1038/ng.3305
- Li, C., Wang, D., PengChen, S. Y., Chen, Y., Su, P., Chen, J., et al. (2019). Genome-wide Association Mapping of Resistance against rice Blast Strains in South China and Identification of a New *Pik* Allele. *Rice* 12, 47. doi:10.1186/s12284-019-0309-7
- Lim, S. D., Cho, H. Y., Park, Y. C., Ham, D. J., Lee, J. K., and Jang, C. S. (2013). The rice RING finger E3 Ligase, *OsHCII*, Drives Nuclear export of Multiple Substrate Proteins and its Heterogeneous Overexpression Enhances Acquired Thermotolerance. *J. Exp. Bot.* 64, 2899–2914. doi:10.1093/jxb/ert143
- Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., et al. (2012). GAPIT: Genome Association and Prediction Integrated Tool. *Bioinform* 28, 2397–2399. doi:10.1093/bioinformatics/bts444
- Liu, X., Huang, M., Fan, B., Buckler, E. S., and Zhang, Z. (2016). Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-wide Association Studies. *Plos Genet.* 12, e1005767. doi:10.1371/journal.pgen.1005767
- Liu, G., Zha, Z., Cai, H., Qin, D., Jia, H., Liu, C., et al. (2020). Dynamic Transcriptome Analysis of Anther Response to Heat Stress during Anthesis in Thermotolerant Rice (*Oryza Sativa* L.). *Ijms* 21, 1155. doi:10.3390/ijms21031155
- Lyman, N. B., Jagadish, K. S. V., Nalley, L. L., Dixon, B. L., and Siebenmorgen, T. (2013). Neglecting rice Milling Yield and Quality Underestimates Economic Losses from High-Temperature Stress. *PLoS ONE* 8, e72157. doi:10.1371/journal.pone.0072157
- Matsui, T., and Omasa, K. (2002). Rice (*Oryza Sativa* L.) Cultivars Tolerant to High Temperature at Flowering: Anther Characteristics. *Ann. Bot.* 89, 683–687. doi:10.1093/aob/mcf112
- Matsui, T., Omasa, K., and Horie, T. (2000). High Temperature at Flowering Inhibit Swelling of Pollen Grains, a Driving Force for Thecae Dehiscence in Rice (*Oryza sativa* L.). *Plant Prod. Sci.* 3, 430–434. doi:10.1626/pp.3.430
- Matsui, T., Omasa, K., and Horie, T. (2001). The Difference in Sterility Due to High Temperatures During the Flowering Period Among Japonica-Rice Varieties. *Plant Prod. Sci.* 4, 90–93. doi:10.1626/pp.4.90
- McCouch, S. R., Wright, M. H., Tung, C.-W., Maron, L. G., McNally, K. L., Fitzgerald, M., et al. (2016). Open Access Resources for Genome-Wide Association Mapping in Rice. *Nat. Commun.* 7, 10532. doi:10.1038/ncomms10532
- Melandri, G., Prashar, A., McCouch, S. R., Van Der Linden, G., Jones, H. G., Kadam, N., et al. (2020). Association Mapping and Genetic Dissection of Drought-Induced Canopy Temperature Differences in Rice. *J. Exp. Bot.* 71, 1614–1627. doi:10.1093/jxb/erz527
- Merrick, L. F., Burke, A. B., Zhang, Z., and Carter, A. H. (2022). Comparison of Single-Trait and Multi-Trait Genome-wide Association Models and Inclusion of Correlated Traits in the Dissection of the Genetic Architecture of a Complex Trait in a Breeding Program. *Front. Plant Sci.* 12, 772907. doi:10.3389/fpls.2021.772907
- Michaely, P., and Bennett, V. (1992). The ANK Repeat: A Ubiquitous Motif Involved in Macromolecular Recognition. *Trends Cel. Biol.* 2, 127–129. doi:10.1016/0962-8924(92)90084-z
- Misra, G., Anacleto, R., Badoni, S., Butardo, V., Jr, Molina, L., Graner, A., et al. (2019). Dissecting the Genome-Wide Genetic Variants of Milling and Appearance Quality Traits in Rice. *J. Exp. Bot.* 70, 5115–5130. doi:10.1093/jxb/erz256
- Mittal, D., Madhyastha, D. A., and Grover, A. (2012). Genome-Wide Transcriptional Profiles During Temperature and Oxidative Stress Reveal Coordinated Expression Patterns and Overlapping Regulons in Rice. *PLoS ONE* 7, e40899. doi:10.1371/journal.pone.0040899
- Morimoto, K., Ohama, N., Kidokoro, S., Mizoi, J., Takahashi, F., Todaka, D., et al. (2017). BPM-CUL3 E3 Ligase Modulates Thermotolerance by Facilitating Negative Regulatory Domain-Mediated Degradation of DREB2A in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* 114, E8528–E8536. doi:10.1073/pnas.1704189114
- Murray, M. G., and Thompson, W. F. (1980). Rapid Isolation of High Molecular Weight Plant DNA. *Nucl. Acids Res.* 8, 4321–4326. doi:10.1093/nar/8.19.4321
- Obaid, A. Y., Sabir, J. S. M., Atef, A., Liu, X., Edris, S., El-Domyati, F. M., et al. (2016). Analysis of Transcriptional Response to Heat Stress in *Rhazya stricta*. *BMC Plant Biol.* 16, 252. doi:10.1186/s12870-016-0938-6
- Paeng, S. K., Kang, C. H., Chi, Y. H., Chae, H. B., Lee, E. S., Park, J. H., et al. (2020). AtTPR10 Containing Multiple ANK and TPR Domains Exhibits Chaperone Activity and Heat-Shock Dependent Structural Switching. *Appl. Sci.* 10, 1265. doi:10.3390/app10041265
- Pandian, B. A., Sathishraj, R., Djanaguiraman, M., Prasad, P. V. V., and Jugulam, M. (2020). Role of Cytochrome P450 Enzymes in Plant Stress Response. *Antioxidants* 9, 454. doi:10.3390/antiox9050454
- Peng, S., Huang, J., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X., et al. (2004). Rice Yields Decline with Higher Night Temperature from Global Warming. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9971–9975. doi:10.1073/pnas.0403720101
- Pradhan, S. K., Barik, S. R., Sahoo, A., Mohapatra, S., Nayak, D. K., Mahender, A., et al. (2016). Population Structure, Genetic Diversity and Molecular Marker-Trait Association Analysis for High Temperature Stress Tolerance in Rice. *PLoS ONE* 11, e0160027. doi:10.1371/journal.pone.0160027
- Prasad, P. V. V., Boote, K. J., Allen, L. H., Sheehy, J. E., and Thomas, J. M. G. (2006). Species, Ecotype and Cultivar Differences in Spikelet Fertility and Harvest Index of Rice in Response to High Temperature Stress. *Field Crops Res.* 95, 398–411. doi:10.1016/j.fcr.2005.04.008
- Rahaman, M., Mamidi, S., and Rahman, M. (2018). Genome-Wide Association Study of Heat Stress-Tolerance Traits in Spring-Type *Brassica napus* L. Under Controlled Conditions. *Crop J.* 6, 115–125. doi:10.1016/j.cj.2017.08.003
- Ravikiran, K. T., Gopala Krishnan, S., Vinod, K. K., Dhawan, G., Dwivedi, P., Kumar, P., et al. (2020). A Trait Specific QTL Survey Identifies NL44, a NERICA Cultivar as a Novel Source for Reproductive Stage Heat Stress Tolerance in Rice. *Plant Physiol. Rep.* 25, 664–676. doi:10.1007/s40502-020-00547-z
- Rohilla, M., Singh, N., Mazumder, A., Sen, P., Roy, P., Chowdhury, D., et al. (2020). Genome-Wide Association Studies Using 50 K Rice Genic SNP Chip Unveil Genetic Architecture for Anaerobic Germination of Deep-Water Rice Population of Assam, India. *Mol. Genet. Genomics* 295, 1211–1226. doi:10.1007/s00438-020-01690-w
- Ryu, M. Y., Cho, S. K., Hong, Y., Kim, J., Kim, J. H., Kim, G. M., et al. (2019). Classification of Barley U-Box E3 Ligases and Their Expression Patterns in Response to Drought and Pathogen Stresses. *BMC Genomics* 20, 1–15. doi:10.1186/s12864-019-5696-z
- Satake, T., and Yoshida, S. (1978). High Temperature-Induced Sterility in Indica Rices at Flowering. *Jpn. J. Crop Sci.* 47, 6–17. doi:10.1626/jcs.47.6
- Segura, V., Vilhjálmsson, B. J., Platt, A., Korte, A., Seren, Ü., Long, Q., et al. (2012). An Efficient Multi-Locus Mixed-Model Approach for Genome-Wide Association Studies in Structured Populations. *Nat. Genet.* 44, 825–830. doi:10.1038/ng.2314
- Sharma, D., Jaiswal, J. P., Gahtyari, N. C., Chauhan, A., Chhabra, R., Saripalli, G., et al. (2020). Population Structure, Marker-Trait Association and Identification of Candidate Genes for Terminal Heat Stress Relevant Traits in Bread Wheat (*Triticum aestivum* L. Em Thell). *Plant Genet. Resour.* 18, 168–178. doi:10.1017/S1479262120000131

- Shen, G., Kuppu, S., Venkataramani, S., Wang, J., Yan, J., Qiu, X., et al. (2010). ANKYRIN REPEAT-CONTAINING PROTEIN 2A is an Essential Molecular Chaperone for Peroxisomal Membrane-Bound ASCORBATE PEROXIDASE3 in *Arabidopsis*. *Plant Cell* 22, 811–831. doi:10.1105/tpc.109.065979
- Singh, N., Jayaswal, P. K., Panda, K., Mandal, P., Kumar, V., Singh, B., et al. (2015). Single-Copy Gene Based 50 K SNP Chip for Genetic Studies and Molecular Breeding in Rice. *Sci. Rep.* 5, 11600. doi:10.1038/srep11600
- Sukkeo, S., Rerkasem, B., and Jamjod, S. (2017). Heat Tolerance in Thai Rice Varieties. *ScienceAsia* 43, 61–69. doi:10.2306/scienceasia1513-1874.2017.43.061
- Tadesse, W., Suleiman, S., Tahir, I., Sanchez-Garcia, M., Jighly, A., Hagras, A., et al. (2019). Heat-Tolerant QTLs Associated with Grain Yield and its Components in Spring Bread Wheat Under Heat-Stressed Environments of Sudan and Egypt. *Crop Sci.* 59, 199–211. doi:10.2135/cropsci2018.06.0389
- Tafesse, E. G., Gali, K. K., Lachagari, V. B. R., Bueckert, R., and Warkentin, T. D. (2020). Genome-Wide Association Mapping for Heat Stress Responsive Traits in Field Pea. *Ijms* 21, 2043. doi:10.3390/ijms21062043
- Tang, L., Zhang, F., LiuSun, A. J., Sun, J., Mei, S., Wang, X., et al. (2019). Genome-Wide Association Analysis Dissects the Genetic Basis of the Grain Carbon and Nitrogen Contents in Milled Rice. *Rice* 12, 101. doi:10.1186/s12284-019-0362-2
- Tazib, T., Kobayashi, Y., Koyama, H., and Matsui, T. (2015). QTL Analyses for Anther Length and Dehiscence at Flowering as Traits for the Tolerance of Extreme Temperatures in Rice (*Oryza sativa* L.). *Euphytica* 203, 629–642. doi:10.1007/s10681-014-1291-1
- Tenorio, F. A., Ye, C., Redona, E., Sierra, S., Laza, M., and Argayoso, M. A. (2013). Screening Rice Genetic Resources for Heat Tolerance. *SABRAO J. Breed. Genet.* 45, 371–381.
- Thomson, M. J., Singh, N., Dwiyantri, M. S., Wang, D. R., Wright, M. H., Perez, F. A., et al. (2017). Large-Scale Deployment of a Rice 6 K SNP Array for Genetics and Breeding Applications. *Rice* 10, 40. doi:10.1186/s12284-017-0181-2
- Wang, D., Qin, B., Li, X., Tang, D., Zhang, Y. e., Cheng, Z., et al. (2016). Nucleolar DEAD-Box RNA Helicase TOGR1 Regulates Thermotolerant Growth as a Pre-rRNA Chaperone in Rice. *Plos Genet.* 12, e1005844. doi:10.1371/journal.pgen.1005844
- Wang, Y., Zhang, Y., Zhang, Q., Cui, Y., Xiang, J., Chen, H., et al. (2019). Comparative Transcriptome Analysis of Panicle Development Under Heat Stress in Two Rice (*Oryza sativa* L.) Cultivars Differing in Heat Tolerance. *PeerJ* 7, e7595. doi:10.7717/peerj.7595
- Wassmann, R., Jagadish, S. V. K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., et al. (2009). Chapter 3 Regional Vulnerability of Climate Change Impacts on Asian Rice Production and Scope for Adaptation. *Adv. Agron.* 102, 91–133. doi:10.1016/S0065-2113(09)01003-7
- Wedow, J. M., Yendrek, C. R., Mello, T. R., Creste, S., Martinez, C. A., and Ainsworth, E. A. (2019). Metabolite and Transcript Profiling of Guinea Grass (*Panicum maximum* Jacq) Response to Elevated [CO₂] and Temperature. *Metabolomics* 15, 51. doi:10.1007/s11306-019-1511-8
- Wu, Y.-S., and Yang, C.-Y. (2019). Ethylene-Mediated Signaling Confers Thermotolerance and Regulates Transcript Levels of Heat Shock Factors in Rice Seedlings Under Heat Stress. *Bot. Stud.* 60, 1–12. doi:10.1186/s40529-019-0272-z
- Xiao, Y. H., Pan, Y., Luo, L., Zhang, G., Deng, H., Dai, L., et al. (2011a). Quantitative Trait Loci Associated with Seed Set Under High Temperature Stress at the Flowering Stage in Rice (*Oryza sativa* L.). *Euphytica* 178, 331–338. doi:10.1007/s10681-010-0300-2
- Xiao, Y. H., Pan, Y., Luo, L. H., Deng, H. B., Zhang, G. L., Tang, W. B., et al. (2011b). Quantitative Trait Loci Associated with Pollen Fertility Under High Temperature Stress at Flowering Stage in Rice (*Oryza sativa* L.). *Rice Sci.* 18, 1–7. doi:10.1016/s1672-6308(11)60028-0
- Xu, Y., Zhang, L., Ou, S., Wang, R., Wang, Y., Chu, C., et al. (2020). Natural Variations of *SLG1* Confer High-Temperature Tolerance in *Indica* Rice. *Nat. Commun.* 11, 1–13. doi:10.1038/s41467-020-19320-9
- Ye, C., Argayoso, M. A., Redoña, E. D., Sierra, S. N., Laza, M. A., Dilla, C. J., et al. (2012). Mapping QTL for Heat Tolerance at Flowering Stage in Rice Using SNP Markers. *Plant Breed* 131, 33–41. doi:10.1111/j.1439-0523.2011.01924.x
- Ye, C., Tenorio, F. A., Argayoso, M. A., Laza, M. A., Koh, H.-J., Redoña, E. D., et al. (2015a). Identifying and Confirming Quantitative Trait Loci Associated with Heat Tolerance at Flowering Stage in Different Rice Populations. *BMC Genet.* 16, 41. doi:10.1186/s12863-015-0199-7
- Ye, C., Tenorio, F. A., Redoña, E. D., Morales-Cortezano, P. S., Cabrega, G. A., Jagadish, K. S. V., et al. (2015b). Fine-Mapping and Validating *qHTSF4.1* to Increase Spikelet Fertility Under Heat Stress at Flowering in Rice. *Theor. Appl. Genet.* 128, 1507–1517. doi:10.1007/s00122-015-2526-9
- Yoshida, S., Satake, T., and Mackill, D. (1981). High Temperature Stress. *IRRI Res. Paper Ser.* 67, 1–15.
- Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., et al. (2006). A Unified Mixed-Model Method for Association Mapping that Accounts for Multiple Levels of Relatedness. *Nat. Genet.* 38, 203–208. doi:10.1038/ng1702
- Yu, H., Xie, W., Li, J., Zhou, F., and Zhang, Q. (2014). A Whole-Genome SNP Array (RICE6K) for Genomic Breeding in Rice. *Plant Biotechnol. J.* 12, 28–37. doi:10.1111/pbi.12113
- Yuan, J., Wang, X., ZhaoKhan, Y. N. U., Khan, N. U., Zhao, Z., Zhang, Y., et al. (2020). Genetic Basis and Identification of Candidate Genes for Salt Tolerance in Rice by GWAS. *Sci. Rep.* 10, 9958. doi:10.1038/s41598-020-66604-7
- Zhang, S., Chen, C., Li, L., Meng, L., Singh, J., Jiang, N., et al. (2005). Evolutionary Expansion, Gene Structure, and Expression of the Rice Wall-Associated Kinase Gene Family. *Plant Physiol.* 139, 1107–1124. doi:10.1104/pp.105.069005
- Zhang, Y.-M., Mao, Y., Xie, C., Smith, H., Luo, L., and Xu, S. (2005). Mapping Quantitative Trait Loci Using Naturally Occurring Genetic Variance Among Commercial Inbred Lines of Maize (*Zea mays* L.). *Genetics* 169, 2267–2275. doi:10.1534/genetics.104.033217
- Zhang, X., Li, J., Liu, A., Zou, J., Zhou, X., Xiang, J., et al. (2012). Expression Profile in Rice Panicle: Insights into Heat Response Mechanism at Reproductive Stage. *Plos ONE* 7, e49652. doi:10.1371/journal.pone.0049652
- Zhang, Y.-W., Tamba, C. L., Wen, Y.-J., Li, P., Ren, W.-L., Ni, Y.-L., et al. (2020). mrMLM v4.0.2: An R Platform for Multi-Locus Genome-Wide Association Studies. *Genomics, Proteomics Bioinformatics* 18, 481–487. doi:10.1016/j.gpb.2020.06.006
- Zhao, K., Tung, C.-W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., et al. (2011). Genome-Wide Association Mapping Reveals a Rich Genetic Architecture of Complex Traits in *Oryza sativa*. *Nat. Commun.* 2, 467. doi:10.1038/ncomms1467
- Zhao, Q., Feng, Q., Lu, H., Li, Y., Wang, A., Tian, Q., et al. (2018). Pan-Genome Analysis Highlights the Extent of Genomic Variation in Cultivated and Wild Rice. *Nat. Genet.* 50, 278–284. doi:10.1038/s41588-018-0041-z

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Copyright © 2022 Ravikiran, Gopala Krishnan, Abhijith, Bollinedi, Nagarajan, Vinod, Bhowmick, Pal, Ellur and Singh. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.