

**MOLECULAR ANALYSIS FOR TRAITS RELATED
TO DROUGHT ADAPTATION IN RICE
(*Oryza sativa* L.)**

**A Thesis
Submitted to the
Assam Agricultural University**

**In partial fulfilment of the requirements for the degree of
DOCTOR OF PHILOSOPHY (AGRICULTURE)**

**IN
AGRICULTURAL BIOTECHNOLOGY**



By

**SUBHROTA BORDOLOI
Regn No. 256 of 2018
Roll No. 2018-ADJ-08**

**DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
ASSAM AGRICULTURAL UNIVERSITY
Jorhat-785013(ASSAM)
AUGUST, 2023**

ASSAM AGRICULTURAL UNIVERSITY
FACULTY OF AGRICULTURE

CERTIFICATE-I

This is to certify that the thesis entitled “**Molecular Analysis for traits related to draught adaptation in Rice (*Oryza sativa* L.)**” submitted to the Faculty of Agriculture, Assam Agricultural University in partial fulfilment of the degree of Doctor of Philosophy (Agriculture) in Agricultural Biotechnology is a record of research work carried out by **Subhrota Bordoloi** under my personal supervision and guidance.

All help received by her have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for any degree.

Place: Jorhat

Dated: The....., 20.....

(Akhil Ranjan Baruah)
Major Advisor
Assistant Professor
Department of Agricultural Biotechnology
College of Agriculture
AAU, Jorhat-13

CERTIFICATE II

This is to certify that the thesis entitled “**Molecular Analysis for traits related to draught adaptation in Rice (*Oryza sativa* L.)**” submitted by **Subhrota Bordoloi**, Roll No. **2018-ADJ-08** to the Assam Agricultural University in partial fulfilment of the requirement for the degree of **Doctor of Philosophy (Agriculture) in Agricultural Biotechnology** has been examined and approved by the Student’s Advisory Committee after viva- voce.

(Akhil Ranjan Baruah)
Major Advisor

Chairman
Board of Examiners

Members of the Advisory committee:

1. -----
(P. Bhorali)

2. -----
(Samindra Baishya)

3. -----
(K. Das)

Professor & Head
Department of Agricultural Biotechnology
Assam Agricultural University
Jorhat-13

Director
Post-graduate studies
Assam Agricultural University
Jorhat-13

Acknowledgements

*First and foremost, I would like to express my sincere gratitude to my esteemed supervisor **Dr. Akhil Ranjan Baruah**, Assistant Professor, Department of Agricultural Biotechnology, AAU, Jorhat, Assam for his constant support, mentoring and guidance. His expertise and support kept me motivated in completing my Ph.D. work. Their generous contributions and valuable suggestions have presented the thesis in its current form.*

*I would also like to express my gratitude to **Dr. M. K. Modi** (Head of Dept and Member of Major Discipline), **Dr. P. Bharali** (Member of Major Discipline), **Dr. S. Baishya** (Member of Minor Discipline), **Dr. K. Das** (Member supporting Discipline), **Dr. B.K. Sarmah** (Director, DBT-NECAB) and all other faculty members of Agricultural Biotechnology for instilling faith and patience during the research work.*

Furthermore, I would like to acknowledge DPGS, ICR farm, RARS, Karimganj, DBT-NECAB for providing different amenities like funds, Field workers and fellowship to smooth conduction of the research work.

I would also like to extend my gratitude to the ERASMUS program for providing the exposure to visit Greece for three months training programme at Academy of Athens under Prof. Nastos.

I also take the privilege to express profound gratitude to Citrus Lab, Marker Lab members, Ms. Gracia, Ms. Bhagyashree, Jayashankar, Ms. Khonang, Ms. Neha Pandey, Dipen, Dr. Rahul Kaldate for their continuous support in completing this research work.

Additionally, I, also extend my sincere thanks to my dear scholar colleagues (Kuntal Da, Manab Sir, Richita, Jyotsna Di, Atsey) and seniors for their valuable suggestions and feedbacks.

I would like to thank my parents, my elder sister and brother-in-law for putting up with me and giving their continuous love and motivation throughout.

Place: Jorhat

Date:....., 202....

The Author

ABSTRACT

Drought is a major abiotic constraint for rice production worldwide. Model based studies revealed reproductive stage drought stress (RSDS) and intermittent drought stress (IMDS) are expected to reduce yield significantly in *Sali* and *Ahu* season, respectively. The present study was conducted with the aim to detect the genetic effects of water stress through quantitative trait locus (QTLs) associated with drought tolerance in rice. The donor and the recipient parent were selected based on the phenotypic evaluation and gene expression analysis using five drought responsive genes in two drought tolerant cultivar (*ARC10372*, *Koimurali*) and two susceptible cultivar (*Disang*, *IR-64*). Based on the results, *Koimurali* was crossed with a drought susceptible cultivar, *Disang* to obtained true F₁ seeds. Subsequently the single F₁ seeds was selfed for and advanced to subsequent generations (F_{5:6}) using single seed descent (SSD) method to develop the mapping population consisting of 247 RILs. Parental polymorphism study was conducted by screening 600 SSR markers; among which 98 SSR markers were found to be polymorphic (16.33%). The RILs along with their parents were genotyped using the 98 polymorphic SSR markers and a linkage map was constructed covering a distance of 2495.31cM spanning across the 12 linkage groups using the software JoinMap 4.0. The phenotyping score of two different stress condition (reproductive stage drought stress stress and intermittent drought stress condition) were compared with the genotypic data for identification of QTLs. The QTL analysis detected eight QTLs at reproductive drought stress located on chromosome number 3(*qGY3* LOD 3.54, *qYSI3* LOD 3.54); 4(*qGY4* LOD 2.57, *qTOL4* LOD 2.56, *qYSI4* LOD 2.56, *qYSI4* LOD 2.59, *qRYR4* LOD 2.58, *qDSI4*, LOD 2.62) and 11 QTLs at intermittent drought stress located on chromosome1 (*qFLW1*, LOD 2.23, *qNOC1* LOD 2.7, *qNOG1* LOD 1.83); 3(*GLW3* LOD 2.55), 4(*qGY4* LOD 2.7, *qRYR4* LOD 2.35, *qTOL4* LOD 3.5, *qTOL4* LOD 2.2, *qYSI4* LOD 2.35), 7(*qNOG7* LOD 2.91, *qTNG7* LOD 2.84). The detected QTL regions were scanned to identify putative genes associated with drought tolerance in rice in both the stress conditions. The putative gene in reproductive drought stress were used to check the expression in four extreme breeding line of different drought tolerant capacities. QTL comparison between both the stress conditions were evaluated and one QTL was found common and few shared the same chromosome but in different marker intervals indicating different genetics might control the underlying stress conditions. The putative QTLs and candidate genes identified in the present study need to be further validated, which will be helpful for the improvement of drought tolerance in rice.

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I	INTRODUCTION	1-5
II	REVIEW OF LITERATURE	6-20
III	RESEARCH METHODOLOGY	21-47
IV	RESULTS AND DISCUSSIONS	48-111
V	SUMMARY AND CONCLUSION	112-115
	BIBLIOGRAPHY	116-136
	APPENDICES	i-xxiv

LIST OF TABLES

Table No.	Title	Page No.
3. 1	List of cultivars used in the study	25
3.2	Reagent with concentrationsfor cDNA Preparation	28
3.3	List of gene specific primers used in RT-qPCR analysis	30
3.4	Components and concentration of qPCR Master mix	31`
3.5	Thermo-cyclicconditions for real time PCR	31
3.6	Timeline of breeding activities performed during the study	38
3.7	Drought score level based on Standard Evaluation System (IRRI, 2013)	39
3.8	Yield attributing traits for Phenotypic Observations	39
3.9	List of chemicals used in genomic DNA isolation	43
3.10	Chemical composition of 25X TBE buffer	45
3.11	Components of PCR master mix used in the study	47
3.12	Thermocyclic PCR profile for amplification of microsatellite (SSR) markers	47
4.1	List of extreme cultivars used in different drought tolerance capacity	52
4.2	Chi square test for segregating of SSR Markers	80
4.3	Linkage Map construction	87
4.4	QTLs detected for drought tolerance under reproductive staged	96
4.5	List of total gene scanned within the QTL regions	99
4.6	Effects of QTLs detected in the study for drought adaptive traits underintermittent drought stress	103
4.7	List of total gene scanned within the QTL regions	108
4.8	Putative gene along with its functions	108
4.9	Comparison between QTLs in intermittent drought stress and reproductive drought stress	111

LIST OF FIGURES

Figure No.	Title	Page No.
4.1	Quantitative Expression of DREB2A in 4 cultivars at 5DWW and 10 DWW	55
4.2	Difference in Quantitate expression profile of DREB2B at 5DWW and 10DWW, *=P, 0.05	57
4.3	Difference in Quantitative expression profiles for OsNAC9	60
4.4	Difference in expression profiles for OsbZIP73	62
4.5	Difference in Quantitative expression profiles for OsAPX1	64
4.6	Phenotypic parameters observed in (a) Non Stress and (b) Stress condition	66
4.7	Correlation between drought responsive genes and drought adaptive traits	67
4.8	Chromosome wise marker distribution of polymorphic markers.	68
4.9	Phenotypic parameter of the parental cultivar	70
4.10	Genotyping of the RIL population of RM 523 of chromosome 2 and chromosome 8	77
4.11	Segregation pattern of 98 SSR markers in 247 F ₆ lines of Disang x Koimurali; (a)Chromosome 1,2,3; (b)Chromosome 4,5,6; (c) Chromosome 7, 8, 9; (d)Chromosome 10,11, 12.	78
4.12	QTL likelyhood curve of different traits under reproductive drought stress	100
4.13	Transcript Profile of (a) OsbZIP 73; (b) OsAPX 1 and (c) OsNAC 9	102
4.14	QTL likelihood curve of different traits under intermittent drought stress	106

LIST OF PLATE

Plate No.	Title	Page No.
3.1	Sowing of the cultivars for parental	27
3.2	Phenotypic variation of Drought tolerance under non-stress ,5 DWW and 10 DWW	27
3.3	Agarose Gel (0.8%) images of total RNA isolated from leaves of control and drought stressed plants	29
3.4	Agarose Gel (3 %) showing expression pattern of endogenous control Ubiquitin	29
3.5	Morphology of the parental genotype	33
3.6	F ₂ :F ₃ population (<i>Ahu</i> ,2020)	34
3.7	F ₃ :F ₄ population (<i>Sali</i> ,2020)	35
3.8	F ₄ :F ₅ population(<i>Ahu</i> ,2021)	36
4.1	Semi quantitative estimation of expression for OsDREB2A at 5DWW and 10DWW (*=P<0.05)	55
4.2	Semi quantitative estimation of expression for OsDREB2B at 5DWW and 10DWW (*=P<0.05)	57
4.3	Semi quantitative estimation of expression for OsNAC9 at 5DWW and 10DWW (*=P<0.05)	60
4.4	Semi quantitative estimation of expression for BZIP73 at 5DWW and 10DWW (*=P<0.05)	62
4.5	Semi quantitative estimation of expression for <i>OsAPX1</i> at 5DWW and 10DWW (*=P<0.05)	64
4.6	Confirmation of Hybridity in F ₁ S using RM167	68
4.7	Parental polymorphism check using SSR markers	71
4.8	Phenotyping of mapping population at rainout shelter F ₅ :F ₆ (<i>Sali</i> ,2021)	72
4.9	Phenotyping of the mapping population at ICR Field F ₅ :F ₆ (<i>Sali</i> ,2021);	73
4.10	Phenotyping of the mapping population under drought stress at the reproductive stage F ₆ :F ₇ (<i>Ahu</i> ,2022)	74
4.11a	Genotypic of the RIL population of RM523 of chromosome 2	77
4.11. b.	Genotypic of the RIL population of RM310 of chromosome 8	78
4.12	Root architecture	102

ABBREVIATIONS AND SYMBOLS

%	: Per cent
&	: and
/	: Per
AAU	: Assam Agricultural University
ABA	: Absciscic Acid
ABRE	: Absciscic Acid Responsive Element Binding
APX	: Ascorbate Peroxidase
BIL	: Backcross Inbred Lines
Bp	: Base Pairs
b-ZIP	: Basic Leucine Zipper
CAT	: Catalase
cDNA	: Complementary DNA
CDS	: Coding Sequence
chr	: Chromosomes
CI	: Choloroformisoamyl alcohol
cm	: centimetre
CRD	: Controlled Random Drought
CTAB	:Cetyltrimethylammonium bromide
DAS	:Days After Sowing
DFF	: Days to 50% Flowering
DH	: Doubled Haploid
DNA	: Deoxyribonucleic Acid
DREB	: Dehydration Responsive Element Binding
DSI	: Drought Susceptibility Index
DWW	: Drought Water Withdrawal

EBT	: Effective booting tillers per plant
EDTA	: Ethylenediamine tetra acetic acid
ERF	: Ethylene Responsive Factor
FLL	: Flag Leaf Length
FLLW	: Flag Leaf Length to Width ratio
FLW	: Flag Leaf Width
GBS	: Genotyping by sequencing
GCV	: Genotypic Coefficient of Variance
GL	: Grain Length
GL	: Grain Length
GLW	: Grain Length-Width ratio
GO	: Gene Ontology
GPCR	: G-protein-coupled receptors
GPX	: Glutathione Peroxidase
GW	: Grain Width
GY	: Grain Yield
HCL	: Hydrochloric Acid
HYV	: High Yielding Varieties
ICR	: Instruction cum Research Field
IMD	: Indian Meteorological Department
IR	: International Rice
IRRI	: International Rice Research Institute
LG	: Linkage Group
LOD	: Logarithm of odds
ml	: Millilitres
NaCl	: Sodium Chloride
NCBI	: National Centre for Biotechnology Information

NER	: North Eastern Region
ng	: Nanogram
NIDIS	: National Integrated Drought Integrated System
NIL	: Near Isogenic Lines
NLS	: Nuclear Localising Signal
NOC	: No. of chaffy grains
NOT	: Number of tillers per plant
°C	: Degree Celsius
PCR	: Polymerase chain reaction
PH	: Plant Height
PL	: Panicle Length
pmol	: Picomole
PVC	: Polyvinyl Chloride
PVE	: Phenotypic Variance Explained
PYR/RCAR	: Pyrabactin Resistance 1/ Regulatory Component of ABA receptor
q-PCR	: Quantitative Polymerase Chain Reaction
QTL	: Quantitative trait locus
RAP-DB	: Rice Annotation Project Database
RARS	: Regional Agricultural Research Stations
RCBD	: Randomized Complete Block Design
RIL	: Recombinant Inbred Line
RLK	: Receptor-Like Kinases
RNA	: Ribonucleic acid
Rpm	: Revolutions per minute
RT-qPCR	: Quantitative reverse transcription PCR
RWC	: Relative Water Content
RYR	: Relative Yield Reduction

S	: Svedberg unit
SF	: Spikelet fertility
SNP	: Single Nucleotide Polymorphism
SOD	: Superoxide Dismutase
SSD	: Single Seed Decent
SSI	: Stress Susceptibility Index
SSR	: Single Sequence Repeat
STI	: Salt Tolerance Index
TBE	: Triclopyr Butotyl Ester
TE	:Tris EDTA
TF	: Transcription factor
TNG	: Total number of grains per panicle
TOL	: Stress tolerance
TPE	: Target Population of Environment
TRI	: Trizol
WUE	: Water Use Efficiency
YSI	: Yield Susceptibility Index
μg	: Microgram (s)
μl	: Microliter (s)
μM	: Micromolar

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is considered to be one of the major staple food that is consumed by more than one-third of the world's population. Rice entitles up to 80% of the daily calories intake of a vast majority of the human population, especially in Asia (Oladosu *et al.*, 2019). It is a source of 21% of global human per capita energy and 15% of per capita protein and makes up quality food for almost half of the world's population (Xu *et al.*, 2011). It is produced at different locations under a variety of climatic conditions. Rice is also the first cereal crop to be sequenced. Although there are a lot of rice databases available with plenty of genomic information, however, a lot needs to be done to improve its production and productivity worldwide as a huge population thrives for this cereal as a staple food. In Assam, rice occupies about two-thirds of the total cropped area. Assam has been traditionally rice dependent state, rice is grown in three distinct seasons: autumn or *Ahu* rice (February/March to June/July), winter or *Sali* rice (June/July to November/December), and summer or *Boro* rice (November/December to May/June). *Sali* rice which is grown in 1.86 million hectares followed by *Boro* rice (0.38 million hectares) and *Ahu* rice (0.11 million hectares). The *Sali* rice has the highest production 396 metric tonnes followed by *Boro* rice with 107 metric tons and *Ahu* has the least production of 18 metric tonnes in Assam. However, the productivity in *Ahu* and *Sali* seasons is less as compared to the *Boro* season (2.1 metric tonne/hac; 1.5 metric tonne/ha and 1.8 metric tonne/ha respectively). (Statistical hand book of Assam, 2022). The reason for the reduction in rice production is due to application of numerous biotic and abiotic stresses for the *Ahu* and *Sali* seasons rice. In general, a rice plant requires plenty of water and it alone receives about 35% of the global surface water (Bouman *et al.*, 2007). Climatic changes cause erratic rainfall patterns, leading to limited availability of surface water for irrigation in field crops, thus creating a water deficit or drought scenario in arid and semi-arid regions (Baisakh *et al.*, 2020). Among various abiotic stresses, drought is the most widespread and damaging factor in the worldwide affecting 23 million hectares of rainfed rice in South and Southeast Asia. In some states in India, severe drought can cause a 40% yield loss (Pandey and Bhandari, 2009).

The term drought refers to a deficiency of precipitation over an extended period (usually a season or more), resulting in a water shortage. Drought is a complex quantitative trait and is one of the major constraints in limiting productivity and yield instability in rice. In India, the rainfed lowland rice area is about 20.4 million ha of rainfed rice area, approximately 7.3 million ha of lowland area are drought-prone (Pandey and Bhandari, 2009). In Assam, reproductive stage and intermittent drought is recognized as the most critical stage at which drought stress can cause a high yield reduction for rice grown in upland and lowland area, respectively under rainfed ecosystem. These two different kinds of drought need to be addressed separately. Rice responds to drought in various physio-chemical ways despite its small root systems, thin cuticular wax, and swift stomatal closure (Sahebi *et al.*, 2018). There are some adaptive traits (osmotic adjustment, deep and thick root system, dehydration tolerance, leaf rolling, green leaf area, water use efficiency, fast stomatal closure), which confer drought tolerance in rice (Pandey and Shukla, 2015) and these phenotypic characters have a strong correlation with drought. It is known that tolerance to drought in plants is under transcriptional regulation and is widely studied in various plant systems. Different breeding programs for drought tolerance aim in developing varieties that are suitable for different ecosystems. The ability of crop cultivars to perform reasonably well under drought-stress situations is the need of the hour and of paramount importance for the stability of rice production. Therefore, the relative yield performance of genotypes in reproductive and intermittent drought stresses and non-stressed conditions is of utmost importance. Rice plants exhibit various responses to drought stresses, such as seeking water, evading scarcity, or managing deficiencies. Another important task in the rice breeding program is to find suitable rice varieties for specific environmental conditions (Target Population of Environments, TPE). The key attributes governing drought tolerance in rice is the development of an intricate, deep-rooted system that facilitates soil penetration to greater depths. The prime objective of drought-tolerance breeding programs is to identify the performance of the cultivars that surpasses the existing ones.

Maintaining the resilience of crop cultivars under prolonged drought conditions is of paramount importance. The exposure of plants to dehydration stress causes increased expression of a variety of functional and regulatory proteins (Yamaguchi-Shinozaki 2007). Molecular analysis of the signal transduction pathways reveals a connection between changes in turgor pressure, synthesis of hormones such as ABA, and the induction of a set of genes involved in dehydration tolerance, while other genes are activated through a cellular dehydration signal transduction pathway that does not involve ABA. Drought adaptation and

adaptive traits are under strong polygenic control, and hence dissection of genetic effects is analysed through Quantitative Trait Loci (QTL) analysis. Previous studies revealed several QTLs and genes on different chromosomes associated with different traits directly or indirectly for drought tolerance by using different mapping populations (Venuprasad *et al.*, 2009; Shamsudin *et al.*, 2016; Swamy *et al.*, 2017; Sollis *et al.*, 2018). However, more markers for QTLs controlling various traits for stress tolerance need to be identified to speed up marker-assisted selection for developing drought-tolerant varieties.

Plants have developed multiple mechanisms to adapt and respond to abiotic stress. There are many molecular mechanisms and highly coordinated changes at the transcriptional level of entire gene networks involved in stress response. There are a lot of studies that focus on different drought tolerance mechanisms to identify the drought-inducible genes and their contribution in imparting drought tolerance. Exposure of a plant to drought stress activates several regulatory pathways which involve a variety of TF and genes (Yamaguchi-Shiozaki, 1992). The highly conserved inducible response of a plant towards stress disrupts cellular homeostasis. Molecular analysis of signal transduction pathways shows a strong connection between the change in turgor pressure and the synthesis of the stress hormone ABA leading to the activation of a series of stress-responsive genes. Transcription factors contain a huge number of regulatory proteins which participate in managing the complex abiotic stress regulatory network. There are several TF/genes from different gene families which act as positive or negative regulators in abiotic stress signalling. APETALA2/ERF (AP2/ERF) family, which is a large family of TFs involved in regulating various biological processes in plants. The AP2/ERF family is known for its role in responding to various environmental stresses, including dehydration. It consists of OsDREB2A and OsDREB2B which impart stress response through an ABA-independent pathway (Singh *et al.*, 2022). In contrast, there are a lot of TFs which impart drought tolerance through ABA-dependent pathway, for example bZIP TF, a huge class of TF which exclusively give tolerance against abiotic stress via ABA-dependent pathway. Furthermore, there are plant-specific TF families which consist of numerous genes involved in changes in plant growth and development leading to the abiotic stress response. (Puranik *et al.*, 2012).

The shifts in climate patterns and the ongoing expansion of the global population, creating rice varieties that yield abundantly and adapt to changing conditions has become increasingly urgent for farmers. To attain this goal, the preferred breeding strategy involves identifying and mapping Quantitative Trait Loci (QTLs) responsible for Grain Yield (GY) under drought stress. This approach is aimed at developing rice varieties that are both high-

yielding and resilient to drought. During the process of locating the genomic regions linked to drought tolerance in rice, multiple factors carry significance, including the plant's growth stage, environment, and the severity of the drought stress (Kamoshita *et al.*, 2002). Numerous studies have highlighted the identification of QTLs exhibiting consistent stability and significant impact on grain yield, especially under reproductive-stage drought stress. These QTLs led to the development of rice varieties that perform better despite reproductive-stage drought exists. The utilization of these robust QTLs might lead to the development of such drought-tolerant, high-yield rice varieties. However, to accelerate the process of producing new varieties with improved grain yield drought conditions, a comprehensive genetic mapping for loci governing drought tolerance in rice is essential.

The breeding rice plants to withstand tough conditions like drought is a challenging task due to the complexity of genetics and unexpected events (Debnath *et al.*, 2023). It is crucial to find rice types that need less water and can still give good harvests during drought to tackle food shortages. Ways to save water in farming, such as direct seeding, aerobic rice, and alternating wetting and drying systems, have been proposed (Salleh *et al.*, 2020). Grain yields often the main factor for selection. Certain studies have detected stable QTLs linked to good grain yield despite drought stress (Ghimire *et al.*, 2012; Mishra *et al.*, 2013; Swamy and Kumar, 2013; Dixit *et al.*, 2014a; Dixit *et al.*, 2014b). Some of these QTLs have been used to develop rice varieties that have better yield and resist drought (Kumar *et al.*, 2014). The reproductive stage is vulnerable to drought and can affect rice yields for farmers. Therefore, it is important to keep searching for QTLs and traits that boost rice yields under varying drought at that stage. Therefore, is crucial.(Kumar *et al.*, 2014). Choosing the right donor and creating a mapping population are the key steps to test if the drought-tolerant type fits in a breeding program.

The North East Regions (NERs) in the recent decade (2000–2014) has experienced more frequent and unprecedented drought conditions than the Western India. The state of Assam witnesses floods nearly every year due to its geographical location, high-intensity rainfall, and inundation of riverine areas caused by the Brahmaputra River. However, the scenario is the opposite in 2005–06, 2009–2011, and 2013 due to scanty rainfall in many districts of Assam. Most of the drought events are associated with the variability of rainfall during the South-West monsoon season. Assam received 30% less rainfall than normal during those years and consequently, the rainfed rice crops were profoundly affected by drought. The districts critically affected were Dhubri, Nalbari, Jorhat, Morigaon, Nowgong and Sonitpur (Parida and Oinam, 2015). Therefore, in the future, researcher has predicted the

occurrence of intense drought in this region is most likely, which will surely have an adverse effect on agriculture and food security in the NER. A study of future drought scenarios in Assam was predicted using the past and future meteorological data from Indian Meteorological Department using the standard precipitation index indicates severe drought in the upcoming years. The early stages of growth in the *Ahu* season and late stages of growth in the case of *Sali* season crops are the most adversely affected by water stress under the rainfed ecosystem in this region. The yield performance of popular mega varieties of this region is drastically reduced if these specific stages of the crop encounter drought-stress environments. Intermittent drought is a phenomenon that causes heavy loss to the crop. Therefore understanding of stage specific drought in rice of Assam is need of the hour and the the present study aims to ascertain the specific genomic positions of QTLs that underlie the genetic factors related to yield and yield-associated traits under drought stress at the reproductive stage and intermittent drought stress. To successfully detect the QTLs with substantial effects, it is imperative to implement stringent screening of the phenotypic traits associated with drought tolerance under both stressed and normal conditions. This detailed assessment serves as the basis for the identification and understanding of significant QTLs to have clear understanding of genetic bases/mechanisms underlying drought tolerance at reproductive stages of growth in the case of *Sali* and *Ahu* rice and to compare with the genetic effects of intermittent drought is inevitable to manipulate in a breeding program It is also equally important to improve the locally grown varieties for this trait. Therefore, based on these considerations, the present research problem is proposed with the following objectives.

1. To assess the correlation between drought-responsive gene expression and drought-adaptive traits at the reproductive stage.
2. To map the Morphological traits for drought tolerance at the reproductive stage in rice.
3. To Compare the genetic effects of drought-related traits under intermittent water stress and drought at the reproductive stage

CHAPTER III

REVIEW OF LITERATURE

Climate change is a major threat to the food and agriculture sector, impacting staple crops like rice, which constitutes 70% to 80% of global daily calorie consumption (Khush, 2005). The industry heavily relies on water, and water scarcity has profound implications for agricultural productivity (Wang *et al.*, 2012). To address this critical situation, there is an urgent need to develop climate-resilient crops (Foley *et al.*, 2011). Among all the abiotic stresses drought is one of the major abiotic stresses leading to yield reduction. Drought tolerance is a multifaceted trait influenced by an interplay of morphological, biochemical, and molecular changes. The mechanisms underlying water-stress resilience and its regulatory systems governing plant responses to drought (Pandey and Shukla, 2015) are being studied extensively.

Drought stress in plants refers to a physiological condition that occurs when the availability of water in the soil falls below the minimum amount required for normal plant growth, development, and functioning. It results from an imbalance between the water uptake by plant roots and the rate of water loss through transpiration (water vapor loss through leaf stomata) and other physiological processes. As a consequence of drought stress, plants experience various adverse effects on their physiological, biochemical, and morphological processes, which can ultimately lead to reduced growth, impaired reproduction, and even plant mortality. Drought stress highly affects rice growth and development in all growth stages (Boonjung *et al.*, 1996). Drought stress influences key aspects including leaf area index, plant height, root length, and effective panicle number (Alou *et al.*, 2018). The initial indication of drought stress is evident in leaf rolling, a mechanism that conserves water by reducing leaf surface area (Ji *et al.*, 2012). Severe drought stress impacts leaf area by mesophyll cell expansion and division leading to reduced photosynthesis and diminished biomass accumulation (Davatgar *et al.*, 2009). Furthermore, drought leads to an increase in plant height (Anjum *et al.*, 2017). Early-stage panicle differentiation under controlled water conditions leads to increase plant height as a compensatory growth response. Conversely, severe drought stresses during the mid-tillering stage markedly decrease plant height. Roots plays an important role in supplying water and nutrients for plant growth. When the plant is subjected to drought

stress, rice roots undergo adaptive changes such as increased hair length and density. These alterations lead to an adaptive strategy. In the initial stages of drought stress, root growth accelerates to enhance water absorption. This process involves the engagement of various proteins in root morphogenesis, as well as carbon and nitrogen metabolism (Jaleel *et al.*, 2009). The reproductive phase of rice is particularly vulnerable to drought stress. Drought disrupts the development of reproductive organs, resulting in diminished fertile pollen count, prolonged flowering periods, and abnormal anther cracking. As the grain-filling stages progress, drought triggers premature leaf senescence, shortens grain-filling duration, restricts assimilate availability, and reduces grain weight (Prathap *et al.*, 2019). Mitigating these challenges requires a deep understanding of the underlying molecular and physiological mechanisms plant under drought stress. The ultimate objective lies in the development of climate-resilient crop cultivars that can cope up with the rapid climate change.

2.1. Drought stress scenario in Assam

During the southwest monsoon season, the distribution of rainfall across Western and North East India exhibits distinct geographical patterns. The North Eastern Region (NER) receives the most substantial seasonal rainfall, while the Western region (including Saurashtra and Kutch in Gujarat, and Rajasthan) witnesses the lowest precipitation. Gujarat is positioned in an arid to semiarid zone, consisting of more drought-prone areas. In contrast, Assam and Meghalaya have humid climates and experience rare occurrences of drought. However, a concerning trend has emerged in recent times. The NER has observed a gradual decline in southwest monsoon rainfall, especially when compared to the levels experienced in the 1980s and 1990s. This shift has led to an increased frequency of unprecedented drought events in these regions, surpassing those in Western India. Notably, in the last decade (2004-2014), the probability of drought occurrences was 54% for the NER, contrasting with 27% for Western India. The increase in drought frequency has a significant impact on agriculture, resulting in a negative impact on rice production during 2005-06 and 2009, particularly impacting Assam. Areas within Assam (including Bogaigaon, Cachar, Dhubri, Goalpara, Golaghat, Hailakandi, Jorhat, Kamrup, Kokrajhar, Lakhimpur, Morigaon, Nagaon, Nalbari, Sivsagar, and Sonitpur) and Meghalaya faced consecutive drought events in 2005 and 2006. Additionally, there are a lot of drought reports that emerged from other states in the NER during 2010-11 and 2013. The rising temperatures and decrease in monsoon season rainfall in NER are integral components of global climate change. This evolving scenario may result in induced droughts, exerting adverse effects on agriculture and food security in the Assam (Parida and Oinam, 2015).

2.2. Drought adaptation mechanism in rice

Drought stress imparts specific responses in rice crops, including leaf rolling, the closure of stomata, and the synthesis of abscisic acid (ABA), which collectively aim to mitigate water scarcity. Rice drought resistance depends on two key physiological factors: water use efficiency (WUE) and osmotic adjustment. WUE facilitates optimal production with minimal water consumption, while osmotic adjustment turgor pressure and preserves the meristematic tissues. Plants employ various mechanisms to adapt to drought stress, categorized as drought escape, drought avoidance, and drought tolerance. These adaptations manifest through molecular reactions and morpho-physiological alterations, ultimately allowing plants to navigate the challenges posed by water deficits.

2.2.1. Drought escape

Drought escape is a mechanism that allows a plant to complete its life cycle prior to substantial water depletion. This phenomenon is based on two distinct mechanisms: rapid phenological development and developmental plasticity. Rapid phenological development entails the production of flowers with minimal vegetative growth, enabling seed production even under constrained water resources. Conversely, developmental plasticity enables plants to have vegetative growth, flowers, and seeds during periods of rainfall. Drought escape is a vital mechanism that empowers rice to achieve grain production under conditions of restricted water availability (Kumar *et al.*, 2008).

2.2.2. Drought avoidance

Drought avoidance allows plant ability to uphold elevated tissue water potential despite encountering inadequate moisture. In response to higher evaporative demand and increasing soil water deficit, plant tissues can choose between two strategies: reducing water loss or sustaining water supply. In drought avoidance, rice genotypes exhibit the capability to uphold water status through various mechanisms, including abscisic acid (ABA) biosynthesis, other biochemical mechanisms, or adaptations in root systems. These mechanisms enable these genotypes to mitigate the detrimental impact of drought on yield. Strategies that enhance water use efficiency (WUE) and reduce water loss also fall within the spectrum of drought avoidance. Rice genotypes that embrace drought avoidance often exhibit specific attributes, such as deep and robust roots with robust branching and soil penetration abilities, an increase root-to-shoot ratio, the ability to roll leaves elastically, stomatal closure, and increase cuticular resistance. (Singh *et al.*, 2012).

2.2.3. Drought tolerance

Drought tolerance is characterized by a plant's ability to endure conditions of low tissue water content. Within the genetic context, drought tolerance in rice emerges as a complex trait, influenced by a polygenic effect and intricate morpho-physiological mechanisms. These mechanisms encompass various processes, including the decrease in turgor through osmotic adjustment, increase cell elasticity, and decrease cell size. The degree of drought tolerance is determined by plants to respond to tissue water potential with traits associated with these responses termed secondary traits. The secondary traits includes osmotic adjustment, relative water content (RWC), leaf rolling, and stomatal conductivity (Singh *et al.*, 2012).

2.3. Impact of drought on growth and development

A plant undergoes drought stress when the roots of the plant is unable to uptake water or has excessively high transpiration rates. Such stress significantly impairs rice growth, development, and ultimately its yield. In response to water scarcity, plants adapt themselves to a survival strategy by limiting their growth and physiological response (Zhu, 2002). This adaptive mechanism leads to diminished root development, compromised leaf-surface attributes, including altered form, shape, cuticular wax composition and colour. These alterations influence sun exposure on the leaf canopy. The slowing of normal plant leads to senescence as maturity approaches and inhibition of stem growth further contribute to growth reduction (Blum, 2011) helps the plant to survive this drought scenario. The reduction in turgor pressure, resulting from stress-induced water deficits, profoundly hampers cell growth (Taiz and Zeiger, 2006). Elongation and expansion both experience impairments due to drought, with cell enlargement being more affected than cell division (Jaleel *et al.*, 2009). Seedling germination and tiller count are reduced and limited plant height is some of the morphological changes a plant undergoes during drought stress (Sokoto and Muhammad, 2014). Continuous drought stress leads to a decrease in biomass production (Farooq *et al.*, 2009a; Farooq *et al.*, 2010). The decline in fresh and dry shoot weights and root weights (Ji *et al.*, 2012) under drought conditions contributes to reductions in shoot and root attributes ultimately resulting in a decline in photosynthetic rate and essential physiological and biochemical processes of rice (Usman *et al.*, 2013)

2.3.1. Impact of drought on leaf and root traits

Leaf growth is constrained due to reduced water potential in plants under drought-stress conditions (Zhu *et al.*, 2020). This is caused by disrupted water movement within cells, leading

to lower turgor pressure owing to insufficient water supply. Consequently, a reduction in cell development results in smaller leaf areas in crops (Hussain *et al.*, 2018). Drought stress brings about changes in the leaf structure and composition. This involves shrinking leaf size, fewer stomata, thicker cell walls, a coating on the leaf surface called cuticle, and impaired conduction system development (Upadhyaya and Panda, 2019). Leaf rolling and premature aging are also notable features under drought stress (Anjum *et al.*, 2011). Several leaf characteristics serve as indicators for screening drought-tolerant varieties, including larger flag leaf area, higher leaf area index, greater leaf relative water content, and increased leaf pigment content (Hussain *et al.*, 2018).

Under drought conditions changes in root characteristics are crucial for enhancing crop production. The root system of rice significantly influences crop performance under dehydration stress. Predicting rice production under such conditions can involve assessing root mass (dry weight) and length (Comas *et al.*, 2013). Responses to water stress vary in terms of root growth characteristics. In some cases, drought stress can lead to longer rice roots due to increased levels of abscisic acid in the roots (Manivannan *et al.*, 2007). Generally, rice varieties with extensive and abundant root systems tend to cope better with drought (Mishra *et al.*, 2019). For rice, varieties with deep, coarse roots that can branch out and maintain a high root-to-shoot ratio are crucial for drought tolerance. Additionally, physical and physiological traits of rice roots significantly influence shoot growth and overall grain yield while facing drought stress (Kim *et al.*, 2020).

2.4. The molecular mechanism of drought stress

Environmental stimuli are detected by membrane receptors and lead to the activation of the signal transduction pathway (Dash *et al.*, 2018, Oladosu *et al.*, 2019). Drought is a multifaceted phenomenon, thus hybridization and selection strategies cannot give precision in achieving drought tolerance. However, employing DNA markers within molecular studies offers a better approach to deal with this complex trait. Molecular markers hold promise for efficiently screening large germplasm for drought tolerance traits, serving as a foundation for subsequent crop improvement. Numerous studies have been done in the identification of qualitative trait loci (QTLs) associated with diverse traits related to drought resistance (Barik *et al.*, 2019; Kumar *et al.*, 2018; Upadhyaya and Panda, 2019). Important methodologies have been identified for drought-responsive genes in rice involving DNA-based studies on marker-assisted phenotyping. Despite the advancements, only a limited number of traits have been validated for drought resistance in rice (Prakash *et al.*, 2019). Hence, molecular breeding holds

the potential to improve crop variety and enhance overall yield of the plant. This approach can generate cultivars with high agronomic traits, contributing to the development of productive and resilient agricultural systems.

2.4.1. Role of drought-responsive gene/transcription factor in drought stress in rice

Drought stress is a complex condition whose severity increases with the decrease in water table in soil. This results in a series of alterations in plant physiology, morphology, and genetics. A series of genes are triggered in response to this stress, serving as important regulators. These genes regulate the survival strategies of plants by governing downstream stress-responsive genes, acting as central coordinators (Singh *et al.*, 2013). The expression of individual or combined genes activates a network of stress-responsive pathways in plants, enabling them to manage adverse conditions. Different types of cell surface receptors are responsible for sensing stress, including receptor-like kinases (RLKs), ion channel-linked receptors, G-protein-coupled receptors (GPCRs), and two-component histidine kinase receptors. RLKs are particularly influential in facilitating the interpretation of a wide range of developmental and environmental signals in plants (Bhargava and Sawant, 2013). Phytohormones play a vital role in the response to abiotic stress. For instance, under water deficit stress, ABA synthesized in roots leads to stomatal closure and decrease plant growth (Wilkinson and Davies, 2010). The synthesis of ABA triggers stress responses, while receptors such as PYR/RCAR induce drought stress responses through the activation of serine-threonine kinase SnRK2 (Fan *et al.*, 2016). A plant drought tolerance can be imparted through cumulative effect of phytohormones, its morphological adaptations, specific genes, and regulatory proteins like transcription factors (TFs). Transcription factors are important in modulating the expression of defense genes (Manna *et al.*, 2021). These TFs interact with cis-acting elements present in the promoter regions of various stress-responsive genes. This interaction triggers cascades or entire networks of genes that collaboratively enhance tolerance to multiple stimuli simultaneously. This characteristic role of TFs is a promising candidate for enhancing abiotic stress tolerance. Overexpression of stress-responsive TFs initiates the activation of a cascade of gene. These TFs fall into diverse families such as *AP2/ERF*, *bZIP*, *NAC*, *MYB*, *MYC*, *Cys2*, *His2*, *zinc finger*, and *WRKY* (Umezawa *et al.*, 2006).

2.4.2. Crosstalks between Transcription factor in response to drought

The *DRE/CRT* motif within drought-responsive gene promoter serves as a binding site for ABA-independent *DREB/CBF* TFs. Simultaneously, it operates as a cis-regulatory element

(CE) for *ABRE* in ABA-dependent gene expression (Narusaka *et al.*, 2003). (Lee *et al.*, 2010) demonstrated physical interactions between *DREB1A/CBF3*, *DREB2A*, and *DREB2C* proteins and *AREB/ABF* proteins. This interaction suggests crosstalk between ABA-dependent and independent stress response pathways. Additionally, other signal transduction pathways exhibit interplay between the two pathways. (Kim *et al.*, 2011) revealed the involvement of an *ABRE* promoter sequence, *AREB/ABF* TFs, and *SnRK2s* in *DREB2A* gene expression under osmotic stress. This leads to interaction between *AREB* and *DREB* in both gene expression and protein interaction levels.

The interaction between *AREB/ABFs* (Absciscic Acid Responsive Element Binding Factor/ABRE Binding Factors) and *NAC* (NAM, ATAF1/2, CUC2) transcription factors leads to an increase in gene expression. A study by (Jensen *et al.* 2013) revealed that the Arabidopsis *SNAC* (Stress-responsive NAC) transcription factor ATAF1 exerts direct control over the ABA (Absciscic Acid) biosynthetic gene *NCED3* in the Arabidopsis. This finding provides evidence that *SNAC* TFs might increase the ABA-induced gene expression of *ABRE* (*ABRE cis-regulatory element*). Another study by (Xu *et al.* 2013) reported a vital association involving Arabidopsis *ANAC096* with *AREB/ABF* factors, namely *ABF2/AREB1* and *ABF4/AREB2*, during responses to dehydration and osmotic stress. These findings collectively show inter-relationship existing between the *AREB/ABF* and *NAC*, TF revealing a sophisticated network of transcriptional coordination that operates in the stress-related molecular responses. Arabidopsis consists of an *ERF1* regulator which binds to two cis-elements i.e. GCC box and *DRE/CRT* that respond to various abiotic stress. The GCC box response to biotic stress and *DRE/CRT* response under abiotic stress. (Cheng *et al.* 2013).

2.5. Development of mapping population

2.5.1. Selection of parental cultivars

Germplasm screening is the initial step before starting any breeding program. The ultimate goal of QTL mapping programs is to identify QTLs that will help in the enhancement of a particular trait. To ensure the success of the breeding program, the important choice is to identify a suitable parents, both donors and recipients (Kumar *et al.*, 2014a). This process involves screening from diverse sources such as landraces (Rajurkar *et al.*, 2019) traditional drought-resistant lines (Palanog *et al.*, 2014; Sandhu *et al.*, 2014; Vikram *et al.*, 2011) and advanced pre-breeding lines (Dixit *et al.*, 2014a; Mishra *et al.*, 2013) in rice breeding programs. Interestingly, rice varieties growing in dry rainfed regions have evolved into robust drought-tolerant landraces. However, many of these landraces exhibit limitations like lower yield

potential, early flowering, reduced tiller numbers, tall stature, longer grains, and more time taking cooking qualities (Kumar *et al.*, 2014a). Despite these shortcomings, researchers are successfully developing extensive breeding populations by crossing these drought-tolerant landraces with high-yield but drought-vulnerable varieties. This strategic approach has effectively merged strong yield potential with drought tolerance. Moreover, (Kumar *et al.*, 2014a) found the importance of selecting parental cultivars based on the specific target environment. Several drought-tolerant donor plants, including N-22 (Vikram *et al.*, 2011), DagadDeshi (Ghimire *et al.*, 2012), Kali Aus (Palanog *et al.*, 2014; Sandhu *et al.*, 2014; Ghimire *et al.*, 2012), Apo (Venuprasad *et al.*, 2012b; Venuprasad *et al.*, 2012a; Venuprasad *et al.*, 2009), IR55419-04 (Dixit *et al.*, 2014a), IR74371-46-1-1 (Mishra *et al.*, 2013), and Way Rarem (Bernier *et al.*, 2007), were strategically crossbred with high-yielding popular recipient varieties. This hybridization approach facilitated the identification and mapping of key QTLs associated with grain yield performance during reproductive-stage drought conditions.

2.5.2. Development of the mapping population

Rice, being a self-pollinating plant that can be used for inbreeding, possesses the capacity to generate diverse population structures. This inherent characteristic makes it particularly well-suited for QTL mapping, as highlighted by (Kumar *et al.*, 2014). In the context of rice, there are several segregating populations have been cultivated. These populations are the result of crossing the drought-tolerant line with widely favoured high-yielding varieties, all to effectively map the genetic loci responsible for conferring drought tolerance.

In rice, a variety of mapping populations have been employed to conduct QTL mapping for both yield and associated traits under drought conditions. These encompass diverse types such as F₂ or F_{2:3} lines, Recombinant Inbred Lines (RILs), Near Isogenic Lines (NILs), and Doubled Haploid Lines (DHs). Recombinant Inbred Lines (RILs) were created by crossing two parent with differing traits of interest and then undergoing successive rounds of self-pollination and selection. This iterative process, known as single seed descent (SSD), leads to the establishment of nearly homozygous lines, thus facilitating the exploration of genetic traits (Kumar *et al.*, 2014). Several researchers have employed RILs for the development of the mapping population. A RIL mapping population was developed by crossing Bala and Azucena to map QTLs for root-penetration ability (Price *et al.*, 2000). (Zou *et al.*, 2005) also generated 187 F₉ Recombinant Inbred Lines (RILs) derived from lowland and upland rice cultivars, specifically Zhenshan 97B and IRAT109. Parallely, 180 RILs from the same population were

employed to dissect the genetics of drought resistance during the reproductive phase (Yue *et al.*, 2005). (Rajurkar *et al.*, 2019) developed 272 F₁₁ RILs generated between IR20 and Nootripathu.

A specialized RIL population was developed by crossing CR143-2-2 and Krishnahama was employed to find the genetic behind relative water content (RWC) (Barik *et al.*, 2018). The same population was used for investigations into morpho-physiological traits under reproductive drought stress (Barik *et al.*, 2019). Furthermore, this population proved in mapping the physiological traits linked to terminal-stage drought tolerance, a vital aspect studied by (Barik *et al.*, 2020). Another approach involved the development of Backcross Inbred Lines (BILs). This was achieved by backcrossing, typically for one to three generations, followed by self-pollination using the SSD method, culminating in the formation of BCnF_{3:4} populations. Doubled Haploid (DH) lines, on the other hand, were designed to combine the benefits of homozygosity while also expediting the creation of early-generation populations. This technique was first proposed by (Paterson, 2002) that involves inducing chromosome doubling via pollen grains. However, it is worth noting that this method applies only to species that can be manipulated through tissue culture. Doubled Haploid (DH) populations have proven invaluable for genomic regions linked to drought tolerance at both the reproductive and seedling stages in rice (Xu *et al.*, 2011; Lanceras *et al.*, 2004). Notably, a DH population derived from the cross between CT9993-5-10-1-M and IR62266-42-6-2 has been used in diverse mapping programs. For instance, it played a key role in uncovering and mapping Quantitative Trait Loci (QTLs) associated with drought resistance (Zhang *et al.*, 2001b). This population has further contributed to investigations into plant water stress indicators, phenology, and production traits, elucidated by (Babu *et al.*, 2003a). In the context of rainfed lowland rice, (Kumar *et al.*, 2008) employed this DH population to explore drought tolerance. Similarly, (Sellamuthu *et al.*, 2011) used reproductive-growth traits and grain yield under drought stress conditions using the same population. These studies collectively showed the importance of DH populations in advancing our understanding of rice adaptive responses to drought challenges. These distinct mapping populations offer valuable tools to dig into the genetics of rice traits under drought conditions, paving the way for enhanced crop resilience.

2.5.3. Phenotyping of the mapping population for drought tolerance

The accurate phenotyping of mapping populations relies on three fundamental factors: the design of the experiments, effective crop management, and ensuring consistent levels of drought stress throughout the trial. (Venuprasad *et al.*, 2012) undertook trials on distinct upland

and lowland conditions, where upland conditions were done through direct seeding, non-flooding, and aerobic growth, while lowland conditions involved flooding, puddling, transplantation, and anaerobic conditions.

(Kumar *et al.*,2017) executed an experiment within lowland regions. Water was deliberately withheld following 30 days of transplantation to mimic water stress during the critical reproductive phase. For approximately two weeks, most population lines exhibited observable symptoms such as leaf rolling. The water table descended beyond 100 cm, but flash flooding was introduced to mitigate stress effects in the trial environment. Several researchers have recommended various experimental designs for such trials, encompassing methodologies like the randomized complete block design (RCBD), augmented RCBD, and controlled random drought (CRD). A method utilizing perforated PVC pipes erected within the experimental field was adopted to monitor the daily fluctuations in water table depth under lowland conditions post-stress initiation (Verulkar and Verma, 2014). In upland scenarios, tensiometers were utilized to gauge soil moisture potential. The soil water tension at a depth of 30 cm fell below -50 kPa in upland settings, or similarly, in lowland conditions where the water table descended beyond 100 cm, rehydration protocols were implemented. Consistency in crop management practices is essential for stress and control conditions. While stress-imposed trials must adhere to the same conditions, control trials necessitate irrigation through maturity within the controlled environment.

2.5.4. Genotyping of the mapping population

The fundamental principle behind the genetic enhancement of crop plants through plant breeding is the selection of superior offspring from the available population, primarily driven by specific desirable traits. These traits include higher yield, enhanced nutritional value, consumer-preferred attributes like color or fragrance, and more. Interestingly, the direct assessment of such traits in plants can be difficult, therefore an alternative approach is necessary. Instead of directly measuring these traits, researchers often rely on correlated markers or tags closely associated with the desired trait. In the case of rice yield, where factors like an increased number of productive tillers and grains per spikelet contribute significantly. Another classical example is found in Mendel's work, where traits such as pea seed size, color, and plant height were studied. These markers serve as flags to facilitate the selection of superior offspring from a diverse population mixture. Additionally, these markers play a crucial role in simplifying and refining the process of identifying and choosing the most promising progenies

with the desired characteristics, ultimately advancing the genetic improvement of crop plants (Boopathi, 2020).

The genotyping of mapping populations constitutes a fundamental process for the detection of Quantitative Trait Loci (QTL). In molecular breeding programs, the selection of optimal and economically efficient marker systems to screen the genome for specific traits is of utmost importance. Therefore, numerous marker systems are available, yet their selection depends upon the specific workflow and the crop species under study. Distinct marker systems such as isozymes, AFLP, BAC, RAPD, RFLP, ISSR, EST, InDelS, and SSRs have been employed by researchers to detect various morpho-physiological and yield-related traits (Sahebi *et al.*, 2018). The gel-based marker systems are time-consuming, labor-intensive, and complex as noted by (Rasheed *et al.*, 2017). Nonetheless, these systems are most common among researchers in the construction of linkage maps and the identification of QTLs using SSR markers (Bernier *et al.*, 2007; Venuprasad *et al.*, 2009; Vikram *et al.*, 2011; Dixit *et al.*, 2012). However, the advancements in next-generation sequencing, gel-based approaches have taken a back seat, as GBS-based SNP markers are more convenient. These SNP markers, characterized by rapidity, precision, and efficiency, have gained momentum for their effectiveness in genotyping.

2.6. Construction of Linkage Map

The construction of a genetic linkage map is important in both fundamental genetic analysis and practical plant molecular breeding. This linkage map serves as a critical tool to know the position of Quantitative Trait Loci (QTLs) and provides valuable information on chromosomes for gene cloning and marker-assisted breeding initiatives. The importance of linkage maps is to know the precise localization of QTLs and to know the accurate arrangement of genetic markers along chromosomes. This detailed linkage map serves as an essential foundation, providing the necessary framework to effectively position and differentiate QTL regions within the genome. The importance of linkage maps cannot be overstated, as they empower researchers to unlock the genetic underlying of the desired traits, thereby helping in advancements of plant breeding and crop improvement strategies (Qu *et al.*, 2020; Palumbo *et al.*, 2019; Meng *et al.*, 2015).

In McCouch *et al.*, 1998 created the first rice linkage map using 135 markers using RFLPs. Since then, researchers worldwide have built many such maps using different populations and markers. (Harushima *et al.*, 1998) constructed a map using 2275 markers from

an F₂ population derived from Nipponbare and Kasalath. This map spanned a genetic distance of 1521.6 cM. (Zhang *et al.*, 2001b) introduced another map based on 154 Doubled Haploid lines developed between IR62266-42-6-2 and CT9993-5-10-1M, covering a total length of 1,788 cM with an average marker interval of 5.7 cM. (Babu *et al.*, 2003a) employed the same population to generate a map containing 134 RFLP, 131 AFLP, and 15 SSR markers. These maps provide invaluable insights into the genetic makeup of rice, supporting research and breeding efforts.

2.7. QTL analyses for drought tolerance

Within the plant genome, a few genes are governed by quantitative traits and are recognized as Quantitative Trait Loci (QTLs). Previous molecular genetic investigations have numerous QTLs linked to various physiological and biochemical traits (Alexandrov *et al.*, 2015, Todaka *et al.*, 2015, Dixit *et al.*, 2017, Barik *et al.*, 2019). However, these studies often struggle to identify the candidate genes regulating these traits due to low mapping resolution and subtle phenotypic effects (Bin Rahman and Zhang, 2015, Singh *et al.*, 2016). The identification of QTLs related to specific traits is important in designing stress-screening programs for plants (Vikram *et al.*, 2011). Many QTLs associated with physiological and growth traits under drought conditions have been successfully identified and have been used extensively for selecting resilient rice genotypes (Vikram *et al.*, 2011, Vikram *et al.*, 2016, Venuprasad *et al.*, 2012, Dixit *et al.*, 2017, Vinod *et al.*, 2019). These QTLs have also been classified according to various growth stages in rice (Dixit *et al.*, 2012, Venuprasad *et al.*, 2012, Ramchander *et al.*, 2016). As yield remains an important trait so ongoing global research predominantly revolves around mapping QTLs associated with rice grain yield under drought stress (Pandey and Shukla, 2015).

(Chen *et al.*, 2023) investigated a population of 253 RILs produced by crossing Miyang 23 and Jileng 1. Their study reported traits linked to drought indicators such as leaf rolling, leaf withering, and leaf chlorophyll content. Through QTL mapping, they reported a total of 28 QTLs. These QTLs were distributed across chromosomes, with four on chromosome 1, two on chromosome 5, and two on chromosome 12. (Satrio *et al.*, 2021) established a comprehensive linkage map for all 12 chromosomes, leveraging a substantial pool of 55,205 SNP markers. They developed a RIL population originating from the cross IR64 x Hawara Bunar, subjecting it to scrutiny for 175 morpho-physiological traits under drought stress. A total of 41 QTLs were detected in response to drought stress, with noteworthy hotspot regions emerging on chromosomes 6, 8, and 9. (Barik *et al.*, 2020) generated 190 F₇ RIL individuals obtained from

CR 143–2-2 and Krishnahamsa, focusing on terminal water stress conditions. In their study, they identified three new QTLs: *qRCC1.1*, *qCHLa1.1*, and *qPRO3.1*, respectively to relative chlorophyll, chlorophyll a, and proline content. *qRCC1.1* was positioned on chromosome 1, residing at a distance of 142.8 cM. The identification of these novel QTLs holds promise for advancing marker-assisted breeding strategies in the context of terminal water stress conditions. (Sandhu *et al.*, 2019) highlighted the urgent need for climate-resilient crops due to unpredictable climate changes and rapid population growth. They stressed the importance of focusing on QTL studies to enhance crop yield under different stress conditions. (Barik *et al.*, 2019) employed BSA to uncover QTLs linked to morpho-physiological traits within a population of 190 F₉ RILs under drought stress. They evaluated 401 SSR primers, of which only 77 exhibited polymorphism. Through Composite interval Mapping (CIM) identified significant QTLs named *qLR9.1*, *qLD9.1*, *qSF9.1*, *qHI9.1*, and *qRWC9.1*, associated with leaf rolling, leaf drying, spikelet fertility, harvest index, and relative water content, respectively. These QTLs offer the potential for enhancing marker-assisted selection of morpho-physiological traits beyond yield, particularly under reproductive stage drought. (Palanog *et al.*, 2014) investigated QTLs associated with grain yield and its related traits in response to drought stress. They employed a mapping population derived from a landrace, Kali Aus, and two common cultivars, IR64 and MTU1010. The study involved phenotyping under both drought-stressed and non-stressed conditions. For genotyping, they utilized six polymorphic markers for the Kali Aus/IR64 mapping population and eight for the Kali Aus/MTU1010 mapping population within the BSA population. This research revealed two significant QTLs, namely *qDTY2.3* and *qDTY2.2*, linked to grain yield, brought to light through the influence of drought stress. These findings hold promise for enhancing drought-tolerant varieties, particularly the widely cultivated mega-cultivars IR64 and MTU1010. Subashri *et al.*, 2009 conducted a study where they examined 22 morpho-physiological traits to gain insights into how drought tolerance genes function under water stress during the reproductive phase. For genotyping, they employed 23 SSR markers. Given that the *SD1* semidwarf gene's location was known on chromosome 1 between markers RM246 and RM315, they validated the QTL for drought-related traits within this region. Through QTL analysis, they uncovered four QTLs associated with 10 characteristics on chromosome 1. Remarkably, traits such as plant height, panicle length, panicle exertion, biological yield, and stomatal conductance under drought conditions were situated near the region marked by the *SD1* gene. Moreover, numerous other QTLs linked to various drought-related traits were also successfully identified. MacMillan *et al.*, 2006 identified 145 QTLs spread across chromosomes. They examined interactions of

QTLs with environmental factors for five traits in the Bala x Azucena cross. Their study affirmed that detecting QTLs for root attributes has advanced marker-assisted breeding. In (Lang *et al.*, 2013) surveyed 229 BC₂F₂ plants from the OM1490/WAB880-1-38-18-20-P1-HB cross to detect QTLs linked to drought traits like flowering drought (DRF), root dry weight (RDW), and root length (RL). They used 232 SSR markers to create a linkage map spanning 2,553.7 cM, with markers about 10.97 cM apart. Employing single-marker analysis (SMA) and interval mapping (IM), they detected two QTLs for RL and two for RDW, accounting for phenotypic variation ranging from 6.2% to 30.8% across different morphological traits.

2.7.1. Important QTL detected in drought stress

Various unique QTLs have been identified for drought tolerance, which could be used in breeding drought-resistant rice varieties. To date, most of the identified drought-tolerance QTLs in rice have originated from non-elite genotypes. Notable QTLs include *qDTY1.1*, widely used as a yield trait under drought stress (Vikram *et al.*, 2011). Other significant QTLs identified in various rice includes *qDTY2.1*, *qDTY2.2*, *qDTH12.3*, *qDTY3.1*, *qDTY6.1*, *qDTR8*, *qDLR8.1*, *qDTY9.1A*, and *qDTY12.1*, each associated with distinct drought tolerance aspects (Lin *et al.*, 2007, Qu *et al.*, 2008, Dixit *et al.*, 2012, Venuprasad *et al.*, 2012, Mishra *et al.*, 2013, Dixit *et al.*, 2014, Ramchander *et al.*, 2016). SSR markers linked to these QTLs have also been reported (Vikram *et al.*, 2011), facilitating the molecular screening of new rice genotypes for drought tolerance. (Barik *et al.* 2019) reported the genetic mapping of morpho-physiological traits associated with drought tolerance during the reproductive stage in rice. They revealed five QTLs (*qLR9.1*, *qLD9.1*, *qHI9.1*, *qSF9.1*, and *qRWC9.1*) controlling leaf rolling, leaf drying, harvest index, spikelet fertility, and relative water content, respectively, under drought stress at the reproductive stage.

2.8. Identification of genes related to QTL

Drought resistance is a complex trait and involves numerous genes that work together. Once QTL is detected, the detected QTL regions is scan for the genes governing these traits. This helps in learning more about drought adaptation in crops. Researchers find these genes by looking at where markers are on the genome. Genome connected to traits like yield. In rice, researchers identified various genes related to stress response by scanning the QTLs regions associated with yield and related traits.

(Zhou *et al.*, 2016) looked at certain parts regions of the genome that help it grow deep roots that resist drought. They focused on two areas on chromosome 11. Using tools to study

genes and their activity, they discovered a new gene called *OsAHL1*. This gene has special parts in its structure. When there is drought stress, this gene becomes more active, helping the plant's roots grow deeper and its seedlings and panicles grow better. This gene doesn't have much effect when there enough water, but it helps the plant handle drought better. (Dwiningsih *et al.*, 2021) investigated a population that was developed by crossing two rice types, japonica Kaybonnet, and indica ZHE733. They aimed to find the gene responsible for the chlorophyll content of a plant under drought. They used 4133 SNPs to find a specific QTL linked to this trait. RAP-DB was used to scan for genes that might be responsible. They found 43 potential genes with those 7 QTLs for chlorophyll content. A study was conducted on roots architecture in two types of rice: IR64, which is sensitive to drought, and Azucena, which can handle drought better. They found specific genes that help plants cope with drought, like *NAC*, *AP2/ERF*, *AUX/IAA*, *EXPANSIN*, *WRKY*, and *MYB*. They also discovered that 288 of these genes are linked to root growth,. The study summarized that Azucena deals with drought by making its roots grow more, while IR64 relies on protecting its cells to handle drought. This study was conducted by (Abdirad *et al.*,2022).

Rice being the staple food feeding almost half the world's population, requires climate-resilient crops due to escalating climate change challenges. This demands a strategic approach involving the identification and manipulation of Quantitative Trait Loci (QTLs) for abiotic and biotic stress like drought tolerance and disease resistance. Molecular techniques such as GWAS and fine mapping are important for detection of these genetic regions, with subsequent integration into breeding programs using gene editing and marker-assisted selection. Collaboration among experts in genomics, physiology, agronomy, and socio-economics ensures the development of climate-smart rice varieties, enhancing global food security and livelihoods by multifaceted impacts of climate change.

CHAPTER III

MATERIAL AND METHODS

The study was undertaken in the department of Agricultural Biotechnology, Assam Agriculture University (AAU). The field work for the research was carried out at Instructional-cum- Research (ICR) farm and in the net house and rain out shelter of DBT-NECAB. The chapter provides insights about the methodologies applied to fulfill the research objectives.

3.1. Planting Material and Location

For the fulfilment of the research objectives, initially, a total of 428 cultivars were screened for drought tolerance in three different field experiments. Out of which, 13 different rice cultivars were chosen based on level of drought tolerance (Parray, 2019). Out of the 13 cultivars, 4 cultivars, two of each tolerant cultivars (*ARC-10372* and *Koimurali*) and two of each susceptible cultivars (*IR-64* and *Disang*) were chosen, the moderately tolerant and susceptible cultivars were eliminated. The cultivars were chosen on the basis of level of tolerance to drought with substantial evidence obtained from morphological, physiological, and molecular analysis. The list of cultivars were listed Table 3.1.

3.1.1. Screening of the cultivars for identification of parents

The four chosen cultivars were screened on the basis of phenotypic parameters and differential gene expression studies using drought responsive genes. The seeds of all the four germplasm were collected from RARS Karimganj and were grown in petri dishes (see Plate 3.1). Once the seeds were germinated, each germplasm were transferred to small plastic cups and allowed it to grow for 21 days. The 21 days old seedlings were then transferred to black poly bags (Plate 3.2) with two replications of each germplasm in both stress and non-stress conditions. Under non-stress conditions, there was continuous supply of water and the drought stress was imposed at reproductive stage whereas the water supply was stopped at the maximum tillering stage till the beginning of reproductive stage. The leaf sample of both stress and non-stress plants of each cultivar with replication were collected after 5 days of withdrawing of water (5 DWW) and 10 days after withdrawing of water (10 DWW) and were stored in -80°C refrigerator to study the expression profile for different drought responsive genes.

TABLE 3. 1: List of cultivar used in the study

Sl no.	Cultivar Name	Source	Description	Drought Response
1.	TepiDumai	RARS, Karimganj	<i>Ahu rice</i>	Tolerant
2.	LeujaAus	RARS, Karimganj	<i>Ahu rice</i>	Moderately Tolerant
3.	IR-64	RARS, Karimganj	<i>Ahu rice</i>	Susceptible
4.	Disang	RARS, Karimganj	HYV	Susceptible
5.	Horina Arai	RARS, Karimganj	<i>Ahu rice</i>	Moderately Tolerant
6.	C6725	RARS, Karimganj	<i>Ahu rice</i>	Moderately Tolerant
7.	Koimurali	RARS, Karimganj	<i>Ahu rice</i>	Tolerant
8.	GaruJum	RARS, Karimganj	<i>Hill rice</i>	Moderately Tolerant
9.	Dumai	RARS, Karimganj	<i>Ahu rice</i>	Tolerant
10.	ARC 10372	RARS, Karimganj	<i>Ahu rice</i>	Tolerant
11.	Ranjit	RARS, Karimganj	HYV <i>Sali</i>	Susceptible
12.	Dimbra	RARS, Karimganj	<i>Ahu rice</i>	Moderately Tolerant

3.2. Isolation of total RNA

The total RNA of all 4 cultivars was isolated under irrigated conditions, at 5 DWW and at 10 DWW in the two replications. The total RNA was extracted under reproductive drought stress following the protocol of Simms *et al.*,1993 with minor modifications (Plate 3.3) and was calibrated using endogenous gene *Ubiquitin*.

Methodology

1. 100mg of fresh leaf sample from both control and stressed plants (5 DWW and 10 DWW) were ground in a mortar-pestle into fine powder using liquid nitrogen.
2. The powdered leaf tissue was transferred to 2ml micro-centrifuge tubes and homogenized in 1ml TRI Reagent (Sigma).
3. To ensure complete disassociation of nucleo-protein complexes, samples were allowed to stand at 4°C for 30 minutes.
4. The samples were centrifuged at 12000 rpm for 15 minutes at 4°C and the supernatant was then transferred to 2ml micro-centrifuge tube.

5. 200ml of chloroform was added to the microcentrifuge tube and was subjected to vigorous shaking for 15 seconds.
6. The resulting mixture was centrifuge at 12000rpm for 15 minutes at 4°C.
7. The centrifugation step separated the mixtures into 3 phases: an organic phase containing proteins, an interphase containing the DNA and white aqueous phase containing RNA.
8. Transferred the aqueous phase to a 1.5 ml fresh tube and add 500µl of 2-propanol. The two phases were mixed well and allowed to stand for 1hour at 4°C.
9. Centrifuged the tube at 12000 rpm for 15 minutes at 4°C so that the RNA was precipitated.
10. Pippeted out the supernatant and wash the pellet with 75% ethanol (RNase molecular grade water) and centrifuged at 7500rpm for 10minutes at 4°C.
11. Discarded the supernatant slowly and allowed the pellet to air dry and re-suspended the pellet at 30µl of RNAase free water
12. Stored the RNA samples at -80°C.

3.3. Quantification of RNA

The concentration of all the RNA samples were measured using a spectrophotometer (Nanodrop 1c). 1µl of RNAase free water (blank) was used as blank for initialization of the instrument. 1µl each of the RNA samples were then loaded on to the reader after initialization and its concentration was recorded in ng/µl, absorbance of the RNA at 260/280 and 260/230 were recorded. The quality of the RNA samples was also checked to know the integrity of the 18S and 28S bands.

3.4. cDNA synthesis for real-time qPCR analysis

The cDNA synthesis was performed using PrimeScript™ RT reagent Kit(Perfect real time) (TAKARA: Catalogue No. RR037A) and the real time PCR protocol was performed as per manufacturer instructions given in PowerUp™ SYBR™ GREEN Master Mix(Applied Biosystem Catalogue No.2631652)(Applied Biosystem USA) and the procedure were as follows:

3.4.1. Preparation of cDNA

The cDNA reaction mixture was prepared on ice. The master mix of the given components (Table 3.2) were prepared in 1.5 ml microcentrifuge tube except the RNA sample in a volume sufficient for the number of reactions. The appropriate volume of the master mix

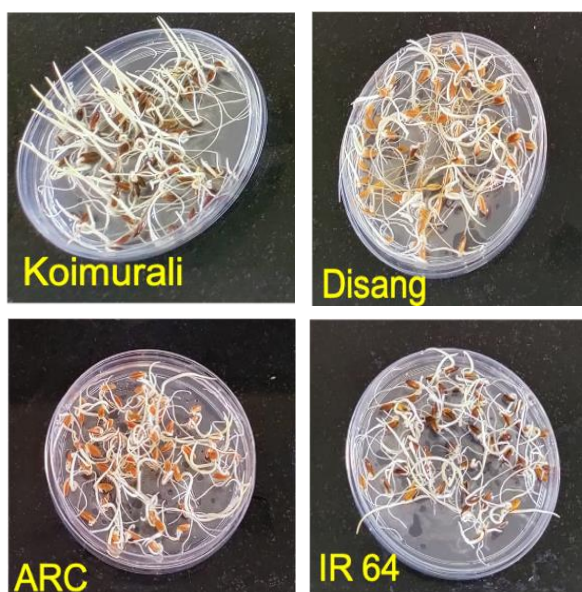


Plate 3. 1: Sowing of the cultivars for parental

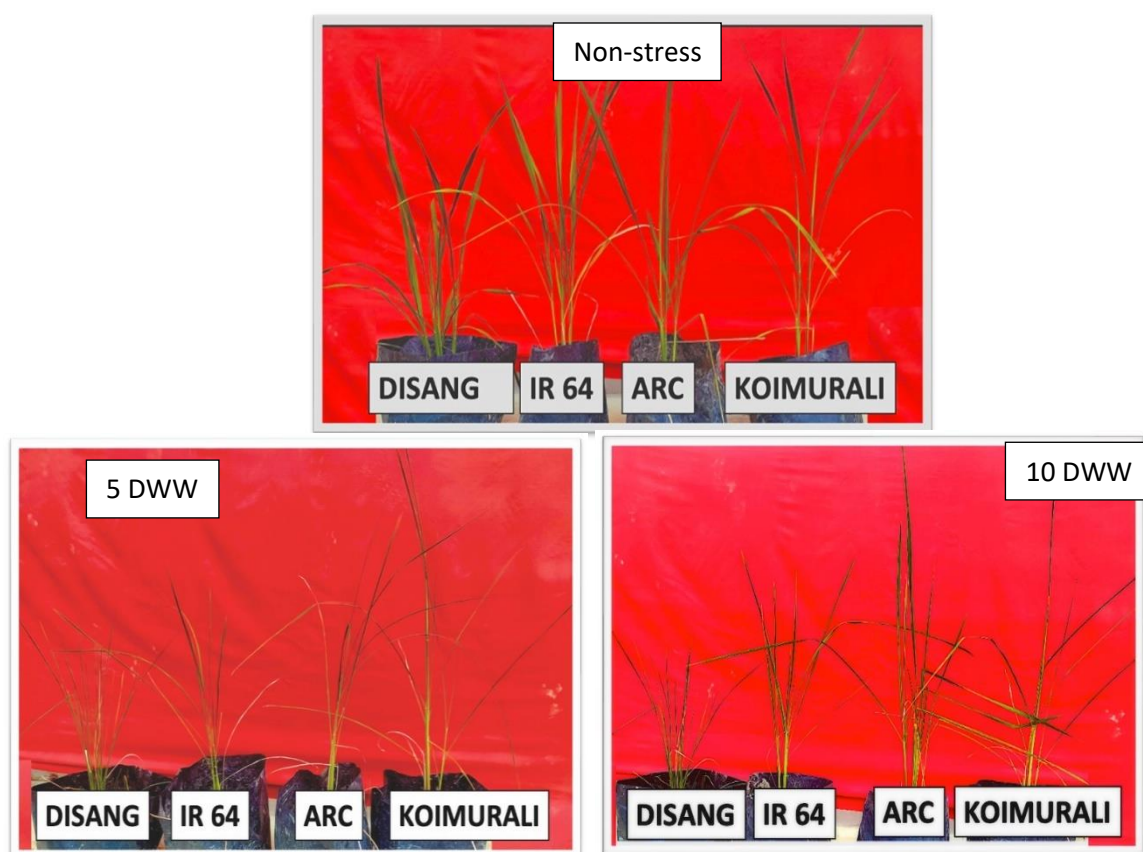


Plate 3. 2: Phenotypic variation for drought tolerance under non-stress, 5 DWW and 10 DWW

TABLE 3. 2: Reagents with concentrations for cDNA Preparation

Reagent	Volume	Final Concentration
5X PrimeScript Buffer (for Real time)	2µl	1X
PrimeScript RT enzyme Mix 1	0.5µl	
Oligo dT Primer (50µM)	0.5µl	25pmol
Random 6mer(100µM)	0.5µl	50pmol
Total RNA	*1	
RNase free dH ₂ O	To adjust the volume	
Total	10µl	

*1µg of the total extracted RNA was used to prepare the cDNA

(9µl) was added to different microcentrifuge tube and then the RNA samples were added to each microcentrifuge. The respective tubes were incubated at 37°C for 15minutes and 85°C for 5 seconds. The samples were stored for further use at 4°C.

3.4.2. Quantification of the cDNA

The concentration of all the cDNA samples were measured using a spectrophotometer (Nanodrop 1c). 1µl of the elution water (blank) was first used to initialize the instrument. 1µl of each cDNA samples was then loaded on to the reader after initialization and its concentration (ng/µl), absorbance of the cDNA at 260/280 and 260/230 were recorded. The quality of the cDNA samples was also checked to know the integrity cDNA bands using *Ubiquitin 10* as endogenous control as shown in Plate 3.4

3.4.3. Designing of Primers for Expression studies

Drought-responsive genes were identified through an extensive review of existing literature. The Coding Sequence (CDS) regions of these genes were retrieved from the National Center for Biotechnology Information (NCBI). To facilitate the assessment of the expression patterns of these selected genes in response to drought, specific primers were designed. This was achieved by utilizing the qPCR primer design tool offered by IDT Technologies UK. The list of the identified genes and is presented in Table 3.3.

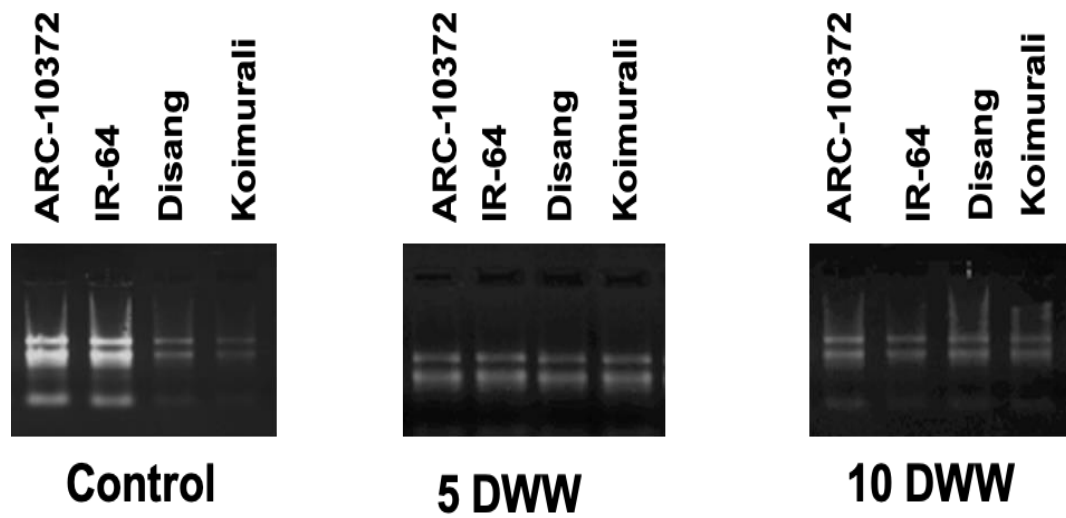


Plate 3. 3:Agarose Gel (0.8%) images of total RNA isolated from leaves of control and drought stressed plants

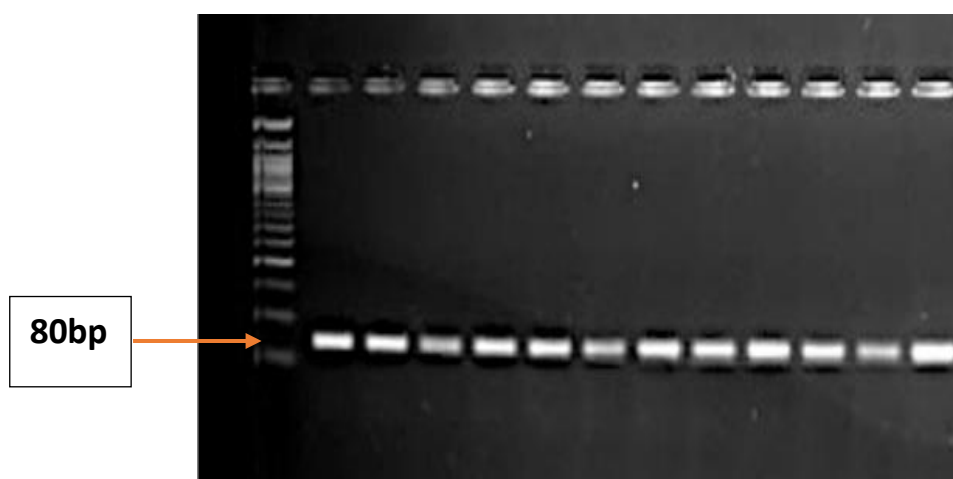


Plate 3. 4: Agarose Gel (3 %) showing expression pattern of endogenous control Ubiquitin

TABLE 3. 3:List of gene specific primers used in RT-qPCR analysis

Sl No	Gene Family	Gene/TF	Sequence(5'-3')	Reference
1	AP2/ERF	OsDREB2A	F- AAAGCGTATCGTTGGGACAG R-TTCTTCTAGTGCCTGGACCT	Cui <i>et al.</i> ,2011
2.	AP2/ERF	OsDREB2B	F-CCAGAGGACCAAGGAGATTA R- GCGTCTGATCCATCTTTG	Nakashima <i>et al.</i> , 2009
3.	NAC	OsNAC9	F-CAAGAAGGGATCTCTCAGGTTG R-CATCTTCTCCCACTCGTTCTTC	Redillas <i>et al.</i> ,2012
4.	bZIP	bZIP73	F- TCAGTTCCCACTACCACAGCAACA R- TACCACAGGGAGCAATTCCTGGAT	Liu <i>et al.</i> , 2019
5.	APX	OsAPX1	F- AGGTGCCACAAGGAAAGATCTGGT R- TCAGCAGGGCTTTGTCACTAGGAA	Caverzan <i>et al.</i> , 2012

3.4.4. Real-time quantitative study for gene expression

Real time quantitative protocol polymerase reaction (qPCR) protocol was followed as per the manual given along with PowerUpTM SYBRTM GREEN Master Mix(Applied Biosystem Catalogue No.2631652)(Applied Biosystem USA)as shown in Table 3.4. The real-time Polymerase Chain reaction was performed in Applied Biosystems RT-PCR system, Quant studio 3 (Applied Biosystem, USA) as shown in Table 3.5. All the qPCR experiment were performed twice using two Biological replicates and four technical replicates using gene specific Primers. *Ubiquitin 10* gene was taken as an endogenous control for normalization of all the gene/transcription specific qPCR primers.

3.5. Correlation between drought responsive genes and drought adaptive traits

The correlation analysis based on Pearson coefficient of the drought responsive genes and drought adaptive traits was studied using R software.

TABLE 3. 4: Components and concentration of qPCR Master mix

Sl No.	Components	Concentration	Quantity per Reaction(μ l)
1.	SYBR Green Master Mix	2X	5
2.	Forward Primer	10 μ M	0.5
3.	Reverse Primer	10 μ M	0.5
4.	Molecular Grade water		3
5.	Template		1
Total			10

TABLE 3. 5: Thermocyclic conditions for real time PCR

Sl No.	Temperature($^{\circ}$ C)	Duration(sec)	Cycles	Functions
1	95	120	1	Initial hold
2	95	30	40	2 step PCR
3	60	34	40	Dissociation

3.6. Criteria for selection of the parental cultivar

Based on the expression profiling and phenotypic parameters the parents chosen for the present investigation were *Disang*, (a HYV) and *Koimurali*, an indigenous landraces of Assam. *Disang* is a short duration (80-90 days) plant showing 35% more yield than any other local variety of Assam (Choudhary *et al.*, 2019). The variety shows a yield of 4.2 tones/ha. Under extreme drought stresses, the yield reduces to 76.09% (Kaldate *et al.*, 2023) therefore it was chosen as the recipient parent. On the other hand, *Koimurali*, was an exotic landrace of Assam which shows tremendous drought tolerance. This landrace of Assam was tall and has a very short growing season (75-80 days). Due to its tolerance this cultivar was chosen as the donor parent for the present study. Morphology of the parental genotype were shown in plate 3.5. The two parents having contrasting characters were used further for the development of a biparental mapping population. The plant material chosen as parents showed high Proline content, water use efficiency (WUE), relative water content (RWC) and root length (Parrray, 2019).

3.7. Development of Biparental Mapping population

The development of biparental mapping population was started from *Ahu* 2019 and ended in *Sali* 2022. The aim was to develop mapping population by crossing a drought tolerant indigenous landrace of Assam *Koimurali* with a semi dwarf short duration variety of Assam named *Disang* that was developed and released by RARS in 2010. The selected parents for further development are sowed six times at five-week intervals to coincide the flowering time for crossing. The pollen grains were afterwards collected from the tolerant donor parent *Koimurali* and dusted onto the emasculated susceptible parent, *Disang* at 5 a.m morning. After the pollination process was completed, the panicles of the susceptible parent were properly covered with a butter paper bags. The seeds from the crossed plant material were then harvested after 30 days and were stored at room temperature. After harvesting, the F_1 seeds were planted in pots with well-dried soil and a low N, P, and K treatment. The leaf samples were collected from each plant after 2 to 3 weeks and stored at $-20\text{ }^{\circ}\text{C}$. True F_1 s were identified using SSR markers. The single F_1 plant with highest number of seeds was chosen to raise F_2 plants. The seeds of each individual F_2 plants were advanced to F_3 population through single seed descent (SSD) method (Plate 3.6). The subsequent generations were also generated using SSD method till $F_{5:6}$ population and $F_{6:7}$ population for the development of Recombinant inbred lines (RILs) (Plate 3.7 and Plate 3.8). The breeding schemes used for the development of the mapping population was presented in Table 3.6.

3.7.1. Phenotypic evaluation of mapping population under intermittent drought stress

The present study aimed to evaluate the phenotypic responses of the F_5 population between *Koimurali* and *Disang* under intermittent drought stress during the *Sali* 2021 season. The experiment was conducted in two distinct environments, namely the ICR farm, AAU (control condition), and the rain-out shelter at DBT-NECAB, AAU (stressed condition). Both environments were thoroughly monitored for drought adaptive traits. F_6 seeds derived from the F_5 generation were germinated in petri plates under dark conditions. Subsequently, one-week-old seedlings from 247 lines were individually transplanted into the nursery bed. At 28th day, the seedlings were tagged and transplanted to both plots with a spacing of 25cm by 20cm. In the rain-out shelter facility, water was provided at 55 DAS (days after sowing) until the field completely dried up and plants showed mild drought symptoms. Following this, water supply was then stopped at 65 DAS until the field again exhibited mild drought symptoms. At 75 DAS, water supply was ceased until the



Plate 3. 5:Morphology of the parental genotype

breeding lines started showing symptoms, after which continuous water supply was resumed. In contrast, no drought stress was imposed in the non-stress condition. The list of phenotypic parameter observed during the study were listed in Table 3.8.

3.7.2. Phenotypic evaluation of mapping population under reproductive drought stress

The study aimed to assess the phenotypic responses and drought adaptive traits of the F_6 population derived from the cross between *Koimurali* and *Disang* under reproductive drought stress during the *Ahu 2022* season. The experiment was conducted using plastic pots, with each F_6 line planted in six different pots. Out of these pots, three were designated as control (non-stress) conditions, while the other three were subjected to induced reproductive drought stress. To initiate the experiment, F_5 -derived F_6 seeds were germinated under dark conditions in petri plates. After one week, 247 F_6 lines seedlings were individually transplanted to a nursery bed. At 28th day, the seedlings were tagged and then transplanted to pots, with three pots of each line assigned as control and the remaining three pots were designated for reproductive drought stress induction. Reproductive drought stress was imposed on the designated stress pots once the transplanted lines reached the maximum tillering stage. At this point, water supply was withheld, and various drought adaptive traits were meticulously observed (Table 3.7) and scoring of the drought symptoms were done as per Systematic evaluation System (SES, IRRI, 2013) (Table 3.8). The experimental design aimed to explore and compare the F_6 population's phenotypic responses to reproductive drought stress, focusing on their ability to tolerate and recover from water scarcity during specific growth stages.

3.8. Phenotypic observation of characteristics in stress and control conditions

3.8.1. Phenotypic data of yield attributing traits:

3.8.2. A total of 16 different yield attributing traits (table 3.7) were observed and recorded.

For both control and stress conditions under artificially induce intermittent and reproductive stress condition along with their control environment. The yield attributing traits taken under consideration were elaborated below.

3.8.1.1. Days to 50 per cent flowering (DFF)

The date at which 50 percent of the plants in each line reached the flowering stage was noted, and the number of days from sowing was recorded for each line.

3.8.1.2. Plant height (PH)

The height of the plants was measured in centimeters (cm) by determining the distance from the ground level to the tip of the main panicle approximately 10 days after flowering. For each line, three plants were randomly selected, and their average height was calculated and used for further analysis.

Season	Location	Activity	Season
Ahu 2019	PBG Net house	Crossing of Parents(Koimurali X Dwasang)	Ahu 2019
Sali 2019	PBG Net house	Development of F ₁ hybrids and Hybridity Confirmation	Sali 2019
Ahu 2020	ICR Farm	Sowing of F ₂ Plants(375 lines) and starting of developing a mapping population and checking segregation ratio	Ahu 2020
Sali 2020	ICR Farm	Sowing of F ₃ Lines by SSD method(350 lines)	Sali 2020
Ahu 2021	ICR Farm	Sowing of F ₄ Lines by SSD method(350 lines)	Ahu 2021
Sali 2021	ICR Farm and Rainout shelter	Phenotyping of F ₅ lines(319 lines) under intermittent drought and Control condition	Sali 2021
Ahu 2022	AAU Net House(Pot experiment)	Phenotyping of F ₆ lines(250 lines), Leaf collection for Genotyping and Polymorphism check	Ahu 2022
Sali 2022-2023	Biotechnology Marker Lab	Genotyping of the mapping population, Construction of linkage map, QTL detection,	Sali 2022-2023
Ahu 2023	AAU Net house (PVC pipe experiment)	Sowing of extreme lines for expression analysis	Ahu 2023

TABLE 3. 6: Timeline of breeding activities performed during the study.

TABLE 3. 7: Yield attributing traits for Phenotypic Observations

Scale	Category	Leaf rolling	Leaf drying	Spikelet Fertility	Scale
0	Highly tolerant	Leaves healthy	No symptoms	More than 80%	1
1	Tolerant	Leaves start to fold (shallow)	Slight tip drying	61-90%	3
3	Moderately tolerant	Leaves folding (deep V-shape)	Tip drying extended up to 1/4	41-60%	5
5	Moderate	Leaves fully cupped (U- shape)	One-fourth to 1/2 of all leaves were fully dried	11-40%	7
7	Moderately Susceptible	Leaf margins touching (O-shape)	More than 2/3 of all leaves were fully dried	Less than 15	9
9	Susceptible	Leaves tightly rolled (V-shale)	All plants were dead. Length in most leaves fully dried		

TABLE 3. 8:Drought score level based on Standard Evaluation System (IRRI, 2013)

Days to 50% flowering (DFF)	Number of chaffy grains per panicle (NOC)
Plant height (PH)	Number of filled grains per panicle (NOG)
Total number of tillers per plant (NOT)	Total number of grains per panicle (TNG)
Effective booting tillers per plant (EBT)	Spikelet Fertility (SF)
Panicle Length (PL)	Grain Yield per plant (GY)
Flag Leaf Length (FLL)	Grain Length (GL)
Flag Leaf Width (FLW)	Grain Width (GW)
Flag Leaf Length to Width ratio (FLLW)	Grain Length to Width ratio (GLW)

3.8.1.3. Panicle length (PL)

The length of the panicle was measured in centimeters (cm) by determining the distance from the neck node of the panicle to the tip of the panicle at maturity. For each line, three panicles were randomly selected from three different plants. These chosen panicles were then used to record observations regarding their length. The length of the panicle was measured in centimeters (cm) using a measuring scale. The mean panicle length in centimeters was calculated for each line using the recorded measurements.

3.8.1.4. *Flag leaf length (FLL)*

The length of the panicle was measured in centimeters (cm) using a measuring scale. The measurement was taken from the first node to the tip of the flag leaf. Three plants were randomly selected from each line, and data regarding the panicle length was recorded for each of these plants. The mean panicle length in centimeters was calculated for each line using the recorded measurements.

3.8.1.5. *Flag leaf width (FLW)*

The leaf width was measured in centimeters (cm) by determining the width at the broadest part of the flag leaf. Measurements were taken from three randomly selected plants from each line, specifically from the main tiller. Data regarding leaf width were recorded for each of these selected plants, and the mean leaf width was analyzed by calculating the average of the recorded measurements.

3.8.1.6. *Number of tillers per plant (NOT)*

The tiller number per plant was determined by counting three randomly selected plants from each line. Mean values of tillers per plant were calculated and used for further analysis.

3.8.1.7. *Effective booting tillers per plant (EBT)*

At harvest, the number of productive tillers was counted from three randomly selected plants in each line. The average number of effective tillers per plant was calculated by dividing the sum of productive tillers by three, providing valuable information on tiller productivity for each line.

3.8.1.8. *Total number of grains per panicle (TNG)*

The total number of filled and chaffy grains in each panicle was calculated by adding the counts of both types of grains. Data were collected from three panicles of three randomly selected plants in each line. The average count of filled and chaffy grains per panicle was obtained by calculating the average across the three panicles.

3.8.1.10 Number of Chaffy Grains per Panicle (NOC):

The count of unfertilized or empty seeds from each panicle was performed by examining three panicles from three randomly selected plants in each line. The average number of unfertilized seeds per panicle was calculated by taking the mean across the three panicles.

3.8.1.11 Number of Filled Grains per Panicle (NOG)

The count of filled or mature grains was determined by examining three randomly selected plants within each line. Additionally, the average number of fertile grains per panicle was calculated by counting the filled grains from three randomly chosen panicles. These values provide the average count of fertile or filled grains per panicle across the three selected panicles. The data can be further analyzed to assess grain yield potential of each line.

3.8.12 Spikelet Fertility (SF)

Spikelet fertility was calculated using the following formula:

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of filled grains per panicle}}{\text{Total Number of spikelets per panicle}} \times 100$$

3.7.13 Grain Yield per Plant (GY)

Grain yield per plant was determined by weighing the grains in grams (g) from three randomly selected plants in each line. The average grain yield per plant was obtained by calculating the mean of the recorded weights across the three selected plants.



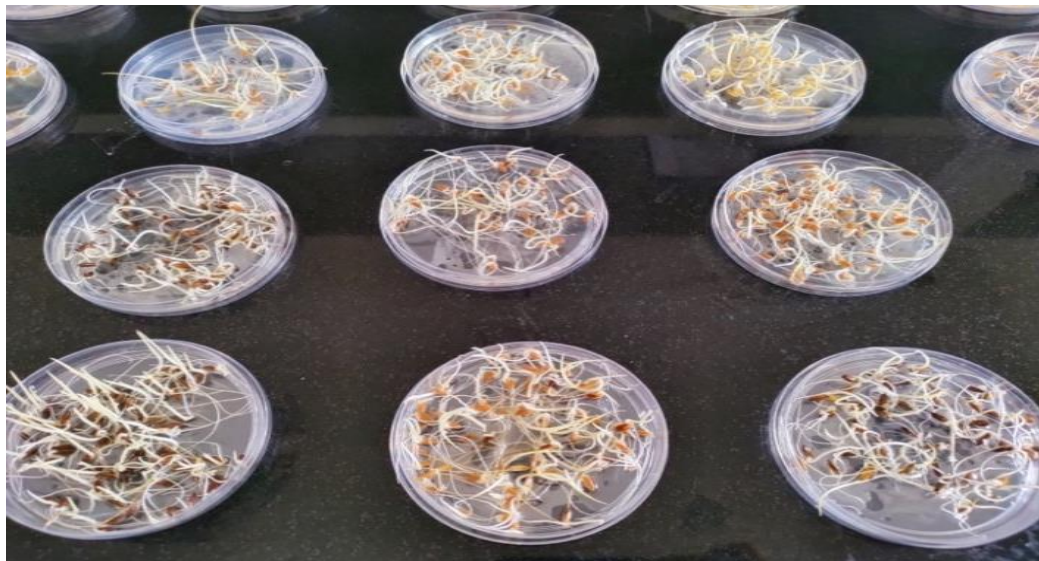
Parents (Dwasang X Koimurali)



F₂ plants

Plate 3. 6:F₂:F₃ population (Ahu,2020)

Sowing of F_3 lines



Nursery preparation of F_3 Lines



F_3 Population

Plate 3. 7: F_3 : F_4 population(Sali,2020)

3.8.14 Grain Length (GL)

The length of three grains was measured in millimeters (mm) using a digital Vernier calliper from three randomly selected plants in each line. These measurements were used to estimate the average grain length for further analysis.

3.8.15 Grain Width (GW)

Using a digital vernier calliper, the width of a grain was measured in millimeters (mm), and the average width was calculated. This average width value provides information about the typical size of the grains and can be further analyzed for comparison.

3.8.16 Grain Length-to-Width Ratio (GLW)

The grain length-to-width ratio was calculated by dividing the grain length by the grain width. This ratio provides insights into the shape and aspect ratio of the grains and can be used for further analysis and interpretation.

3.8. Phenotypic Scoring (Leaf rolling, leaf drying and spikelet fertility)

The leaf rolling and drying scale was observed under the artificial induce stress (pot experiment) conditions once the irrigation was withheld and drought symptoms became visible. The scale level of all the observations were presented in Table 3.8.

3.9. Screening of F₅ and F₆ population for using different drought indices

The 247 F₅ and F₆ lines along with their parents were screened for different drought tolerance parameters using different drought indices for both stress and non -tress conditions. The indices used were discussed below:

Relative Yield Reduction percentage (RYR %) of Fischer and Maurer (1978):

$$RYR\% = \left(1 - \frac{GY \text{ stress}}{GY \text{ control}}\right) \times 100$$

Drought Susceptibility Index (DSI) of Fischer and Maurer (1978):

$$DSI = \left(1 - \frac{GY \text{ stress}}{GY \text{ control}}\right) / SII$$

Stress Tolerance (TOL) of Rosielle and Hamblin (1981):

$$TOL = (GY \text{ control} - GY \text{ stress})$$



Plate 3. 8: F4:F5 population(*Ahu*,2021)

Yield Stability Index (YSI) of Bouslama and Schapaugh (1984):

$$YSI = (GY \text{ stress} / GY \text{ control})$$

Stress Intensity Index (SII) of Fischer and Maurer (1978):

$$SSI = 1 - (GY \text{ stress}) / (GY \text{ control})$$

Where,

GY stress= yield under stress for a genotype,

GY control= yield under control for a genotype,

(GY stress) = mean yield in stress for all genotypes , and

(GY control) = mean yield in control for all genotypes.

3.10 Genotyping

3.10.1. Genotyping of the F₅:F₆ RIL population

The genomic DNA was isolated for the parents and used to check the parental polymorphism using 600 SSR markers (Appendix 4) out of which 98 SSR markers were found to polymorphic. These 98 polymorphic SSR marker were further used to check the segregation in the developed RIL population containing 248 Lines. The procedure used for isolation of parental DNA and 248 lines were listed below:

3.10.2. Extraction of genomic DNA

The leaf sample of 248 lines along with their parents were collected and the total genomic DNA of all the sample were isolated using (Doyle 1991) with minor modification. The list of chemicals used for genomic DNA isolation were listed in Table 3.9

TABLE 3. 9:List of chemicals used in genomic DNA isolation

Composition	Quantity(ml)
Trwas HCL(1M)	100
EDTA(0.5 M)	100

Nacl (5M)	300
CTAB(10%)	300
Beta marcaptoethanol	40

- a) DNA extraction buffer
- b) The final volume was made to 1000ml using dH₂O
- c) Choloform: isoamylachohol (24:1) (v/v)
- d) Ethanol (100% and 70%)
- e) 1 X TE buffer: 10 mM Tris, 1 mM EDTA, PH 8.0
- f) RNase (10 mg/ml) stored at -20°C

Procedure

1. Young leaf sample was collected and cut into small pieces using sterile scissors.
2. The leaf pieces were transferred into a mortar, and liquid nitrogen was added.
3. Using a pestle, the leaf samples were crushed into a fine powder.
4. The powdered sample was transferred to 2 mL Eppendorf tubes, and 700 ul of extraction buffer was added.
5. The tubes were placed in a 65 °C water bath for 30 minutes with occasional mixing at 5- to 6-minute intervals for homogeneity.
6. After the sample were removed from waterbath,an equal amount of CI at 24:1 v/v was added to each tube and mixed gently for 5 minutes.
7. The mixture was then centrifuged at 10,000 rpm for 20 minutes at 40 °C.
8. The clear aqueous liquid (supernatant) was carefully pipetted and transferred into a fresh tube.
9. To the supernatant, 500 ml of chilled isopropanol was added, and the mixture was stored overnight at -20 °C.
10. The sample was then centrifuged at 10,000 rpm for 10 minutes, and the supernatant was discarded.

11. The pellets were washed with 70 per cent ethanol by centrifuging for 5 minutes at 10,000 rpm. Afterward, the ethanol was discarded, and the pellets were allowed to air dry completely.

12. Finally, the dried pellet was dissolved in TE buffer (50 μ l) and stored at 4° $\pm$ ∞ C.

3.10.3. Observations on quality and quantity of genomic DNA

3.10.3.1. Qualification of DNA using Gel electrophoresis

Gel electrophoresis was used to check the quality of the all isolated genomic DNA. For the analysis, 0.8 % agarose gel was prepared using 1X TBE buffer solution. The chemical used during the process were listed below:

TABLE 3. 10: Chemical composition of 25X TBE buffer

Composition	Amount
Tris Base (pH 7.8)	270 g
Boric acid	137.5 g
EDTA	100 ml

- 1X TBE buffer was used in the procedure was prepared from the 25X stock TBE buffer solution

Procedure

1. Agarose gels of 0.8% were prepared by melting 0.8 g of agarose in 100 ml of 1X TBE buffer by heating in a microwave oven until it completely dissolved and formed a crystal-clear solution, and it was allowed to cool down.
2. When the solution temperature reaches 55–60 °C, an EtBr solution with a 10 μ l/ml concentration was added.
3. The solution was poured into a pre-set levelled casting tray with a comb fitted to it, and the gel was allowed to dry.
4. After solidification, the comb was carefully removed from the casting tray.

5. The gel was then placed into an electrophoresis tank with the wells facing the cathode, and the whole gel was submerged in 1 x TBE buffer of pH 8.0 to a depth of about 1 cm.
6. The DNA sample of 2 μ l, with 5 μ l of loading dye remaining, and 3 μ l of dH₂O were loaded into the wells along with the known concentration of a 1 kb DNA ladder.

The gel was run for 60–90 minutes at 50 V (5 v/cm), and the bands were visualised using the gel documentation system (Bio-Rad,). The DNA sample with good lower shearing bands was considered for the present investigation.

3.10.3.2. Quantification of genomic DNA

The genomic DNA was quantified, and its purity was checked using a NanoDrop One spectrophotometer (Thermo Scientific). In the sampling arm, 1 μ l of DNA was loaded, and the absorbance of DNA was measured at 260 nm to find the concentration of DNA was observed in ng/microlitre . At 280 nm absorbance, the extent of protein contamination during the DNA extraction was observed. The sample value was recorded and stored in an image file, and the DNA was diluted according to the concentration found. After checking the quality and quantity of the isolated sample, the sample were diluted to the required concentration.

3.11. Dilution of DNA

The DNA was diluted using molecular grade water, making the final concentration of the DNA 50 ng μ l⁻¹ for PCR amplification.

3.12. Primer Dilution

In this experiment, PCR primers was prepared for genotyping by diluting the stock solutions. A 100 pmol/ μ l stock solution of each forward and reverse primer was used. To achieve a working concentration of 10 pmol/ μ l, the primers were diluted with an appropriate amount of distilled water. Specifically, 15 μ l of the forward primer and 15 μ l of the reverse primer were carefully mixed with 270 μ l of distilled water, resulting in a total volume of 300 μ l for each primer mixture. The prepared primer mixtures were then used for PCR amplification to target and amplify the specific DNA regions of interest in the genotyping process namely 3.10 PCR amplification, PCR profile and agarose gel electrophoresis.

The thermo cycler that was used for amplification was Veriti 94 (Applied Biosystems, CA, USA) and a 2720 Thermal Cycler (Applied Biosystems, CA, USA) using

the thermocyclic profile as in Table 3.12 The components of the reaction mixture used to PCR amplification was given table:

TABLE 3. 11: Components of PCR master mix used in the study

Reagent	Quantity
Template	1
Forward Primer	0.5
Reverse Primer	0.5
Emerald Master Mix(Takara)	5
Molecular Grade Water	3
Total	10

TABLE 3. 12: Thermocyclic profile for amplification of microsatellite (SSR) markers

Step	Temperature (°C) and time	Cycle
Initial denaturation	94°C for 5 minutes	1
Denaturation	94°C for 1 minute	35
Annealing	55*°C for 1 minute	
Extension	72°C for 1 minute	
Final Extension	72°C for 5 minutes	1
Holding	4°C for ∞	

The PCR amplification was performed using the PCR profile as mentioned in Table 3.12 for all the 248 lines with 98 polymorphic markers. The amplified PCR products and a 100bp DNA ladder were analysed using gel electrophoresis on a 3% agarose gel. To prepare the gel, 9g of agarose was dissolved in 300 ml of 1x TBE buffer and heated in an oven until completely melted. Once the solution reached a safe temperature, 12 µl of Ethidium Bromide was added for gel staining. The mixture was poured into gel casting tray with the appropriate comb placement to create wells for sample loading. After the gel solidified and the comb was removed, the gel was carefully transferred into a gel electrophoresis tank. The PCR-amplified product and the 100bp DNA ladder are loaded into separate wells of the gel. The

electrophoresis system was operated at a constant voltage of 85–90 volts for a duration of 2 hours to facilitate the separation and migration of DNA fragments based on their size. After completion of the electrophoresis, the gel was visualized and documented using a Gel Documentation System) to capture digital images of the separated DNA fragments and the DNA ladder bands. This documentation allows for precise analysis and identification of the PCR products and estimation of their sizes relative to the DNA ladder standard.

3.13. Detection of polymorphism in parents

The detection of polymorphism between parents was mandatory for marker-assisted selection and QTL mapping. Paternal polymorphism between *Disang* and *Koimurali* was investigated using 600 SSR markers of dinucleotide repeats distributed across the 12 linkage groups in the study. Out of which, 98 markers were polymorphic between the parents.

3.14. Hybridity confirmation

The F₁ seed developed from the cross combination between *Disang* and *Koimurali* was germinated in sterile Petri plates, and once it started sprouting, it was transferred into properly labelled pots. The leaf samples were collected from all the putative F₁s along with the parentals. The true F₁ were selected based on the results found after DNA extraction, PCR amplification, agarose gel electrophoresis, and gel documentation of the product. The selected F₁ plants were further advanced to develop the RIL mapping population.

3.15. Segregation analysis of polymorphic SSR markers in various F₅ and F₆ RILs

The segregation patterns of the F₆ population resulting from the cross between *Disang* and *Koimurali* using polymorphic SSR markers. SSR markers were molecular tools commonly employed in genetic studies to detect variations in DNA sequences between individuals. The genotypes of the F₆ individuals were scored based on the presence of specific alleles at each SSR marker locus. For alleles identical to the drought-susceptible parent, *Disang*, the genotype was designated as "a" in a homozygous state. Conversely, when the alleles matched the drought-tolerant parent, *Koimurali*, the genotype was designated as "b" in a homozygous condition. In instances where the alleles differed between the two parents (heterozygous state), the genotype was recorded as "h or ab," indicating the allelic combination from both parents. To determine the segregation patterns at each SSR marker locus, we performed a Chi-square test. The Chi-square test was a statistical method used to compare observed data with expected data and assess whether any significant deviation from expected ratios occurs. In this context, the Chi-square test allows us to analyze whether the observed genotypic frequencies at each SSR marker locus follow the expected Mendelian segregation ratios. The

observed segregations were tested against the expected Mendelian ratio of 1:1 in the F₆ population by the formula:

$$\chi^2 = \sum_i \frac{(O_i - E_i)^2}{E_i}$$

Where, O = observed value (s)

E = expected value (s)

Statistically, a 0.01 probability level was used as a critical value. The null hypothesis was accepted if the chi-square value at two degrees of freedom was less than 0.01; however, if the value was more significant than 0.01, the null hypothesis was rejected, and an alternate theory was accepted, lacking any significant difference between the expected and observed results.

3.16. Linkage map construction

The linkage map was constructed using JointMap version 4 based on the segregation data of the marked polymorphic population developed from the F₆ population derived from *Disang* x *Koimurali*. For each segregating marker, a chi-square analysis was performed to test for deviation from the expected segregation ratio of 1:1 in the F₆ RIL mapping population. The chi square was calculated on the basis of maximum linkage. The critical LOD scores of 2 between two markers were used to establish linkage groups based on permutation test, and Kosambi's 1944 mapping function was used to determine map distance and locus order.

3.17. QTL analysis

In this study, a Quantitative Trait Locus (QTL) analysis was conducted using Map QTL 6.0 software. The analysis aimed to identify genetic loci associated with various yield-related traits in the RILs. Phenotypic data for the yield-related traits were recorded from the RILs and evaluated under two contrasting environmental conditions: 1) an artificial drought created using a rain-out shelter, and 2) irrigated field conditions representing intermittent drought stress. For reproductive drought stress, each line in the population was subjected to both conditions, with three pots per line under irrigated conditions and another three pots subjected to reproductive drought stress. To detect QTLs, a LOD score threshold of greater than equal to 2.0 was employed based on permutation test, indicating a significant linkage between the genetic marker and the trait of interest. The Kosambi mapping function was used to determine the genetic distances between markers on the chromosome and to

construct the genetic linkage map. The interval or composite interval mapping methods were used for QTL detection, allowing the researchers to identify regions in the genome associated with the expression of specific yield-related traits under different hydrological conditions. For each detected QTL, a nomenclature convention was followed, starting with a lowercase "q" followed by three capital letters denoting the specific trait and the chromosome number. This naming convention simplifies the identification and reference of QTLs for different yield-related traits.

3.18. *In silico* identification of genes for QTLs

QTLs associated with yield-related traits under drought stress conditions were identified, and *in-silico* analysis was performed to identify genes or proteins located within the QTL regions. To retrieve genes within the QTL regions, the flanking markers' base pair information was used to delineate the QTL boundaries. These flanking markers served as genetic signposts to define the regions of interest on the genome. The gene information was obtained from the Rice Annotation Project Database (RAP-DB) or the Rice Genome Annotation Project (<http://rapdb.dna.affrc.go.jp>, <http://rice.uga.edu>). These databases contain comprehensive information about the rice genome, including gene sequences, annotations, and functional data. Using the base pair information of the flanking markers, relevant genes within the QTL regions were extracted from the databases. Each gene's annotated function was then obtained from sources like Google Scholar, which provides a vast collection of scientific literature and research articles. The extracted gene information included gene descriptions, locus numbers, and gene ontology (GO) terms. The GO terms offer valuable insights into the biological processes, molecular functions, and cellular components associated with each gene, aiding in the understanding of their potential roles in the observed yield-related traits under drought stress. By correlating the QTL regions with the annotated gene functions, this *in-silico* analysis aims to identify candidate genes that may play significant roles in conferring drought tolerance and influencing yield performance in rice. Few of the putative genes were utilised to check the expression profile of the extreme breeding line sorted out from the reproductive drought stress conditions.

CHAPTER IV

RESULT AND DISCUSSION

Drought is one of the important abiotic stresses affecting crop yield adversely in rice. Drought is a complex trait that imparts detrimental effects on the growth and productivity of rice crops. There is a need to improve drought tolerance in rice suitable to Assam's situations, most particularly at the reproductive stage. Moreover, intermittent drought has become common in Assam due to irregular rainfall and also needs special attention. However, drought tolerance in rice is a multifaceted trait, influenced by the interaction of multiple genetic and environmental factors. The present study was conducted to choose best donor parents based on gene expression analysis for drought responsive genes among four drought tolerant and susceptible cultivars screened previously. The chosen parents (*Disang* and *Koimurali*) were used to develop recombinant inbred lines (RILs) and marker-based detection of quantitative trait loci (QTLs) was performed for tolerance to intermittent drought and reproductive stage's drought in rice. The results obtained from the current work has been discussed in detail in the section below.

4.1. Criteria for selection of cultivars

As discussed on the previous section, out of 428 cultivars, 13 extreme cultivars were selected based on the morpho-physiological and biochemical parameters for drought tolerance (Pararay; PhD Thesis, AAU, 2019). Out of these cultivars, four cultivars were chosen based on trait performance and yield attributing traits and utilized for gene expression profiling. The cultivars, *IR-64* and *Disang* were high yielding but lacked drought tolerance whereas *ARC10372* and *Koimurali* were found drought tolerant but poor yielder (Table 4.1). 4 different cultivars selected for the research work are discussed in Table 4.1.

Table 4. 1: List of cultivars with different drought tolerance capacity

Sl no.	Cultivar Name	Source	Season	Drought response
1.	<i>ARC 10372</i>	RARS, Karimganj	<i>Ahu</i>	Tolerant
2.	<i>IR 64</i>	RARS, Karimganj	<i>Sali</i>	Susceptible
3.	<i>Disang</i>	RARS, Karimganj	<i>Ahu</i>	Susceptible
4.	<i>Koimurali</i>	RARS, Karimganj	<i>Ahu</i>	Tolerant

4.2 Relative Expression for drought responsive

Drought involves a series of physiological, morphological, and molecular changes due to activation of certain number of genes in plants. These genes play an important role in survival of plants under stress situations by serving as master regulator. (Singh *et al.*, 2013). Therefore, single or cumulative gene expression leads to activation of a number of stress-responsive pathways in plants to cope up with water stress. There are several kinds of cell surface receptors that response to environmental stimuli which includes serine/threonine-like receptor kinases known as receptor-like kinases (RLKs), ion channel-linked receptors, G-protein-coupled receptors (GPCRs), and two-component histidine kinase receptors. RLKs play a significant role in processing of a wide range of plant developmental and environmental signals (Bhagava *et al.*, 2013). Phytohormones such abscisic acid (ABA) in roots under water deficit stress condition leads to translocation of ABA synthesis in roots to leaves, leading to stomatal closure and inhibits plant growth (Wilkinson and Davis, 2010). As the synthesis of ABA induces stress response in plant, ABA binding receptors like PYR/RCAR induces drought stress response by activating serine/threonine kinase SnRK2 (Fan *et al.*, 2016). Moreover, transcription factors (TFs) play an important role in regulating the expression of genes (Manna *et al.*, 2021). The TFs interact with cis-acting elements found in the promoter region of several stress-responsive genes, activating cascades or whole networks of genes that work together to improve tolerance to numerous stimuli at a given time. Over expression of the stress responsive TFs leads to activation of a cascade of genes. There are several TFs grouped under large families such as *AP2/ERF*, *bZIP*, *NAC*, *MYB*, *MYC*,

Cys2, *His2* and *WRKY* (Umezawa *et al.*,2006). In the present study relative gene expression was studied in two droughts tolerant and two drought susceptible cultivars for *OsDREB2A*, *OsDREB2B*, *OsZIP73*, *OsNAC9* and *OsAPX1*.

4.2.1 Gene expression analysis for *OsDREB2A* under Drought stress

Dehydration responsive element binding (DREB) transcription factors (TF) are well known for imparting abiotic stress in different plant systems. The *DREB* TF can be broadly classified into *DREB1* and *DREB2* associated with cold and dehydration stress response. These TFs are mostly following two separate signaling pathways that are found to associate with Ethylene responsive element binding factor (ERF) ERF proteins consists of ERF domain which is conserved in the region between 58-59 amino acid sequences that connect to two cis-elements (Singh and Chandra.,2021). *DREB* TF works independent of the ABA and therefore impart better stress response to various abiotic stresses. In a study conducted by Matsukura *et al.*,2010 and Dubouzet *et al.*, 2003 found that all five *OsDREB2* in rice works under dehydration stresses and the expression of *OsDREB2A* was observed exclusively higher compared to others. The present study attempts to analyze the expression of *OsDREB2A* at the reproductive stage in two time points; five days of withholding water (5DWW) and ten days of withholding water (10DWW).

At 5DWW of drought tolerance, an increase in the expression of *OsDREB2A* in both the selected tolerant cultivars *ARC10372* and *Koimurali* was observed. On the other hand, a significant decrease in expression for susceptible cultivar *IR-64* was observed. However, susceptible cultivar *Disang* did not show any significant decrease in gene expression as compared to *IR-64* but there was a considerably lower gene expression than the control (Fig. 4.1). At 10DWW, there was slight decrease in expression of *OsDREB2A* in the tolerant cultivars namely *ARC-10372* and *Koimurali* but the gene accumulation in this two cultivars were found to be higher (0.88 folds and 0.65 folds at $p < 0.05$ respectively) than that of *IR-64* and *Disang* (0.2 folds and 0.25 folds at $p < 0.05$) (Fig 4.1). In a study conducted by (Cui *et al.*,2011) to observe the expression of *DREB2A* under drought stress using a stress-inducible promoter (*4ABRC*) to improve tolerance against *japonica* rice variety *TNG67*, it was observed that there was an improve in tolerance of *japonica* than the non-stressed plants. Overexpression of *OsDREB2A* led to increase in drought tolerance (Dubouzet *et al.*,2003). From the discussion it may be estimated that *DREB2A* acts as a negative regulator as it is present in the central region

and in any deletion portion that converts *DREB2A* into an active form (Qin *et al.*, 2007; Mizoi *et al.*, 2012). Transgenic Arabidopsis also indicated an increase in expression of *DREB2A* under drought, salt and heat stress conditions (Mizio *et al.*, 2012). (Ganguly *et al.*, 2019) reported upregulation of *DREB2A* gene at 8DWW at the pre-flowering stage. From the different expression analyses of *DREB2A* gene, it maybe inferred that the expression pattern of *OsDREB2A* was more pronounced at early stress (5DWW) and decreased during later part of stress (10DWW). However, both the tolerant cultivars showed considerable upregulation of *OsDREB2A* as compared to the susceptible ones.

4.2.2 Gene expression analysis for *OsDREB2B* under Drought stress

OsDREB2B is a transcription factor (TF) that is associated with dehydration stress in plants. It belongs to the *APETALA2/ERF* (*AP2/ERF*) family, which is a large family of TFs involved in regulating various biological processes in plants. The *AP2/ERF* family is known for its role in various environmental stress responses, including dehydration. A study conducted by (Song *et al.*, 2013) provided evidence of *OsDREB2B* involvement in response to dehydration stress in rice (*Oryza sativa*). *OsDREB2B* play a crucial role in regulating gene expression during environmental stresses, allowing plants to adapt and survive under adverse conditions. The GCC box is a cis-acting element found in the promoter regions of ethylene-responsive genes. Ethylene is a plant hormone involved in various growth and developmental processes, including responses to stress in plants. The binding of *OsDREB2B* to the GCC box suggests that it is also involved in ethylene-responsive pathways, likely contributing to the regulation of gene expression under ethylene-related stresses. In addition, *OsDREB2B* has been implicated in various other biological processes at different stages of plant growth and development.

In the present study, at 5 days after withholding water (5DWW), *Koimurali* exhibited a significant increase in transcript accumulation compared to the control and other tolerant cultivar, *ARC-10372*. However, after 10DWW, both *Koimurali* and *ARC-10372* showed significant upregulation in gene expression (5-fold and 6.5-fold increase, respectively, at $p < 0.05$). On the other hand, the susceptible variety *IR-64* displayed a 0.5-fold decrease, and the cultivar *Disang* showed a 2-fold increase in gene expression compared to the tolerant cultivars at both 5DWW and 10DWW (Fig.4.2). Chakraborty *et al.*, 2021 studied the correlation of all *DREB* genes in both *japonica* and *indica* rice varieties and found that *DREB2* genes are highly effective in abiotic stress

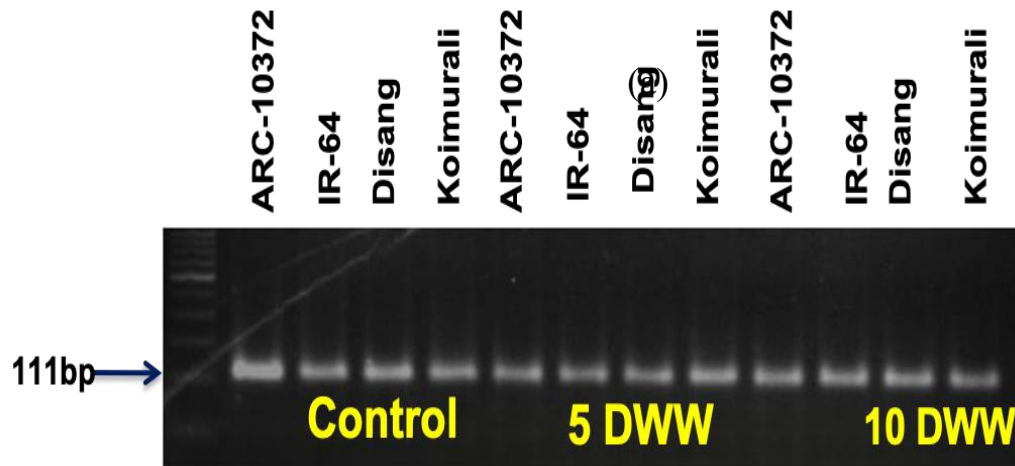


Plate 4. 1: Semi quantitative estimation of expression for *OsDREB2A* at 5DWW and 10DWW (*= $P < 0.05$)

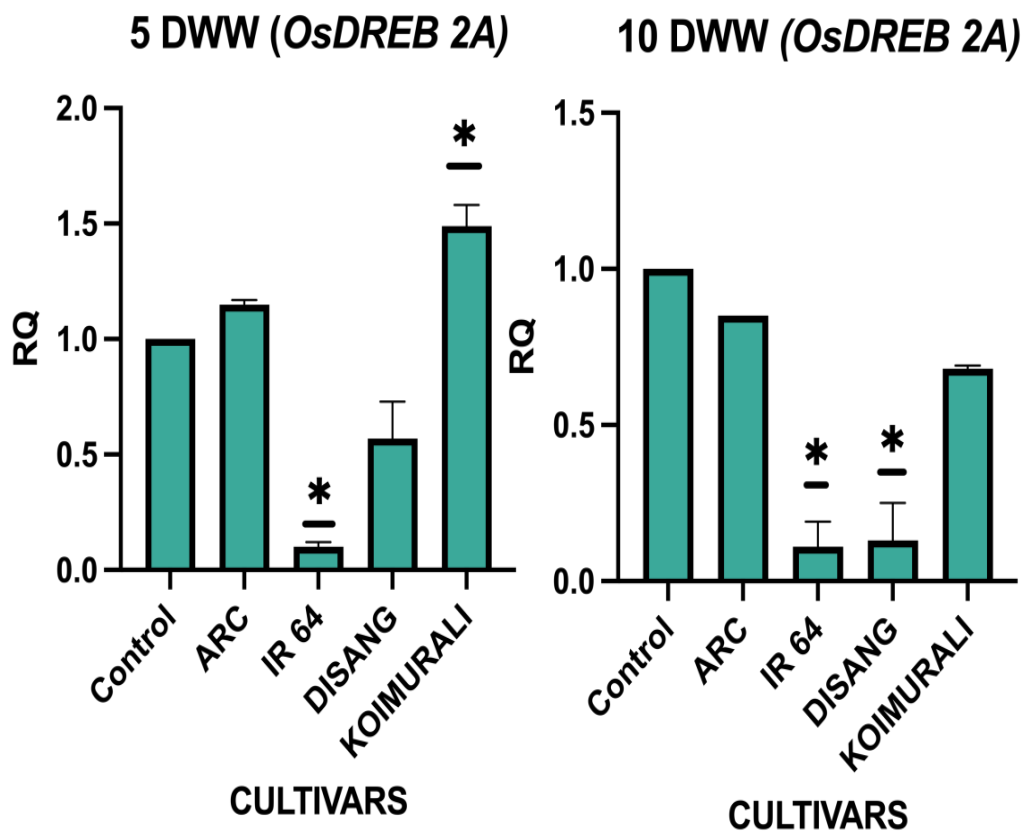


FIGURE 4. 1: QUANTITATIVE EXPRESSION OF *DREB2A* IN 4 CULTIVARS AT 5DWW AND 10DWW

Response. Interestingly, the overexpression of OsDREB2B in transgenic rice demonstrated enhanced tolerance to drought without any visible phenotypic changes (Chen et al.,2008). Furthermore, the gene accumulation of OsDREB2B reached its peak after 10 hours of the onset of stress but exhibited weak expression in irrigated plants (Nakashima et al.,2000). These findings align with the current results, where both tolerant rice cultivars showed increased gene expression under severe drought stress conditions.

4.2.3 Gene expression analysis for *OsNAC9* under drought stress

NAC (NAM, ATAF1/2, and CUC2) transcription factors (TFs) play pivotal roles in various aspects of plant biology, including growth, development, and responses to both biotic and abiotic stresses. The NAC TFs are characterized by DNA-binding NAC domain situated in the N-terminal region. This domain is composed of 150 amino acids, exhibiting five conserved regions. Unlike the classical helix-turn-helix motif, the NAC domain in *Arabidopsis* comprised of beta-pleated sheets surrounded by a limited number of helical motifs (Ernst *et al.*,2004). The corresponding structure in rice, *OsNAC* protein, follows a similar architecture (Kikuchi *et al.*,2000). A subgroup of NAC transcription factors has been identified as crucial player in to diverse abiotic stress responses such as salinity, drought, and low temperature (Hao *et al.*,2011, Mao *et al.*,2012; Wu *et al.*, 2012;).In rice, the stress-responsive NAC1 and 2 (SNAC1 and SNAC2) genes are very important for stress responses. For instance, SNAC1 has been demonstrated to confer drought resistance when introduced into transgenic rice (Hu *et al.*, 2006). Another member, *OsNAC9*, belonging to subgroup 1 of the NAC family, responds to drought stress and believed to have role in root diameter enlargement thereby helping withstand drought, more particularly at the reproductive stage (Redillas *et al.*,2012).The present study investigated the expression of *OsNAC9* in the selected cultivars at reproductive drought stress.

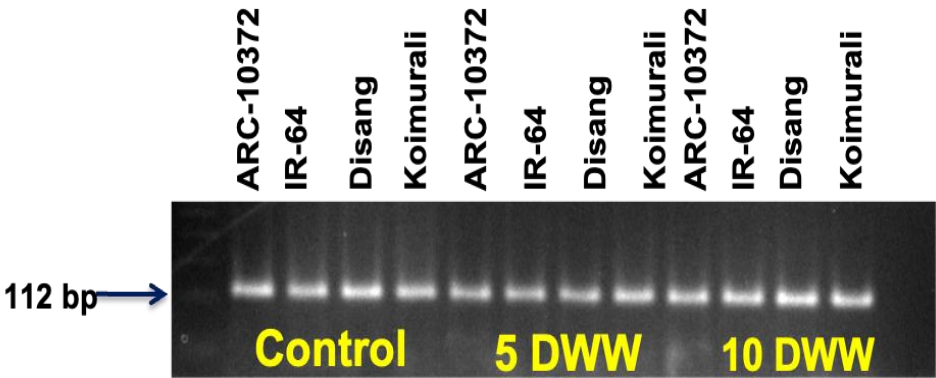


Plate 4. 2: Semi quantitative estimation of expression for *OsDREB2B* 5DWW and 10DWW, P=0.05

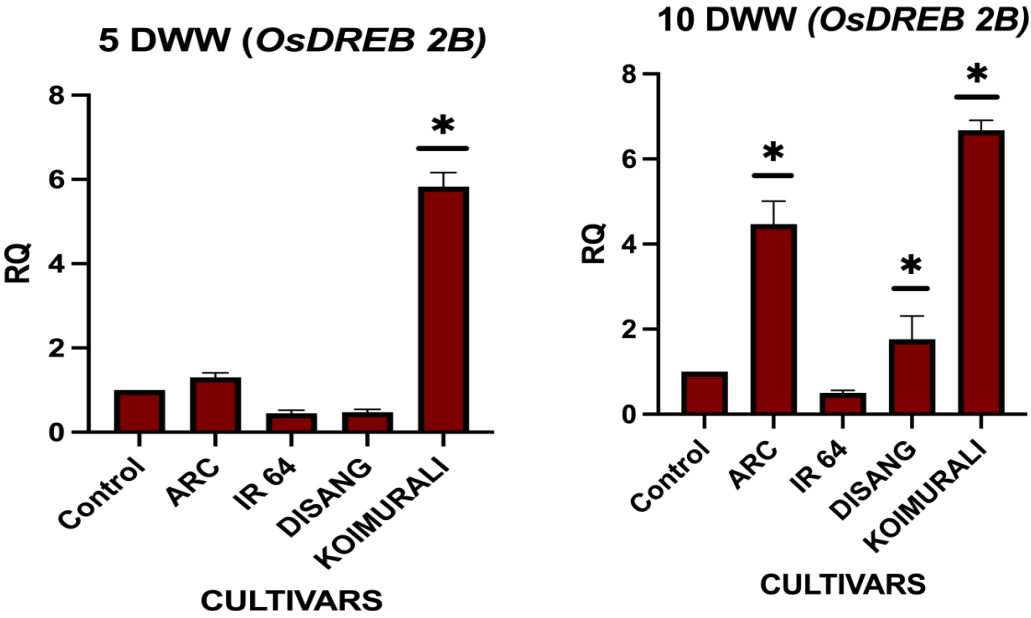


FIGURE 4. 2: DIFFERENCE IN QUANTITATE EXPRESSION PROFILE OF *DREB2A*= AT 5DWW AND 10DWW, P=0.05

A distinct gene expression pattern was observed in response to drought stress across the rice cultivars. At a short duration of drought stress (5 days after withholding water, 5DWW), both drought-tolerant cultivars displayed a notable increase in gene expression when compared to the susceptible varieties, *IR-64* and *Disang*. Under prolonged drought conditions, the drought-tolerant cultivar *ARC-10372* exhibited a marginal decline in gene expression relative to the control, but the *Koimurali* cultivar demonstrated enhanced tolerance by exhibiting a 1.5-fold increase in gene expression at $p < 0.05$. On the other hand, the susceptible varieties *IR-64* and *Disang* indicated a reduction in gene expression by 0.3-fold and 0.35-fold respectively at $p < 0.05$ (Fig 4.3). A different study focusing on rice calcium-dependent protein kinase under osmotic and drought stress, it was found that the transcript levels of *OsNAC9* were considerably higher in the transgenic line (*OsCPK9*) compared to their wild-type counterparts (Wei *et al.*, 2014). Despite conducting drought stress assessments during the reproductive stage of the plants, both the *ARC-10372* and *Koimurali* cultivars displayed significant increases in gene expression at both 5DWW and 10DWW (Fig.4.3). This observation suggests a crucial role for *OsNAC* genes in orchestrating reproductive stress responses. This underlines the diverse responses to drought stress among rice cultivars. The drought-tolerant cultivars, *ARC-10372* and *Koimurali* exhibited early and sustained increases in gene expression, particularly during the reproductive stage, indicative of the prime role played by *OsNAC* genes in enabling adaptive responses to drought-induced stress.

4.2.4 Gene expression analysis for *OsZIP73* under drought stress

The *bZIP* (basic leucine zipper) transcription factor family stands out as one of the largest and prime transcription factor families in plants in inducing responses to abiotic stresses. In rice, the *bZIP* TF group comprises 13 members, with Groups A, C, and S being implicated in abiotic stress signaling. Numerous *bZIP* TFs have been identified as key players in stress responses (Choi *et al.*, 2000; Correa *et al.*, 2008). A distinctive *bZIP* TF named *OsHBP1b* was reported by (Das *et al.*, 2019), which, upon expression, triggers the activation of multiple abiotic stress responses through various molecular pathways. Furthermore, overexpression of *OsZIP71* has been shown by Liu *et al.*, (2014) which conferred increase tolerance to salt, drought, and other osmotic stresses. Another example is *OSBZIP73*, known for conferring tolerance to abiotic stresses, particularly cold stress (Liu *et al.*, 2019). Although *OsZIP73* is expressed mostly

in response to cold, we intended to validate if the expression pattern of *OsZIP73* under drought is similar in the cultivars under study.

In the current study, the expression levels of *bZIP73* under varying drought stress conditions are analyzed. At 5 days after withholding water (5DWW), it was observed that there was significant upregulation of *bZIP73* in the drought-tolerant cultivars, *ARC-10372* and *Koimurali*. As the intensity of drought stress escalated by 10 days after withholding water (10DWW), the gene expression in both susceptible cultivars significantly decreased. In the case of *ARC-10372*, there was decrease in gene expression by 0.59-fold at $p < 0.05$ compared to its expression at 5DWW.

Nevertheless, the gene remained substantially upregulated relative to the susceptible plants. Intriguingly, the drought-tolerant cultivar *Koimurali* displayed significant increase in gene expression by 2.9-fold at 5DWW and at 10 DWW the gene expression increase by 6 folds. The results depicted in Fig. 4.4, underscored the responsiveness of *bZIP73* to drought stress, particularly in the drought-tolerant cultivars. It is worth noting that *bZIP73^{Jap}*, a related TF, has been linked to conferring cold stress tolerance during the reproductive phase. The variant was found to be upregulated under cold treatment, primarily expressing in panicles at the early binucleate and flowering stages (Liu *et al.*, 2018). Furthermore, *bZIP73* TF's role might seem to be influenced the differential responses of these cultivars to drought stresses. This understanding can aid in unraveling the complex genetic mechanisms that govern stress responses and ultimately pave the way for strategies aimed at enhancing drought tolerance in rice varieties.

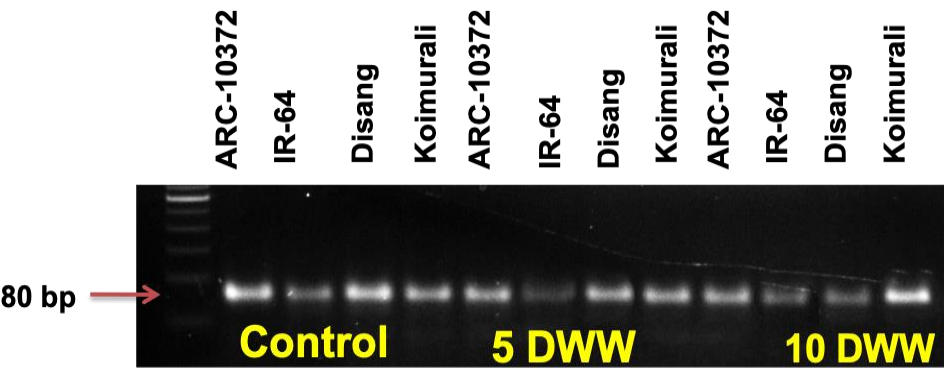


Plate 4. 3: Semi quantitative estimation of expression for OsNAC9 5DWW and 10DWW, *=P,0.05

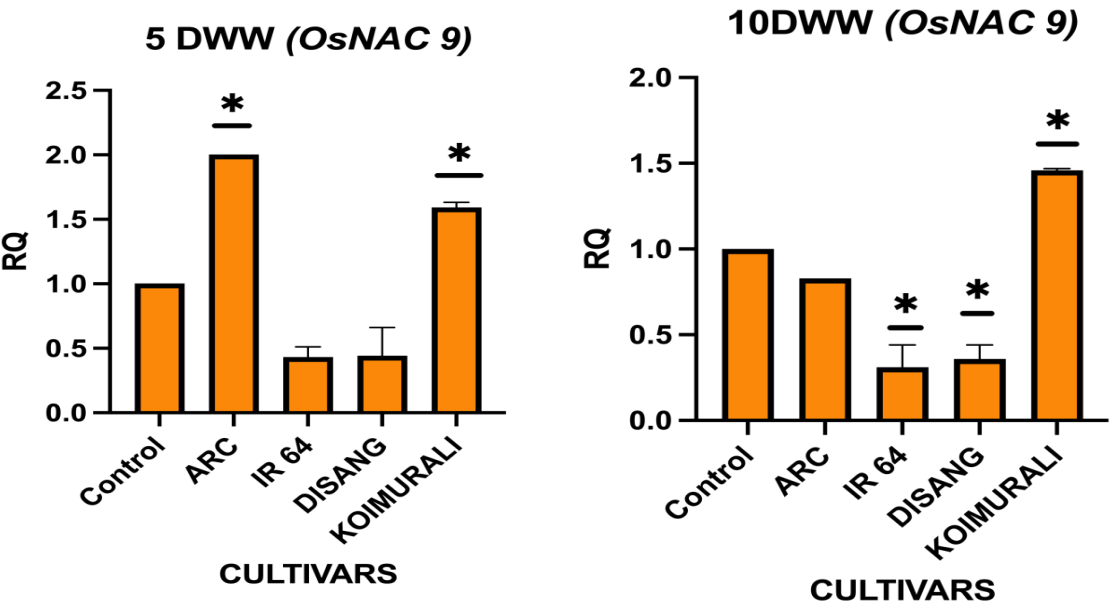


FIGURE 4. 3: DIFFERENCE IN QUANTITATIVE EXPRESSION PROFILES FOR OSNAC9

4.2.5 Gene expression analysis for *OsAPX1* under drought stress

When plants experience drought stress, there is an accumulation of reactive oxygen species (ROS) within cells, leading to the generation of free radicals that initiate cellular damage (Wang *et al.*, 2005; Ubaidillag *et al.*, 2013). These free radicals, specifically superoxide, undergo conversion to hydrogen peroxide and hydroxy radicals, cause lipid peroxidation within cell membranes (Nahar *et al.*, 2016). The survival of plants under such conditions hinges on the production of antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and others (Miller *et al.*, 2010; Farssi *et al.*, 2022). The detoxification of ROS primarily occurs through the ascorbate-glutathione cycle, with APX functioning as a key enzyme. APX acts as an electron donor, reducing hydrogen peroxide to water. Its functional scope extends beyond chloroplasts, encompassing various cell organelles like mitochondria, peroxisomes, and the cytosol (Mittler *et al.*, 2004). Within the *APX* gene family of rice, there exist eight members, categorized into two cytosolic, two peroxisomal, two chloroplast, and two mitochondrial members (Menezes *et al.*, 2004). The expression of genes associated with *APX* is triggered by a range of abiotic stimuli including drought, exhibiting tissue and stage-specific patterns (Agrawal *et al.*, 2003; Teixeira *et al.*, 2006) chaperones, and the activation of pathways that helps to detoxify harmful substances which are crucial for the plant's survival (Mahajan and Tuteja, 2005). In a study by (Kim *et al.*, 2015) it showed that when the *OsAPX1* gene was overexpressed in rice, the plants developed flowers and produced seeds just like the normal, healthy plants. This suggests that the ascorbate peroxidase in rice plays a role in regulating seed development by dealing with harmful substances called ROS.

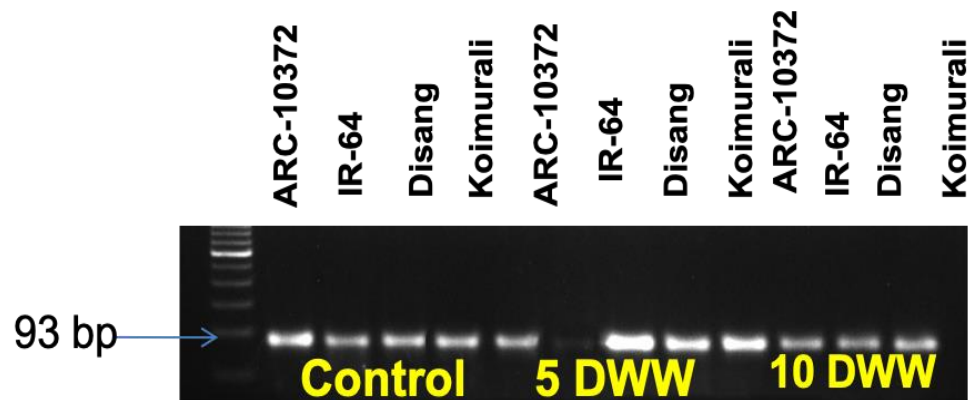


Plate 4. 4 Semi quantitative estimation of expression for *bZIP73*, 5DWW and 10DWW, $P=0.05$

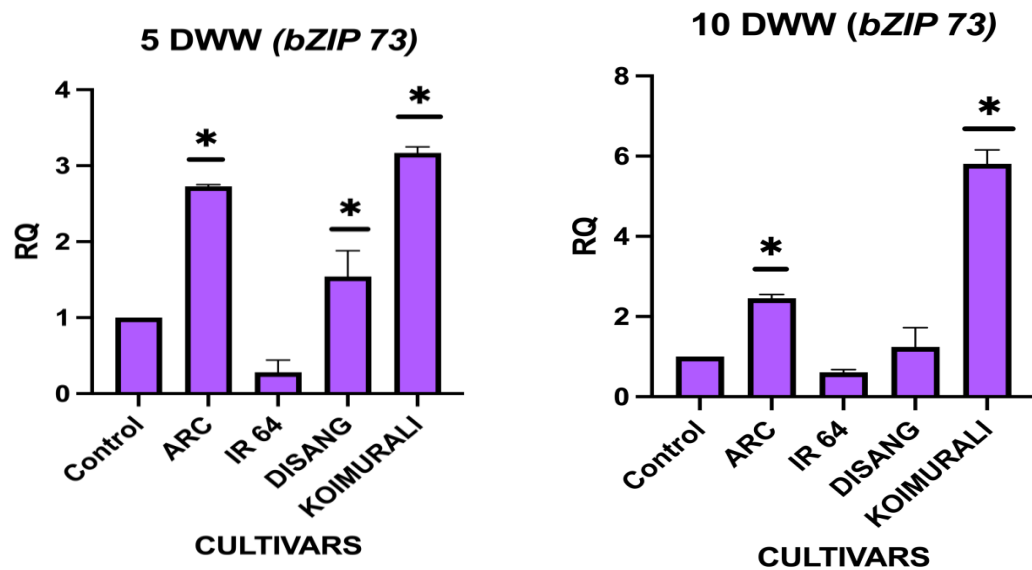


FIGURE 4. 4: DIFFERENCE IN EXPRESSION PROFILES FOR *OSBZIP73*

In the present study, at 5DWW, the gene expression of *OsAPX1* in the drought-tolerant *ARC10372* cultivar exhibited a significant decrease followed by diminished gene accumulation in both the susceptible cultivars, *IR-64* and *Disang*. It may be due to

difference in genetic potential to withstand drought and symptoms of dehydration was not acute at 5DWW in *ARC10372*. However, as the intensity of the drought stress escalated, it indicated a distinct shift in gene expression (Fig 4.5). Both of the tolerant cultivars demonstrated a substantial increase in *OsAPX1* expression: *ARC-10372* exhibited a 1.5-fold increase, while *Koimurali* exhibited a remarkable 2-fold increase, both at $p < 0.05$. On the other hand, the susceptible cultivars showed a contrasting trend, experiencing a decrease in gene accumulation. *Disang* exhibited a downregulation of *OsAPX1* by 0.55-fold, and *IR-64* displayed a more significant downregulation by 1-fold, both at $p < 0.05$. The gene expression of APX is stimulated by drought stress, and it was found that the plants without enough APX were more sensitive to the stress compared to the regular plants (Koussevitzky *et al.*, 2008). The findings offer insights about the *OsAPX1* gene responds to varying levels of drought stress across four different rice cultivars.

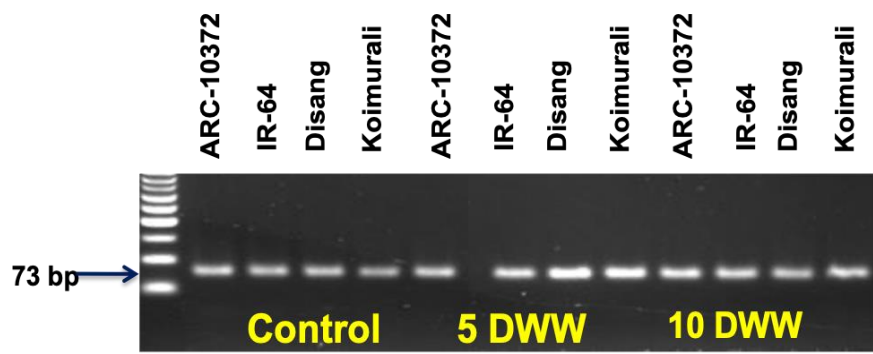


Plate 4. 5: Semi- Quantitative expression profiles for OsAPX1

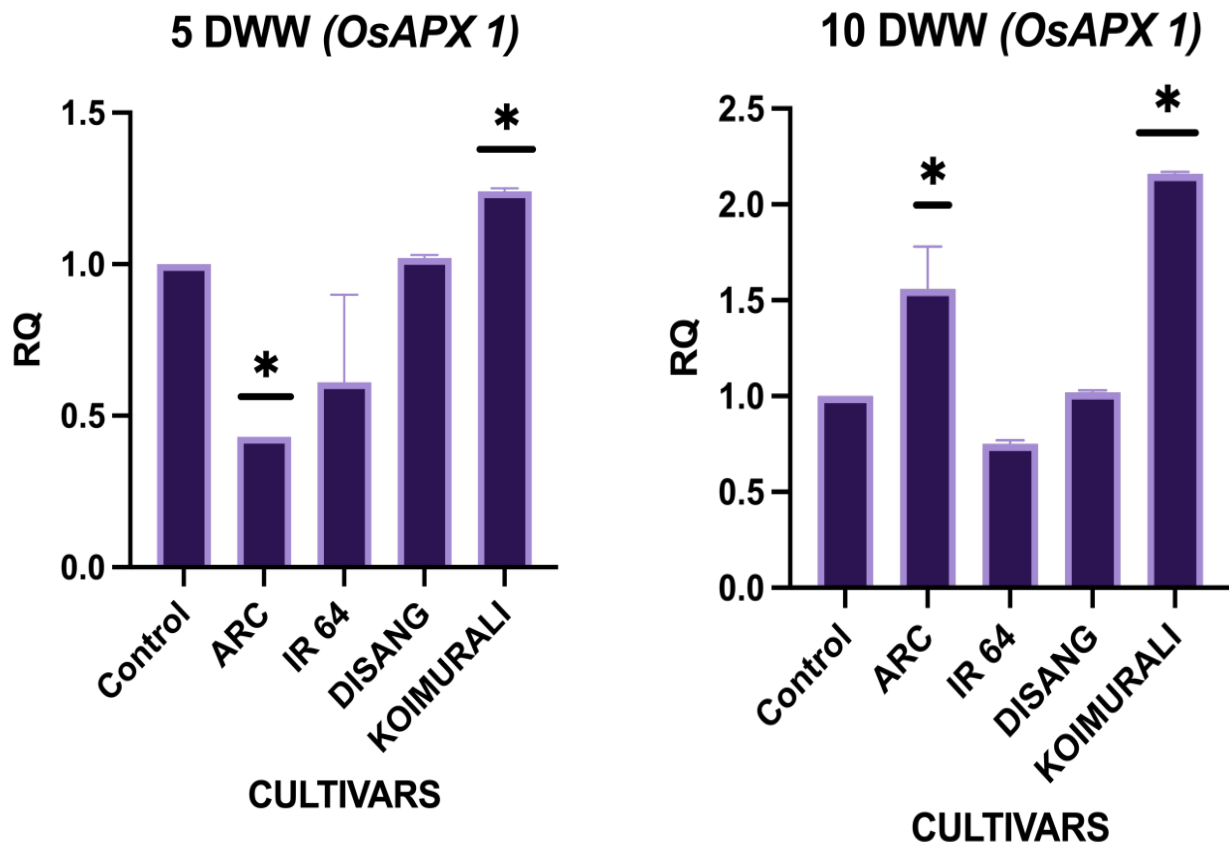


FIGURE 4. 5: DIFFERENCE IN QUANTITATIVE EXPRESSION PROFILES FOR OSAPX1

4.3 Correlation between drought responsive genes and drought adaptive traits

The performances of various drought adaptive traits are presented in Fig 4.6. Pearson's correlation analysis was performed to understand how expression of the genes was associated with drought responsive traits at the reproductive stage. The significant correlations obtained in the study are discussed gene-wise below.

***OsNAC9*:** In spikelet fertility, the number of fertile grains compared to the total number of grains, was examined. Under controlled conditions, a significant positive correlation between the *OsNAC9* gene and spikelet fertility (0.99*). However, under drought stress although positive correlation was displayed yet it was not significant, indicating that higher expression of this gene was linked to better spikelet fertility. .

Number of Grains (NOG): The number of grains produced by each plant was investigated. Under non-stress and drought stress condition, a positive correlation between the *OsAPX1* gene and the number of grains (0.96*, 0.95*, respectively at $p < 0.05$) was observed. This suggests that increased *OsNAC9* expression influences enhancement of overall grain number.

Plant Height (PH): Plant height typically decreases under drought stress. In non-stress conditions, a negative correlation between plant height and genes *OsNAC9*, *DREB2A*, and *DREB2B* (Fig.4.7) was observed. Interestingly, as drought stress intensified, these genes showed a significantly positive correlation with plant height. This suggests that as the drought-responsive gene expression increased, plant height was maintained, potentially due to restricted water movement between the xylem and elongation cells.

Leaf Rolling (LR): Under drought stress conditions, a significant positive correlation between leaf rolling and *OsDREB2A* was observed. This aligns with the general observation that increased drought stress leads to reduced leaf area as a part of drought avoidance mechanism and rolling occurs. In the present study, higher gene expression during extreme drought conditions led to reduced leaf surface area and photosynthetic content. Furthermore, the study also revealed how certain genes influence

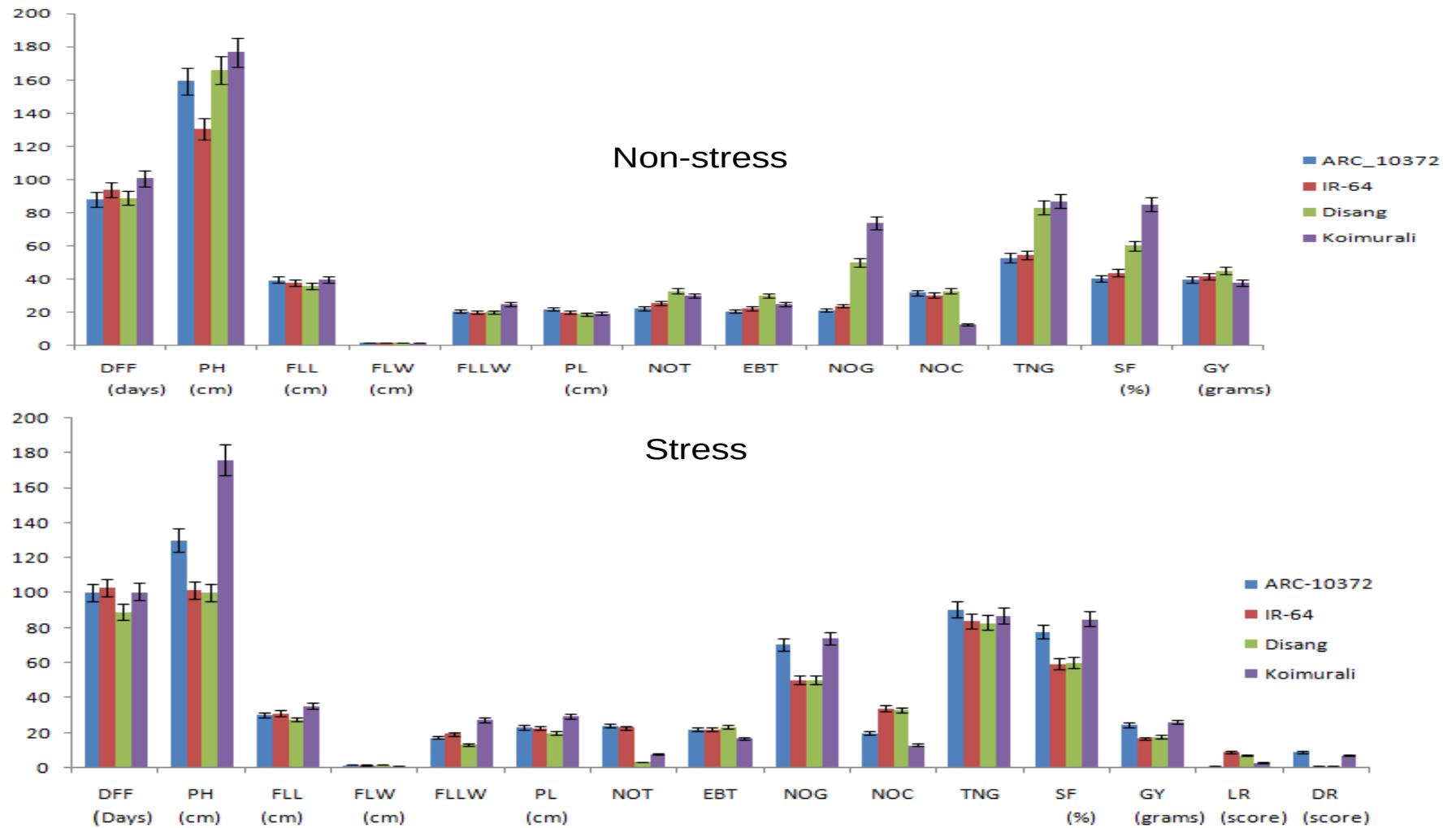
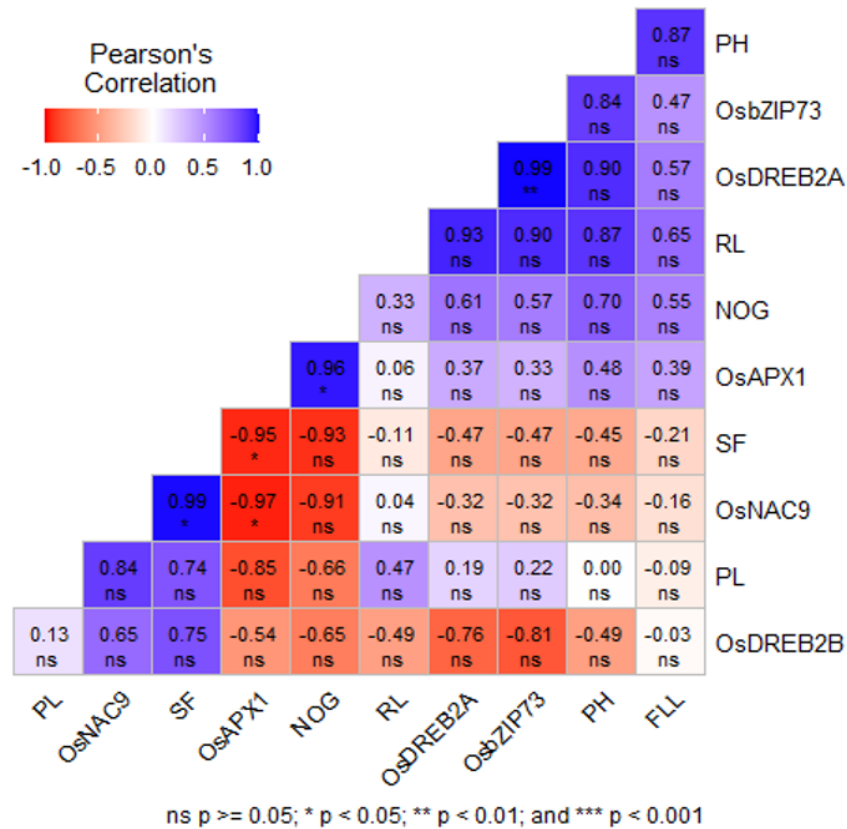
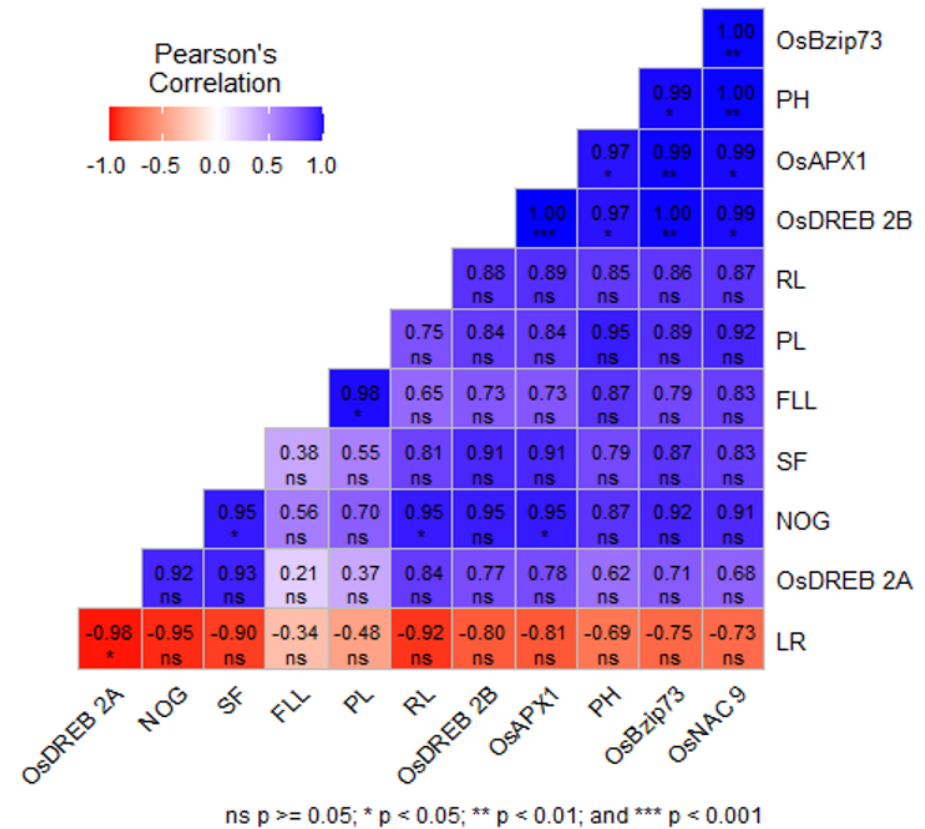


FIGURE 4. 6:PHENOTYPIC PARAMETERS OBSERVED IN (A) NON STRESS AND (B) STRESS CONDITION



Non- stress



Stress

FIGURE 4. 7: CORRELATION BETWEEN DROUGHT RESPONSIVE GENES AND DROUGHT ADAPTIVE TRAITS

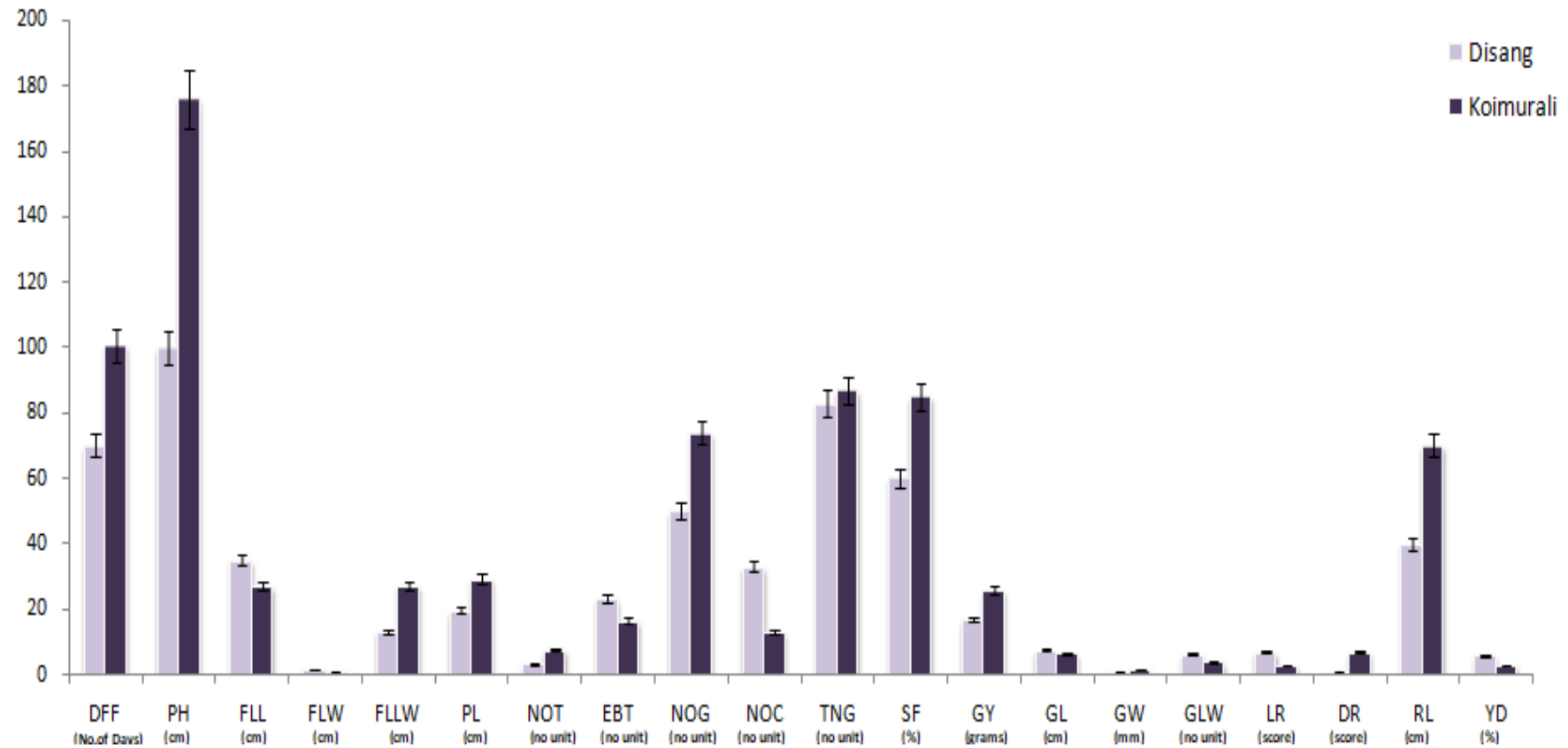


FIGURE 4. 8: PHENOTYPIC PARAMETER OF THE PARENTAL CULTIVAR

various traits in response to drought. These insights help us to understand the complex interactions between genes and traits to adapt and cope with drought stress.

4.4 Selection of the parents

Based on the expression profiling (as shown in fig 4.1,4.2,4.3,4.4 and 4.5) and phenotypic observation (as shown in Fig: 4.6) *Koimurali* was chosen as the donor parent and *Disang* as the recipient Parent. The phenotypic observation of yield attributing traits of both the cultivar are shown in Fig 4.8.

4.5 Development of mapping population

A mapping population was developed by crossing drought tolerant cultivar *Koimurali* with a high-yielding but drought-susceptible cultivar *Disang*. Six F₁ hybrids were obtained from crossing "*Disang*" and "*Koimurali*" as discussed in section 3.7 of chapter III. The single F₁ with the highest seed count was selected to advance further generations. These F₂ plants were cultivated in plastic pots after germination. A total of 315 F₂ plants from a single F₁ seed, which was planted in irrigated field conditions at the ICR Field, Assam Agricultural University. The F₂ plants were individually harvested and seeds from each plant were germinated and transplanted, leading to the F_{2:3} population (as shown in Plate 3.6). Subsequently, individual harvests were carried out for each F_{2:3} population, comprising 315 F₃ lines. These F₃ lines were collected in paper bags (Plate 3.7). F_{3:4} populations were advanced consisting of 247 F₄ plants, each derived from a single F₃ seed per plant (Plate 3.8). This breeding approach was continued through F_{5:6} and F_{6:7} generations with 247 lines (RILs) using single seed descend (SSD) method. The number of lines in subsequent generations were reduced due to various biotic and abiotic factors, which is very common (Sudheesh *et al.*, 2015). RILs as a mapping population to construct linkage map and detect QTL has been used extensively as it explores recombination among the lines to the best possible way and the chromosome segments of all the lines from both the parents are nearly homozygous (Shanmugavadivel *et al.*, 2013). During the F_{5:6} stage, the breeding lines were subjected to distinct hydrological conditions such as intermittent drought stress within a rainout shelter (Plates 4.8) and well-irrigated field conditions in ICR farm AAU (Plate 4.9). The assessment of various yield-related traits was performed; data as recorded in Appendix 1. The subsequent F_{6:7} lines were subjected to two droughts in a pot experiment (Plate 4.10). Here, each line was represented by three replicates : three pots were under continuous watering as a control, while the other three were imposed drought stress during the reproductive phase. Data

were recorded for 16 distinct yield-related traits for the RILs, outlined in Appendix I and II. The use of Recombinant Inbred Line (RILs) populations to map various quantitative traits offer advantages over other mapping populations due to their repeatability and the ease of dissecting quantitative trait loci through powerful statistical analysis (Sudheesh *et al.*, 2015).

4.6 Parental polymorphism survey

A total of 600 SSR markers(Appendix IV) were screened across 12 rice chromosomes for *Koimurali* and *Disang*. It was observed that only 98 markers (16.33%) exhibited polymorphism. (Plate 4.7) The highest polymorphic markers was observed on chromosome number 8 with 17 (out of 66). On the contrary, chromosome 10 showed the lowest level of polymorphism, with only two markers showing polymorphism although a total of 28 markers has been used on chromosome 10. The list of these polymorphic markers is shown in Appendix III while the distribution of markers across individual chromosomes are graphically depicted in Fig 4.9. This relatively lower degree of polymorphism between the parent varieties could be due the cross between two *indica* type cultivars, as reported by (Javed *et al.*,2013). Furthermore, the reduction in parental polymorphism might be due to the limited genetic diversity at the specific recombination points between the chosen parental lines.

4.7 Confirmation of F₁

To confirm F₁ between *Koimurali* and *Disang*, polymorphic SSR markers between parents were used. The procedure for confirming hybridity was carried out following the methodology outlined in section 3.7. Among the six F₁ plants examined, five exhibited distinct hybrid characteristics by displaying marker alleles from both the parents at RM 167. On the other hand, one plant showed only recipient allele, indicating that crossing was unsuccessful and hence selfed seeds were obtained (Plate 4.6.).

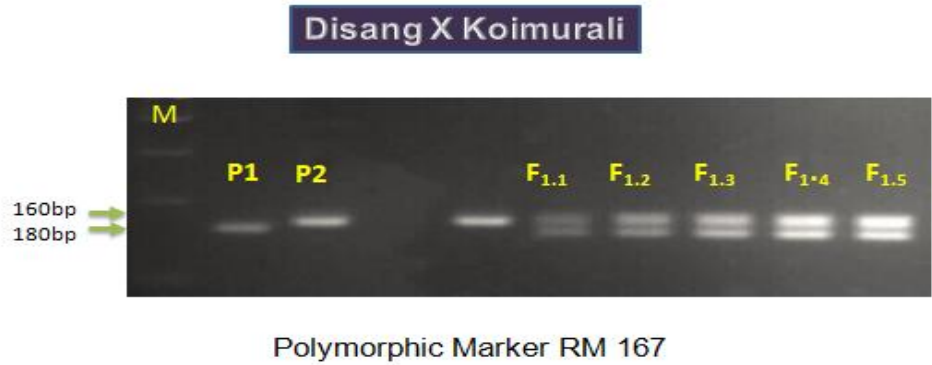


Plate 4. 6: Confirmation of hybridity in F₁s using RM 167

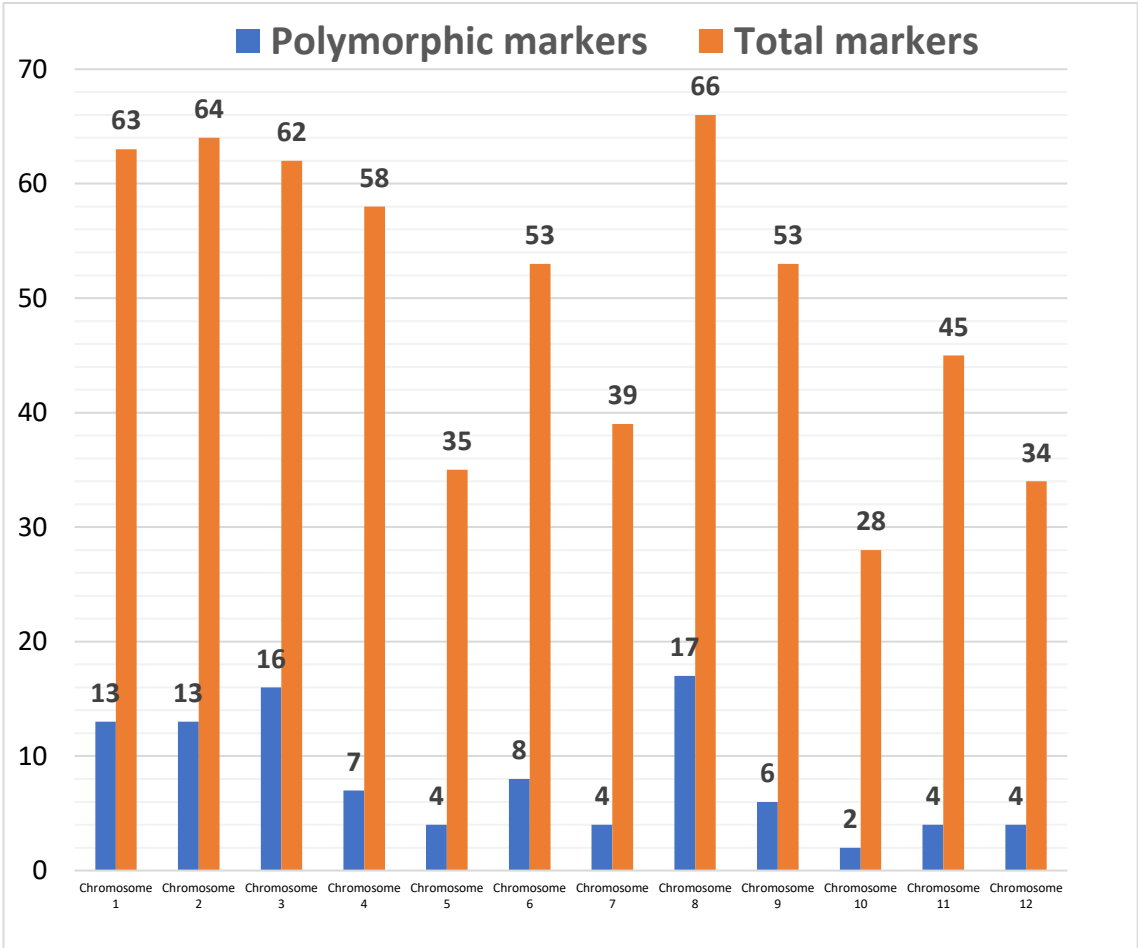


Figure 4. 6: Chromosome wise marker distribution of polymorphic markers

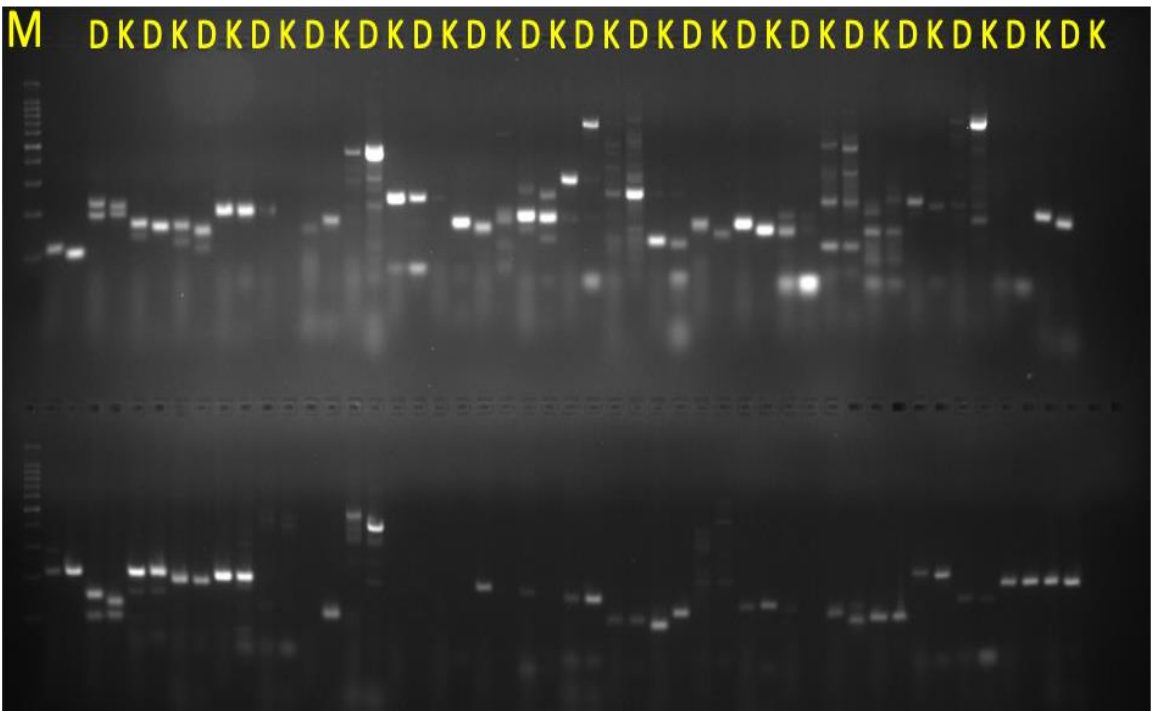


Plate 4. 7: Parental polymorphism check using SSR markers

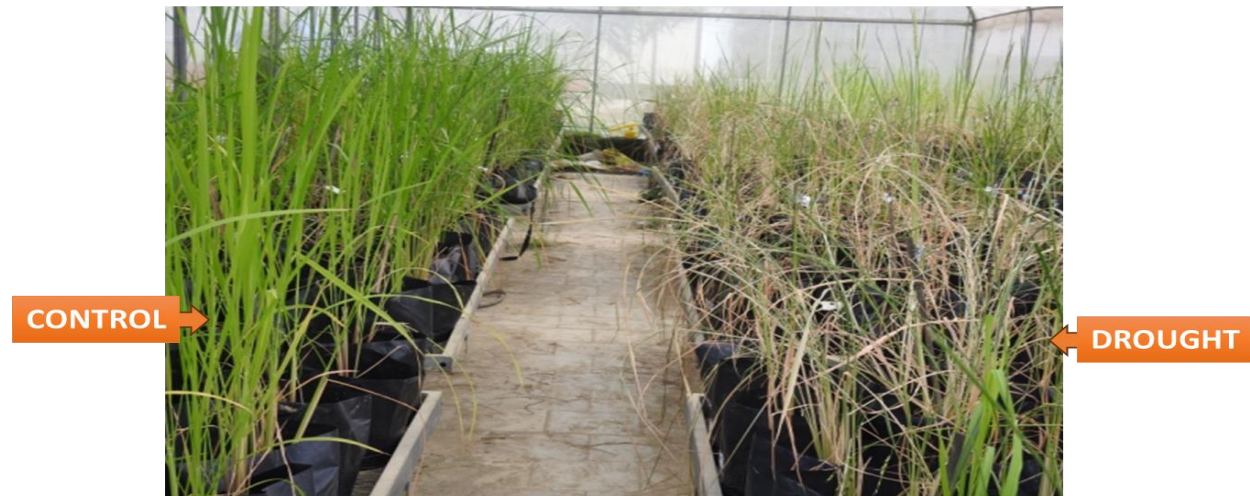


**Water
Withhold
55 DAS
65 DAS
75 DAS**

Plate 4. 8: Phenotyping of F₅:F₆ mapping population at rainout shelter (*Sali,2021*)



(a)



(b)

Plate 4. 9: Phenotyping of the mapping population at ICR Field F5:F6 (Sali,2021);

Plate 4. 10: Phenotyping of the mapping population under drought stress at the reproductive stage F6:F7 (Ahu,2022)

4.8 Construction of linkage map using SSR markers

4.8.1. Genotyping of the mapping population

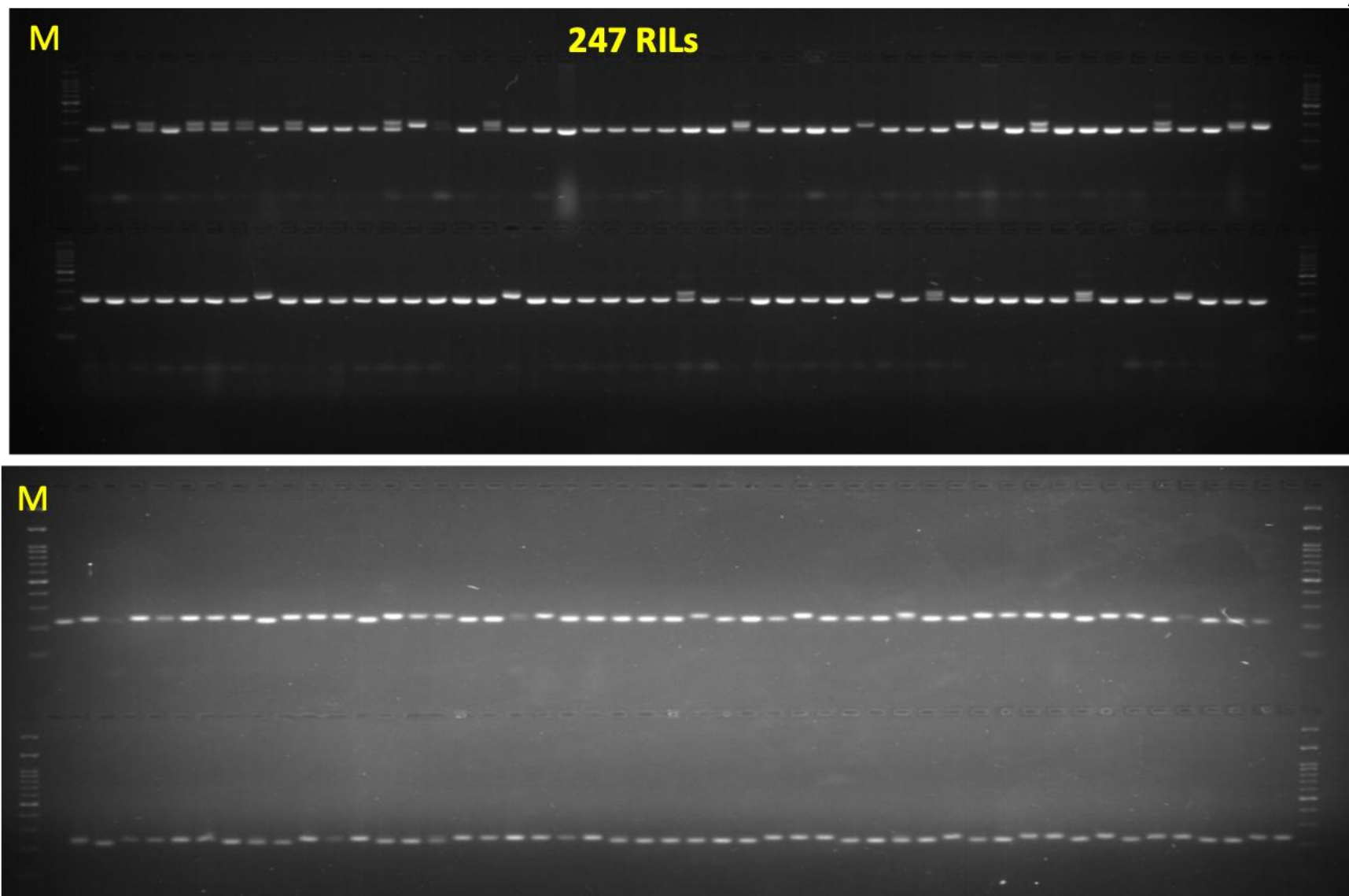
A total of 247 RILs were genotyped using 98 SSR markers across 12 chromosomes (Plate 4.11 a, b). The marker data for RILs were analyzed to fit into the expected Mendelian ratio of 1:1. To assess the same, Chi square (χ^2) test was performed. The table 4.2 illustrates the segregation patterns of SSR markers. Out of the 98 markers 25 markers (25.51%) followed the predicted Mendelian ratio while the remaining 73 markers (74.48%) showed significant deviations from it at $p < 0.01$ and $p < 0.05$, respectively. It was also revealed that 18 markers (18.36%) exhibited a pattern biased to *Disang* while 80 markers (81.63%) were biased to *Koimurali*. The Segregation pattern of F₆ lines (RILs) are shown in Figure 4.10(a, b,c,d). Out of the markers, 89% (sixty five) displayed the *Koimurali* allele and 10.9% (eight) showed the *Disang* allele. The chromosome 3 had the highest number of distorted markers followed by chromosome 8. Segregation distortion in RILs is a common phenomenon and is reported throughout. (Longkho, 2023) reported that in a set of 82 polymorphic markers, 30 markers (36.58%) conformed to the anticipated Mendelian ratio while a notable deviation was observed in 52 markers (about 63.41%).

Segregation distortion is resulted from a combination of physiological and genetic factors. It can be influenced by statistical biases, errors during genotyping and scoring, as highlighted by (Plomion *et al.*, 1995). However, genotyping and scoring has been performed repeatedly to avoid misinterpretation of results in the present study. Other factors include competition among male gametes for fertilization, the occurrence of abortion in male or female gametes or zygotes, and the involvement of incompatibility genes (Dia *et al.*, 2017). This deviation in segregation can play a significant role in either conscious or unconscious selection against one of the parental alleles during the establishment of mapping populations (Lorieux *et al.*, 1995). The presence of loci with notable segregation distortion can lead to challenges in accurately constructing the linkage maps due to errors in estimating map distances and determining marker order. This, in turn, may influence the mapping of QTLs (Lorieux *et al.*, 1995). However, as stated by (Hackett *et al.*, 2002), distorted segregation does not necessarily disrupt the overall genetic map order.

Traditionally, it is recommended to exclude Segregation Distortion (SD) markers, but the removal of these skewed markers can result in reduced genome coverage for linked markers. (Han *et al.*, 2018) further indicated that skewed markers have minimal

impact on assessing recombination rates within co-dominant markers. Furthermore, continuous selfing across generations was a significant genetic cause for segregation distortion in RIL populations and more pronounced in RIL populations of the indica subspecies compared to the japonica (Wu *et al.*, 2010).

Likewise, (Shanmugavadivel *et al.*, 2013) obtained segregation distortion of 54 percent in RILs. However, rather than discarding these markers, those were utilized to construct linkage map through the application of appropriate statistical tools. In the present study, the linkage map was constructed using all the 98 polymorphic SSR markers in a 247 F₆ RILs. It also signifies that even though the genetic data do not exactly match with the expectations, however, a lot of other information which may be lost if the distorted marker can be discarded.



(a)

Plate 4. 11: (a) Genotyping of the RIL population of RM 523 of chromosome 2

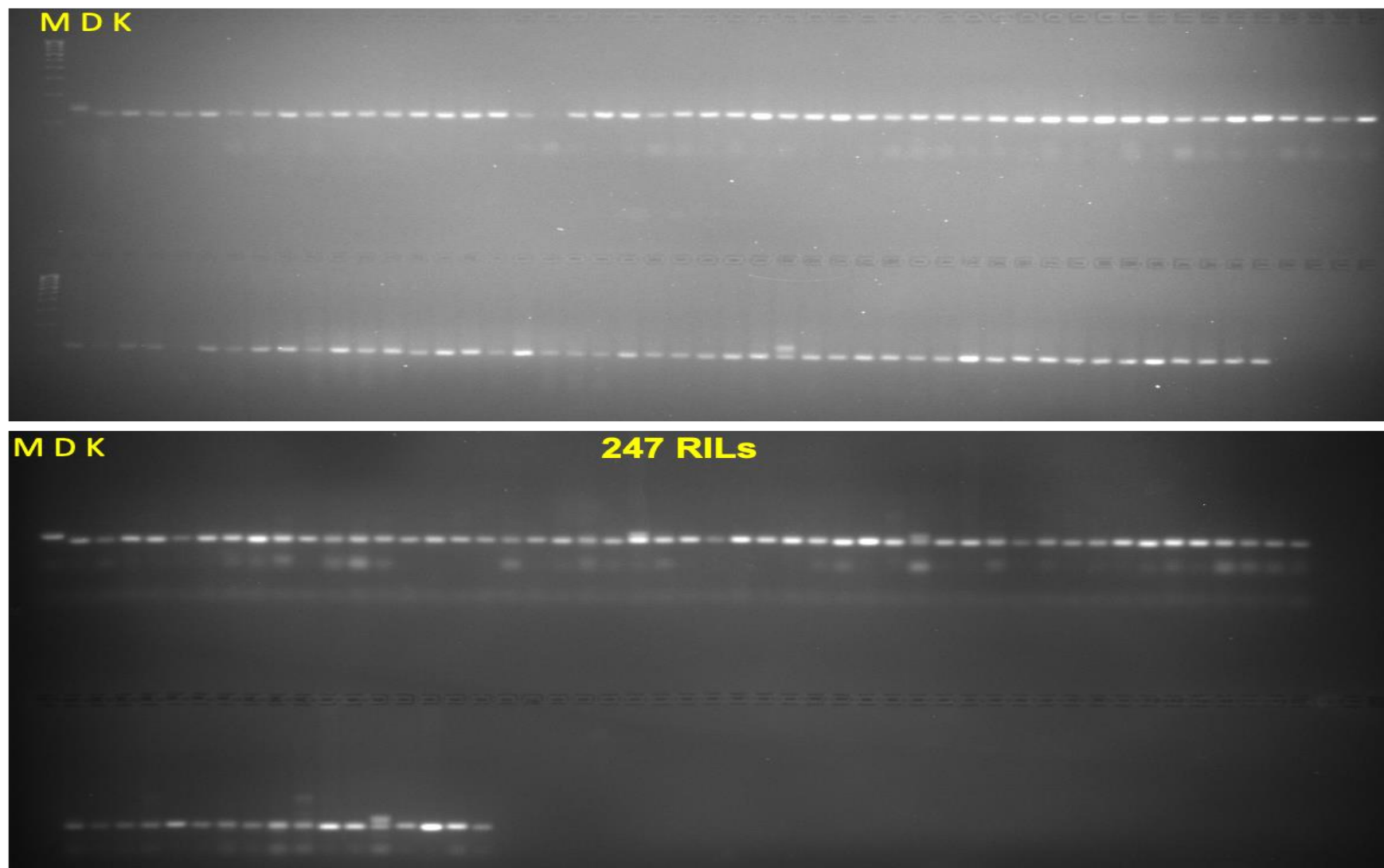
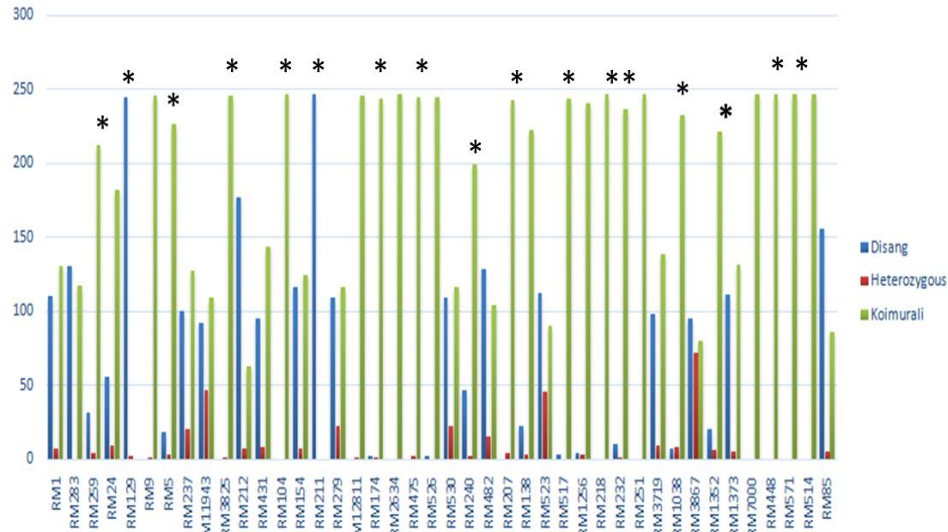
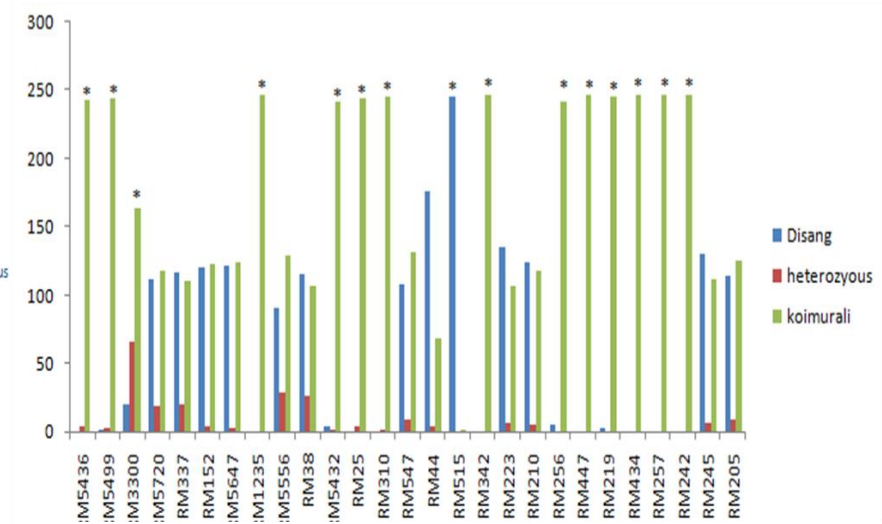


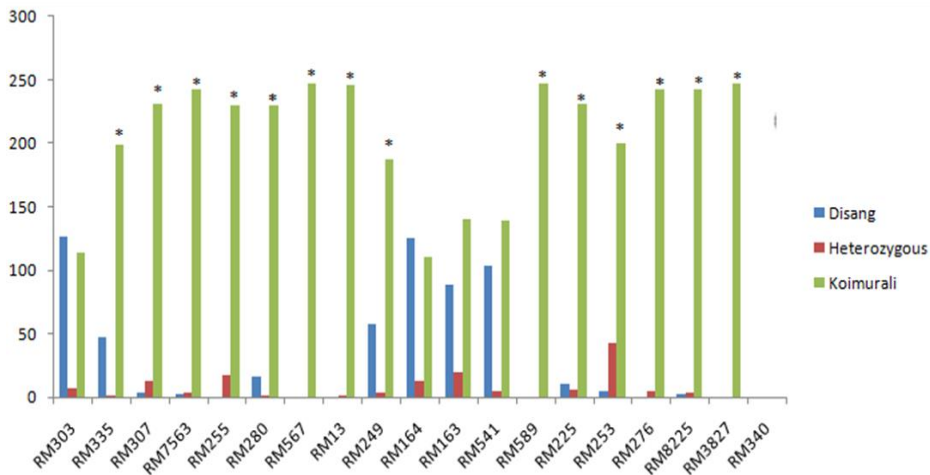
Plate 4. 11 :(b) Genotyping of the RIL population RM 310 of Chromosome 8



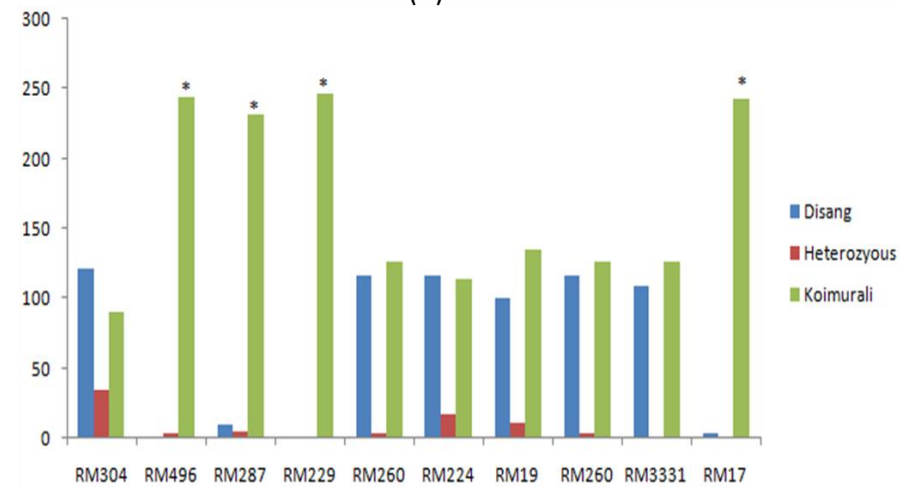
(a)



(b)



(c)



(d)

Figure 4. 10: Segregation pattern of 98 SSR markers in 247 F_6 lines of *Disang* x *Koimurali*; (a)Chromosome 1,2,3; (b)Chromosome 4,5,6; (c) Chromosome 7, 8, 9; (d)Chromosome 10,11, 12.

Table 4. 2: Chi square test for segregating of SSR Markers

S/n	Locus	Chromosome no.	Disang(a)	heterozygote(h)	Koimurali(b)	BASEPAIR	X2	Df	Classification	Signif.	Direction of Skewness
1	RM1	1	110	7	130	4568457	1.67	1	[a:b]	-	
2	RM283	1	130	0	117	22993785	0.68	1	[a:b]	-	
3	RM259	1	31	4	212	24298288	134.82	1	[a:b]	**	Koimurali
4	RM24	1	56	9	182	19738458	66.71	1	[a:b]	**	Koimurali
5	RM129	1	245	2	0	19738458	245	1	[a:b]	**	Disang
6	RM9	1	0	1	246	34138065	246	1	[a:b]	**	Koimurali
7	RM5	1	18	3	226	37517249	177.31	1	[a:b]	**	Koimurali
8	RM237	1	100	20	127	27865492	3.21	1	[a:b]	*	Koimurali
9	RM11943	1	92	46	109	41175917	1.44	1	[a:b]	-	
10	RM3825	1	0	1	246	4812644	246	1	[a:b]	**	Koimurali
11	RM212	1	177	7	63	38775892	54.15	1	[a:b]	**	Disang
12	RM431	1	95	8	144	758037	10.05	1	[a:b]	**	Koimurali
13	RM104	1	0	0	247	39861138	247	1	[a:b]	**	Koimurali
14	RM154	2	116	7	124	28326124	0.27	1	[a:b]	-	

15	RM211	2	247	0	0	21727602	247	1	[a:b]	**	Disang
16	RM279	2	109	22	116	21631465	0.22	1	[a:b]	-	
17	RM1281 1	2	0	1	246	37466179	246	1	[a:b]	**	Koimurali
18	RM174	2	2	1	244	37090281	238.07	1	[a:b]	**	Koimurali
19	RM263 4	2	0	0	247	32314410	247	1	[a:b]	**	Koimurali
20	RM475	2	0	2	245	7022559	245	1	[a:b]	**	Koimurali
21	RM526	2	2	0	245	1028624	239.06	1	[a:b]	**	Koimurali
22	RM530	2	109	22	116	37187760	0.22	1	[a:b]	-	
23	RM240	2	46	2	199	1966335	95.55	1	[a:b]	**	Koimurali
24	RM482	2	128	15	104	33241470	2.48	1	[a:b]	-	
25	RM207	2	0	4	243	2836363	243	1	[a:b]	**	Koimurali
26	RM138	2	22	3	222	7890177	163.93	1	[a:b]	**	Koimurali
27	RM523	3	112	45	90	30667358	2.4	1	[a:b]	-	
28	RM517	3	3	0	244	1332286	235.15	1	[a:b]	**	Koimurali
29	RM1256	3	4	3	240	10289586	228.26	1	[a:b]	**	Koimurali
30	RM218	3	0	0	247	10083660	247	1	[a:b]	**	Koimurali

31	RM232	3	10	1	236	36773519	207.63	1	[a:b]	**	Koimurali
32	RM251	3	0	0	247	35433741	247	1	[a:b]	**	Koimurali
33	RM3719	3	98	9	138	10095197	6.78	1	[a:b]	**	Koimurali
34	RM1038	3	7	8	232	38354880	211.82	1	[a:b]	**	Koimurali
35	RM3867	3	95	72	80		1.29	1	[a:b]	-	
36	RM1352	3	20	6	221	36279079	167.64	1	[a:b]	**	Koimurali
37	RM1373	3	111	5	131	36915762	1.65	1	[a:b]	-	
38	RM7000	3	0	0	247	34856087	247	1	[a:b]	**	Koimurali
39	RM448	3	0	0	247	8702745	247	1	[a:b]	**	Koimurali
40	RM571	3	0	0	247	35731912	247	1	[a:b]	**	Koimurali
41	RM514	3	0	0	247	34495121	247	1	[a:b]	**	Koimurali
42	RM85	3	156	5	86	6298762	20.25	1	[a:b]	**	Disang
43	RM303	4	126	7	114	16865934	0.6	1	[a:b]	-	
44	RM335	4	47	1	199	22942121	93.92	1	[a:b]	**	Koimurali
45	RM307	4	3	13	231	34983463	222.15	1	[a:b]	**	Koimurali
46	RM7563	4	2	3	242	763604	236.07	1	[a:b]	**	Koimurali
47	RM255	4	0	17	230	35462222	230	1	[a:b]	**	Koimurali

48	RM280	4	16	1	230	31289565	186.16	1	[a:b]	**	Koimurali
49	RM567	4	0	0	247	28908370	247	1	[a:b]	**	Koimurali
50	RM13	5	0	1	246	11041719	246	1	[a:b]	**	Koimurali
51	RM249	5	57	3	187	20464758	69.26	1	[a:b]	**	Koimurali
52	RM164	5	125	12	110	2076455	0.96	1	[a:b]	-	
53	RM163	5	88	19	140	20457690	11.86	1	[a:b]	**	Koimurali
54	RM541	6	103	5	139	9239206	5.36	1	[a:b]	**	Koimurali
55	RM589	6	0	0	247	3315378	247	1	[a:b]	**	Koimurali
56	RM225	6	10	6	231	6075303	202.66	1	[a:b]	**	Koimurali
57	RM253	6	5	42	200	5206122	185.49	1	[a:b]	**	Koimurali
58	RM276	6	0	5	242	20304998	242	1	[a:b]	**	Koimurali
59	RM8225	6	2	3	242	1259100	236.07	1	[a:b]	**	Koimurali
60	RM3827	6	0	0	247	30043709	247	1	[a:b]	**	Koimurali
61	RM340	6	0	0	247	23378465	247	1	[a:b]	**	Koimurali
62	RM5436	7	0	4	243	19571420	243	1	[a:b]	**	Koimurali
63	RM5499	7	1	2	244	10134228	241.02	1	[a:b]	**	Koimurali
64	RM3300	7	19	65	163	9171773	113.93	1	[a:b]	**	Koimurali

65	RM5720	7	111	18	118	29184213	0.21	1	[a:b]	-	
66	RM337	8	117	20	110	23883089	0.22	1	[a:b]	-	
67	RM152	8	120	4	123	705151	0.04	1	[a:b]	-	
68	RM5647	8	121	2	124	4371944	0.04	1	[a:b]	-	
69	RM1235	8	0	0	247	4371988	247	1	[a:b]	**	Koimurali
70	RM5556	8	90	28	129	4531189	6.95	1	[a:b]	**	Koimurali
71	RM38	8	115	26	106	2913740	0.37	1	[a:b]	-	Disang
72	RM5432	8	4	1	242	25738966	230.26	1	[a:b]	**	Koimurali
73	RM25	8	0	3	244	22021511	244	1	[a:b]	**	Koimurali
74	RM310	8	0	1	246	21639843	246	1	[a:b]	**	Koimurali
75	RM547	8	108	8	131	4964359	2.21	1	[a:b]	-	
76	RM44	8	176	3	68	27941903	47.8	1	[a:b]	**	Disang
77	RM515	8	246	0	1	11686201	243.02	1	[a:b]	**	Disang
78	RM342	8	0	0	247	184425	247	1	[a:b]	**	Koimurali
79	RM223	8	135	6	106	5461947	3.49	1	[a:b]	*	Disang
80	RM210	8	124	5	118	2038831	0.15	1	[a:b]	-	
81	RM256	8	5	0	242	1232681	227.4	1	[a:b]	**	Koimurali

82	RM447	8	0	0	247	21350354	247	1	[a:b]	**	Koimurali
83	RM219	9	2	0	245	11046418	239.06	1	[a:b]	**	Koimurali
84	RM434	9	0	0	247	24479344	247	1	[a:b]	**	Koimurali
85	RM257	9	0	0	247	19184712	247	1	[a:b]	**	Koimurali
86	RM242	9	0	0	247	20324402	247	1	[a:b]	**	Koimurali
87	RM245	9	130	6	111	9010430	1.5	1	[a:b]	-	
88	RM205	9	114	8	125	23964362	0.51	1	[a:b]	-	
89	RM304	10	122	35	90	24703987	4.83	1	[a:b]	**	Disang
90	RM496	10	0	3	244	20860234	244	1	[a:b]	**	Koimurali
91	RM287	11	10	5	232	19188007	203.65	1	[a:b]	**	Koimurali
92	RM229	11	0	0	247	25045284	247	1	[a:b]	**	Koimurali
93	RM260	11	117	3	127	21261527	0.41	1	[a:b]	-	
94	RM224	11	116	17	114	30450718	0.02	1	[a:b]	-	
95	RM19	12	100	11	135	25980202	5.21	1	[a:b]	**	Koimurali
96	RM260	12	117	3	127	8229359	0.41	1	[a:b]	-	
97	RM3331	12	109	1	127	22341707	1.37	1	[a:b]	-	
98	RM17	12	4	0	243	3937986	231.26	1	[a:b]	**	Koimurali

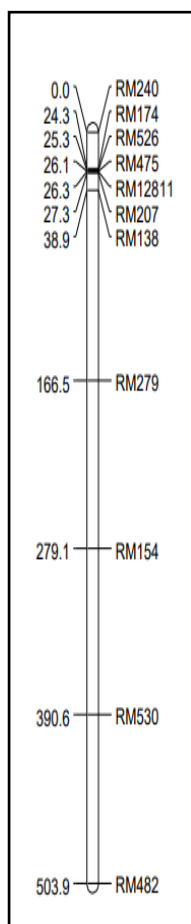
4.8.2. SSR Marker based linkage map

The genetic linkage map was constructed using 98 polymorphic SSR markers distributed across 12 chromosomes using JoinMap Version 4.1. The main aim was to create a linkage map to check the pattern of marker distribution throughout all chromosomes in rice. The fundamental role of a high-resolution genetic linkage map is to detect QTL at specific position on certain chromosome. The basic prerequisite for constructing a high-resolution linkage map is the availability of polymorphic DNA markers (Li *et al.*, 2015). In the present study, 600 SSR markers were used to detect polymorphism between the parents. Among the tested markers, 98 (16.33%) were found to be polymorphic between the parents.

A segregating population comprises a combination of original parental genotypes and newly formed recombinant genotypes. The recombinant genotypes can be employed to compute recombination fractions, which in turn aids in estimating the genetic separation between markers as units of genetic distance. These procedures collectively allow the construction of genetic maps, illustrating the arrangement of markers along chromosomes and facilitating the understanding of genetic relationships and inheritance patterns. In the present study, the linkage map was constructed using 98 polymorphic SSR markers in a 247 F₆ RIL population derived from the parental cultivars *Disang* and *Koimurali*.

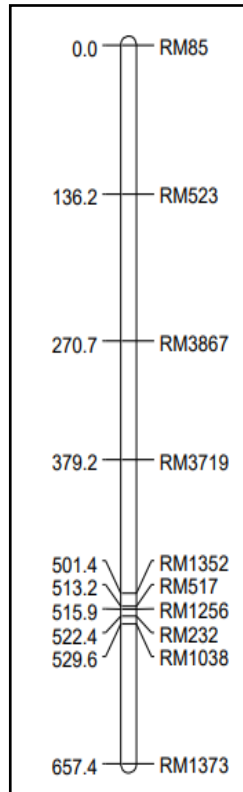
Table 4. 3: Linkage Map construction**(a) Chromosome 1**

	S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
	1	1	RM1	4	RM24	0.435	0.37
	2	1	RM1	7	RM5	0.4375	0.07
	3	9	RM11943	2	RM283	0.4537	0.19
	4	9	RM11943	10	RM3825	0.4637	0.07
	5	5	RM129	11	RM212	0.2733	0.03
	6	5	RM129	2	RM283	0.4738	0
	7	11	RM212	5	RM129	0.2733	0.03
	8	11	RM212	8	RM237	0.4597	0.41
	9	8	RM237	12	RM431	0.4411	0.11
	10	8	RM237	10	RM3825	0.4447	0.52
	11	4	RM24	10	RM3825	0.247	0
	12	4	RM24	6	RM9	0.247	0
	13	3	RM259	6	RM9	0.1316	0.36
	14	3	RM259	10	RM3825	0.1356	0
	15	2	RM283	9	RM11943	0.4537	0.19
	16	2	RM283	11	RM212	0.4637	0.01
	17	10	RM3825	6	RM9	0.0041	0
	18	10	RM3825	7	RM5	0.0769	0.56
	19	12	RM431	7	RM5	0.3948	0.06
	20	12	RM431	6	RM9	0.3989	0.07
	21	7	RM5	10	RM3825	0.0769	0.56
	22	7	RM5	6	RM9	0.0809	0
	23	6	RM9	10	RM3825	0.0041	0
	24	6	RM9	7	RM5	0.0809	0

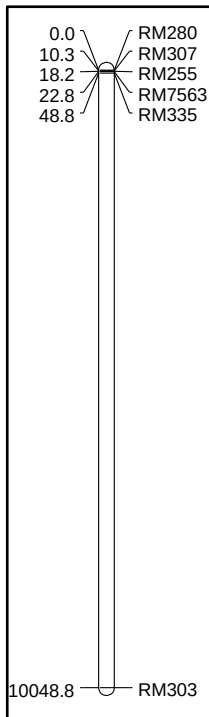
(b) Chromosome 2

S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	4	RM12811	7	RM475	0.002	2.22
2	4	RM12811	8	RM526	0.0061	2.22
3	13	RM138	4	RM12811	0.0932	1.35
4	13	RM138	7	RM475	0.0952	0.82
5	1	RM154	9	RM530	0.4463	1.02
6	1	RM154	3	RM279	0.4472	0.75
7	5	RM174	7	RM475	0.0101	0.99
8	5	RM174	8	RM526	0.0101	0.99
9	12	RM207	4	RM12811	0.0101	0.01
10	12	RM207	7	RM475	0.0121	0.01
11	10	RM240	8	RM526	0.1903	0.01
12	10	RM240	5	RM174	0.1923	0.01
13	3	RM279	1	RM154	0.4472	0.75
14	3	RM279	13	RM138	0.4614	0.31
15	7	RM475	4	RM12811	0.002	2.22
16	7	RM475	8	RM526	0.0081	1.62

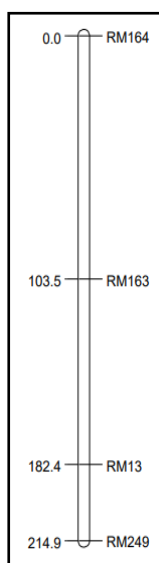
(c)Chromosome 3



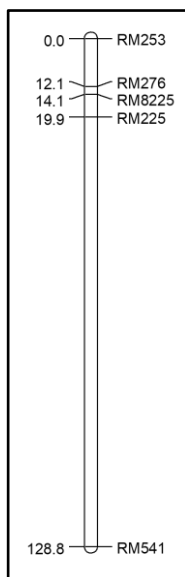
S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	8	RM1038	2	RM517	0.0567	0
2	8	RM1038	5	RM232	0.0668	0.96
3	3	RM1256	2	RM517	0.0263	0.47
4	3	RM1256	5	RM232	0.0608	0.04
5	10	RM1352	2	RM517	0.1052	0
6	10	RM1352	3	RM1256	0.1114	0.15
7	11	RM1373	8	RM1038	0.4614	0.07
8	11	RM1373	1	RM523	0.4675	0.76
9	5	RM232	2	RM517	0.0546	0
10	5	RM232	3	RM1256	0.0608	0.04
11	7	RM3719	3	RM1256	0.4286	0
12	7	RM3719	2	RM517	0.4304	0.25
13	9	RM3867	7	RM3719	0.4427	1.38
14	9	RM3867	1	RM523	0.4662	0.01
15	2	RM517	3	RM1256	0.0263	0.47
16	2	RM517	5	RM232	0.0546	0
17	1	RM523	9	RM3867	0.4662	0.01
18	1	RM523	11	RM1373	0.4675	0.76
19	16	RM85	1	RM523	0.4675	0.05
20	16	RM85	9	RM3867	0.499	0

(d) Chromosome 4

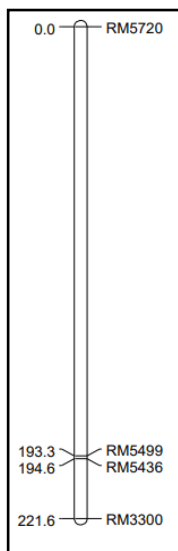
S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	5	RM255	4	RM7563	0.0446	0.08
2	5	RM255	3	RM307	0.0729	0.11
3	6	RM280	4	RM7563	0.0809	0
4	6	RM280	3	RM307	0.0931	0.02
5	1	RM303	2	RM335	0.499	0
6	1	RM303	5	RM255	0.499	0
7	3	RM307	4	RM7563	0.0526	0
8	3	RM307	5	RM255	0.0729	0.11
9	2	RM335	4	RM7563	0.2024	0.98
10	2	RM335	5	RM255	0.2228	0.15
11	4	RM7563	5	RM255	0.0446	0.08
12	4	RM7563	3	RM307	0.0526	0

(e)Chromosome 5

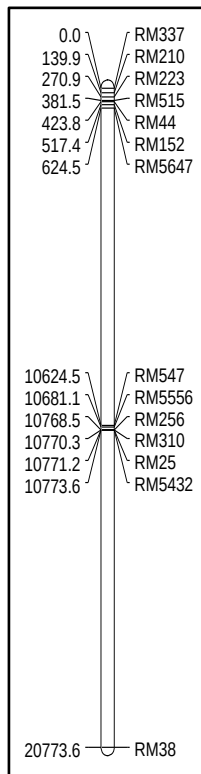
S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	1	RM13	2	RM249	0.2389	0
2	1	RM13	4	RM163	0.3969	0.02
3	4	RM163	1	RM13	0.3969	0.02
4	4	RM163	3	RM164	0.437	0.3
5	3	RM164	4	RM163	0.437	0.3
6	3	RM164	2	RM249	0.499	0
7	2	RM249	1	RM13	0.2389	0
8	2	RM249	4	RM163	0.4779	0.14

(f) Chromosome 6

S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	3	RM225	5	RM276	0.0506	1.63
2	3	RM225	6	RM8225	0.0546	0.31
3	4	RM253	5	RM276	0.1072	1.74
4	4	RM253	6	RM8225	0.1113	0.3
5	5	RM276	6	RM8225	0.0202	0.57
6	5	RM276	3	RM225	0.0506	1.63
7	1	RM541	6	RM8225	0.4291	0.37
8	1	RM541	5	RM276	0.4375	0.66
9	6	RM8225	5	RM276	0.0202	0.57
10	6	RM8225	3	RM225	0.0546	0.31

(g) Chromosome 7

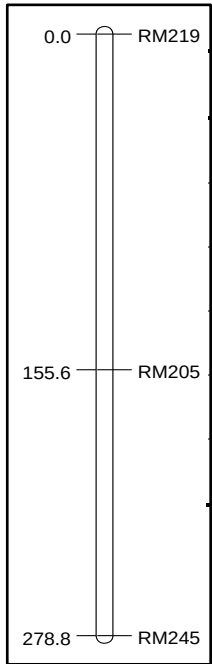
S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	3	RM3300	1	RM5436	0.2088	0.02
2	3	RM3300	2	RM5499	0.2126	0.08
3	1	RM5436	2	RM5499	0.0121	0.65
4	1	RM5436	3	RM3300	0.2088	0.02
5	2	RM5499	1	RM5436	0.0121	0.65
6	2	RM5499	3	RM3300	0.2126	0.08
7	4	RM5720	2	RM5499	0.4898	1.02
8	4	RM5720	1	RM5436	0.4898	1.12

(h) Chromosome 8

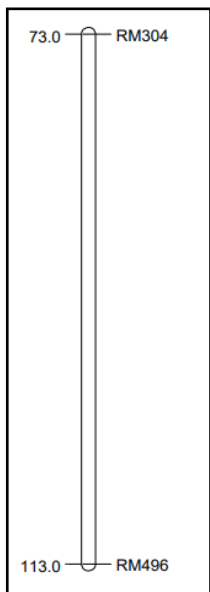
S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	2	RM152	11	RM44	0.4229	0.96
2	2	RM152	3	RM5647	0.4411	0.36
3	15	RM210	11	RM44	0.4496	0.26
4	15	RM210	14	RM223	0.4637	0.06
5	14	RM223	12	RM515	0.4451	0.02
6	14	RM223	15	RM210	0.4637	0.06
7	8	RM25	9	RM310	0.0081	0.01
8	8	RM25	16	RM256	0.0223	0.92
9	16	RM256	9	RM310	0.0182	1.74
10	16	RM256	8	RM25	0.0223	0.92
11	9	RM310	8	RM25	0.0081	0.01
12	9	RM310	16	RM256	0.0182	1.74
13	1	RM337	15	RM210	0.4695	0.09
14	1	RM337	11	RM44	0.4718	0.19
15	6	RM38	14	RM223	0.4779	0.15
16	6	RM38	12	RM515	0.4859	0.04
17	11	RM44	12	RM515	0.2855	0
18	11	RM44	2	RM152	0.4229	0.96
19	12	RM515	11	RM44	0.2855	0
20	12	RM515	14	RM223	0.4451	0.02
21	7	RM5432	9	RM310	0.0202	0
22	7	RM5432	8	RM25	0.0243	0
23	10	RM547	5	RM5556	0.3387	5.01
24	10	RM547	7	RM5432	0.4431	0.05
25	5	RM5556	10	RM547	0.3387	5.01
26	5	RM5556	16	RM256	0.4128	1.66

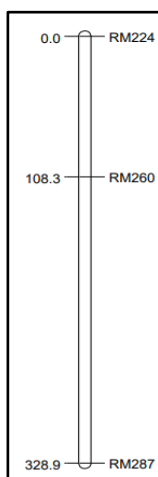
27	3	RM5647	2	RM152	0.4411	0.36
28	3	RM5647	11	RM44	0.4597	0.04

(i) Chromosome 9

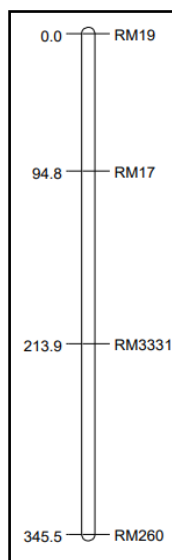
	S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
	1	6	RM205	5	RM245	0.4577	0.06
	2	6	RM205	1	RM219	0.4779	0
	3	1	RM219	6	RM205	0.4779	0
	4	1	RM219	5	RM245	0.499	0
	5	5	RM245	6	RM205	0.4577	0.06
	6	5	RM245	1	RM219	0.499	0

(j) Chromosome 10

	S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
	1	1	RM304	2	RM496	0.499	0
	2	2	RM496	1	RM304	0.499	0

(k) Chromosome 11

S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	4	RM224	3	RM260	0.4427	0.45
2	4	RM224	1	RM287	0.499	0
3	3	RM260	4	RM224	0.4427	0.45
4	3	RM260	1	RM287	0.494	0.01
5	1	RM287	3	RM260	0.494	0.01
6	1	RM287	4	RM224	0.499	0

(l) Chromosome 12

S/n	Nr1	Locus1	Nr2	Locus2	Rec.Freq	LOD
1	4	RM17	1	RM19	0.4247	0.15
2	4	RM17	3	RM3331	0.4538	0.03
3	1	RM19	4	RM17	0.4247	0.15
4	1	RM19	2	RM260	0.499	0
5	2	RM260	3	RM3331	0.4643	0.03
6	2	RM260	4	RM17	0.492	1.25
7	3	RM3331	4	RM17	0.4538	0.03
8	3	RM3331	2	RM260	0.4643	0.03

The 98 polymorphic markers in 12 linkage groups (LGs) spanned a distance of 2495.31 cM of the rice genome at an average density of 31.99 cM. The LOD (logarithm of odds) threshold value of >3 are usually preferred for the construction of linkage maps (Collard *et al.*, 2005), which indicate that linkage is 1000 times more likely than no linkage. Marker pairs with recombination frequency greater than 0.01 are considered linked, while those with less than 0.50 are considered unlinked. However, the LOD value may be lowered to detect a greater level of linkage (Collard *et al.*, 2005). Therefore, for the current study, the LOD threshold value was declared as ≥ 2 based on permutation test. The Joint Map software was used to visualize the final charts with locus and position in cM for the *Disang* x *Koimurali* population. The number of linked markers in each linkage group were 12 (LG 1); 11 (LG 2); 10 (LG 3), 6 (LG 4), 4 (LG 5), 4 (LG 5), 5 (LG 6), 4 (LG 7), 14 (LG 8), 3 (LG 9), 2 (LG 10), 3 (LG 11), 4 (LG 12), respectively.

4.9 QTL Analysis for drought tolerance at reproductive stage

The principle of QTL analysis is the association between phenotypic traits and the genetic composition of marker alleles within a segregating population. This requires accurate recording of both phenotypic and genotypic data, coupled with the utilization of statistical methods to facilitate the analytical process. (Lander & Botstein, 1989). One of the most effective approaches for QTL mapping is interval mapping. This technique systematically scans the intervals established between adjacent pairs of markers along a chromosome. The objective is to determine the likelihood profile, often quantified as a logarithmic odds (LOD) score, representing the potential location of a QTL within each interval. The present study employed interval mapping approach to detect QTL in the RILs. Interval mapping results in detection of possible QTL positions relative to that linkage map. The generated LOD scores identify the most probable QTL position in relation to the linkage map. To declare a potential QTL out of different interval combinations, threshold LOD score is determined through permutation test. In this procedure, phenotypic values are shuffled, while marker genotypic values are held constant to break marker-trait associations. The QTL analysis is subsequently revised multiple times (e.g., 500 or 1000) to ascertain false positive marker-trait associations. (Collard *et al.*, 2005; Churchill & Doerge, 1994; Haley & Andersson, 1997; Hackett, 2002). In the present study LOD score is declared as ≥ 2 based on the permutation test.

4.9.1 Detection of QTLs for drought tolerance at reproductive stage

The QTL analysis was performed based on the linkage map and the trait values of the RILs using MapQTL 6. A total of 8 QTLs were detected for yield related traits under drought. (Table 4.4). The mapping function used for QTL analysis was the Kosambi mapping function and mapping approach was interval mapping, To ensure the reliability of results, a permutation test involving 500 bootstraps was performed, and QTLs were declared to be significant when their associated LOD score reached or exceeded a threshold of 2. QTL nomenclature was given based on (McCouch,1997).The LOD scores and percent variance explained (PVE) of QTLs detected under drought stress varied from 2.56 to 3.54 and 4.7 to 6.4, respectively. The details of QTLs detected in the study are discussed below.

4.9.1.1. Grain yields

Two QTLs were detected for the trait grain yield on chromosome 3 (*qDGY3*) and chromosome 4(*qDGY4*) at 124.6 cM and 125.8 cM with a LOD score of 3.54 and 2.57 and phenotypic variance of 6.4 and 4.7, respectively.(Fig 4.11a and 4.11b). In the present study, the additive effect for *qDGY3* and *qDGY4* were 0.51 and 0.78, respectively, indicating both the traits were being contributed by *Disang*, in a study conducted by (Venuprasad *et al.*,2009), a major QTL for grain yield under drought (*qDTY3.1*) was detected. The QTL contributed to grain yield under conditions of severe lowland stress during the dry Season (DS). The study was conducted for a mapping population comprising 490 F_{4:5}RILs derived from a cross between *Apo*(tolerant) and Swarna (susceptible) rice varieties. The QTL, *qDTY3.1* was responsible for explaining approximately 31% of the genetic variance associated with this trait ($p < 0.0001$). However, the location of the QTL, *qDGY3* detected in the present study was different from *qDTY3.1*, indicating two different genetics for drought tolerance; the previous one being a minor effect QTL.

Numerous QTLs associated with grain yield under reproductive drought stress have been reported, demonstrating their capacity to positively impact various agronomic traits such as tiller number, harvest index, flowering time, and overall crop yield. (Bernier *et al.*,2007) reported a major effect QTL, *qDTY12.1*, between the marker interval of RM 12460-RM19. In a subsequent study, (Oo *et al.*,2021) introduced the *qDTY12.1*, observed for its influence on tiller number augmentation and crop biomass enhancement, along with the reduction in growth duration and elevation of harvest index, into the *O. sativa* cultivar Pusa 44 known for its susceptibility to drought. The process of marker-assisted

backcrossing facilitated the successful introgression of *qDTY12.1* into the genetic background of Pusa 44, enhancing tolerance without inducing any deleterious effects. (Vikram *et al.*, 2011) reported another QTL, *qDTY1.1*, while (Ghimire *et al.*, 2012) affirmed its consistent impact on grain yield under drought stress. (Palanog *et al.*, 2014) elucidated the existence of two major QTLs, namely *qDTY2.3* and *qDTY2.2*. (Sahu *et al.*, 2017) reported multiple QTLs *qYLD1.1*, *qYLD2.1*, and *qYLD5.1* linked to yield traits. (Bhattarai and Subudhi, 2018) detected *qGY1.42*, while (Baisaikh *et al.*, 2020) reported a series of QTLs, including *qGY1.1*, *qGY7.1*, *qGY8.1*, and *qGY11.1*. Together, these findings indicated that various QTLs from different donors were contributing to yield under drought suggesting its complex inheritance. In the present study detected two QTLs for grain yield under drought at the reproductive stage with two different effects.

Table 4. 4: QTLs detected for drought tolerance under reproductive stage

Trait	Chr	POSITION (cM)	Marker	LOD	PVE	Additive effect
GY	3	124.6	RM1038-RM3867	3.54	6.4	0.515352
	4	125.8	RM255-RM280	2.57	4.7	0.78572
TOL	4	120.8	RM255-RM280	2.56	4.7	-0.76341
YSI	4	127.8	RM255-RM280	2.59	4.7	0.610128
	4	127.8	RM355-RM7563	2.58	4.7	-9.37005
RYR	4	127.8	RM225-RM280	2.58	4.7	-60.9004
DSI	3	124.6	RM1038-RM3867	3.54	6.4	0.515352
	4	127.8	RM255-RM280	2.62	4.8	0.825924

4.9.1.2. QTLs on drought indices

Assessment of drought tolerance through various drought indices such as relative yield index (RYR), yield susceptibility index (STI), drought susceptibility index (DSI), stress tolerance index (TOL), and stress susceptibility index (SSI) has been a standard approach. Therefore, these tolerance indices were used to evaluate the drought tolerance in rice. The tolerance index is an important character to study the plant response to abiotic stress including drought stress (Moosavi *et al.*, 2008); however, there are few studies that used the tolerance index for QTL identification. In the present study, four QTLs were detected for drought tolerance at the reproductive stage. Four out of five QTLs were detected on chromosome 4 and one on chromosome 3 as mentioned in table 4.4. The QTLs detected on chromosome 4 are for stress tolerance index (*qTOL4*), relative yield reduction index (*qRYR4*), drought susceptibility index (*qDSI4*) and two QTLs for yield susceptibility index (*qYSI4*) in two different marker intervals (plate 4.11 c,d,e,f,g). Out of the five QTLs detected in chromosome, four had LOD score ranged from 2.56-3.54 and phenotypic variance ranged from 4.7-6.4. The additive effect of QTL for stress tolerance was -0.76, yield susceptibility index was -9.3 and for relative yield reduction index was -6.0. All these traits were contributed by the donor parent, *Koimurali* whereas QTL for drought susceptibility index and another QTL for yield susceptibility index showed

additive effect of 0.82 and 0.61, respectively, indicating that these traits were contributed by the recipient parent, *Disang*. Another QTL detected using drought indices was detected on chromosome 3 for drought susceptibility index within the marker interval of RM1038-RM3867 with a LOD score of 3.54 and phenotypic variance of 6.4. The additive effect for this QTL was 0.51, indicating that the trait is contributed by the recipient parent *Disang*. From this, it has become clear that various indices for drought tolerance are under the control of multiple QTLs with varied genetic effects. The effects of three QTLs have been contributed by the recipient parent but the phenotype of the recipient parent is drought susceptible. This may be due to various gene interactions underlying the trait because of which the actual effect could not be visible.

Satrio *et al.* (2021) also observed multiple QTLs (41) for various drought indices on rice chromosomes 1, 3, 6, 8, 9, and 12 in RILs developed between *IR64* and *Hawara Bunar* using SNPs. They revealed prominent QTL hotspot regions on chromosomes 6 and 8 several significant QTLs, namely *qTR.TOL-8*, *qCT.TOL-8*, *qCW.TOL-8*, *qCC.TOL-8*, *qCT.DRI-8*, *qCC.DRI-8*, and *qCW.DRI-8*. Yue *et al.* (2005) detected QTL for drought tolerance in rice related to drought response index at the reproductive stage. using a recombinant inbred population derived from a cross between an lowland *indica* rice and upland rice. A total of 39 QTLs for these traits were detected with individual QTL ranging from 5.1–32.1% of phenotypic variation. It is apparent that numerous QTLs are involved in imparting drought tolerance as measured using various indices.

4.9.1.3 Identification of putative genes within the QTL regions for drought tolerance at the reproductive stage

The QTLs detected at reproductive stage for drought tolerance were utilized for *in silico* gene identification. All the genes present within the selected QTLs marker interval (10 Mb region) were retrieved from RAP-DB (<https://rapdb.dna.affrc.go.jp>). (Table 4.5). A total of 22576 genes were scanned across the 8 QTL regions out of which the highest number of annotated genes was found for *qGY3* with 758 annotated genes and the lowest was found for *qRYR4* with 200 annotated genes. Some of the putative genes were identified whose function was possibly related to the respective QTL. Few of the putative genes have been found related to confer grain yield or yield related traits. A locus identified in this process, *Os03g0214200*, belongs to the third family of bZIP TF, *OsZIP46* is induced by drought,

heat, hydrogen peroxide and elevated levels of ABA. Over-expression of this TF leads to increase in ABA sensitivity (Tang *et al.* 2012). The gene, *Os03g0225100*, belongs to MAPK super family, *OsMPKK10.2*: it improves rice's capacity to withstand drought stress. It is possible by initiating a process called phosphorylation, which activates two specific MAPK proteins: MPK6 for disease resistance against blast and MPK3 for enhancing drought tolerance (Ma *et al.*, 2017). Another gene, *Os03g032710* belongs to NAC family, *OsNAC17*, a specific protein, is situated within the nucleus of cells, and its production is notably increased when subjected to drought conditions. *OsNAC17* functions as a transcriptional activator, influencing gene expression. This activating capability is attributed to a particular region located at the C-terminal end of the protein (Jung *et al.*, 2022).

In silico identification of putative genes related to QTL detected for Yield susceptibility index on chromosome 4(*qYSI4*) is MYB protein (*Os04g0583900*) MYB transcription factors have been demonstrated to play key regulatory role in plant growth, development and abiotic stress response. There are numerous reports which suggest that over expression of *OsMYB* gene leads to increase in drought tolerance (Tang *et al.*, 2019). *OsZEP* (*Os04g0448900*) is triggered by drought stress, an elevation of ZEP (zeaxanthin epoxidase) levels in roots, while causing a breakdown of ZEP in leaves, accompanied by similar changes in ABA concentrations (Zhang *et al.*, 2019). (Teng *et al.*, 2014) demonstrated that the gene *OsZEP*, associated with ABA biosynthesis, exhibited increased activity in rice when exposed to drought stress. Another gene, *OsGPX1* (*Os04g0556300*) is an important contributor to plant stress reactions, yet the precise ways through which plants detect and convey redox signals remain to be fully understood. This gene, encoding thiol peroxidase GPX1, occupies a crucial role in rice's response to osmotic stress, such as drought and high salinity. In this context, it acts as both a redox sensor and a signal transmitter, effectively enhancing the plant's ability to withstand

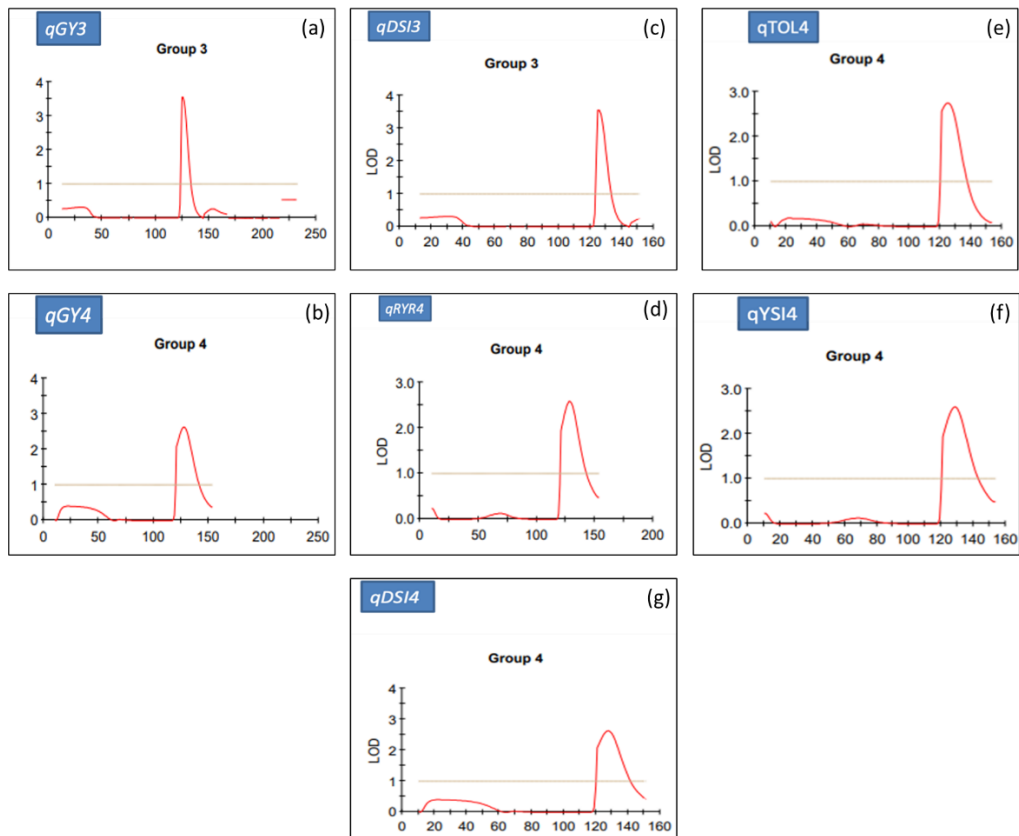


FIGURE 4. 7: QTL LIKELYHOOD CURVE FOR DIFFERENT TRAITS UNDER REPRODUCTIVE DROUGHT STRESS

Table 4. 5:List of total gene scanned within the QTL regions

Sr. no	TraitName	QTL Name	Chr	QTL interval (Mb)	Total number of genes	Number of annotated genes ^a
1	Grain Yield	<i>qGY3</i>	3	13	3206	758
2	Grain Yield	<i>qGY4</i>	4	12	3106	256
3	Yield susceptible index	<i>qYSI4</i>	4	10	3531	456
4	Yield susceptible index	<i>qYSI4</i>	4	13	3106	389
5	Stress Tolerance	<i>qTOL4</i>	4	10	3307	675
6	Relative yield reduction index	<i>qRYR4</i>	4	6	3106	200
7	Drought susceptible index	<i>qDSI3</i>	3	10	2106	350

drought conditions. The challenge of osmotic stress, stemming from drought and elevated salinity, poses a substantial threat to plant growth and agricultural productivity (Zhou *et al.*, 2022). If expression of the genes identified within the QTL regions is studied in RILs, we can improve our understanding of drought responsive genes. Therefore, expression pattern of three of such genes were studied.

4.9.2. Gene expression studies for *OsZIP73*, *OsAPX1* and *OsNAC9* in drought tolerant and susceptible RILs

Among the RILs, developed two drought tolerant (T174 and T67) and two susceptible (S 262 and S14) lines were chosen (Plate 4.12) on the basis of phenotypic observation for gene expression analysis using primers as mentioned in Table 3.3. The expression profiling of the RILs along with their parents are discussed below:

As discussed in section 4.2.4, *OsZIP73* plays an important role in imparting abiotic stress tolerance; the current study revealed a 2.5-fold increase in gene expression under mild drought stress conditions (5DWW). Conversely, susceptible RILs exhibited down regulation of the gene when compared to the tolerant counterparts (Fig. 4.12a). As drought stress intensity escalated (10DWW), there were no significant shifts in gene expression patterns observed among either the tolerant or susceptible RILs (see Fig 4.12 a). The expression pattern of *OsAPX1* was also studied.

As mentioned in section 4.2.5, *OsAPX1* plays an important role in mitigating the effects of reactive oxygen species (ROS) generated under stress conditions by actively participating in the APX-GPX cycle. In the study, the tolerant RILs revealed a substantial upregulation in the gene expression, more particularly in the case T174, under conditions of mild drought stress (5DWW). The observed upregulation in T174 gene expression was particularly remarkable, amounting to a 23-fold increase (Fig.4.12b). Interestingly, as the stress conditions escalated to a more severe level (10DWW), the gene expression of T174 exhibited an even more pronounced increase, reaching a remarkable 50-fold upregulation at $p < 0.05$ (Fig. 4.12b). The expression of another gene, *OsNAC9* was also investigated as this particular transcription factor regulate the root architecture making it more fibrous and longer under drought stress condition. In the present study, the root length

Plate 4. 12: Root architecture

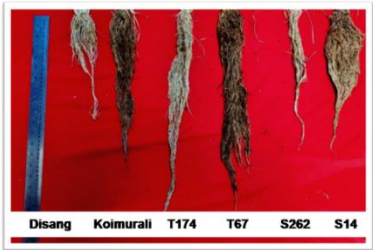
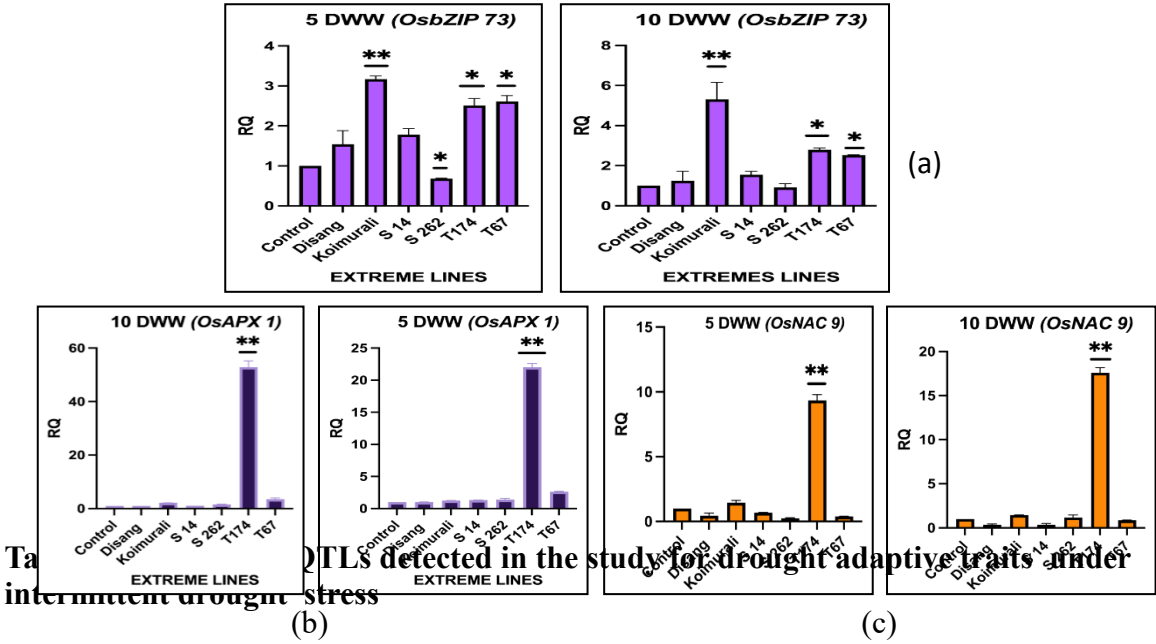


Figure 4. 12:Transcript Profile of (a) OsbZIP 73; (b) OsAPX 1 and (c) OsNAC 9



TRAIT*	CHR	MARKER	POSITION (cM)	LOD	PVE*	ADDITIVE EFFECT
FLW	1	RM212-RM431	159	2.23	4.1	-1.45
GLW	3	RM1352-RM1373	150.6	2.55	4.6	0.23
GY	4	RM7563-RM335	49.5	2.7	4.9	-1.84
NOC	1	RM283-RM259	35.4	2.7	4.9	1.93
	7	RM3300-RM5720	84.22	2.91	5.3	-9.27
TNG	7	RM3300-RM5720	83.2	2.84	5.2	-9.66
SF	1	RM283-RM259	128	2.82	5.1	-2.64
RYR	4	RM335-RM7563	39.5	2.35	4.1	73.33
	4	RM335-RM307	54.5	3.5	6.3	3.14
TOL	4	RM335- RM7563	24.2	2.2	4	0.73
YSI	4	RM335-RM7563	47.5	2.35	4.3	-2.24

susceptibility and tolerant lines were examined (Plate 4.12). The gene expression of the breeding lines along with its parents showed higher accumulation of transcript in T174 by 9-fold in mild drought stress (5DWW) and 18-fold in severe drought stress condition (10DWW) at $p < 0.05$ significance (Fig. 4.12 c). The observed trend suggests that the RIL, T174 may potentially be a transgressive segregant, exhibiting genetic traits superior to the parents. The expression pattern of *OsAPX1* and *OsNAC9* in T174 was found to be significantly higher that underlines its potentially vital role in conferring tolerance under drought stress.

4.9.3. Detection of QTLs for intermittent drought stress

In the present study, QTLs related to intermittent drought stress were detected. using 98 SSR markers and the phenotyping data for yield related traits generated during *Sali*, 2021. A total of 11 QTLs were detected for various drought related traits across 4 chromosomes.

The LOD score values corresponding to these QTLs ranged from 2.2 to 3.5 and PVE from 4% to 6.3% (Table 4.6). Comprehensive details concerning each identified QTL, their mapping positions in the current study as well as any previous work, and the graphical representation of LOD scores are elaborated in the subsequent sections.

4.9.3.1 QTLs for Flag leaf width

A specific QTL (*qFLW1*) was detected on chromosome 1, between RM212 and RM431. This QTL was associated with Flag Leaf Width (FLW) and exhibited a LOD score of 2.23, accounting for 4.1% of the phenotypic variance. It demonstrated an additive effect of -1.45, indicating the effect was contributed by the donor parent, *Koimurali* (Fig. 4.13). Interestingly, (Zhang *et al.*, 2015) also reported a significant QTL, denoted

qFLW7.2, associated with FLW on chromosome 7. This QTL displayed a substantial phenotypic variance of 14%. Notably, *qFLW7.2* and another QTL, *qPY7*, were found to be co-localized on chromosome 7, displaying a positive correlation. This correlation indicated that FLW has a direct impact on enhancing the overall yield of the plant. Additionally, (Tian *et al.*, 2015) identified two major QTLs, *qGNPPI-1* and *qFLW1-1*, linked to grain number per panicle and flag leaf width, respectively. These QTLs were detected in the same chromosomal region of chromosome 1, located between markers RM3521 and RM8111. In another study, five QTLs governing flag leaf width were detected and one major QTL, *qFLW4* was fine-mapped to a specific interval on chromosome 4, housing the gene NAL1 (Narrow leaf 1) within a 74.8 kb region (Chen *et al.*, 2012). Although various QTLs have been reported for flag leaf width on chromosome 1, the location of the QTL detected in the present study was distantly apart, indicating different genetic control for drought tolerance of the RILs.

4.9.3.2 QTLs for Grain length to width ratio

A QTL, for grain length to width ratio (*qGLW3*), was detected on chromosome 3, between RM1352-RM1373 at 150.6 cM. This QTL displayed an LOD score of 2.55, displaying phenotypic variance of 4.6% and was contributed by *Disang* (additive effect:0.23). (Fig. 4.13 b). The enhancement of grain quality is an important objective in rice breeding programs. A QTL was mapped to the chromosomal region between RM6283 and RM16 on chromosome 3 for grain length width ratio. This QTL, known as *qLWR-3*, accounted for 36.09% of the trait variation, as substantiated by a high LOD score of 43.1 for a draught experiment (Lou *et al.*, 2009). (Guang *et al.*, 2004) detected a total of 18 QTLs for traits related to grain shape on chromosomes 1, 2, 3, 4, 6, 8, and 12 .Out of the QTLs detected, six QTLs were observed for length-width ratio. These QTLs accounted from 9.8% to 22.7% of phenotypic variance with LOD score ranged from 2.44 to 6.02. These findings collectively provide valuable insights into the genetic factors governing grain shape traits and their variations across diverse environments However, in the present study, only one QTL was detected for grain length to width ratio in the RILs and it was contributed by the recipient parent. This was desired as *Disang* has better trait performance than the donor parent, *Koimurali*.

4.9.3.3 QTLs for Grain yield

In present study, a QTL for grain yield under intermittent drought (*QTLqGY4*) was detected on chromosome 4 between RM7563 and RM335. The LOD score for *qGY 4* was

2.7, and its corresponding phenotypic variance accounted for 4.9% of the trait variation. Furthermore, the additive effect was -1.84, and was contributed by the donor parent, *Koimurali* (Fig4.13d). Grain yield is an important trait in rice, and numerous QTLs associated with this trait has been successfully characterized (Vikram *et al.*, 2011). These QTLs have played a vital role in the advancement of marker-assisted breeding strategies, contributing to the development of rice varieties with enhanced grain yield through informed genetic selection. However, the present study could not detect major effect QTL for grain yield as it was found in other studies reported before.

4.9.3.4 QTLs for number of chaffy grains

In the context of detection of QTLs for intermittent drought stress, the investigation revealed two QTLs influencing the number of chaffs per panicle. Designated as *qNOC1* and *qNOC7*, these QTLs were positioned on chromosome 1 (RM283-RM259) and chromosome 7 (RM3300-RM5720), respectively, with corresponding LOD scores of 2.7 and 2.91. and PVE of 4.9% and 5.3%. The additive effect values indicated contribution of the recipient parent, *Disang* for *qNOC1* (additive effect: 1.93), and from the donor parent, *Koimurali* for *qNOC7* (additive effect: -9.27) towards the trait Fig4.13e).

Similar results were reported by Vermireddy *et al.* (2015), who detected three QTLs linked to chaffy grains—denoted as *qCG3.1*, *qCG9.1*, and *qCG12.1*—each situated on chromosomes 1, 9, and 12, respectively. However the location of *qNOC1* was different from the *qCG3.1* Collectively, these

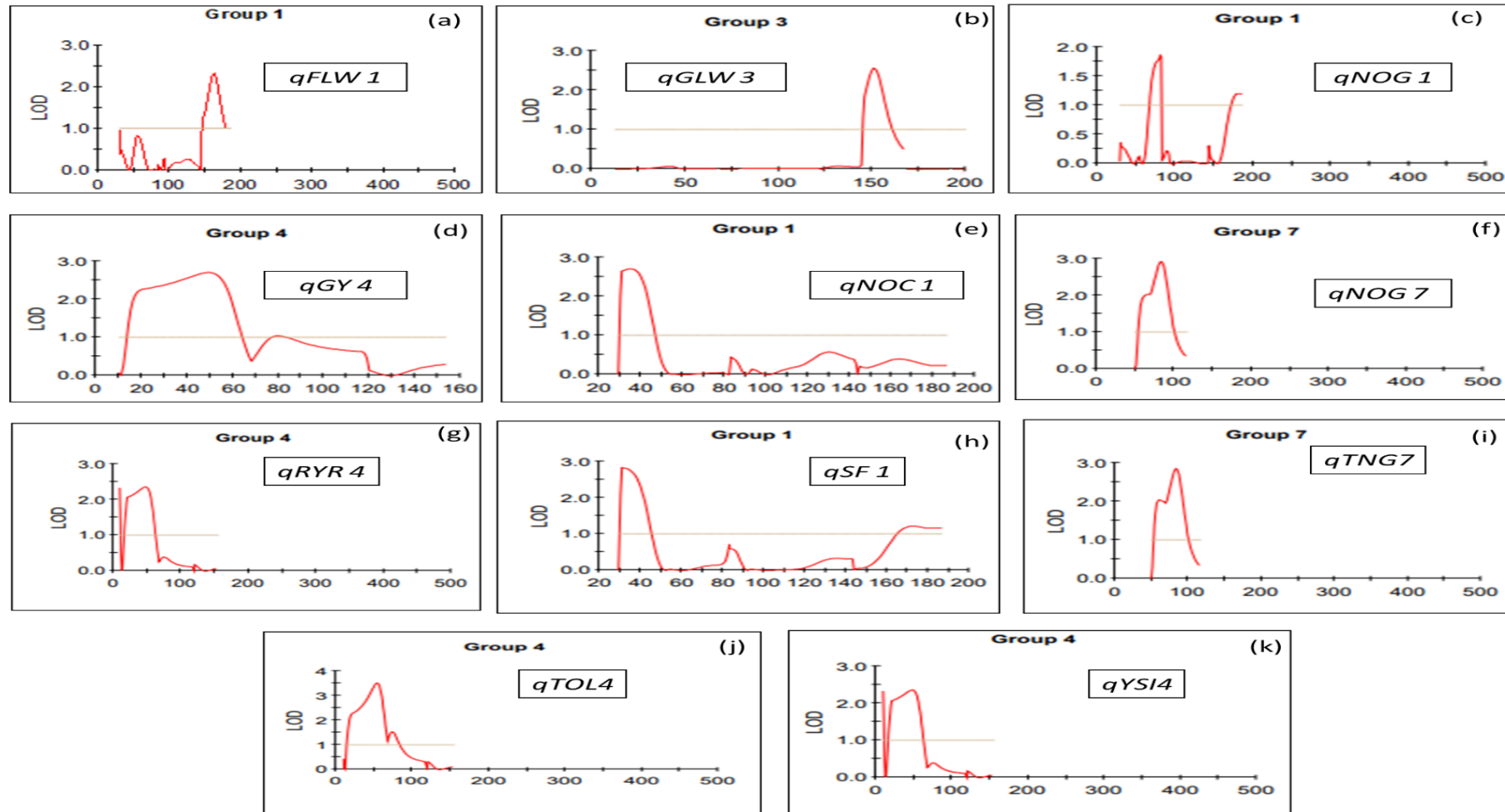


FIGURE 4. 13:. QTL LIKELYHOOD CURVE OF DIFFERENT TRAITS UNDER INTERMITTENT DROUGHT STRESS

QTLs accounted for 32.46% of the observed phenotypic variation, higher than the present study.

QTLs for total number of grain per panicle

The QTL named as *qTNG 7*, was detected on chromosome 7. This QTL was detected between the marker interval of RM3300 and RM5720. The LOD score of *qTNG7* was 2.84, and its corresponding phenotypic variance accounted for 5.2% of the trait variation. Additionally, the additive effect of the QTL (-9.66) revealed that the phenotype has been contributed by the donor parent, *Koimurali* (Fig.4.13c and 4.13f). Similar results were also reported by Lin *et al.* ,2007, Dixit *et al.*, 2014, Xu *et al.*,2015.

4.9.3.5 QTLs for Spikelet fertility

A QTL was detected for spikelet fertility. This QTL, referred to as *qSF3*, was located on chromosome 1 at 128 cM, with a LOD score of 2.82. The *qSF3* was flanked by the SSR markers RM283-RM259, and it exhibited a PVE of 5.1%. The additive effect of *qSF3* was -2.64, signifying contribution of donor parent towards the trait (Fig 4.14h). Similar results were reported by (Sahu *et al.*, 2017) and detected two QTLs, namely *qSF1.1* and *qSF3.1*, which influenced spikelet fertility within an RIL population under intermittent drought. Moreover, (Bhattarai and Subudhi, 2018) reported four QTLs governing spikelet fertility: *qSF1.38*, *qSF6.23*, *qSF7.04*, and *qSF11.19*. However, the present study detected only one QTL for spikelet fertility. These findings might contribute to the understanding of the genetics of spikelet fertility and its responses to varying water stress conditions.

4.9.3.6 QTLs for Relative yield reduction (%) (RYR)/Stress tolerance (TOL)/yield stability index (YSI)

The QTLs were detected for relative yield reduction (%), stress tolerance, and yield stability index (*qRYR4*, *qTOL4*, and *qYSI4*, respectively), all on chromosome 4. Two QTLs were found within the same flanking markers, RM335 and RM7563, one QTL was found within the marker interval RM 335-RM307. However, the LOD score values were 4.1 for *qRYR4*, 3.5 and 2.2 for *qTOL 4* and 2.35 for *qYSI4*. The PVE were 4.1 for *qRYR2*, 6.3 and 4 for *qTOL4* and 4.3 for *qYSI4*. (Fig 4.13g, 4.13j and 4.13k, respectively; Table 4.6)

Table 4. 7: List of total gene scanned within the QTL regions

Sr no	TraitName	QTL Name	Chr	QTL interval (Mb)	Total number of genes	Number of annotated genes ^a
1	FLW	<i>qFLW1</i>	1	12	2609	600
2	GLW	<i>qGLW3</i>	3	10	1654	445
3	GY	<i>qGY4</i>	4	10	1900	985
4	NOG	<i>qNOG1</i>	7	10	1321	408
5	NOG	<i>qNOG1</i>	1	10	1757	445
6	TNG	<i>qTNG7</i>	7	12	2710	629
7	SF	<i>qSF1</i>	1	10	1930	765
8	RYS	<i>qRYS4</i>	4	11	2563	398
9	TOL	<i>qTOL4</i>	4	11	2784	678
10	YSI	<i>qYSI4</i>	4	12	2456	654
11	NOC	<i>qNOC1</i>	1	10	1346	550

Table 4. 8: Putative gene along with its functions

Locus ID	Gene name /TF	Description	Reference
<i>qFLW1</i>			
Os01g0104200	<i>Os NAC</i>	Stress-responsive NAC transcription factor, Positive regulation of BR-regulated rice architecture, Negative modulation of ABA-mediated drought tolerance (Os01t0104200-01)	(Li <i>et al.</i> ,2021)
<i>qGLW3</i>			
Os01g0135700	<i>CaM</i>	Calmodulin-like protein, Calcium signaling during abiotic stress, Promotion of root growth and drought tolerance	(Xu <i>et al.</i> ,2016)
<i>qGY4</i>			
Os04g0398000	<i>ERF</i>	ERF transcription factor, A member of APETALA2/Ethylene-Responsive Element Binding Protein(AP2/EREBP) family, Regulation of drought stress response	(Quan <i>et al.</i> ,2010)
Os07g0680400	<i>WRKY</i>	WRKY transcription factor Interaction with MAPK to promote drought tolerance	(Zhang <i>et al.</i> ,2022)
Os07g0686100	<i>bZIP</i>	Drought stress-related bZIP transcription factor, Positive regulation of drought and oxidative tolerance	(Liu <i>et al.</i> ,2014)
<i>qRYS4</i>			
Os04g0448900	<i>OsZEP</i>	Zeaxanthin epoxidase, Absciscic acid (ABA) biosynthesis, "Resistance to osmotic and drought stresses, seed development and dormancy",	(Teng <i>et al.</i> , 2014)
<i>qYSI4</i>			
Os04g0583900	<i>MYB</i>	MYB-related transcription factor plays a key role in plants growth and development and increases drought tolerance	(Tang <i>et al.</i> ,2019)

The additive effects indicated the contribution of drought-tolerant indices from the recipient parent *Disang*, whereas in *qYSI4* the additive effect value of -2.23 was contributed by the donor parent *Koimurali*. Similar QTLs were detected by (Satrio *et al.*, 2021; Yu *et al.* 2015).

4.9.3.7 Identification of putative genes within the detected QTL regions for intermittent drought

There were 11 QTLs detected for intermittent drought stress, searched *in-silico* in RAP-DB (<https://rapdb.dna.affrc.go.jp>) for presence of genes related to drought stress, if any. A total of 23030 gene were scanned across the 11 QTL regions; out of which the highest number of annotated genes was found for *qGY4* with 985 annotated genes and the lowest was observed for *qRYR4* with 398 annotated genes (Table 4.6). Some of the putative genes were identified which function was possibly related to the respective QTL. The functions of the putative genes have been listed in the Table 4.8.

4.9.3.8 Comparison of QTLs detected under intermittent drought and reproductive drought stress

The purpose of the comparative study was to investigate whether the genetic controls for drought stress responses were similar in two different stages of drought stress, namely intermittent drought stress and drought stress at the reproductive stage. To conduct the study, four different experimental plots were used. The intermittent drought stress was performed in *Sali* 2021, in which non-stress RILs were maintained at the ICR farm with adequate water. The intermittent stress treatment was given in the rainout shelter of DBT, NECAB. On the other hand, the reproductive drought stress experiment was performed as a pot experiment in *Ahu* 2022, and both the control and stress conditions were maintained in pots.

During the analysis of QTL in *Sali* 2021, a total of 11 QTLs were detected under intermittent drought stress. These QTLs were associated with various drought-related traits under intermittent drought stress (*qFLW*, *qGLW*, *qGY*, *qNOC*, *qTNG*, *qSF*, *qRYR*, *qTOL*, and *qYSI*). Similarly, analysis for trait performance under drought at reproductive stage, a total of 8 QTLs (*qGY3*, *qTOL*, *qYSI*, *qRYR*, *qDSI*). The comparative study of the QTL detected in both the stress conditions are presented in Table 4.9

The comparison of two different drought stresses (reproductive and intermittent), only one common QTL was observed, namely *qYSI4*, located between the marker interval RM335-RM7564, with a LOD score of 2.58, PVE of 4.7, and an additive effect of -9.37

(Table 4.9). This suggests that the same QTL affect both stress conditions, indicating similar physiological mechanisms may contribute to drought response under both stress environments. The results of the analysis based on the findings from (Foolad *et al.*, 2008), imply that there are common genetic controls for drought responses in plants under both intermittent drought stress and drought stress at the reproductive stage. This indicates that similar genetic pathways and physiological mechanisms are involved in the plants' response to these different drought stress conditions and influenced by specific locations on chromosomes. However, it may be too early to draw a general statement.

4.10. Trait wise comparison of similar QTLs detected in both intermittent and drought stress conditions

QTL for Grain yield: One QTL for grain yield was detected at intermittent stress at chromosome 4 having a LOD score of 2.7 whereas two QTL were detected in reproductive drought stress in chromosome 3 and 4 having LOD score of 3.54 and 2.57, respectively. As the QTLs associated with grain yield in both the stress condition has almost equal LOD scores, it implies that similar kinds of gene governs both the traits although other factors may be associated. The consistency of a QTL in different environmental conditions reflects that this QTL can perform across environments. Similar kinds of results have been observed by (Ferris *et al.*, 2002).

Co-localized QTL: Several genomic regions with QTL (*qGY4*, *qYSI4*, *qTOL4* and *qRYR4*) associated with different traits were collocated within the same marker interval with varied LOD score and PVE percentage. These QTLs were associated with grain yield related traits (Table 4.10). The existence of QTLs in the same genomic region for various traits is projected for highly correlated traits and genetic interdependency between these traits (Adam *et al.*, 2023; Ali *et al.*, 2000). Similar results were reported by (Rajurkar *et al.*, 2019) as they detected several co-located QTL regions associated with various traits on different chromosomes in rice using SSR markers on mapping populations developed from locally adapted landraces and evaluated under drought conditions. While comparing the two drought stresses, it was evident that there were few QTLs sharing the same chromosome but in different marker intervals. In traits like *RYR*, *TOL*, *GLW*, QTLs were not found in intermittent stress but were detected in reproductive stress. In the case of *FLW*, QTL was found in intermittent stress (Table 4.9). This indicates that two different genetics might underlie the drought tolerance in RILs.

Table 4.9: Comparison between QTLs in intermittent drought stress and reproductive drought stress

Trait	Intermittent Drought stress			Reproductive Drought stress		
	QTL	LOD	Candidate gene	QTL	LOD	Candidate gene
Grain yield	<i>qGY4</i>	2.7	<i>ERF, WRKY, bZIP</i>	<i>qGY4</i>	2.57	<i>ERF</i>
	-	-	-	<i>qGY3</i>	3.54	<i>Os bZIP, MAPK, OsNAC</i>
Grain length width ratio	-	-	-	<i>qGLW3</i>	2.55	<i>CaM</i>
Yield susceptibility index	<i>qYSI4</i>	2.58	<i>MYB</i>	<i>qYSI4</i>	2.58	<i>MYB</i>
Stress tolerance	-	-	-	<i>qTOL4</i>	2.56	<i>GPX1</i>
Relative yield reduction	-	-	-	<i>qRYR4</i>	2.58	<i>OsZEP</i>
Flag leaf width	<i>qFLW1</i>	2.23	<i>OsNAC</i>	--	-	-

CHAPTER V

SUMMARY AND CONCLUSION

Drought scenario in Assam will be getting severe in the upcoming years and will have a devastating effect on the paddy industry. Reproductive drought stress in the *Ahu* season and intermittent drought stress in the *Sali* season led to a decrease in the yield of the plant henceforth there is a need to develop varieties that can cope with both kinds of stresses. To better understand the genetic basis of drought tolerance it is very important to employ a breeding program to detect significant QTL. The present study attempts to assess the correlation between drought-responsive gene expression and drought-adaptive traits at the reproductive stage. Additionally, to map the Morphological traits for drought tolerance at the reproductive stage in rice. Finally, a comparative study of the genetic effects of drought-related traits under intermittent water stress and drought at the reproductive stage. A series of experimental analyses were carried out and the key findings of the research are summarized below.

- ❖ A total of 428 cultivars were screened for drought tolerance in the field initially and 13 cultivars were chosen based on levels of drought tolerance (Pararay, R.A., Ph.D Thesis, 2019). Out of 13, two tolerant and susceptible cultivars were chosen and moderately drought-responsive cultivars were eliminated. The four cultivars were chosen based on the level of tolerance to drought along with substantial evidence obtained from morphological, physiological, and molecular analysis.
- ❖ Expression profiling of all four cultivars was performed using 5 drought-responsive genes. Expression profiling of the drought-responsive genes *OsDREB2B*, *OsNAC9*, *bZIP73*, and *APX1* showed up-regulation in both the tolerant cultivars ARC 10372 and Koimurali. The transcript profile of *OsDREB2A*, *OsDREB2B*, *OsNAC9*, and *OsAPX1* indicated a decrease in gene expression in both the susceptible cultivars *IR 64* and *Disang*.
- ❖ Based on the expression profiling and phenotypic observations, Disang and Koimurali were selected to employ in the development of the mapping population. A high-yielding, drought-susceptible variety was crossed with a landrace of Assam, selected as a donor, Koimurali.
- ❖ A hybridity confirmation test was performed and single F1s were utilized for the advancement of the mapping population. A total of 247 RILs were generated using the

SSD method. Polymorphism of the parental cultivars was screened using 600 SSR markers and only 98(16.33%) SSR markers were found to be polymorphic. Phenotyping and genotyping of these RIL populations were done in F₅:F₆ generation for intermittent drought stress and F₆:F₇ in reproductive drought stress.

- ❖ A linkage map was constructed using 98 SSR markers, which covered a distance of 2491.31 cM across 12 rice chromosomes, with Chromosome 1 having the highest coverage of 786 cM and Chromosome10 having the lowest coverage of 113 cM. Among 98 polymorphic markers 25 markers(25.51%) fit the expected Mendelian ratio, and 73 markers(74.48%) deviate from the expected ratio with $p < 0.01$ and $P < 0.05\%$ respectively.
- ❖ 65 out of 73(89%) markers deviates towards the donor parent Koimurali while 8(8%) markers deviates towards the recipient parent Disang. Chromosome 3 has the highest distorted marker followed by chromosome 8.
- ❖ QTL analysis detected 8 QTLs at reproductive drought stress located on chromosome number 3(*qGY3*, LOD 3.54;*qYSI3*, LOD 3.54); 4(*qGY4*, LOD 2.57;*qTOL4* LOD 2.56;*qYSI4*, LOD 2.56;*qYSI4* LOD 2.59;*qRYR4*, LOD 2.58;*qDSI4*, LOD 2.62).11 QTLs were detected at intermittent drought stress located on chromosome 1(*qFLW1*, LOD2.23;*qNOC1*, LOD 2.7;*qNOG1*, LOD 1.83), 3(*GLW3*, LOD 2.55),4(*qGY4* 2.7;*qRYR4* 2.35;*qTOL4*, LOD 3.5;*qTOL4* LOD 2.2; *qYSI4*, LOD 2.35),7(*qNOG7*, LOD 2.91;*qTNG7*, LOD 2.84).
- ❖ The QTL detected under reproductive drought stress were scanned to identify candidate genes associated with drought. A 10Mb region for each QTL was scanned. A total of 34,796 genes were scanned across the 8 QTL regions out of which the highest number of annotated genes was found for *qGY3* with 758 annotated genes and the lowest was found for *qRYR4* with 200 annotated genes. From the annotated genes 63 genes were found to be stress-related genes and from these, seven (7) putative genes associated with drought stress were selected(*OsZIP46*, *MAPk*, *OsNAC6*, *ERF*, *MYB*, *OsZEP*, *OsGPX1*).
- ❖ 11 QTLs detected in intermittent drought stress conditions were utilized for in silico gene identification. All the genes present within the selected QTLs marker interval region were retrieved from RAP-DB (<https://rapdb.dna.affrc.go.jp>). 10mb region of the nearest marker was explored in RAP-DB. A total of 49840 genes were scanned across the 11 QTL regions out of which the highest number of annotated genes was found for *qGY4* with 985 annotated genes and the lowest was found for *qRYR4* with 398 annotated

genes. From the annotated genes 98 genes were found to be stress-related genes and from these, seven (7) putative genes associated with drought stress were selected (OsNAC, CaM, ERF, WRKY, bZIP, OsZEP, MYB).

- ❖ Out of the 247 RILs generated by crossing Koimurali and Disang, 4 extreme lines (T174, T67, S262, S14) were selected based on phenotypic observation. Gene expression analysis of 4 breeding lines was observed using previously designed primers. Expression analysis of the 4 extremes lines using OsbZIP73, OsAPX1 and OsNAC9 TF showed upregulation of these drought-responsive genes in T174 and T67 and downregulation in S262 and S14.
- ❖ The observed trend suggests that T174, a breeding line, may potentially represent a transgressive segregant, exhibiting genetic traits that go beyond those present in the parental lines. This noteworthy expression pattern of OsAPX1 and OsNAC9 in T174 underlines its potentially vital role in conferring tolerance to drought stress.
- ❖ Trait performance in intermittent drought stress and reproductive drought stress seems to be regulated by a few QTLs with minor effects as reflected in the phenotypic variation. Only one QTL was found to be common between the two different drought stress conditions. QTL (*qGY4*, *qYSI4*, *qTOL* and *qRYR*) associated with different traits were co-located within the same marker interval with varied LOD scores and PVE percentages indicating highly correlated traits and genetic interdependency between these traits.

Through the series of comprehensive experimental analyses, distinct minor QTLs were identified as contributors to conferring drought tolerance under two specific conditions of drought stress: reproductive drought stress and intermittent drought stress. Moreover, the investigation encompassed the examination of expression patterns among extreme lines using candidate genes, revealing significant up-regulation in the tolerant lines (T174). Remarkably, a comparative assessment between intermittent and continuous drought stress conditions indicates that different genetic pathways and physiological mechanisms are involved in governing the plant's adaptive response to these drought stress scenarios with an exception for the QTL (*qYSI4*) detected in both the stresses. The observed differences in responses between intermittent and continuous drought stress further emphasize the adaptability of plants and the need for a better understanding of their response to varying environmental challenges.

Future scope of work

1. The inclusion of more markers in QTL mapping improves resolution and facilitates fine mapping, aiding in the precise identification and isolation of the gene responsible for the trait of interest.
2. The QTL regions can be sequenced to identify Single Nucleotide Polymorphisms (SNPs), which can then be utilized for the development of functional markers.
3. The QTLs have to be validated further by fine-mapping the QTL to find utility for Marker Assisted breeding.

BIBLIOGRAPHY

- Abdirad, S.; Ghaffari, M.R.; Majd, A.; Irian, S.; Soleymaniniya, A.; Daryani, P. and Salekdeh, G.H. (2022). Genome-wide expression analysis of root tips in contrasting rice genotypes revealed novel candidate genes for water stress adaptation. *Frontiers in Plant Science*, **13**: 792079.
- Adam, H.; Gutierrez, A.; Coudert, M.; Sabot, F.; Ntakirutimana, F.; Serret, J. and Lorieux, M. (2023). African rice (*Oryza glaberrima*) genomic introgressions impacting upon panicle architecture in Asian rice (*O. sativa*) lead to the identification of key QTLs. *bioRxiv*, p. 04.
- Agrawal, G.K.; Jwa, N.; Iwahashi, H. and Rakwal, R. (2003). Importance of ascorbate peroxidases OsAPx1 and OsAPx2 in the rice pathogen response pathway and growth and reproduction revealed by their transcriptional profiling. *Gene*. **322**: 93-103.
- Alexandrov, N.; Tai, S.; Wang, W.; Mansueto, L.; Palis, K.; Fuentes, R.R. and McNally, K.L. (2015). SNP-Seek database of SNPs derived from 3000 rice genomes. *Nucleic Acids Research*, **43**(1): 1023-1027.
- Ali, M.L.; Pathan, M.S.; Zhang, J.; Bai, G.; Sarkarung, S. and Nguyen, H.T. (2000). Mapping QTLs for root traits in a recombinant inbred population from two indica ecotypes in rice. *Theoretical and Applied Genetics*, **101** : 756-766.
- Alou, I.N.; Steyn, J.M.; Annandale, J.G. and Van der Laan, M. (2018). Growth, phenological, and yield response of upland rice (*Oryza sativa* L. cv. Nerica 4®) to water stress during different growth stages. *Agricultural Water Management*, **198**: 39-52.
- Anjum, S.A.; Ashraf, U.; Zohaib, A.; Tanveer, M.; Naeem, M.; Ali, I.; Tabassum, T. and Nazir, U. (2017). Growth and development responses of crop plants under drought stress: a review. *Zemdirbyste*, **104**: 267-276.
- Anjum, S.A.; Xie, X.; Wang, L.C.; Saleem, M.F.; Man, C. and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, **6**(9): 2026-2032.
- Babu, R.C.; Nguyen, B.D.; Chamarerk, V.; Shanmugasundaram, P.; Chezian, P.; Jeyaprakash, P. and Nguyen, H.T. (2003). Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. *Crop Science*, **43**(4): 1457-1469.
- Bai, X.; Luo, L.; Yan, W.; Kovi, M.R.; Zhan, W. and Xing, Y. (2010). Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping a pleiotropic quantitative trait locus qGL7. *BMC Genetics*, **11**: 1-11.

- Baisakh, N.; Yabes, J.; Gutierrez, A.; Mangu, V.; Ma, P.; Famoso, A. and Pereira, A. (2020). Genetic mapping identifies consistent quantitative trait loci for yield traits of rice under greenhouse drought conditions. *Genes*, **11**(1): 62.
- Barik, S.R.; Pandit, E.; Mohanty, S.P.; Nayak, D.K. and Pradhan, S.K. (2020). Genetic mapping of physiological traits associated with terminal stage drought tolerance in rice. *BMC Genet*, **21**(1): 76.
- Barik, S.R.; Pandit, E.; Pradhan, S.K.; Mohanty, S.P. and Mohapatra, T. (2019). Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. *PloS one*, **14**(12): 0214979.
- Barik, S.R.; Pandit, E.; Pradhan, S.K.; Singh, S.; Swain, P. and Mohapatra, T. (2018). QTL mapping for relative water content trait at reproductive stage drought stress in rice. *Indian Journal of Genetics and Plant Breeding*, **78**(04): 401-408.
- Barik, S.R.; Pandit, E.; Pradhan, S.K.; Singh, S.; Swain, P. and Mohapatra, T. (2018). QTL mapping for relative water content trait at reproductive stage drought stress in rice. *Indian Journal of Genetics and Plant Breeding*, **78**(04): 401-408.
- 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): 507-516
- Bhargava, S. and Sawant, K. (2013). Drought stress adaptation: metabolic adjustment and regulation of gene expression. *Plant Breeding*, **132**(1): 21-32.
- Bhattarai, U. and Subudhi, P.K. (2018). Identification of drought responsive QTLs during vegetative growth stage of rice using a saturated GBS-based SNP linkage map. *Euphytica*, **214**(2): 38.
- Bin Rahman, A.R. and Zhang, J. (2016). Flood and drought tolerance in rice: opposite but may coexist. *Food and Energy Security*, **5**(2): 76-88.
- Blum, A. (2011). Drought resistance is it really a complex trait?. *Functional Plant Biology*, **38**(10): 753-757
- Boonjung, H. and Fukai, S. (1996). Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. *Field Crops Research*, **48**(1): 47-55.
- Boopathi, N.M. and Boopathi, N.M. (2020). Genotyping of mapping population. *Genetic Mapping and Marker Assisted Selection: Basics, Practice and Benefits*, pp. 107-178.
- Bouman, B. (2007). A conceptual framework for the improvement of crop water productivity at different spatial scales. *Agricultural Systems*, **93**(1-3): 43-60.

- Bousslama, M. and Schapaugh Jr, W.T. (1984). Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance 1. *Crop Science*, **24**(5): 933-937.
- Caverzan, A.; Passaia, G.; Rosa, S.B.; Ribeiro, C.W.; Lazzarotto, F. and Margis-Pinheiro, M. (2012). Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. *Genetics and Molecular Biology*, **35**: 1011-1019.
- Chakraborty, K.; Jena, P.; Mondal, S.; Dash, G.K.; Ray, S.; Baig, M.J. and Swain, P. (2021). Relative contribution of different members of OsDREB gene family towards osmotic stress tolerance in indica and japonica ecotypes of rice. pp. 356-366.
- Chen, J.Q.; Meng, X.P.; Zhang, Y.; Xia, M. and Wang, X.P. (2008). Over-expression of OsDREB genes lead to enhanced drought tolerance in rice. *Biotechnology Letters*, **30**: 2191-2198.
- Chen, L.; Ma, J.; Ma, X.; Cui, D.; Han, B.; Sun, J. and Han, L. (2023). QTL analysis of drought tolerance traits in rice during the vegetative growth period. *Euphytica*, **219**(3): 33.
- Chen, M.; Luo, J.; Shao, G.; Wei, X.; Tang, S.; Sheng, Z. and Hu, P. (2012). Fine mapping of a major QTL for flag leaf width in rice, qFLW4, which might be caused by alternative splicing of NAL1. *Plant Cell Reports*, **31**: 863-872.
- Cheng, M.C.; Liao, P.M.; Kuo, W.W. and Lin, T.P. (2013). The Arabidopsis ETHYLENE RESPONSE FACTOR1 regulates abiotic stress-responsive gene expression by binding to different *cis*-acting elements in response to different stress signals. *Plant Physiol.* **162**: 1566-1582.
- Choi, H.I.; Hong, J.H.; Ha, J.O.; Kang, J.Y. and Kim, S.Y. (2000). ABFs, a family of ABA-responsive element binding factors. *Journal of Biological Chemistry*, **275**(3): 1723-1730.
- Choudhury, B.; Khan, M.L. and Dayanandan, S. (2013). Genetic structure and diversity of indigenous rice (*Oryza sativa*) varieties in the Eastern Himalayan region of Northeast India. *Springer Plus*, **2**: 1-10.
- Chowdhury, P.; Pathak, P.K.; Tripathy, A.K.; Neog, M.; Saud, R.K.; Saharia, R.R.; Bharali, M.; Nath, D.; Saikia, N.; Borah, D.; Rahman, A.; Saikia, S.; Sarkar, L. and Biswas, S. (2019). Disang- A Promising Short Duration Rice Variety Suitable as Early Ahu (pre flood) and for Post Flood Situation in Cachar district of Assam. *International Journal of Agriculture Sciences*, **11**(15): 8881-8882.
- Churchill, G.A. and Doerge, R. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, **138**(3): 963-971.

- Collard, B.C.; Jahufer, M.Z.Z.; Brouwer, J.B. and Pang, E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, **142**: 169-196.
- Comas, L.H.; Becker, S.R.; Cruz, V.M.V.; Byrne, P.F. and Dierig, D.A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*, **4**: 442.
- Corrêa, L.G.G.; Riaño-Pachón, D.M.; Schrago, C.G.; Vicentini dos Santos, R.; Mueller-Roeber, B. and Vincentz, M. (2008). The role of bZIP transcription factors in green plant evolution: adaptive features emerging from four founder genes. *PloS one*, **3**(8): 2944.
- Cui, M.; Zhang, W.; Zhang, Q.; Xu, Z.; Zhu, Z.; Duan, F. and Wu, R. (2011). Induced over-expression of the transcription factor OsDREB2A improves drought tolerance in rice. *Plant Physiology and Biochemistry*, **49**(12): 1384-1391
- Cui, M.; Zhang, W.; Zhang, Q.; Xu, Z.; Zhu, Z.; Duan, F. and Wu, R. (2011). Induced over-expression of the transcription factor OsDREB2A improves drought tolerance in rice. *Plant Physiology and Biochemistry*, **49**(12): 1384-1391.
- Dai, B.; Guo, H.; Huang, C.; Ahmed, M.M. and Lin, Z. (2017). Identification and characterization of segregation distortion loci on cotton chromosome 18. *Frontiers in Plant Science*, **7**: 2037.
- Das, P.; Lakra, N.; Nutan, K.K.; Singla-Pareek, S.L. and Pareek, A. (2019). A unique bZIP transcription factor imparting multiple stress tolerance in Rice. *Rice*, **12**: 1-16.
- Dash, P.K.; Rai, R.; Rai, V. and Pasupalak, S. (2018). Drought induced signaling in rice: delineating canonical and non-canonical pathways. *Frontiers in Chemistry*, **6**: 264.
- Davatgar, N.; Neyshabouri, M.; Sepaskhah, A.R. and Soltani, A.F.S.H.I.N. (2009). Physiological and morphological responses of rice (*Oryza sativa* L.) to varying water stress management strategies. **3**(4): 1735-8043.
- Debnath, S.; Kant, A.; Bhowmick, P.; Malakar, A.; Purkaystha, S.; Jena, B.K. and Hasan, R. (2023). The Enhanced Affinity of WRKY Reinforces Drought Tolerance in *Solanum lycopersicum* L.: *An Innovative Bioinformatics Study*. **12**: 762.
- Dellaporta, S.L.; Wood, J. and Hicks, J.B. (1983). A plant DNA miniprep: version II. *Plant Molecular Biology Reporter*, **1**: 19-21.
- Dixit, S.; Grondin, A.; Lee, C.R.; Henry, A.; Olds, T.M. and Kumar, A. (2015). Understanding rice adaptation to varying agro-ecosystems: trait interactions and quantitative trait loci. *BMC Genetics*, **16**: 1-14.

- Dixit, S.; Huang, B.E.; Sta Cruz, M.T.; Maturan, P.T.; Ontoy, J.C.E. and Kumar, A. (2014). QTLs for tolerance of drought and breeding for tolerance of abiotic and biotic stress: an integrated approach. *PLoS One*, **9**(10): 109574.
- Dixit, S.; Singh, A.; Sandhu, N.; Bhandari, A.; Vikram, P. and Kumar, A. (2017). Combining drought and submergence tolerance in rice: marker-assisted breeding and QTL combination effects. *Molecular Breeding*, **37**: 1-12.
- Dixit, S.; Singh, A.; Sta Cruz, M.T.; Maturan, P.T.; Amante, M. and Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *Bmc Genetics*, **15**: 1-13.
- Dixit, S.; Swamy, B.M.; Vikram, P.; Ahmed, H.U.; Sta Cruz, M.T.; Amante, M. and Kumar, A. (2012). Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. *Theoretical and Applied Genetics*, **125**: 155-169.
- Doyle, J. (1991). DNA protocols for plants. In *Molecular techniques in taxonomy* (pp. 283-293). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Dubouzet, J.G.; Sakuma, Y.; Ito, Y.; Kasuga, M.; Dubouzet, E.G.; Miura, S. and Yamaguchi-Shinozaki, K. (2003). OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt-and cold-responsive gene expression. *The Plant Journal*, **33**(4): 751-763.
- Ellenberger, T. (1994). Getting a grip on DNA recognition: structures of the basic region leucine zipper, and the basic region helix-loop-helix DNA-binding domains. *Current Opinion in Structural Biology*, **4**(1): 12-21.
- Ernst, H.A.; Nina Olsen, A.; Skriver, K.; Larsen, S. and Lo Leggio, L. (2004). Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors. *EMBO Reports*, **5**(3): 297-303.
- Fan, W.; Zhao, M.; Li, S.; Bai, X.; Li, J.; Meng, H. and Mu, Z. (2016). Contrasting transcriptional responses of PYR1/PYL/RCAR ABA receptors to ABA or dehydration stress between maize seedling leaves and roots. *BMC Plant Biology*, **16**: 1-14.
- Farooq, M.; Kobayashi, N.; Ito, O.; Wahid, A. and Serraj, R. (2010). Broader leaves result in better performance of indica rice under drought stress. *Journal of Plant Physiology*, **167**(13): 1066-1075.
- Farooq, M.; Wahid, A.; Kobayashi, N.S.M.A.; Fujita, D.B.S.M.A. and Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. *Sustainable Agriculture*, pp. 153-188.
- Farssi, O.; Mouradi, M.; Aziz, F. and Berrougui, H. (2022). Role of acid phosphatase and enzymatic and non-enzymatic antioxidant systems in tolerance of alfalfa (*Medicago sativa* L.) populations to low phosphorus availability. **20**(1).

- Ferris, R.; Long, L.; Bunn, S.M.; Robinson, K.M.; Bradshaw, H.D.; Rae, A.M. and Taylor, G. (2002). Leaf stomatal and epidermal cell development: identification of putative quantitative trait loci in relation to elevated carbon dioxide concentration in poplar. *Tree Physiology*, **22**(9): 633-640.
- Fischer, R.A. and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*, **29**(5): 897-912.
- Foley, J.A.; Ramankutty, N.; Brauman, K.A.; Cassidy, E.S.; Gerber, J.S.; Johnston, M. and Zaks, D.P. (2011). Solutions for a cultivated planet. *Nature*, **478**(7369): 337-342.
- Foolad, M.R.; Subbiah, P. and Zhang, L. (2008). Common QTL affect the rate of tomato seed germination under different stress and nonstress conditions. *International Journal of Plant Genomics*, 2007.
- Ganguly, M.; Roychoudhury, A.; Sengupta, D.N.; Datta, S.K. and Datta, K. (2020). Independent overexpression of OsRab16A and AtDREB1A exhibit enhanced drought tolerance in transgenic aromatic rice variety PusaSugandhi 2. *Journal of Plant Biochemistry and Biotechnology*, **29**: 503-517.
- Ghimire, K.H.; Quiatchon, L.A.; Vikram, P.; Swamy, B.M.; Dixit, S.; Ahmed, H. and Kumar, A. (2012). Identification and mapping of a QTL (qDTY1. 1) with a consistent effect on grain yield under drought. *Field Crops Research*, **131**: 88-96.
- Ghimire, K.H.; Quiatchon, L.A.; Vikram, P.; Swamy, B.M.; Dixit, S.; Ahmed, H. and Kumar, A. (2012). Identification and mapping of a QTL (qDTY1. 1) with a consistent effect on grain yield under drought. *Field Crops Research*, **131**: 88-96.
- Goswami, A.; Sharma, M.; Mrishra, M.; Kalita, D.; Das, B. and Buragohain, D. (2022). Statistical handbook of assam. **54**.
- Grant, V. (1975). Genetics of Flowering plants. Ed.1. Columbia University Press, New York
- Guang-heng, Z.H.A.N.G.; Guo-ping, Z.H.A.N.G.; Qian, Q.I.A.N.; Lu-ping, X.U.; Da-li, Z.E.N.G.; Sheng, T.E.N.G. and Jin-song, B.A.O. (2004). QTL analysis of grain shape traits in different environments. *Chinese Journal of Rice Science*, **18**(1): 16.
- Hackett, C.A. (2002). Statistical methods for QTL mapping in cereals. *Plant Molecular Biology*, **48**: 585-599.
- Haley, C.S. and Andersson, L. (1997). Linkage mapping of quantitative trait loci in plants and animals. *Genome mapping: a practical approach*. pp. 59-99.

- Han, B.; Wang, J.; Li, Y.; Ma, X.; Jo, S.; Cui, D. and Han, L. (2018). Identification of quantitative trait loci associated with drought tolerance traits in rice (*Oryza sativa* L.) under PEG and field drought stress. *Euphytica*, **214**: 1-16.
- Hao, Y.J.; Wei, W.; Song, Q.X.; Chen, H.W.; Zhang, Y.Q.; Wang, F. and Chen, S.Y. (2011). Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. *The Plant Journal*, **68**(2): 302-313.
- Harushima, Y.; Yano, M.; Shomura, A.; Sato, M.; Shimano, T.; Kuboki, Y. and Sasaki, T. (1998). A high-density rice genetic linkage map with 2275 markers using a single F2 population. *Genetics*, **148**(1): 479-494.
- Hu, H.; Dai, M.; Yao, J.; Xiao, B.; Li, X.; Zhang, Q. and Xiong, L. (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Sciences*, **103**(35): 12987-12992.
- Hussain, H.A.; Hussain, S.; Khaliq, A.; Ashraf, U.; Anjum, S.A.; Men, S. and Wang, L. (2018). Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. *Frontiers in Plant Science*, **9**: 393.
- Jaleel, C.A.; Manivannan, P.; Wahid, A.; Farooq, M.; Al-Juburi, H.J.; Somasundara, R. and Panneerselvam, R. (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, **11**: 100-105.
- Javed, M.A.; Huyop, F.Z.; Ishii, T.; ABD, A.; Samad, T.; Haider, M. S. and Saleem, M. (2013). Construction of microsatellite linkage map and detection of segregation distortion in indica rice (*Oryza sativa* L.). *Pakistan Journal of Botany*, **45**: 2085-2092.
- Jensen, M.K.; Lindemose, S.; de Masi, F.; Reimer, J.J.; Nielsen, M.; Perera, V. *et al.* (2013). ATAF1 transcription factor directly regulates abscisic acid biosynthetic gene NCED3 in *Arabidopsis thaliana*. *FEBS Open Bio*. **3**: 321-327.
- Jeong, J.S.; Park, Y.T.; Jung, H.; Park, S.H. and Kim, J.K. (2009). Rice NAC proteins act as homodimers and heterodimers. *Plant Biotechnology Reports*, **3**: 127-134.
- Ji, K.; Wang, Y.; Sun, W.; Lou, Q.; Mei, H.; Shen, S. and Chen, H. (2012). Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *Journal of Plant Physiology*, **169**(4): 336-344.

- Kaldate, R.; Verma, R.K.; Chetia, S.K.; Dey, P.C.; Mahalle, M.D.; Singh, S.K. and Modi, M.K. (2023). Mapping of QTLs associated with yield and related traits under reproductive stage drought stress in rice using SNP linkage map. *Molecular Biology Reports*, pp. 1-11.
- Kamoshita, A.; Wade, L.; Ali, M.; Pathan, M.; Zhang, J.; Sarkarung, S. and Nguyen, H. (2002). Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. *Theoretical and Applied Genetics*, **104**: 880-893.
- Khush, G.S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, **59**: 1-6.
- Kikuchi, K.; Ueguchi-Tanaka, M.; Yoshida, K.T.; Nagato, Y.; Matsusoka, M. and Hirano, H.Y. (2000). Molecular analysis of the NAC gene family in rice. *Molecular and General Genetics MGG*, **262**: 1047-1051.
- Kim, J.S.; Mizoi, J.; Yoshida, T.; Fujita, Y.; Nakajima, J.; Ohori, T. and Yamaguchi-Shinozaki, K. (2011). An ABRE promoter sequence is involved in osmotic stress-responsive expression of the DREB2A gene, which encodes a transcription factor regulating drought-inducible genes in Arabidopsis. *Plant and Cell Physiology*, **52**(12): 2136-2146.
- Kim, Y.; Chung, Y.S.; Lee, E.; Tripathi, P.; Heo, S. and Kim, K.H. (2020). Root response to drought stress in rice (*Oryza sativa* L.). *International Journal of Molecular Sciences*, **21**(4): 1513.
- Kim, Y.J.; Kim, S.I.; Kesavan, M.; Kwak, J.S.; Song, J.T. and Seo, H.S. (2015). Ascorbate peroxidase OsAPx1 is involved in seed development in rice. **11**(2).
- Koussevitzky, S.; Suzuki, N.; Huntington, S.; Armijo, L.; Sha, W.; Cortes, D. and Mittler, R. (2008). Ascorbate peroxidase 1 plays a key role in the response of Arabidopsis thaliana to stress combination. *Journal of Biological Chemistry*, **283**(49): 34197-34203.
- Kumar, A.; Basu, S.; Ramegowda, V. and Pereira, A. (2017). Mechanisms of drought tolerance in rice. *BurleighDodds Sci. Publ. Ltd*, pp. 131-163.
- Kumar, A.; Basu, S.; Ramegowda, V. and Pereira, A. (2017). Mechanisms of drought tolerance in rice. *BurleighDodds Sci. Publ. Ltd*, pp. 131-163.
- Kumar, A.; Bernier, J.; Verulkar, S.; Lafitte, H.R. and Atlin, G.N. (2008). Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crops Research*, **107**(3): 221-231.

- Kumar, A.; Dixit, S.; Ram, T.; Yadav, R.B.; Mishra, K.K. and Mandal, N.P. (2014). Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. *Journal of Experimental Botany*, **65**(21): 6265-6278.
- Kumar, A.; Sandhu, N.; Dixit, S.; Yadav, S.; Swamy, B.P.M. and Shamsudin, N.A.A. (2018). Marker-assisted selection strategy to pyramid two or more QTLs for quantitative trait-grain yield under drought. *Rice*, **11**(1): 1-16.
- Lanceras, J.C.; Pantuwan, G.; Jongdee, B. and Toojinda, T. (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiology*, **135**(1): 384-399.
- Lander, E.S. and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, **121**(1): 185-199.
- Lang, N.T.; Nha, C.T.; Ha, P.T.T. and Buu, B.C. (2013). Quantitative trait loci (QTLs) associated with drought tolerance in rice (*Oryza sativa* L.). *SABRAO Journal of Breeding and Genetics*, **45**(3): 409-421.
- Lazzarotto, F.; Menguer, P.K.; Del-Bem, L.E.; Zámocký, M. and Margis-Pinheiro, M. (2021). Ascorbate peroxidase neofunctionalization at the origin of APX-R and APX-L: Evidence from basal Archaeplastida. *Antioxidants*, **10**(4): 597.
- Lee, S. J., Kang, J. Y., Park, H. J., Kim, M. D., Bae, M. S., Choi, H. I., & Kim, S. Y. (2010). DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression and its overexpression affects abscisic acid sensitivity. *Plant Physiology*, **153**(2): 716-727.
- Li, C.; Bai, G.; Chao, S. and Wang, Z. (2015). A high-density SNP and SSR consensus map reveals segregation distortion regions in wheat. *BioMed Research International*,
- Li, M.; Hou, L.; Liu, S.; Zhang, C.; Yang, W.; Pang, X. and Li, Y. (2021). Genome-wide identification and expression analysis of NAC transcription factors in *Ziziphus jujuba* Mill. reveal their putative regulatory effects on tissue senescence and abiotic stress responses. *Industrial Crops and Products*, **173**: 114093.
- Lin, M.H.; Lin, C.W.; Chen, J.C.; Lin, Y.C.; Cheng, S.Y.; Liu, T.H. and Ku, H.M. (2007). Tagging rice drought-related QTL with SSR DNA markers. **4**(1): 65-76.
- Liu, C.; Mao, B.; Ou, S.; Wang, W.; Liu, L.; Wu, Y. and Wang, X. (2014). OsbZIP71, a bZIP transcription factor, confers salinity and drought tolerance in rice. *Plant Molecular Biology*, **84**: 19-36.
- Liu, C.; Schläppi, M.R.; Mao, B.; Wang, W.; Wang, A. and Chu, C. (2019). The bZIP 73 transcription factor controls rice cold tolerance at the reproductive stage. *Plant Biotechnology Journal*, **17**(9): 1834-1849.

- Longkho, K. (2023). QTL mapping for drought tolerance in rice (*Oryza sativa* L.) Ph.D Thesis, Assam Agricultural University, Jorhat.
- Lorieux, M.; Goffinet, B.; Perrier, X.; De Leon, D.G. and Lanaud, C. (1995). Maximum-likelihood models for mapping genetic markers showing segregation distortion. 1. Backcross populations. *Theoretical and Applied Genetics*, **90**: 73-80.
- Lou, J.; Chen, L.; Yue, G.; Lou, Q.; Mei, H.; Xiong, L. and Luo, L. (2009). QTL mapping of grain quality traits in rice. *Journal of Cereal Science*, **50**(2): 145-151.
- Lyttle, T.W. (1991). Segregation distorters. *Annual Review of Genetics*, **25**(1): 511-581.
- Ma, H.; Chen, J.; Zhang, Z.; Ma, L.; Yang, Z. Zhang, Q. and Wang, S. (2017). MAPK kinase 10.2 promotes disease resistance and drought tolerance by activating different MAPKs in rice. *The Plant Journal*, **92**(4): 557-570.
- MacMillan, K.; Emrich, K.; Piepho, H.P.; Mullins, C.E. and Price, A.H. (2006). Assessing the importance of genotype× environment interaction for root traits in rice using a mapping population II: conventional QTL analysis. *Theoretical and Applied Genetics*, **113**: 953-964.
- Mahajan, S. and Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*, **444**(2): 139-158.
- Mahalle, M.D.; Dey, P.C.; Chetia, S.K.; Baruah, A.R.; Ahmed, T.; Sarma, R.N. and Modi, M.K. (2021). Association mapping for yield traits under drought stress in Autumn rice germplasm collection of Assam. *Journal of Plant Biochemistry and Biotechnology*, **30**: 26-36.
- Manivannan, P.; Jaleel, C.A.; Sankar, B.; Kishorekumar, A.; Somasundaram, R.; Lakshmanan, G.A. and Panneerselvam, R. (2007). Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. *Colloids and Surfaces B: Biointerfaces*, **59**(2): 141-149.
- Manna, M.; Thakur, T.; Chirom, O.; Mandlik, R.; Deshmukh, R. and Salvi, P. (2021). Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. *Physiologia Plantarum*, **172**(2): 847-868.
- Mao, X.; Zhang, H.; Qian, X.; Li, A.; Zhao, G. *et al.* (2012). TaNAC2, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in *Arabidopsis*. *J. Exp. Bot.* **63**: 2933-2946.
- Mather, K. (1938). The measurement of linkage in heredity. *The measurement of linkage in heredity*. p. 132.
- Matsukura, S.; Mizoi, J.; Yoshida, T.; Todaka, D.; Ito, Y.; Maruyama, K. and Yamaguchi-Shinozaki, K. (2010). Comprehensive analysis of rice DREB2-type genes that encode transcription factors involved in the expression of abiotic stress-responsive genes. *Molecular Genetics and Genomics*, **283**: 185-196.

- 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**:11-3.
- Menezes-Benavente, L.; Teixeira, F.K.; Kamei, C.L.A. and Margis-Pinheiro, M. (2004). Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.). *Plant Science*, **166**(2): 323-331.
- Meng, C.; Cai, C.; Zhang, T. and Guo, W. (2009). Characterization of six novel NAC genes and their responses to abiotic stresses in *Gossypium hirsutum* L. *Plant Science*, **176**(3): 352-359.
- 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): 269-283.
- Miller, G.A.D.; Suzuki, N.; Ciftci-Yilmaz, S.U.L.T.A.N. and Mittler, R.O.N. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment*, **33**(4): 453-467.
- Mishra, K.K.; Vikram, P.; Yadaw, R.B.; Swamy, B.M.; Dixit, S.; Cruz, M.T.S.; Maturan, P.; Marker, S. and Kumar, A. (2013). qDTY 12. 1: a locus with a consistent effect on grain yield under drought in rice. *BMC Genetics*, **14**(1): 12.
- Mishra, K.K.; Vikram, P.; Yadaw, R.B.; Swamy, B.P.; Dixit, S.; Cruz, M.T.S. and Kumar, A. (2013). qDTY12. 1: a locus with a consistent effect on grain yield under drought in rice. *BMC Genetics*, **14**(1): 1-10.
- Mishra, K.K.; Vikram, P.; Yadaw, R.B.; Swamy, B.P.; Dixit, S.; Cruz, M.T.S. and Kumar, A. (2013). qDTY12. 1: a locus with a consistent effect on grain yield under drought in rice. *BMC Genetics*, **14**(1): 1-10.
- Mishra, S.S.; Behera, P.K. and Panda, D. (2019). Genotypic variability for drought tolerance-related morpho-physiological traits among indigenous rice landraces of Jeypore tract of Odisha, India. *Journal of Crop Improvement*, **33**(2): 254-278.
- Mittler, R.; Vanderauwera, S.; Gollery, M. and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science*, **9**(10): 490-498.
- Mizoi, J.; Shinozaki, K. and Yamaguchi-Shinozaki, K. (2012). AP2/ERF family transcription factors in plant abiotic stress responses. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, **1819**(2): 86-96.
- Monkham, T.; Jongdee, B.; Pantuwan, G.; Sanitchon, J.; Mitchell, J.H. and Fukai, S. (2015). Genotypic variation in grain yield and flowering pattern in terminal and intermittent drought screening methods in rainfed lowland rice. *Field Crops Research*, **175**: 26-36.

- Mousavi, S.S.; Yazdi, S.B.; Naghavi, M.R.; Zali, A.A.; Dashti, H. and Pourshahbazi, A. (2008). Introduction of new indices to identify relative drought tolerance and resistance in wheat genotypes. pp. 165-178.
- Nahar, S.; Kalita, J.; Sahoo, L. and Tanti, B. (2016). Morphophysiological and molecular effects of drought stress in rice. *Ann Plant Sci*, **5**(9): 1409-1416.
- Nakashima, K.; Fujita, Y.; Kanamori, N.; Katagiri, T.; Umezawa, T.; Kidokoro, S. and Yamaguchi-Shinozaki, K. (2009). Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2. 2, SRK2E/SnRK2. 6/OST1 and SRK2I/SnRK2. 3, involved in ABA signaling are essential for the control of seed development and dormancy. *Plant and Cell Physiology*, **50**(7): 1345-1363.
- Nakashima, K.; Ito, Y. and Yamaguchi-Shinozaki, K. (2009). Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiology*, **149**(1): 88-95.
- Nakashima, K.; Shinwari, Z.K.; Sakuma, Y.; Seki, M.; Miura, S.; Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000). Organization and expression of two Arabidopsis DREB2 genes encoding DRE-binding proteins involved in dehydration-and high-salinity-responsive gene expression. *Plant Molecular Biology*, **42**: 657-665.
- Nakashima, K.; Takasaki, H.; Mizoi, J.; Shinozaki, K. and Yamaguchi-Shinozaki, K. (2012). NAC transcription factors in plant abiotic stress responses. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, **1819**(2): 97-103.
- Nakashima, K.; Takasaki, H.; Mizoi, J.; Shinozaki, K. and Yamaguchi-Shinozaki, K. (2012). NAC transcription factors in plant abiotic stress responses. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, **1819**(2): 97-103.
- Narusaka, Y.; Nakashima, K.; Shinwari, Z.K.; Sakuma, Y.; Furihata, T.; Abe, H. and Yamaguchi Shinozaki, K. (2003). Interaction between two cisacting elements, ABRE and DRE, in ABA dependent expression of Arabidopsis rd29A gene in response to dehydration and high salinity stresses. *The Plant Journal*, **34**(2): 137-148.
- Oladosu, Y.; Rafii, M.Y.; Samuel, C.; Fatai, A.; Magaji, U.; Kareem, I. and Kolapo, K. (2019). Drought resistance in rice from conventional to molecular breeding: a review. *International Journal of Molecular Sciences*, **20**(14): 3519.
- Oo, K.S.; Krishnan, S.G.; Vinod, K.K.; Dhawan, G.; Dwivedi, P.; Kumar, P. and Singh, A.K. (2021). Molecular breeding for improving productivity of *Oryza sativa* L. cv. Pusa 44 under reproductive stage drought stress through introgression of a major QTL, qDTY12. 1. *Genes*, **12**(7): 967.

- Palanog, A.D.; Swamy, B.M.; Shamsudin, N.A.A.; Dixit, S.; Hernandez, J.E.; Boromeo, T.H. and Kumar, A. (2014). Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. *Field Crops Research*, **161**: 46-54.
- Palumbo, F.; Qi, P.; Pinto, V.B.; Devos, K.M. and Barcaccia, G. (2019). Construction of the first SNP-based linkage map using genotyping-by-sequencing and mapping of the male-sterility gene in leaf chicory. *Frontiers in Plant Science*, **10**: 276.
- Panda, D.; Mishra, S.S. and Behera, P.K. (2021). Drought tolerance in rice: focus on recent mechanisms and approaches. *Rice Science*, **28**(2): 119-132.
- Pandey, S. and Bhandari, H. (2009). Drought: economic costs and research implications. In: *Drought frontiers in rice: crop improvement for increased rainfed production*. World Scientific, pp. 3-17.
- Pandey, V. and Shukla, A. (2015). Acclimation and tolerance strategies of rice under drought stress. *Rice Science*, **22**(4): 147-161.
- Parida, B.R. and Oinam, B. (2015). Unprecedented drought in North East India compared to western India. *Current Science*, pp. 2121-2126.
- Parray, R.A. (2019). Genetic study for improving under drought stress environment in rice of Assam. Ph.D Thesis. Assam Agricultural University, Jorhat.
- Paterson, A.H. (2002). What has QTL mapping taught us about plant domestication? *New Phytologist*, **154**(3): 591-608
- Plomion, C.; O'Malley, D.M. and Durel, C.E. (1995). Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. *Theoretical and Applied Genetics*, **90**: 1028-1034.
- Prakash, C.; Sevanthi, A.M. and Shanmugavadivel, P.S. (2019). Use of QTLs in developing abiotic stress tolerance in rice. In *Advances in Rice Research for Abiotic Stress Tolerance*. pp. 869-893.
- Prathap, V.; Ali, K.; Singh, A.; Vishwakarma, C.; Krishnan, V.; Chinnusamy, V. and Tyagi, A. (2019). Starch accumulation in rice grains subjected to drought during grain filling stage. *Plant Physiol. Biochem.* **142**: 440-451.
- Prathap, V.; Ali, K.; Singh, A.; Vishwakarma, C.; Krishnan, V.; Chinnusamy, V. and Tyagi, A. (2019). Starch accumulation in rice grains subjected to drought during grain filling stage. *Plant Physiology and Biochemistry*, **142**: 440-451.

- Price, A.H.; Steele, K.A.; Moore, B.J.; Barraclough, P.P. and Clark, L.J. (2000). A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability. *Theoretical and Applied Genetics*, **100**: 49-56.
- Puranik, S.; Sahu, P.P.; Srivastava, P.S. and Prasad, M. (2012). NAC proteins: regulation and role in stress tolerance. *Trends in Plant Science*, **17**(6): 369-381.
- Qin, F.; Kakimoto, M.; Sakuma, Y.; Maruyama, K.; Osakabe, Y.; Tran, L.S.P. and Yamaguchi-Shinozaki, K. (2007). Regulation and functional analysis of ZmDREB2A in response to drought and heat stresses in *Zea mays* L. *The Plant Journal*, **50**(1): 54-69.
- Qu, P.; Shi, J.; Chen, T.; Chen, K.; Shen, C.; Wang, J. and Zhang, L. (2020). Construction and integration of genetic linkage maps from three multi-parent advanced generation intercross populations in rice. *Rice*, **13**: 1-16.
- Qu, X.; Zou, J.; Wang, J.; Yang, K.; Wang, X. and Le, J. (2022). A rice R2R3-type MYB transcription factor OsFLP positively regulates drought stress response via OsNAC. *International Journal of Molecular Sciences*, **23**(11): 5873.
- Qu, Y.; Mu, P.; Zhang, H.; Chen, C.Y.; Gao, Y.; Tian, Y. and Li, Z. (2008). Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica*, **133**: 187-200.
- Quan, R.; Hu, S.; Zhang, Z.; Zhang, H.; Zhang, Z. and Huang, R. (2010). Overexpression of an ERF transcription factor TSRF1 improves rice drought tolerance. *Plant Biotechnology Journal*, **8**(4): 476-488.
- Qun, X.U.; Yuan, X. P.; Yu, H.Y.; Wang, Y.P.; Tang, S.X. and Wei, X.H. (2011). Mapping QTLs for drought tolerance at seedling stage in rice using doubled haploid population. *Rice Science*, **18**(1): 23-28.
- Rajurkar, A.B.; Muthukumar, C.; Bharathi, A.; Thomas, H.B. and Babu, R.C. (2019). Saturation mapping of consistent QTLs for yield and days to flowering under drought using locally adapted landrace in rice (*Oryza sativa* L.). *NJAS-Wageningen Journal of Life Sciences*, **88**: 66-75.
- Raman, A.; Verulkar, S.; Mandal, N.; Variar, M.; Shukla, V.; Dwivedi, J. and Kumar, A. (2012). Drought yield index to select high yielding rice lines under different drought stress severities. *Rice*, **5**(1): 1-12.
- Ramchander, S.; Raveendran, M. and Robin, S. (2016). Mapping QTLs for physiological traits associated with drought tolerance in rice (*Oryza sativa* L.). *J. Investig Genom*, **3**(3): 00052.
- Rasheed, A.; Hao, Y.; Xia, X.; Khan, A.; Xu, Y.; Varshney, R.K. and He, Z. (2017). Crop Breeding Chips and Genotyping Platforms: Progress, Challenges, and Perspectives. *Molecular Plant*, **10**(8): 1047-1064.

- Redillas, M.C.; Jeong, J.S.; Kim, Y.S.; Jung, H.; Bang, S.W.; Choi, Y.D. and Kim, J.K. (2012). The overexpression of OsNAC9 alters the root architecture of rice plants enhancing drought resistance and grain yield under field conditions. *Plant Biotechnology Journal*, **10**(7): 792-805.
- Rehman, M. and Tanti, B. (2021). Screening of boro rice varieties of Assam, India to estimate their potential resistance to cold and heat stresses. *Vegetos*, **34**(3): 540-554.
- Rosielle, A.A. and Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non stress environment 1. *Crop Science*, **21**(6): 943-946.
- Sahebi, M.; Hanafi, M.M.; Rafii, M.Y.; Mahmud, T.M.M.; Azizi, P.; Osman, M. and Atabaki, N. (2018). Improvement of drought tolerance in rice (*Oryza sativa* L.): genetics, genomic tools and the WRKY gene family. *BioMed Research International*, pp. 1-20.
- Sahu, V.K.; Nair, K.; Vishwakarma, A.K.; Verulkar, S.B. and Chandel, G. (2017). QTL hotspots detected for yield contributing traits in rice (*Oryza Sativa* L.) using composite interval mapping analysis. *Biosciences Biotechnology Research Asia*, **14**(1): 329.
- Salleh, M.S.; Malek, R.A.; Shahari, R. and Nordin, M.S. (2020). Screening rice (*Oryza sativa* L.) genotypes for resistance against drought. *Water Conservation & Management (WCM)*, **4**(2): 78-82.
- Sandhu, N.; Dixit, S.; Swamy, B.P.M.; Raman, A.; Kumar, S.; Singh, S.P. and Kumar, A. (2019). Marker assisted breeding to develop multiple stress tolerant varieties for flood and drought prone areas. *Rice*, **12**: 1-16.
- 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**: 1-15.
- Sandhu, N.; Torres, R.O.; Sta Cruz, M.T.; Maturan, P.C.; Jain, R.; Kumar, A. and Henry, A. (2015). Traits and QTLs for development of dry direct-seeded rainfed rice varieties. *Journal of Experimental Botany*, **66**(1): 225-244.
- Sandhya, J.; Ashwini, T.; Manisha, R.; Vinodha, M. and Srinivas, A. (2021). Drought tolerance enhancement with co-Overexpression of DREB2A and APX in indica rice (*Oryza sativa* L.). *American Journal of Plant Sciences*, **12**(2): 234-258.
- Satrio, R.D.; Fendiyanto, M.H.; Supena, E.D.J.; Suharsono, S. and Miftahudin, M. (2021). Genome-wide SNP discovery, linkage mapping, and analysis of QTL for morpho-physiological traits in rice during vegetative stage under drought stress. *Physiology and Molecular Biology of Plants*, **27**(11): 2635-2650.

- Sellamuthu, R.; Liu, G.F.; Ranganathan, C.B. and Serraj, R. (2011). Genetic analysis and validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid line population of rice (*Oryza sativa* L.). *Field Crops Research*, **124**(1): 46-58.
- Seo, P.J.; Kim, S.G. and Park, C.M. (2008). Membrane-bound transcription factors in plants. *Trends in Plant Science*, **13**(10): 550-556.
- SES, I. (2013). Standard evaluation system for rice. *International Rice Research Institute, Philippines*.
- Shamsudin, N.A.A.; Swamy, B.M.; Ratnam, W.; Sta. Cruz, M.T.; Sandhu, N.; Raman, A.K. and Kumar, A. (2016). Pyramiding of drought yield QTLs into a high quality Malaysian rice cultivar MRQ74 improves yield under reproductive stage drought. *Rice*, **9**: 1-13.
- Shanmugavadivel, P.S.; Mithra, S.A.; Dokku, P.; Kumar, K.A.R.; Rao, G.J.N.; Singh, V. P. and Mohapatra, T. (2013). Mapping quantitative trait loci (QTL) for grain size in rice using a RIL population from Basmati× indica cross showing high segregation distortion. *Euphytica*, **194**: 401-416.
- Shanmugavadivel, P.S.; Mithra, S.A.; Dokku, P.; Kumar, K.A.R.; Rao, G.J.N.; Singh, V.P. and Mohapatra, T. (2013). Mapping quantitative trait loci (QTL) for grain size in rice using a RIL population from Basmati× indica cross showing high segregation distortion. *Euphytica*, **194**: 401-416.
- Shao, H.; Wang, H. and Tang, X. (2015). NAC transcription factors in plant multiple abiotic stress responses: progress and prospects. *Frontiers in Plant Science*, **6**: 902.
- Simms, D.; Cizdziel, P.E. and Chomczynski, P. (1993). TRIzol: A new reagent for optimal single-step isolation of RNA. *Focus*, **15**(4): 532-535.
- Singh, A.; Sengar, K. and Sengar, R.S. (2013). Gene regulation and biotechnology of drought tolerance in rice. *Int. J. Biotechnol. Bioeng. Res.*, **4**(6): 547-552.
- Singh, C.S.; Kumar, B.; Mehandi and Chandra K. (2012). Effect of Drought Stress in Rice: A Review on Morphological and Physiological Characteristics. *Trends in Biosciences*. **5**(4): 261-265.
- Singh, K. and Chandra, A. (2021). DREBs-potential transcription factors involve in combating abiotic stress tolerance in plants. *Biologia*, **76**(10): 3043-3055.
- Singh, P.K.; Indoliya, Y.; Agrawal, L.; Awasthi, S.; Deeba, F.; Dwivedi, S. and Tripathi, R.D. (2022). Genomic and proteomic responses to drought stress and biotechnological interventions for enhanced drought tolerance in plants. *Current Plant Biology*, **29**: 100239.

- Singh, R.; Singh, Y.; Xalaxo, S.; Verulkar, S.; Yadav, N.; Singh, S. and Singh, N.K. (2016). From QTL to variety-harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network. *Plant Science*, **242**: 278-287.
- Sokoto, M.B. (2014). Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. *Journal of Biosciences and Medicines*, **2**(01): 68.
- Solis, J.; Gutierrez, A.; Mangu, V.; Sanchez, E.; Bedre, R.; Linscombe, S. and Baisakh, N. (2018). Genetic mapping of quantitative trait loci for grain yield under drought in rice under controlled greenhouse conditions. *Frontiers in Chemistry*, **5**: 129.
- Song, X.; Li, Y. and Hou, X. (2013). Genome-wide analysis of the AP2/ERF transcription factor superfamily in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *BMC Genomics*, **14**: 1-15.
- Subashri, M.; Robin, S.; Vinod, K.K.; Rajeswari, S.; Mohanasundaram, K. and Raveendran, T.S. (2009). Trait identification and QTL validation for reproductive stage drought resistance in rice using selective genotyping of near flowering RILs. *Euphytica*, **166**: 291-305.
- Sudheesh, S.; Lombardi, M.; Leonforte, A.; Cogan, N.O.; Materne, M.; Forster, J.W. and Kaur, S. (2015). Consensus genetic map construction for field pea (*Pisum sativum* L.), trait dissection of biotic and abiotic stress tolerance and development of a diagnostic marker for the *er1* powdery mildew resistance gene. *Plant Molecular Biology Reporter*, **33**: 1391-1403.
- Swamy, B.P.; Shamsudin, N.A.A.; Rahman, S.N.A.; Mauleon, R.; Ratnam, W.; Cruz, S. and Kumar, A. (2017). Association mapping of yield and yield-related traits under reproductive stage drought stress in rice (*Oryza sativa* L.). *Rice*, **10**(1): 1-13.
- Swamy, B.P.M.; Ahmed, H.U.; Henry, A.; Mauleon, R.; Dixit, S.; Vikram, P. and Kumar, A. (2013). Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. *PloS one*, **8**(5): 62795.
- Taiz, L. and Zeiger, E. (2006). Secondary metabolites and plant defense. *Plant Physiology*, **4**: 315-344.
- Tang, N.; Zhang, H.; Li, X.; Xiao, J. and Xiong, L. (2012). Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice. *Plant Physiology*, **158**(4): 1755-1768.
- Tang, Y.; Bao, X.; Zhi, Y.; Wu, Q.; Guo, Y.; Yin, X. and Liu, K. (2019). Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice. *Frontiers in Plant Science*, **10**: 168.

- Teixeira, F.K.; Menezes-Benavente, L.; Galvão, V.C.; Margis, R. and Margis-Pinheiro, M. (2006). Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. *Planta*, **224**: 300-314.
- Teng, K.; Li, J.; Liu, L.; Han, Y.; Du, Y.; Zhang, J. and Zhao, Q. (2014). Exogenous ABA induces drought tolerance in upland rice: the role of chloroplast and ABA biosynthesis-related gene expression on photosystem II during PEG stress. *Acta Physiologiae Plantarum*, **36**: 2219-2227.
- Tian, Y.; Zhang, H.; Xu, P.; Chen, X.; Liao, Y.; Han, B. and Wu, X. (2015). Genetic mapping of a QTL controlling leaf width and grain number in rice. *Euphytica*, **202**: 1-11.
- Todaka, D.; Shinozaki, K. and Yamaguchi-Shinozaki, K. (2015). Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Frontiers in Plant Science*, **6**: 84.
- Ubaidillah, M.; Kim, K.A.; Kim, Y.H.; Lee, I.J.; Yun, B.W.; Kim, D.H. and Kim, K.M. (2013). Identification of a drought-induced rice gene, OsSAP, that suppresses Bax-induced cell death in yeast. *Molecular Biology Reports*, **40**: 6113-6121.
- Umezawa, T.; Fujita, M.; Fujita, Y.; Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006). Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology*, **17**(2): 113-122.
- Upadhyaya, H. and Panda, S.K. (2019). Drought stress responses and its management in rice. In *Advances in Rice Research for Abiotic Stress Tolerance*. pp. 177-200.
- Usman, M.; Raheem, Z.; Ahsan, T.; Iqbal, A.; Sarfaraz, Z.N. and Haq, Z. (2013). Morphological, physiological and biochemical attributes as indicators for drought tolerance in rice (*Oryza sativa* L.). *European Journal of Biological Sciences*, **5**(1): 23-28.
- Vemireddy, L.R.; Noor, S.; Satyavathi, V.V.; Srividhya, A.; Kaliappan, A.; Parimala, S.R. N. and Nagaraju, J. (2015). Discovery and mapping of genomic regions governing economically important traits of Basmati rice. *BMC Plant Biology*, **15**: 1-19.
- Venuprasad, R.; Bool, M.; Quiatchon, L. and Atlin, G. (2012a). A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds. *Theoretical Applied Genetics*, **124**(2): 323-332.
- Venuprasad, R.; Bool, M.; Quiatchon, L.; Cruz, M. S.; Amante, M. and Atlin, G. (2012b). A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. *Molecular Breeding*, **30**(1): 535-547.

- Venuprasad, R.; Dalid, C.O.; Del Valle, M.; Zhao, D.; Espiritu, M.; Sta Cruz, M.T. 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**: 177-190.
- 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 largeeffect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *TAG. Theoretical and applied genetics. Theoretische Undangewandte Genetik*, **120**(1): 177-190.
- Verulkar, S.B. and Verma, S.K. (2014). Screening protocols in breeding for drought tolerance in rice. *Agricultural Research*, **3**: 32-40.
- Vikram, P.; Swamy, B.M.; Dixit, S.; Ahmed, H.U.; Teresa Sta Cruz, M.; Singh, A.K. and Kumar, A. (2011). *qDTY 1.1*, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics*, **12**: 1-15.
- Vikram, P.; Swamy, B.M.; Dixit, S.; Ahmed, H.U.; Teresa Sta Cruz, M.; Singh, A.K. and Kumar, A. (2011). *qDTY 1.1*, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics*, **12**: 1-15.
- Vikram, P.; Swamy, B.M.; Dixit, S.; Singh, R.; Singh, B.P.; Miro, B. and Kumar, A. (2015). Drought susceptibility of modern rice varieties: an effect of linkage of drought tolerance with undesirable traits. *Scientific Reports*, **5**(1): 14799.
- Vikram, P.; Swamy, B.M.; Dixit, S.; Trinidad, J.; Sta Cruz, M.T.; Maturan, P.C. and Kumar, A. (2016). Linkages and interactions analysis of major effect drought grain yield QTLs in rice. *PLoS One*, **11**(3): 0151532.
- Villalta, I.; Reina-Sanchez, A.; Cuartero, J.; Carbonell, E.A. and Asins, M.J. (2005). Comparative microsatellite linkage analysis and genetic structure of two populations of F 6 lines derived from *Lycopersicon pimpinellifolium* and *L. cheesmanii*. *Theoretical and Applied Genetics*, **110**: 881-894.
- Vinod, K.K.; Krishnan, S.G.; Thribhuvan, R. and Singh, A.K. (2019). Genetics of drought tolerance, mapping QTLs, candidate genes and their utilization in rice improvement. *Genomics Assisted Breeding of Crops for Abiotic Stress Tolerance*, **II**: 145-186.
- Wang, F.Z.; Wang, Q.B.; Kwon, S.Y.; Kwak, S.S. and Su, W.A. (2005). Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. *Journal of Plant Physiology*, **162**(4): 465-472.

- Wang, J.H.; Geng, L.H. and Zhang, C.M. (2012). Research on the weak signal detecting technique for crop water stress based on wavelet denoising. *Advanced Materials Research*, **424**: 966-970.
- Wei, S.; Hu, W.; Deng, X.; Zhang, Y.; Liu, X.; Zhao, X. and He, G. (2014). A rice calcium-dependent protein kinase OsCPK9 positively regulates drought stress tolerance and spikelet fertility. *BMC Plant Biology*, **14**(1): 1-13.
- Wilkinson, S. and Davies, W.J. (2010). Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant, Cell and Environment*, **33**(4): 510-525.
- Wu, A.; Allu, A.D.; Garapati, P.; Siddiqui, H.; Dortay, H. *et al.* (2012). Jungbrunnen1, a reactive oxygen species responsive NAC transcription factor, regulates longevity in *Arabidopsis*. *Plant Cell*. **24**: 482-506.
- Wu, Y.P.; Ko, P.Y.; Lee, W.C.; Wei, F.J.; Kuo, S.C.; Ho, S.W. and Lin, Y.R. (2010). Comparative analyses of linkage maps and segregation distortion of two F₂ populations derived from japonica crossed with indica rice. *Hereditas*, **147**(5): 225-236.
- Xu, F.; Huang, Y. and Bao, J. (2015). Identification of QTLs for agronomic traits in indica rice using an RIL population. *Genes & Genomics*, **37**: 809-817.
- Xu, G.Y.; Rocha, P.S.; Wang, M.L.; Xu, M.L.; Cui, Y.C.; Li, L.Y. and Xia, X. (2011). A novel rice calmodulin-like gene, OsMSR2, enhances drought and salt tolerance and increases ABA sensitivity in *Arabidopsis*. *Planta*, **234**: 47-59.
- Xu, Z.S.; Chen, M.; Li, L.C. and Ma, Y.Z. (2011). Functions and application of the AP2/ERF transcription factor family in crop improvement F. *Journal of Integrative Plant Biology*, **53**(7): 570-585.
- Xu, Z.Y.; Kim, S.Y.; Hyeon, D.Y.; Kim, D.H.; Dong, T.; Park, Y. and Hwang, I. (2013). The *Arabidopsis* NAC transcription factor ANAC096 cooperates with bZIP-type transcription factors in dehydration and osmotic stress responses. *The Plant Cell*, **25**(11): 4708-4724.
- Xu, Z.Y.; Kim, S.Y.; Hyeon, Do, Y.; Kim, D.H.; Dong, T.; Park, Y. *et al.* (2013). The *Arabidopsis* NAC transcription factor ANAC096 cooperates with bZIP-type transcription factors in dehydration and osmotic stress responses. *Plant Cell*. **25**: 4708-4724.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (2007). Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor. In *Novartis Foundation Symposium 236-Rice Biotechnology: Improving Yield, Stress Tolerance and Grain Quality: Rice Biotechnology: Improving Yield, Stress Tolerance and Grain Quality: Novartis Foundation Symposium*. **236**: 176-189.

- Yamaguchi-Shinozaki, K.; Koizumi, M.; Urao, S. and Shinozaki, K. (1992). Molecular cloning and characterization of 9 cDNAs for genes that are responsive to desiccation in *Arabidopsis thaliana*: Sequence analysis of one cDNA clone that encodes a putative transmembrane channel protein. *Plant and Cell Physiology*, **33**(3): 217-224.
- Yheni, D.; Kumar, A.; Thomas, J.; Ruiz, C.; Alkahtani, J.; Baisakh, N. and Pereira, A. (2021). "Quantitative trait loci and candidate gene identification for chlorophyll content in RIL rice population under drought conditions". *Indonesian Journal of Natural Pigments*. **3**(2): 54-54.
- Yue, B.; Xiong, L.; Xue, W.; Xing, Y.; Luo, L. and Xu, C. (2005). Genetic analysis for drought resistance of rice at reproductive stage in field with different types of soil. *Theoretical and Applied Genetics*, **111**: 1127-1136.
- Zhang, B.; Ye, W.; Ren, D.; Tian, P.; Peng, Y.; Gao, Y. and Gao, Z. (2015). Genetic analysis of flag leaf size and candidate genes determination of a major QTL for flag leaf width in rice. *Rice*, **8**: 1-10.
- Zhang, J.; Huang, D.; Zhao, X.; Zhang, M.; Wang, Q.; Hou, X. and Sun, P. (2022). Drought-responsive WRKY transcription factor genes IgWRKY50 and IgWRKY32 from *Iris germanica* enhance drought resistance in transgenic *Arabidopsis*. *Frontiers in Plant Science*, **13**: 983600.
- Zhang, J.; Zheng, H.G.; Aarti, A.; Pantuwan, G.; Nguyen, T.T.; Tripathy, J.N. and Nguyen, H.T. (2001). Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theoretical and applied Genetics*, **103**: 19-29.
- Zhang, M.; Fu, M.M.; Qiu, C.W.; Cao, F.; Chen, Z.H.; Zhang, G. and Wu, F. (2019). Response of Tibetan wild barley genotypes to drought stress and identification of quantitative trait loci by genome-wide association analysis. *International Journal of Molecular Sciences*, **20**(3): 791.
- Zhou, H.; Zhang, F.; Zhai, F.; Su, Y.; Zhou, Y.; Ge, Z. and Xie, Y. (2022). Rice GLUTATHIONE PEROXIDASE1-mediated oxidation of bZIP68 positively regulates ABA-independent osmotic stress signaling. *Molecular Plant*, **15**(4): 651-670.
- Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, **53**(1): 247-273.
- Zhu, R.; Wu, F.; Zhou, S.; Hu, T.; Huang, J. and Gao, Y. (2020). Cumulative effects of drought flood abrupt alternation on the photosynthetic characteristics of rice. *Environmental and Experimental Botany*, **169**: 103901.
- Zou, G.H.; Mei, H.W.; Liu, H.Y.; Liu, G.L.; Hu, S.P.; Yu, X.Q. and Luo, L.J. (2005). Grain yield responses to moisture regimes in a rice population: association among traits and genetic markers. *Theoretical and Applied Genetics*, **112**: 106-113.

APPENDIX I

Mean Performance of Phenotypic traits for 247 RILs under intermittent drought stress.

line no:	DFF	PH	FLL	FLW	FLLW	PL	NOT	EBT	NOG	NOC	TNG	SF	GY	GL	GW	GLW
2	80.00	119.67	43.67	1.37	31.95	22.00	5.33	4.67	55.67	6.33	62.00	89.78	4.25	8.03	2.13	3.77
3	82.33	106.67	34.67	1.33	26.00	20.00	3.33	2.67	66.33	20.00	86.33	76.83	6.42	7.87	2.63	2.99
4	80.67	136.33	26.00	1.00	26.00	20.33	8.33	7.33	25.00	18.00	43.00	58.14	3.98	8.10	2.53	3.20
5	80.67	137.33	41.00	1.20	34.17	26.67	4.00	4.00	26.00	6.00	32.00	81.25	1.90	7.50	2.17	3.46
6	80.33	122.00	26.33	2.10	12.54	20.00	7.00	6.00	58.00	17.67	75.67	76.65	1.58	7.73	2.40	3.22
7	79.00	112.33	41.00	2.03	20.16	22.00	6.00	6.00	48.67	2.67	51.33	94.81	1.78	7.87	2.33	3.37
8	80.67	134.00	20.00	2.50	8.00	19.00	7.33	5.33	42.33	13.00	55.33	76.51	3.92	7.80	2.77	2.82
9	82.00	109.33	23.67	1.03	22.90	19.00	7.00	6.67	37.67	7.67	45.33	83.09	4.78	7.40	2.40	3.08
10	82.00	103.00	32.00	1.33	24.00	20.00	5.33	5.00	40.33	29.33	69.66	57.90	6.76	7.60	2.60	2.92
11	79.00	138.67	26.00	2.00	13.00	19.00	9.67	9.00	64.00	9.33	73.33	87.27	1.15	6.80	2.60	2.62
12	82.33	116.33	36.00	2.03	17.70	22.67	4.33	3.67	25.00	8.00	33.00	75.76	3.11	8.00	2.70	2.96
13	82.33	110.33	33.67	1.27	26.58	25.00	5.67	4.67	37.67	10.67	48.33	77.93	6.52	7.70	2.33	3.30
14	82.00	117.67	25.67	1.33	19.25	19.00	3.33	2.67	40.67	17.00	57.67	70.52	7.23	6.63	2.23	2.97
15	74.67	101.33	22.67	1.00	22.67	19.00	6.67	6.00	49.00	14.00	63.00	77.78	3.48	7.47	2.57	2.91
19	78.67	132.67	42.00	1.33	31.50	33.67	5.00	4.67	34.67	19.00	53.67	64.60	3.33	7.67	2.23	3.43
22	79.67	127.67	25.00	1.20	20.83	24.50	7.33	5.67	32.33	16.00	48.33	66.90	7.61	7.20	2.40	3.00
24	78.00	117.67	36.33	1.33	27.25	24.00	2.67	2.67	41.00	18.00	59.00	69.49	7.57	7.83	2.30	3.41
27	80.00	140.33	24.67	1.50	16.44	19.00	4.00	3.67	26.33	6.00	32.33	81.44	4.99	7.03	2.40	2.93
28	81.00	114.00	34.00	1.20	28.33	19.33	6.00	5.67	36.33	11.33	47.67	76.22	1.56	7.80	2.03	3.84
30	82.67	97.00	38.00	1.00	38.00	19.00	3.67	5.00	51.33	12.00	63.33	81.05	1.72	6.33	2.10	3.02
31	82.00	120.33	49.67	1.17	42.57	26.33	5.67	4.00	25.00	2.67	27.67	90.36	4.07	7.43	2.33	3.19
32	79.33	117.67	21.33	2.00	10.67	26.33	5.33	4.00	53.33	3.67	57.00	93.57	3.28	7.73	2.63	2.94
33	80.67	114.33	29.00	1.33	21.75	19.00	5.33	4.00	24.00	4.67	28.67	83.72	2.07	7.30	2.50	2.92
34	74.00	121.67	29.67	2.23	13.28	19.00	6.00	4.33	20.00	15.00	35.00	57.14	5.31	7.77	2.73	2.84
35	80.33	118.33	34.50	2.07	16.69	21.00	5.33	3.67	37.00	10.33	47.33	78.17	2.23	7.43	2.27	3.28
36	79.00	119.00	25.33	2.33	10.86	19.00	5.00	5.00	52.67	1.67	54.33	96.93	4.02	8.13	2.73	2.98
37	81.00	119.67	39.00	1.33	29.25	21.67	2.67	1.33	55.00	6.00	61.00	90.16	5.21	6.63	1.57	4.23
40	80.67	137.00	27.00	1.00	27.00	19.00	4.00	3.67	25.00	26.67	51.67	48.39	3.76	8.07	2.73	2.95
41	80.67	116.00	27.33	1.73	15.77	19.00	2.67	2.67	30.33	6.67	37.00	81.98	2.50	7.80	1.33	5.85

47	78.00	124.00	16.67	2.00	8.33	19.00	4.67	3.67	55.33	13.33	68.67	80.58	1.88	5.87	1.83	3.20
48	79.67	101.33	51.00	2.00	25.50	19.67	7.33	5.33	27.33	30.00	57.33	47.67	4.34	8.30	2.50	3.32
49	82.67	113.00	31.33	1.00	31.33	19.33	5.67	5.00	24.00	15.00	39.00	61.54	2.70	7.77	2.30	3.38
51	80.67	110.33	24.00	1.00	24.00	21.00	8.00	7.33	46.33	2.33	48.67	95.21	2.51	7.80	2.40	3.25
55	80.00	119.67	33.00	1.73	19.04	26.33	4.67	4.33	50.33	8.00	58.33	86.29	3.30	8.57	2.03	4.21
56	82.33	122.00	33.33	1.00	33.33	19.00	5.33	5.67	41.00	23.33	64.33	63.73	7.19	6.90	1.83	3.76
57	81.33	114.67	40.33	1.50	26.89	20.00	6.00	5.67	31.00	2.67	33.67	92.08	3.17	8.17	2.73	2.99
64	80.00	125.33	34.67	2.00	17.33	25.67	5.33	5.33	20.00	7.67	27.67	72.29	3.18	7.87	2.63	2.99
67	81.00	137.33	26.00	1.87	13.93	19.00	5.00	4.00	15.00	11.33	26.33	56.96	2.33	8.13	2.27	3.59
68	75.67	106.67	34.00	1.83	18.55	19.33	4.67	4.00	20.00	9.00	29.00	68.97	6.93	7.40	2.60	2.85
70	82.67	102.67	31.00	1.77	17.55	19.33	4.33	4.00	30.33	13.00	43.33	70.00	2.33	8.30	2.70	3.07
71	74.67	108.00	19.33	0.93	20.71	19.00	5.33	4.67	66.33	6.33	72.67	91.28	4.16	8.40	2.73	3.07
73	79.67	88.67	29.00	1.40	20.71	19.67	2.00	1.67	26.33	14.00	40.33	65.29	2.63	7.40	10.43	0.71
75	75.33	102.67	22.33	3.00	7.44	19.00	2.67	2.67	29.33	11.00	40.33	72.73	4.36	7.97	2.50	3.19
76	79.33	128.00	41.00	1.33	30.75	24.00	6.33	5.67	53.67	9.33	63.00	85.19	9.22	15.00	2.70	5.56
77	74.33	101.33	33.67	1.73	19.42	19.00	6.33	4.67	39.67	12.00	51.67	76.77	3.55	8.13	2.33	3.49
79	74.33	104.00	32.33	2.00	16.17	19.00	2.33	2.33	39.00	5.00	44.00	88.64	4.52	6.70	1.17	5.74
82	80.00	112.33	34.67	1.67	20.80	18.00	3.00	2.00	35.00	8.67	43.67	80.15	1.74	8.40	2.80	3.00
83	82.33	117.33	28.00	1.00	28.00	19.67	4.00	2.67	48.33	8.33	56.67	85.29	1.82	7.53	2.47	3.05
84	83.67	103.33	32.33	1.20	26.94	18.33	2.67	2.67	20.00	2.67	22.67	88.24	2.43	7.10	2.67	2.66
86	83.67	104.67	24.33	1.07	22.81	24.33	3.67	3.33	23.33	9.00	32.33	72.16	2.10	7.70	2.57	3.00
88	82.00	99.67	39.67	1.13	35.00	20.00	4.33	4.67	36.67	3.33	40.00	91.67	7.39	7.80	2.90	2.69
92	82.00	110.00	42.50	1.33	31.88	20.00	4.67	3.33	37.33	11.00	48.33	77.24	6.02	8.47	2.57	3.30
95	82.00	123.33	24.67	1.00	24.67	18.00	6.33	5.33	37.00	9.67	46.67	79.29	5.60	7.77	2.50	3.11
97	74.00	119.00	53.67	1.27	42.37	22.00	4.67	4.00	57.33	9.00	66.33	86.43	4.25	7.97	2.57	3.10
98	75.33	103.00	25.67	2.17	11.85	18.00	5.00	3.33	71.00	9.67	80.67	88.02	2.97	6.37	2.73	2.33
99	82.33	128.67	35.00	1.07	32.81	20.67	5.33	4.67	54.33	3.00	57.33	94.77	9.13	8.23	2.63	3.13
100	82.00	114.00	30.67	1.17	26.29	23.00	4.67	4.33	39.67	10.67	50.33	78.81	4.32	6.87	2.53	2.71
101	77.00	110.67	34.33	2.00	17.17	18.00	3.33	3.33	40.00	12.00	52.00	76.92	6.39	8.10	2.73	2.96
105	82.67	116.33	29.00	2.50	11.60	20.00	5.33	4.67	41.33	15.67	57.00	72.51	2.23	8.20	2.53	3.24
107	82.00	110.67	33.33	1.67	20.00	20.33	6.00	4.67	40.00	21.33	61.33	65.22	9.50	7.77	2.27	3.43
108	82.00	106.00	25.00	1.47	17.05	19.33	3.67	3.33	20.67	7.67	28.33	72.94	9.04	8.10	2.57	3.16
109	81.00	106.33	36.00	1.50	24.00	19.00	2.67	2.33	31.00	3.67	34.67	89.42	5.35	7.20	2.40	3.00
110	80.00	130.00	28.00	2.00	14.00	19.00	5.00	5.00	44.00	10.33	54.33	80.98	3.46	7.83	2.50	3.13
112	82.33	114.33	24.67	1.27	19.47	19.00	5.00	4.33	50.67	7.33	58.00	87.36	5.01	7.73	2.53	3.05
113	79.00	126.33	42.50	1.17	36.43	22.00	5.00	3.33	43.00	12.33	55.33	77.71	2.53	6.67	2.20	3.03

116	81.67	123.33	57.67	1.20	48.06	25.67	6.00	6.00	32.00	13.67	45.67	70.07	6.33	8.63	2.93	2.94
117	80.00	120.00	45.00	1.17	38.57	20.00	4.00	3.67	20.00	10.00	30.00	66.67	9.65	6.87	1.60	4.29
118	82.33	122.00	36.00	1.07	33.75	26.00	3.67	3.33	51.33	12.67	64.00	80.21	3.77	8.30	2.57	3.23
119	78.00	146.00	28.00	2.07	13.55	25.00	4.33	4.33	29.00	12.00	41.00	70.73	6.32	8.10	2.57	3.16
120	80.67	110.33	32.67	2.00	16.33	18.33	4.00	4.00	38.50	5.33	43.83	87.83	4.40	8.20	2.57	3.19
121	80.33	117.33	22.67	2.20	10.30	28.00	7.33	6.00	46.67	21.33	68.00	68.63	4.27	8.40	2.60	3.23
122	82.00	121.00	28.33	1.17	24.29	31.00	3.00	3.00	34.00	18.67	52.67	64.56	3.87	8.07	2.63	3.06
123	78.00	142.67	26.33	1.33	19.75	28.00	4.00	4.00	33.33	15.00	48.33	68.97	5.52	8.17	2.47	3.31
124	79.00	125.33	16.33	1.17	14.00	17.33	3.67	3.33	35.00	8.67	43.67	80.15	3.93	7.67	2.47	3.11
125	82.00	128.67	29.67	1.07	27.81	18.67	3.33	3.33	43.33	5.33	48.67	89.04	9.46	8.00	2.57	3.12
128	82.00	156.67	23.00	1.17	19.71	25.00	4.67	4.33	21.67	15.00	36.67	59.09	4.48	7.40	2.53	2.92
129	82.33	112.67	42.67	1.00	42.67	19.00	4.00	4.00	40.67	15.00	55.67	73.05	4.02	8.03	2.60	3.09
131	81.33	115.67	39.33	1.13	34.71	19.00	4.67	2.67	45.67	1.33	47.00	97.16	10.55	7.57	2.87	2.64
132	80.00	94.33	32.33	2.00	16.17	18.00	4.00	4.00	39.00	5.67	44.67	87.31	6.92	8.17	2.50	3.27
133	80.33	109.67	35.67	1.27	28.16	22.00	5.00	4.67	57.33	6.00	63.33	90.53	7.53	6.70	2.30	2.91
134	80.33	102.67	30.67	1.00	30.67	21.00	4.00	3.67	49.33	4.00	53.33	92.50	5.30	7.47	2.63	2.84
136	82.33	108.00	34.33	1.33	25.75	17.33	3.67	3.00	38.33	18.67	57.00	67.25	3.32	6.83	2.47	2.77
138	79.33	118.33	33.00	1.67	19.80	19.33	4.67	4.67	32.33	1.00	33.33	97.00	4.04	7.80	2.60	3.00
139	82.33	120.67	53.00	1.47	36.14	20.67	5.67	5.00	40.00	16.00	56.00	71.43	5.25	8.10	2.80	2.89
141	78.67	119.67	33.00	2.17	15.23	22.33	6.00	6.00	59.00	5.00	64.00	92.19	4.85	7.97	2.60	3.06
142	82.67	112.67	32.67	1.00	32.67	13.33	3.67	2.67	20.00	12.00	32.00	62.50	6.67	8.17	2.53	3.22
143	74.00	122.67	34.33	2.50	13.73	20.33	6.67	6.33	39.33	18.33	57.67	68.21	4.00	8.20	2.80	2.93
144	82.00	132.00	40.33	1.40	28.81	24.33	5.00	3.00	46.00	12.33	58.33	78.86	11.13	5.77	2.13	2.70
146	79.67	110.00	26.67	1.33	20.00	15.33	6.00	6.00	23.67	15.33	39.00	60.68	6.04	7.27	2.40	3.03
147	82.33	119.67	37.00	1.33	27.75	19.00	5.67	5.00	32.00	8.33	40.33	79.34	1.90	7.87	2.37	3.32
148	74.67	99.67	48.67	1.30	37.44	17.67	6.33	6.33	44.33	0.00	44.33	100.00	4.35	8.83	2.63	3.35
149	78.33	113.00	21.33	1.93	11.03	19.00	7.00	6.33	41.00	5.00	46.00	89.13	5.96	7.57	2.47	3.07
151	82.33	110.33	29.33	1.50	19.56	19.00	5.00	2.33	52.33	11.67	64.00	81.77	5.49	7.97	2.70	2.95
152	82.00	119.67	27.00	1.93	13.97	18.00	9.33	7.33	90.00	3.00	93.00	96.77	6.79	7.63	2.37	3.23
153	82.33	110.33	36.67	1.83	20.04	19.33	6.00	6.00	52.33	11.00	63.33	82.63	5.98	3.26	3.49	0.93
155	79.00	101.33	30.67	1.77	17.33	21.67	5.00	4.67	38.67	7.33	46.00	84.06	4.49	8.40	2.50	3.36
156	80.33	119.67	34.00	1.33	25.50	19.33	4.00	4.00	35.33	19.67	55.00	64.24	5.73	6.50	2.50	2.60
157	79.00	117.67	38.67	1.93	20.00	18.67	4.33	3.33	20.00	6.00	26.00	76.92	9.14	8.37	2.17	3.86
158	82.33	127.33	35.67	1.20	29.72	23.67	3.67	3.33	25.67	3.67	29.33	87.50	3.90	7.73	2.70	2.86
159	81.33	96.00	33.00	2.00	16.50	19.76	2.67	1.33	53.67	4.67	58.33	92.00	4.85	8.43	2.57	3.29
160	79.00	122.33	20.67	1.47	14.09	19.00	4.67	4.33	33.33	10.67	44.00	75.76	5.92	8.20	2.80	2.93

161	82.00	126.33	40.00	1.93	20.73	23.33	3.33	3.33	57.33	11.33	68.67	83.50	3.28	7.27	2.50	2.91
162	79.00	114.33	22.67	2.17	10.46	19.00	3.67	2.33	57.33	4.67	62.00	92.47	8.62	8.10	2.17	3.74
163	80.33	108.00	27.00	2.00	13.50	19.00	4.67	4.67	61.00	7.67	68.67	88.83	6.10	8.53	2.73	3.12
164	81.33	96.00	33.00	2.00	16.50	19.57	2.67	1.33	53.67	4.67	58.33	92.00	4.85	8.43	2.57	3.29
165	79.33	123.00	32.33	1.33	24.25	25.67	6.00	4.00	62.00	2.33	64.33	96.37	10.44	7.43	2.50	2.97
166	82.33	113.33	25.00	1.40	17.86	18.00	5.67	5.00	53.67	17.67	71.33	75.23	5.13	7.80	2.67	2.93
167	81.00	108.33	25.33	1.67	15.20	18.67	2.67	2.67	62.67	13.00	75.67	82.82	9.23	7.47	2.13	3.50
168	79.33	113.00	26.67	1.07	25.00	18.00	3.67	3.33	61.33	21.33	82.67	74.19	3.43	7.17	2.67	2.69
169	82.33	124.00	34.33	1.17	29.43	28.00	3.67	3.67	76.67	11.33	88.00	87.12	4.51	7.87	2.50	3.15
170	79.67	111.00	24.33	1.07	22.81	19.33	3.33	3.00	20.00	12.33	32.33	61.86	2.93	8.33	2.67	3.13
171	77.00	103.33	49.00	1.63	30.00	19.33	2.33	2.33	24.00	8.67	32.67	73.47	5.28	8.30	2.63	3.15
172	78.67	98.00	30.00	1.20	25.00	19.33	2.67	2.33	23.00	8.67	31.67	72.63	3.60	7.50	2.37	3.17
173	79.33	104.00	34.33	1.00	34.33	19.00	4.00	3.67	41.33	19.67	61.00	67.76	4.40	8.67	2.80	3.10
174	82.00	96.00	18.00	1.00	18.00	19.23	8.00	6.33	34.33	7.67	42.00	81.75	9.16	8.07	2.53	3.18
176	83.33	128.33	34.67	1.67	20.80	18.33	6.00	6.00	47.67	12.00	59.67	79.89	5.47	8.17	2.40	3.40
178	79.33	128.00	41.00	1.33	30.75	24.00	6.33	5.67	53.67	9.33	63.00	85.19	9.22	15.77	2.70	5.84
180	80.67	120.33	41.67	1.17	35.71	19.33	5.67	4.67	60.00	6.67	66.67	90.00	9.70	8.53	3.00	2.84
181	81.00	116.67	38.17	1.93	19.78	19.67	5.67	4.33	77.67	21.67	99.33	78.19	8.38	7.73	2.57	3.01
182	80.00	115.67	46.33	1.20	38.61	24.00	4.67	4.00	47.67	12.00	59.67	79.89	4.82	8.67	2.80	3.10
183	79.00	126.00	38.67	1.00	38.67	21.67	6.33	6.00	48.00	1.33	49.33	97.30	3.43	9.60	2.53	3.79
184	79.00	125.33	36.00	1.00	36.00	18.33	7.00	6.00	44.00	2.67	46.67	94.29	2.50	7.07	2.27	3.12
185	78.33	132.67	53.33	1.07	50.00	19.00	7.00	7.00	73.67	16.00	89.67	82.16	3.25	6.80	2.03	3.34
186	80.00	129.67	41.00	1.37	30.00	33.33	5.67	5.00	28.33	4.00	32.33	87.63	4.54	7.83	2.53	3.09
187	78.00	126.67	40.67	2.00	20.33	25.33	7.00	6.33	48.33	6.67	55.00	87.88	6.72	7.87	2.57	3.06
188	79.67	118.67	27.00	1.07	25.31	19.33	5.67	5.67	29.67	9.33	39.00	76.07	2.67	7.67	2.47	3.11
189	80.00	126.67	34.33	2.03	16.89	20.67	5.33	4.67	20.33	22.67	43.00	47.29	6.24	8.27	2.70	3.06
190	79.00	126.00	34.67	1.33	26.00	19.33	6.33	5.67	27.33	9.00	36.33	75.23	4.93	8.23	2.70	3.05
191	82.00	136.67	39.67	1.83	21.64	24.33	5.33	5.33	40.00	24.33	64.33	62.18	3.86	6.97	2.53	2.75
192	80.33	118.00	34.00	1.83	18.55	24.67	5.00	5.00	51.33	3.67	55.00	93.33	4.00	8.43	2.87	2.94
193	79.33	110.67	25.00	1.63	15.31	19.00	8.33	6.33	69.67	7.67	77.33	90.09	5.57	7.97	2.63	3.03
194	79.33	110.67	31.33	1.30	24.10	19.33	4.00	4.00	45.67	5.33	51.00	89.54	3.81	6.93	2.57	2.70
195	82.33	102.67	38.67	1.77	21.89	18.00	5.00	4.33	38.50	1.67	40.17	95.85	5.33	7.43	2.43	3.05
197	80.67	109.00	37.00	1.67	22.20	21.00	8.67	5.33	53.00	18.33	71.33	74.30	3.15	7.53	2.53	2.97
198	79.00	128.67	37.33	1.93	19.34	19.33	6.00	6.00	41.67	13.67	55.33	75.30	5.93	8.27	2.60	3.18
200	79.00	136.67	35.67	2.00	17.83	19.33	4.00	4.00	52.00	7.67	59.67	87.15	4.47	7.77	2.63	2.95
201	84.33	113.33	22.67	1.67	13.60	18.33	7.33	7.00	48.00	17.00	65.00	73.85	1.37	7.33	2.57	2.86

202	82.00	135.00	40.33	1.23	32.70	24.33	5.00	3.33	50.00	10.67	60.67	82.42	4.04	7.10	2.57	2.77
203	79.00	132.33	35.33	1.00	35.33	25.33	6.33	6.33	50.00	14.33	64.33	77.72	4.87	6.50	2.47	2.64
204	82.33	123.67	37.00	2.00	18.50	21.67	5.33	4.33	22.00	2.33	24.33	90.41	4.90	6.77	2.27	2.99
205	82.33	128.67	35.67	1.00	35.67	21.00	4.33	4.00	32.33	4.33	36.67	88.18	4.68	6.90	2.50	2.76
206	82.00	108.33	24.50	1.20	20.42	20.00	6.00	4.00	49.33	8.33	57.67	85.55	5.43	7.20	2.67	2.70
208	82.33	114.00	23.33	1.47	15.91	22.00	8.00	8.67	37.00	15.33	52.33	70.70	4.06	8.53	2.60	3.28
209	83.67	118.33	29.33	1.67	17.60	19.00	4.67	4.67	28.67	12.33	41.00	69.92	6.90	7.30	2.50	2.92
210	74.00	121.67	33.67	1.70	19.80	19.00	5.67	4.00	52.00	8.67	60.67	85.71	3.33	7.70	2.37	3.25
211	78.00	121.00	47.67	1.37	34.88	21.67	6.00	4.67	51.33	12.67	64.00	80.21	2.29	8.17	2.43	3.36
213	81.33	114.67	43.00	1.00	43.00	19.33	1.67	1.33	39.67	12.00	51.67	76.77	5.96	7.63	2.47	3.09
215	75.00	109.67	20.00	1.50	13.33	19.33	4.00	4.00	59.67	5.00	64.67	92.27	3.34	7.97	2.63	3.03
217	82.33	128.67	33.00	1.67	19.80	19.00	4.00	4.00	20.00	4.67	24.67	81.08	2.28	7.63	2.40	3.18
218	78.00	134.33	42.67	1.33	32.00	23.67	8.00	7.67	32.33	13.67	46.00	70.29	7.18	8.33	2.80	2.98
219	80.67	106.00	25.00	1.23	20.27	19.33	3.67	3.33	28.67	0.00	28.67	100.00	1.39	8.40	2.63	3.19
221	81.33	128.33	24.00	1.27	18.95	19.00	6.33	5.67	48.00	17.33	65.33	73.47	4.16	8.10	2.63	3.08
222	74.33	127.67	42.67	2.20	19.39	21.67	3.67	3.33	48.67	19.00	67.67	71.92	1.40	6.80	1.80	3.78
223	80.00	117.33	40.00	1.03	38.71	18.33	5.67	4.33	45.00	4.33	49.33	91.22	4.16	8.07	2.70	2.99
224	80.67	109.67	30.00	1.70	17.65	19.67	4.67	3.67	49.00	17.00	66.00	74.24	2.73	8.13	2.47	3.30
225	80.00	89.67	30.67	1.00	30.67	21.67	4.33	2.00	47.67	4.67	52.33	91.08	7.00	7.60	2.47	3.08
227	80.00	120.33	38.00	1.77	21.51	18.00	5.00	4.67	20.00	11.67	31.67	63.16	3.95	8.50	2.87	2.97
229	82.33	137.00	26.67	2.20	12.12	17.33	6.00	5.67	51.67	9.00	60.67	85.16	3.81	7.50	2.50	3.00
230	82.00	108.00	36.67	1.50	24.44	18.33	4.67	3.67	42.33	17.67	60.00	70.56	4.09	7.73	2.60	2.97
232	79.67	119.33	33.67	1.40	24.05	20.67	4.33	4.33	31.67	29.00	60.67	52.20	4.65	7.50	2.60	2.88
233	81.67	109.33	38.00	1.50	25.33	15.67	4.33	3.67	26.33	15.67	42.00	62.70	4.69	7.57	2.33	3.24
235	82.33	101.33	31.00	2.00	15.50	18.67	5.33	4.67	35.33	13.67	49.00	72.11	11.12	7.03	2.60	2.71
236	74.00	132.33	38.33	2.00	19.17	22.67	6.33	3.00	28.00	10.67	38.67	72.41	7.77	8.33	2.77	3.01
237	80.00	132.33	25.33	1.17	21.71	17.33	4.33	4.33	33.00	13.00	46.00	71.74	8.87	8.03	2.60	3.09
238	81.33	116.00	26.33	2.00	13.17	17.33	4.00	3.67	45.33	14.33	59.67	75.98	5.44	7.33	2.30	3.19
240	74.33	94.33	21.00	2.70	7.78	18.00	4.33	4.33	72.67	22.33	95.00	76.49	3.35	7.30	2.47	2.96
241	78.67	116.67	26.67	1.00	26.67	19.67	4.67	4.33	35.33	11.00	46.33	76.26	5.37	8.13	2.63	3.09
242	78.67	128.00	28.67	1.07	26.88	17.33	4.00	4.00	14.00	9.00	23.00	60.87	2.84	7.97	2.60	3.06
243	80.67	116.67	32.67	1.07	30.63	19.00	5.67	5.67	39.67	9.33	49.00	80.95	4.32	8.83	2.63	3.35
245	79.00	120.00	40.00	1.33	30.00	21.33	6.67	6.00	45.00	0.00	45.00	100.00	1.99	8.17	2.47	3.31
246	79.00	108.33	34.00	1.77	19.21	23.33	8.67	5.33	42.33	7.33	49.67	85.23	1.80	7.90	2.63	3.00
247	80.67	105.67	23.00	1.67	13.80	18.67	2.67	2.33	63.00	3.33	66.33	94.97	3.02	8.40	2.73	3.07
248	80.67	114.67	33.67	1.37	24.63	19.67	4.67	4.67	35.33	5.33	40.67	86.89	2.61	8.50	2.77	3.07

249	82.00	116.00	31.67	1.27	25.00	18.00	7.33	5.00	30.00	19.33	49.33	60.81	4.28	7.77	2.70	2.88
250	82.00	140.67	47.67	2.00	23.83	26.83	5.33	4.00	71.67	19.33	91.00	78.75	2.10	7.37	2.53	2.91
251	74.00	114.33	36.67	2.00	18.33	19.67	4.33	3.00	42.33	8.33	50.67	83.55	1.96	7.63	2.47	3.09
252	81.33	101.33	33.33	1.83	18.18	19.00	5.33	4.67	42.33	11.00	53.33	79.38	1.70	7.50	2.43	3.08
253	78.67	128.00	28.67	1.07	26.88	19.33	4.00	4.00	20.00	9.00	29.00	68.97	2.84	7.97	2.60	3.06
254	81.67	98.67	30.00	1.00	30.00	19.88	2.67	2.67	43.67	6.67	50.33	86.75	1.72	8.00	2.53	3.16
256	80.67	114.00	31.00	1.33	23.25	19.00	4.67	2.67	49.00	11.33	60.33	81.22	6.73	8.90	2.50	3.56
258	83.33	109.67	24.00	2.00	12.00	17.00	6.00	5.67	58.33	15.33	73.67	79.19	3.93	8.37	2.43	3.44
259	81.67	139.33	48.67	1.70	28.63	36.00	4.00	4.00	46.00	3.33	49.33	93.24	3.83	8.27	2.43	3.40
260	76.67	119.67	24.67	1.33	18.50	18.00	5.67	5.00	78.67	29.00	107.67	73.07	1.89	8.23	2.57	3.21
261	78.33	94.33	34.33	1.47	23.41	19.00	7.33	7.00	36.00	29.00	65.00	55.38	3.08	7.70	2.43	3.16
262	78.00	135.33	40.33	1.33	30.25	21.33	4.33	3.67	20.00	9.00	29.00	68.97	2.82	8.03	2.60	3.09
263	82.67	119.00	36.67	1.00	36.67	19.67	6.00	6.00	41.33	24.00	65.33	63.27	3.65	8.20	2.60	3.15
263	78.67	128.00	28.67	1.07	26.88	18.00	4.00	4.00	21.00	9.00	30.00	70.00	2.84	7.97	2.60	3.06
265	78.33	117.00	26.33	1.33	19.75	18.67	5.33	4.33	24.67	5.67	30.33	81.32	2.51	7.80	2.47	3.16
266	81.33	115.67	25.00	1.37	18.29	18.00	4.33	4.00	47.67	11.33	59.00	80.79	1.76	7.47	2.50	2.99
267	81.33	137.00	44.33	1.00	44.33	25.67	8.33	7.00	33.00	30.33	63.33	52.11	4.89	8.23	2.37	3.48
268	82.33	117.33	37.67	1.33	28.25	20.67	5.00	3.33	33.33	6.67	40.00	83.33	5.32	7.93	2.27	3.50
271	80.67	118.33	38.67	1.50	25.78	18.83	5.00	3.67	48.67	19.00	67.67	71.92	3.41	6.97	2.53	2.75
272	79.00	110.33	27.67	1.80	15.37	18.00	5.67	4.33	50.67	17.33	68.00	74.51	3.78	7.93	2.33	3.40
273	81.67	127.33	32.33	1.93	16.75	17.67	5.00	5.00	31.33	3.00	34.33	91.26	2.02	7.17	2.33	3.07
274	78.33	112.67	23.33	1.20	19.44	18.00	5.00	4.00	68.00	5.33	73.33	92.73	1.70	7.63	2.43	3.14
275	80.33	117.33	35.33	2.00	17.67	20.67	4.67	4.33	44.00	7.00	51.00	86.27	5.47	8.23	2.57	3.21
276	80.67	93.33	32.00	1.37	23.41	18.00	4.67	3.67	28.00	18.33	46.33	60.43	1.63	7.53	2.23	3.37
277	78.33	110.33	43.33	1.40	30.95	30.67	6.00	6.00	45.67	14.00	59.67	76.54	2.21	7.20	2.37	3.04
278	78.33	125.33	27.33	1.13	24.12	19.33	6.00	6.00	26.67	29.67	56.34	47.33	2.63	7.33	2.40	3.06
282	78.33	104.00	21.67	2.00	10.83	18.00	8.00	8.00	21.00	26.33	47.33	44.37	2.66	7.73	2.47	3.14
283	75.00	110.67	32.00	1.67	19.20	19.67	5.67	5.67	21.00	15.67	36.67	57.27	5.87	7.67	2.50	3.07
285	81.33	121.67	45.67	1.00	45.67	34.67	5.00	4.33	13.33	16.00	29.33	45.45	3.85	8.30	2.53	3.28
286	78.33	135.33	21.33	2.00	10.67	17.67	6.33	6.00	30.00	7.67	37.67	79.65	5.46	7.73	2.43	3.18
287	81.33	108.00	33.33	1.07	31.25	21.00	8.33	6.33	32.00	13.33	45.33	70.59	4.23	8.30	2.63	3.15
288	82.00	118.33	33.00	1.00	33.00	19.33	4.67	3.00	43.67	18.67	62.33	70.05	6.61	7.90	2.63	3.00
289	75.67	115.67	39.00	1.77	22.08	24.67	6.00	4.33	53.00	14.00	67.00	79.10	2.36	7.37	2.37	3.11
290	74.33	128.00	44.33	2.50	17.73	30.67	6.33	6.33	49.00	5.00	54.00	90.74	4.39	7.90	2.57	3.08
291	81.00	115.33	39.33	2.17	18.15	25.00	6.00	3.67	52.00	6.33	58.33	89.14	1.58	7.53	2.23	3.37
293	80.00	101.67	32.00	1.00	32.00	17.00	5.00	4.67	44.33	12.33	56.67	78.24	3.29	8.23	2.40	3.43

294	80.67	114.33	35.67	1.40	25.48	24.00	4.67	3.67	65.67	17.67	83.33	78.80	3.81	7.27	2.30	3.16
296	82.00	132.33	44.00	1.50	29.33	24.33	4.67	4.67	64.00	9.67	73.67	86.88	4.84	9.20	2.90	3.17
297	81.33	117.67	28.67	1.00	28.67	18.33	5.00	4.00	34.00	9.67	43.67	77.86	5.19	7.33	2.37	3.10
298	79.67	137.33	38.00	1.33	28.50	23.67	5.33	4.67	61.33	17.00	78.33	78.30	3.82	7.40	2.53	2.92
299	81.33	122.00	30.00	1.20	25.00	22.33	6.00	5.67	53.33	5.00	58.33	91.43	7.64	8.03	2.53	3.17
300	79.00	112.67	37.00	2.23	16.57	20.00	5.33	3.67	44.00	4.00	48.00	91.67	3.37	7.67	2.33	3.29
301	78.33	119.67	37.67	1.67	22.60	17.00	6.33	6.00	45.67	5.33	51.00	89.54	1.89	6.77	2.20	3.08
303	79.33	140.33	41.33	1.33	31.00	41.67	7.33	7.00	35.67	11.33	47.00	75.89	4.98	7.97	2.50	3.19
304	78.33	112.67	20.00	1.50	13.33	18.00	5.67	5.67	54.00	4.33	58.33	92.57	3.90	7.97	2.60	3.06
305	80.67	114.33	32.00	1.47	21.82	20.33	6.67	6.67	44.00	6.67	50.67	86.84	5.22	7.20	2.30	3.13
307	80.67	127.00	33.00	1.07	30.94	22.00	7.00	6.67	78.00	22.00	100.00	78.00	6.11	7.40	2.80	2.64
308	79.33	128.33	24.67	1.47	16.82	19.67	5.33	4.33	46.00	5.00	51.00	90.20	3.78	8.13	2.60	3.13
309	80.67	121.00	35.33	2.33	15.14	18.00	4.67	4.33	47.33	11.67	59.00	80.23	3.14	7.23	2.37	3.06
310	82.00	116.67	33.67	1.50	22.44	19.67	5.33	6.00	25.33	4.33	29.67	85.39	3.49	8.27	2.73	3.02
311	79.00	106.00	34.67	1.87	18.57	21.67	6.33	6.33	59.33	5.33	64.67	91.75	2.18	7.27	1.93	3.76
312	82.00	137.67	25.33	1.67	15.20	17.00	7.67	6.67	52.33	11.67	64.00	81.77	1.42	7.67	2.50	3.07
313	82.33	142.33	35.33	1.33	26.50	22.67	6.67	6.67	56.33	13.33	69.67	80.86	4.98	8.00	2.73	2.93
314	78.33	109.33	44.33	1.50	29.56	20.00	4.33	4.00	25.33	4.33	29.67	85.39	3.14	6.67	2.57	2.60
315	82.00	125.67	31.00	1.00	31.00	18.67	5.33	3.67	85.33	8.67	94.00	90.78	3.00	7.13	2.50	2.85
317	79.67	113.67	33.33	1.50	22.22	25.00	3.67	2.33	61.00	21.00	82.00	74.39	4.11	7.67	2.57	2.99
318	78.00	130.00	35.00	1.00	35.00	24.00	7.33	6.67	59.33	13.33	72.67	81.65	3.54	7.63	2.57	2.97
319	80.00	132.00	44.00	1.83	24.00	31.67	5.67	5.33	69.33	6.33	75.67	91.63	6.43	7.47	2.40	3.11
320	79.33	137.33	32.00	1.20	26.67	19.33	8.00	7.00	52.67	3.33	56.00	94.05	4.22	8.13	2.63	3.09
323	79.00	134.33	39.33	1.07	36.88	23.67	7.00	4.67	57.67	5.67	63.33	91.05	3.59	7.50	2.77	2.71
324	78.33	118.00	23.00	1.80	12.78	17.00	7.33	7.00	51.33	8.33	59.67	86.03	3.85	7.93	2.80	2.83
325	81.00	119.67	21.67	1.33	16.25	15.67	4.33	4.33	47.00	9.00	56.00	83.93	3.58	7.37	2.63	2.80
327	78.00	130.00	35.00	1.00	35.00	24.00	7.33	6.67	59.33	13.33	72.67	81.65	3.54	7.63	2.57	2.97
328	81.33	125.33	36.00	1.50	24.00	31.00	4.33	4.33	67.67	12.33	80.00	84.58	5.34	7.93	2.60	3.05
329	81.33	115.67	31.67	2.23	14.18	19.67	6.33	4.67	55.33	15.33	70.67	78.30	2.31	7.10	2.57	2.77
330	80.67	117.33	23.33	2.50	9.33	17.33	4.67	4.67	36.67	9.33	46.00	79.71	2.99	7.87	2.60	3.03
331	80.67	135.00	41.33	1.50	27.56	21.67	5.67	5.33	27.00	6.67	33.67	80.20	2.79	8.00	2.43	3.29
332	80.67	129.33	25.67	1.50	17.11	24.00	5.33	4.67	74.33	2.00	76.33	97.38	5.75	7.77	2.63	2.95
333	80.67	114.33	28.33	2.00	14.17	17.33	3.33	3.00	78.33	9.33	87.67	89.35	4.31	8.40	2.70	3.11
334	81.33	134.00	39.33	1.33	29.50	36.67	4.67	4.33	60.33	5.33	65.67	91.88	6.23	7.73	2.73	2.83
335	81.67	137.67	47.33	1.00	47.33	18.67	4.33	4.00	40.00	14.67	54.67	73.17	3.36	8.60	2.57	3.35
336	76.00	112.67	37.33	1.37	27.32	24.93	7.67	6.67	22.00	9.67	31.67	69.47	3.81	7.37	2.33	3.16

338	79.00	95.37	39.33	1.93	20.38	23.00	5.33	5.00	20.00	26.67	46.67	42.86	4.21	7.67	2.40	3.19
356	78.33	112.33	25.00	2.00	12.50	15.67	6.00	5.67	49.33	8.00	57.33	86.05	2.22	7.67	2.63	2.91
disang	76.67	98.50	21.00	1.77	11.89	19.00	3.50	6.00	26.00	18.33	44.33	58.65	4.73	4.60	1.33	3.45
kali	79.00	132.33	35.33	1.00	35.33	25.33	6.33	6.33	50.00	14.33	64.33	77.72	4.87	6.50	2.47	2.64

APPENDIX II

Mean Performance of Phenotypic traits for 247 RILs under reproductive stage drought stress.

PL	NOT	EBT	NOG	NOC	TNG	SF	GY	GL	GW	GLW	Leaf rolling	Recovery
13.00	2.33	1.33	40.00	28.33	68.33	58.54	1.21	7.40	2.30	3.22	0.00	1.00
12.33	4.67	4.33	13.67	12.33	26.00	52.56	2.17	6.20	1.70	3.65	7.00	7.00
11.33	3.67	2.33	36.67	42.33	79.00	46.41	1.55	7.40	2.33	3.17	3.00	3.00
13.00	4.00	2.00	14.00	7.00	21.00	66.67	1.58	6.17	1.70	3.63	5.00	7.00
19.00	8.33	6.67	16.33	8.33	24.67	66.22	1.40	6.33	1.93	3.28	1.00	3.00
19.17	2.33	1.33	14.67	31.33	46.00	31.88	1.61	7.73	1.53	5.04	7.00	3.00
18.17	5.33	5.00	50.33	28.00	78.33	64.26	1.44	6.83	2.30	2.97	1.00	1.00
15.17	4.33	4.00	62.33	28.33	90.67	68.75	1.67	6.43	2.27	2.84	1.00	1.00
14.00	2.67	1.67	10.00	7.67	17.67	56.60	1.25	6.73	2.10	3.21	7.00	9.00
16.00	3.67	3.00	18.00	5.33	23.33	77.14	1.82	7.10	1.30	5.46	5.00	7.00
12.67	3.67	3.33	18.67	6.00	24.67	75.68	1.50	7.77	2.10	3.70	0.00	1.00
14.33	3.33	1.67	53.33	25.33	78.67	67.80	1.67	4.93	1.40	3.52	1.00	1.00
18.33	3.67	3.00	18.00	4.33	22.33	80.60	1.86	6.67	1.97	3.39	0.00	1.00
19.57	3.00	3.00	21.67	6.33	28.00	77.38	1.40	7.20	1.97	3.66	1.00	1.00
14.33	5.67	5.00	26.67	22.67	49.33	54.05	1.34	7.07	2.20	3.21	3.00	1.00
14.33	2.67	1.00	50.33	17.33	67.67	74.38	2.08	6.93	1.93	3.59	3.00	3.00
16.67	5.00	3.33	22.33	35.33	57.67	38.73	1.13	7.63	2.20	3.47	0.00	1.00
15.67	3.33	2.33	21.33	17.00	38.33	55.65	1.26	7.30	1.70	4.29	0.00	3.00

17.50	2.67	2.00	15.33	15.67	31.00	49.46	1.92	6.83	2.10	3.25	0.00	1.00
17.33	1.67	1.00	15.00	23.33	38.33	39.13	1.91	7.43	2.13	3.48	3.00	3.00
11.23	2.33	1.67	26.67	4.00	30.67	86.96	1.39	8.00	2.30	3.48	1.00	1.00
15.67	3.00	2.33	22.00	36.33	58.33	37.71	1.65	8.20	1.73	4.73	0.00	1.00
10.33	1.33	1.00	17.33	7.67	25.00	69.33	1.90	7.17	2.10	3.41	1.00	5.00
17.17	5.00	3.33	57.33	50.33	107.67	53.25	1.91	7.00	2.20	3.18	3.00	1.00
15.33	2.67	1.33	35.67	24.67	60.33	59.12	1.61	7.30	2.07	3.53	0.00	1.00
12.33	3.33	1.00	16.00	14.67	30.67	52.17	1.49	6.50	2.03	3.20	5.00	3.00
15.00	3.33	2.67	21.33	18.33	39.67	53.78	1.07	8.00	2.17	3.69	0.00	1.00
10.00	2.67	2.00	17.00	35.33	52.33	32.48	1.28	6.30	2.00	3.15	3.00	5.00
15.00	4.00	4.00	91.33	25.00	116.33	78.51	1.85	6.10	2.00	3.05	5.00	5.00
13.00	6.67	4.00	15.67	27.67	43.33	36.15	1.72	6.20	2.07	3.00	0.00	1.00
10.67	3.33	2.33	38.33	12.67	51.00	75.16	1.45	7.37	1.93	3.81	5.00	3.00
12.33	3.33	2.67	17.00	1.67	18.67	91.07	1.37	6.93	2.13	3.25	3.00	3.00
13.17	3.00	2.67	31.33	21.00	52.33	59.87	1.46	7.00	2.20	3.18	1.00	3.00
12.33	3.33	3.00	33.00	37.67	70.67	46.70	1.29	6.27	1.90	3.30	7.00	3.00
14.67	2.67	2.33	18.00	9.33	27.33	65.85	1.46	5.70	1.00	5.70	1.00	3.00
12.67	3.00	2.33	52.00	37.33	89.33	58.21	1.70	6.53	2.13	3.06	3.00	1.00
13.67	6.33	6.00	46.00	53.00	99.00	46.46	1.59	7.60	1.97	3.86	0.00	1.00
14.00	2.67	1.33	14.33	13.33	27.67	51.81	1.56	6.27	2.13	2.94	9.00	7.00
12.33	3.67	3.33	12.00	8.33	20.33	59.02	1.41	7.00	1.83	3.82	0.00	3.00

21.83	2.67	2.00	28.00	43.33	71.33	39.25	1.36	7.30	1.90	3.84	9.00	7.00
15.67	3.67	3.33	57.67	40.00	97.67	59.04	1.78	6.80	2.20	3.09	0.00	1.00
13.00	3.67	1.33	24.00	15.33	39.33	61.02	1.89	6.80	2.30	2.96	0.00	3.00
12.00	4.67	3.33	29.67	9.33	39.00	76.07	1.14	6.77	1.53	4.41	5.00	1.00
18.17	2.33	2.00	50.67	46.33	97.00	52.23	1.89	8.20	2.53	3.24	5.00	7.00
13.00	1.67	1.00	13.00	5.33	18.33	70.91	1.87	6.90	1.60	4.31	1.00	9.00
18.33	2.33	1.67	24.67	26.00	50.67	48.68	1.37	7.87	2.30	3.42	3.00	1.00
15.83	3.67	2.67	38.33	25.67	64.00	59.90	1.44	7.33	1.87	3.93	1.00	1.00
12.00	3.33	2.67	20.33	10.33	30.67	66.30	1.38	7.30	2.20	3.32	1.00	1.00
15.00	5.00	5.00	42.00	37.33	79.33	52.94	1.62	7.00	2.07	3.39	5.00	7.00
15.67	1.67	1.33	28.00	15.67	43.67	64.12	1.30	1.26	1.20	1.05	3.00	1.00
12.67	1.67	1.00	14.00	27.00	41.00	34.15	1.03	5.73	2.17	2.65	3.00	3.00
14.67	2.67	2.33	19.00	19.67	38.67	49.14	1.96	5.73	2.23	2.57	0.00	3.00
14.33	2.33	2.00	35.67	20.33	56.00	63.69	1.67	7.63	2.37	3.23	1.00	5.00
15.33	5.67	4.67	87.33	70.00	157.33	55.51	2.54	7.47	2.23	3.34	3.00	1.00
15.33	3.67	3.33	38.00	27.33	65.33	58.16	1.22	6.97	2.10	3.32	1.00	1.00
13.33	3.00	3.00	30.33	22.33	52.67	57.59	1.69	7.03	2.13	3.30	9.00	7.00
13.33	4.33	4.00	40.00	68.00	108.00	37.04	1.30	7.13	2.30	3.10	1.00	1.00
14.33	3.67	2.33	51.00	41.67	92.67	55.04	1.90	6.23	1.60	3.90	3.00	3.00
14.50	4.67	4.00	17.33	14.00	31.33	55.31	1.88	7.57	1.40	5.40	0.00	1.00
11.33	3.00	1.33	26.67	31.67	58.33	45.71	1.14	7.30	2.20	3.32	1.00	1.00

14.00	5.00	4.00	36.67	28.00	64.67	56.70	1.47	6.90	2.10	3.29	3.00	3.00
14.50	4.33	3.33	30.00	24.00	54.00	55.56	1.23	7.57	2.07	3.66	3.00	1.00
14.00	3.33	3.33	30.00	20.00	50.00	60.00	1.31	6.97	1.93	3.60	1.00	1.00
14.67	3.33	2.67	76.00	30.33	106.33	71.47	1.76	6.57	2.17	3.03	5.00	1.00
16.33	4.33	3.33	32.33	26.33	58.67	55.11	1.65	7.10	2.13	3.33	5.00	3.00
20.67	1.00	1.00	32.33	35.33	67.67	47.78	1.11	6.97	2.07	3.37	5.00	1.00
14.33	3.00	2.33	36.67	52.00	88.67	41.35	1.48	7.30	2.03	3.59	3.00	3.00
14.50	4.00	3.00	25.33	39.00	64.33	39.38	1.25	7.20	1.93	3.72	7.00	3.00
10.50	2.00	1.00	30.00	5.67	35.67	84.11	1.64	6.40	1.80	3.56	0.00	3.00
19.17	4.00	3.33	20.33	21.33	41.67	48.80	1.53	7.20	2.37	3.04	0.00	1.00
12.33	4.00	3.67	11.67	13.00	24.67	47.30	1.16	7.07	2.07	3.42	1.00	1.00
14.50	4.67	2.00	55.33	26.33	81.67	67.76	2.61	6.47	1.93	3.34	5.00	7.00
11.00	3.33	2.67	41.00	29.67	70.67	58.02	1.64	6.80	1.43	4.74	7.00	9.00
10.67	5.33	2.33	24.33	25.67	50.00	48.67	1.36	6.30	1.80	3.50	7.00	7.00
19.83	2.00	1.67	16.00	7.67	23.67	67.61	1.23	6.80	1.57	4.34	9.00	7.00
15.33	1.33	1.00	33.33	12.67	46.00	72.46	2.00	7.00	2.47	2.84	5.00	1.00
13.67	3.33	3.00	26.33	32.00	58.33	45.14	1.32	6.30	2.03	3.10	1.00	3.00
21.33	4.67	3.00	22.33	14.00	36.33	61.47	2.00	8.23	2.03	4.05	3.00	5.00
27.67	2.33	1.00	18.00	8.67	26.67	67.50	2.00	6.57	2.20	2.98	3.00	5.00
16.67	3.67	2.67	12.67	14.67	27.33	46.34	2.00	7.03	1.93	3.64	7.00	3.00
12.33	3.00	2.00	16.33	19.67	36.00	45.37	1.56	6.37	1.67	3.82	9.00	9.00

12.33	3.33	2.67	25.67	16.67	42.33	60.63	1.37	6.47	1.33	4.85	1.00	1.00
12.83	7.00	4.00	11.00	9.00	20.00	55.00	1.69	7.17	2.17	3.31	9.00	9.00
15.67	5.00	3.00	15.00	16.00	31.00	48.39	1.80	7.33	1.03	7.10	7.00	5.00
13.67	3.00	2.33	26.33	2.33	28.67	91.86	1.46	6.67	1.90	3.51	1.00	1.00
18.33	1.00	1.67	16.00	4.00	20.00	80.00	1.73	7.93	2.53	3.13	1.00	1.00
13.83	3.00	2.33	24.00	28.33	52.33	45.86	1.16	7.33	5.00	1.47	3.00	5.00
16.33	6.33	6.00	51.00	8.00	59.00	86.44	1.42	7.87	2.23	3.52	1.00	1.00
14.00	4.33	4.00	14.67	12.67	27.33	53.66	1.58	7.53	1.53	4.91	0.00	5.00
15.33	4.00	3.67	42.33	32.00	74.33	56.95	1.69	6.83	2.17	3.15	5.00	7.00
14.37	3.00	2.00	36.00	15.67	51.67	69.68	1.45	7.37	2.23	3.30	1.00	1.00
17.50	3.00	2.00	17.33	13.00	30.33	57.14	1.56	7.57	2.17	3.49	1.00	1.00
18.00	1.33	1.67	17.33	18.33	35.67	48.60	1.02	7.27	2.00	3.63	7.00	5.00
17.67	3.67	2.33	17.00	9.00	26.00	65.38	1.36	6.47	2.00	3.23	0.00	3.00
13.33	1.67	1.00	25.67	9.33	35.00	73.33	1.32	6.63	2.23	2.97	9.00	3.00
15.33	2.67	2.00	12.67	10.33	23.00	55.07	1.20	7.30	2.00	3.65	5.00	3.00
19.77	3.33	2.67	71.00	17.67	88.67	80.08	2.15	7.53	2.37	3.18	5.00	3.00
16.83	2.67	2.67	23.33	16.00	39.33	59.32	1.56	7.00	2.63	2.66	3.00	7.00
18.33	3.67	3.00	18.00	4.33	22.33	80.60	2.00	6.67	1.97	3.39	9.00	9.00
14.00	2.00	1.33	15.33	5.67	21.00	73.01	1.03	6.80	1.42	4.78	9.00	7.00
13.00	3.67	2.67	24.00	14.33	38.33	62.61	1.47	8.20	1.50	5.47	3.00	5.00
13.50	2.67	1.33	27.00	3.67	30.67	88.04	2.00	7.07	2.20	3.21	7.00	5.00

19.93	2.67	2.67	37.00	33.67	70.67	52.36	1.27	6.73	1.77	3.81	5.00	5.00
16.50	2.67	2.33	21.67	21.67	43.33	50.00	1.40	7.87	2.27	3.47	3.00	7.00
17.67	3.00	2.33	20.67	24.67	45.33	45.59	2.00	7.10	2.13	3.33	3.00	5.00
13.13	3.33	3.33	42.67	21.33	64.00	66.67	1.41	6.77	2.00	3.38	5.00	3.00
13.33	2.00	2.00	25.67	16.00	41.67	61.60	1.00	7.10	1.40	5.07	3.00	5.00
12.33	3.67	1.67	16.67	35.33	52.00	32.05	2.00	7.00	2.06	3.40	1.00	1.00
15.83	3.67	2.67	20.33	21.00	41.33	49.19	1.17	7.23	2.10	3.44	5.00	5.00
12.00	3.00	2.33	26.67	30.33	57.00	46.78	1.41	6.77	2.13	3.17	5.00	1.00
12.33	3.00	2.00	39.67	36.67	76.33	51.97	1.80	5.77	2.10	2.75	9.00	7.00
19.33	3.33	3.00	17.33	29.00	46.33	37.41	1.49	6.87	2.40	2.86	5.00	1.00
15.67	3.67	2.00	38.00	37.67	75.67	50.22	2.00	6.80	2.10	3.24	9.00	3.00
13.33	3.33	3.33	37.00	28.67	65.67	56.35	1.49	6.67	2.43	2.74	9.00	9.00
20.33	3.00	3.00	29.33	24.67	54.00	54.32	1.48	7.33	1.87	3.93	1.00	7.00
16.33	4.00	3.33	30.33	33.33	63.67	47.64	1.10	6.70	1.93	3.47	1.00	1.00
17.83	2.33	1.33	15.33	21.33	36.67	41.82	1.16	6.87	2.07	3.32	5.00	1.00
13.67	4.67	2.67	53.33	15.67	69.00	77.29	1.33	5.63	1.45	3.89	1.00	7.00
13.33	3.67	3.33	53.67	40.33	94.00	57.09	1.56	6.97	2.07	3.37	1.00	7.00
12.67	3.67	4.00	51.67	52.67	104.33	49.52	1.52	6.47	2.00	3.23	9.00	7.00
12.67	2.33	1.67	27.67	25.33	53.00	52.20	1.20	6.63	2.07	3.21	3.00	1.00
20.17	4.00	2.33	29.67	62.00	91.67	32.36	1.35	7.67	1.90	4.04	9.00	5.00
14.33	4.00	3.00	45.00	69.00	114.00	39.47	1.34	7.20	2.02	3.56	5.00	5.00

12.17	3.00	2.33	27.33	25.67	53.00	51.57	1.20	6.83	2.20	3.11	1.00	1.00
15.17	3.67	3.00	16.33	12.67	29.00	56.32	1.80	6.00	2.20	2.72	1.00	3.00
10.00	1.67	1.00	12.00	27.33	39.33	30.51	1.20	6.37	1.93	3.29	3.00	1.00
17.67	2.00	1.33	25.00	14.33	39.33	63.56	2.00	6.83	1.10	6.21	5.00	1.00
18.17	2.33	1.33	18.00	5.00	23.00	78.26	1.50	6.63	1.86	3.57	7.00	7.00
17.33	3.33	3.00	37.33	39.33	76.67	48.70	1.62	7.40	2.23	3.31	1.00	1.00
15.33	2.67	2.33	36.33	41.33	77.67	46.78	1.76	7.80	2.20	3.55	7.00	5.00
11.83	1.67	1.33	57.67	67.67	125.33	46.01	1.25	7.00	2.07	3.39	1.00	7.00
13.67	2.67	1.67	12.33	8.33	20.66	59.67	1.25	7.53	2.20	3.42	1.00	1.00
17.33	4.67	3.67	13.33	13.00	26.33	50.63	1.62	7.17	1.90	3.77	7.00	3.00
22.17	5.33	3.67	66.00	32.67	98.67	66.89	2.21	7.30	2.37	3.08	9.00	1.00
10.67	2.00	1.00	69.33	38.33	107.67	64.40	1.58	7.57	1.80	4.20	9.00	7.00
18.33	2.67	2.00	18.00	11.67	29.67	60.67	1.92	7.00	2.13	3.28	1.00	1.00
15.33	3.33	2.33	14.00	8.67	22.67	61.76	1.81	6.23	2.13	2.92	1.00	5.00
16.23	3.33	3.00	32.33	6.33	38.67	83.62	2.00	7.37	2.07	3.56	5.00	1.00
15.67	4.00	4.00	15.67	22.67	38.33	40.87	1.25	6.93	1.77	3.92	1.00	1.00
15.83	5.33	4.00	28.00	30.00	58.00	48.28	1.27	6.67	2.17	3.08	1.00	1.00
16.67	4.67	4.33	36.00	30.67	66.67	54.00	1.38	6.10	3.00	2.03	1.00	5.00
17.33	4.00	2.33	26.33	2.67	29.00	90.80	2.00	6.33	2.03	3.11	1.00	1.00
14.83	4.33	3.00	36.33	35.67	72.00	50.46	1.03	8.33	1.79	4.66	1.00	1.00
14.67	7.00	4.33	16.67	12.67	29.33	56.82	1.25	7.10	1.90	3.74	3.00	3.00

16.83	5.00	4.33	30.33	44.00	74.33	40.81	1.74	6.83	2.20	3.11	1.00	1.00
15.67	2.33	2.00	72.67	19.33	92.00	78.99	1.24	6.77	2.27	2.99	5.00	3.00
19.50	3.00	2.33	28.00	17.33	45.33	61.76	5.03	6.50	2.17	3.00	3.00	3.00
17.50	4.33	2.33	37.00	39.67	76.67	48.26	1.37	6.50	8.73	0.74	1.00	5.00
16.33	4.33	2.67	29.67	19.67	49.33	60.14	1.15	7.23	2.20	3.29	5.00	5.00
14.62	6.00	5.33	26.33	20.00	46.33	56.83	2.00	6.50	1.97	3.31	5.00	9.00
10.00	2.00	1.00	26.67	9.33	36.00	74.07	2.00	5.27	2.00	2.63	5.00	1.00
16.67	2.67	2.67	19.00	16.00	35.00	54.29	1.63	7.27	1.83	3.96	1.00	1.00
17.83	3.00	3.00	91.67	16.00	107.67	85.14	2.06	7.40	1.93	3.83	1.00	3.00
14.33	3.00	2.67	69.67	41.00	110.67	62.95	2.08	6.10	2.33	2.61	5.00	5.00
19.60	6.00	4.67	63.33	34.67	98.00	64.63	1.66	6.80	1.33	5.10	1.00	1.00
14.00	3.67	3.00	25.67	14.00	39.67	64.71	1.68	8.00	2.33	3.43	1.00	1.00
12.83	1.33	1.33	12.67	9.67	22.33	56.72	2.20	7.03	2.17	3.25	1.00	3.00
20.00	2.00	1.67	54.33	49.00	103.33	52.58	1.28	6.67	1.88	3.54	1.00	7.00
15.67	2.67	1.33	44.00	12.67	56.67	77.65	2.23	7.40	1.90	3.89	1.00	3.00
12.50	2.33	2.00	37.67	27.67	65.33	57.65	1.32	7.00	2.30	3.04	5.00	5.00
13.00	2.33	1.67	21.67	45.33	67.00	32.34	1.75	6.47	1.53	4.22	7.00	3.00
18.13	2.67	2.67	20.33	13.00	33.33	61.00	1.92	7.63	2.13	3.58	1.00	3.00
19.67	6.00	6.00	13.00	8.67	21.67	60.00	2.00	7.37	1.00	7.37	1.00	5.00
21.50	4.67	4.67	19.67	29.00	48.67	40.41	1.68	7.73	2.33	3.31	1.00	5.00
11.00	2.33	2.33	26.00	19.00	45.00	57.78	1.26	7.03	2.13	3.30	0.00	1.00

16.67	2.00	1.33	31.33	21.00	52.33	59.87	1.30	7.60	2.03	3.74	5.00	1.00
10.33	3.00	2.00	36.33	25.00	61.33	59.24	1.22	6.50	1.60	4.06	7.00	5.00
17.67	3.67	2.33	34.00	39.00	73.00	46.58	1.49	7.33	2.17	3.38	0.00	1.00
10.83	3.67	2.33	59.67	46.67	106.33	56.11	1.72	7.77	2.33	3.33	0.00	1.00
16.33	3.33	1.00	49.00	22.67	71.67	68.37	1.93	7.10	2.17	3.28	0.00	3.00
17.33	4.00	2.33	15.33	8.33	23.67	64.79	2.18	7.17	2.20	3.26	1.00	1.00
16.00	1.00	1.00	38.67	41.33	80.00	48.33	1.62	6.73	1.43	4.70	1.00	5.00
12.33	3.00	2.67	25.00	2.00	27.00	92.59	1.59	7.03	2.00	3.52	1.00	1.00
12.00	3.00	3.00	18.00	14.67	32.67	55.10	2.02	7.00	2.43	2.88	5.00	3.00
15.00	5.33	1.33	23.33	17.67	41.00	56.91	1.89	7.63	2.20	3.47	7.00	5.00
11.00	2.00	1.67	16.67	5.67	22.34	74.63	1.12	6.90	1.87	3.70	7.00	5.00
14.33	3.00	3.00	19.00	8.33	27.33	69.51	1.32	7.40	1.90	3.89	0.00	5.00
19.17	5.00	5.00	28.00	18.00	46.00	60.87	2.09	6.47	2.10	3.08	5.00	3.00
10.33	3.33	2.33	71.33	50.67	122.00	58.47	1.40	7.70	2.37	3.25	1.00	3.00
15.67	3.33	3.33	24.67	18.67	43.33	56.92	1.60	6.40	1.70	3.76	9.00	1.00
11.50	1.67	1.00	48.33	47.67	96.00	50.35	1.26	6.67	2.07	3.23	1.00	1.00
17.33	4.33	2.67	22.00	25.00	47.00	46.81	2.00	7.17	2.07	3.47	1.00	5.00
17.50	5.33	4.00	33.67	11.00	44.67	75.37	1.49	2.80	2.17	1.29	1.00	1.00
17.17	3.33	3.00	29.33	16.67	46.00	63.77	2.30	6.20	1.50	4.13	5.00	3.00
19.17	5.00	4.33	50.33	27.00	77.33	65.09	2.25	8.40	2.23	3.76	1.00	0.00
19.67	3.33	2.67	74.00	56.00	130.00	56.92	1.43	6.83	1.53	4.46	1.00	1.00

13.50	4.67	3.67	26.00	11.00	37.00	70.27	1.98	6.83	2.20	3.11	5.00	0.00
13.00	1.00	1.00	60.00	53.33	113.33	52.94	1.36	7.97	1.73	4.60	7.00	7.00
19.00	3.00	1.67	18.00	14.67	32.67	55.10	1.40	7.07	1.87	3.79	0.00	3.00
10.00	1.67	2.00	41.00	20.67	61.67	66.49	2.00	6.87	2.13	3.22	1.00	7.00
19.33	5.67	4.33	26.33	15.33	41.67	63.20	1.37	6.80	1.97	3.46	3.00	1.00
14.00	2.00	1.00	18.00	16.33	34.33	52.43	2.10	5.80	1.50	3.87	0.00	9.00
15.00	2.33	1.00	70.00	18.33	88.33	79.25	1.49	7.30	1.93	3.78	1.00	1.00
18.33	5.33	5.00	22.67	18.67	41.33	54.84	1.72	7.47	2.23	3.34	1.00	1.00
14.50	3.00	2.33	42.67	34.00	76.67	55.65	1.26	7.50	3.07	2.45	5.00	0.00
15.50	8.00	3.67	16.00	21.67	37.67	42.48	1.25	6.57	2.03	3.23	3.00	5.00
10.00	2.33	2.00	29.67	16.33	46.00	64.49	1.64	6.70	2.13	3.14	1.00	1.00
15.83	5.67	4.67	31.67	18.67	50.33	62.91	1.91	7.07	2.27	3.12	1.00	7.00
12.67	4.00	3.00	16.67	25.33	42.00	39.69	1.39	6.77	1.83	3.69	7.00	5.00
12.67	3.33	2.33	11.67	14.33	26.00	44.87	1.85	7.40	1.87	3.96	9.00	9.00
14.50	3.33	2.33	25.33	20.00	45.33	55.88	1.60	6.83	1.77	3.87	1.00	5.00
13.33	2.33	1.00	16.00	33.33	49.33	32.43	11.80	3.80	1.57	2.43	1.00	0.00
16.17	3.00	1.67	13.33	11.00	24.33	54.79	1.40	6.97	2.30	3.03	0.00	3.00
17.00	3.67	2.33	22.00	20.67	42.67	51.56	1.32	6.57	1.83	3.58	9.00	5.00
15.67	1.67	1.00	15.00	11.67	26.67	56.25	1.85	6.00	1.20	5.00	7.00	5.00
14.33	2.67	2.00	48.67	21.00	69.67	69.86	1.11	6.97	1.90	3.67	5.00	5.00
17.00	10.00	5.00	16.00	7.00	23.00	69.57	1.94	5.20	1.57	3.32	9.00	9.00

12.00	4.67	4.00	21.00	25.00	46.00	45.65	0.97	6.97	1.94	3.58	9.00	7.00
16.07	3.00	2.33	28.67	43.33	72.00	39.81	1.26	7.30	2.17	3.37	5.00	5.00
23.33	2.00	1.00	22.67	15.67	38.33	59.13	1.78	6.40	1.00	6.40	1.00	3.00
10.83	3.67	3.00	25.00	22.67	47.67	52.45	1.14	6.90	1.97	3.51	1.00	5.00
12.67	8.67	5.67	19.00	19.33	38.33	49.57	1.34	6.20	1.67	3.72	9.00	9.00
14.33	1.33	1.67	13.00	26.00	39.00	33.33	1.31	5.57	1.50	3.71	1.00	3.00
14.00	4.00	2.00	36.33	33.00	69.33	52.40	1.33	7.53	2.17	3.48	3.00	3.00
13.33	2.33	1.67	14.67	30.33	45.00	32.60	1.49	6.70	1.90	3.53	5.00	3.00
12.00	4.00	3.00	19.67	21.67	41.33	47.58	1.93	7.10	2.07	3.44	5.00	9.00
17.50	5.00	5.00	12.67	70.00	82.67	15.32	1.49	6.70	2.00	3.35	3.00	3.00
12.00	4.33	3.00	28.00	17.67	45.67	61.31	1.77	7.17	1.53	4.67	3.00	3.00
13.67	3.00	2.67	30.00	7.00	37.00	81.08	1.29	6.97	1.86	3.74	1.00	1.00
16.67	2.67	1.67	31.33	15.33	46.67	67.14	1.96	7.40	2.00	3.71	5.00	5.00
11.83	4.33	4.33	17.00	23.67	40.67	41.80	1.13	6.73	2.30	2.93	5.00	5.00
13.20	6.33	4.33	28.00	25.00	53.00	52.83	1.38	6.80	2.03	3.34	5.00	3.00
10.33	3.67	1.67	22.67	37.00	59.67	37.99	1.50	6.77	1.33	5.08	5.00	5.00
14.33	2.67	2.00	19.67	20.67	40.34	48.76	1.07	6.20	1.00	6.20	5.00	3.00
16.00	2.67	2.33	21.67	17.33	39.00	55.56	1.27	6.03	1.90	3.18	5.00	5.00
8.97	2.33	1.67	48.00	28.33	76.33	62.88	1.05	7.10	1.37	5.20	0.00	5.00
13.00	1.33	1.00	10.00	17.67	27.67	36.14	1.57	7.13	1.90	3.75	0.00	3.00
10.00	3.00	1.67	33.00	54.33	87.33	37.79	1.08	6.93	2.10	3.30	0.00	3.00

13.00	3.00	1.67	37.00	14.00	51.00	72.55	1.08	6.80	2.07	3.29	7.00	7.00
15.33	2.33	1.67	14.33	45.67	60.00	23.88	1.32	6.60	2.20	3.00	0.00	1.00
13.67	1.67	1.67	11.00	39.00	50.00	22.00	1.85	6.93	2.27	3.06	1.00	1.00
14.67	2.67	2.67	27.00	10.33	37.33	72.32	1.97	6.60	1.83	3.60	1.00	1.00
19.33	4.33	2.67	15.00	41.67	56.67	26.47	1.52	7.30	1.37	5.34	5.00	3.00
10.83	2.67	2.33	32.67	26.67	59.33	55.06	0.92	6.67	1.77	3.77	1.00	1.00
13.33	1.00	1.00	43.00	50.00	93.00	46.24	1.45	7.13	2.20	3.24	1.00	3.00
18.00	3.33	2.00	25.00	29.33	54.33	46.01	1.66	7.10	2.10	3.38	1.00	1.00
10.00	3.33	2.00	9.33	14.00	23.33	40.00	1.81	4.92	5.00	0.98	9.00	3.00
11.00	3.67	1.00	28.00	27.00	55.00	50.91	1.77	7.07	2.17	3.26	1.00	1.00
38.00	19.67	4.00	31.00	32.33	63.33	48.95	1.46	7.50	2.23	3.36	1.00	1.00
11.33	2.33	1.33	18.00	23.67	41.67	43.20	1.68	6.67	3.70	1.80	1.00	1.00
10.67	2.00	1.33	28.33	24.67	53.00	53.46	1.56	6.93	2.17	3.20	3.00	3.00
14.33	2.00	1.67	11.00	13.00	24.00	45.83	1.59	7.07	2.20	3.21	1.00	1.00
12.67	4.33	2.33	37.67	28.33	66.00	57.07	1.33	6.33	2.07	3.06	9.00	3.00
10.33	2.67	1.67	26.00	33.33	59.33	43.82	1.03	7.17	1.97	3.64	0.00	1.00
12.00	2.00	2.00	35.67	11.33	47.00	75.89	1.82	5.70	2.00	2.85	0.00	1.00
10.33	3.33	2.00	17.00	11.00	28.00	60.71	1.64	7.10	2.20	3.23	0.00	1.00
15.00	3.00	2.67	24.33	12.67	37.00	65.77	1.39	5.20	1.57	3.32	0.00	1.00
19.66667	3.33	23.33333	50	33	83	60.24096	26	7.466667	1.2	6.222222	7	1
29.33333	7.6	16.66667	74	13	87	85.05747	17	6.566667	1.666666667	3.94	3	7

APPENDIX III

List of Polymorphic Markers used in the research work.

PRIMER NO	CHROMOSOME NO.	FORWARD	RESERVE	BASEPAIR
RM 1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	4568457
RM 5	1	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	22993785
RM 9	1	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC	24298288
RM 24	1	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG	19738458
RM 129	1	TCTCTCCGGAGCCAAGGCGAGG	CGAGCCACGACGCGATGTACCC	19738458
RM 212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	34138065
RM 3825	1	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTTCG	37517249
RM 237	1	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	27865492
RM 104	1	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC	41175917
RM 283	1	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG	4812644
RM 11943	1	CTTGTTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC	38775892
RM 259	1	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	758037
RM 431	1	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	39861138
RM 526	2	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG	28326124
RM 2634	2	GATTGAAAATTAGAGTTTGCAC	TGCCGAGATTTAGTCAACTA	21727602
RM 475	2	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC	21631465
RM 138	2	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG	37466179
RM 482	2	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC	37090281
RM 530	2	GCACTGACCACGACTGTTTG	ACCGTAACCCGGATCTATCC	32314410
RM 174	2	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG	7022559
RM 154	2	ACCCTCTCCGCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	1028624

RM 207	2	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC	37187760
RM 211	2	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	1966335
RM 240	2	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCTTCCATCC	33241470
RM 279	2	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	2836363
RM 1281	2	ACAGTACACTAGGACATGGTTACG	GTCTAATTGCAGAATGCAGACC	7890177
RM 85	3	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC	30667358
RM 523	3	AAGGCATTGCAGCTAGAAGC	GCACTTGGGAGGTTTGCTAG	1332286
RM 251	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTGATC	10289586
RM 1256	3	ACGCGAAGCAACGGAGATAG	CTAGCCTCGATGCGAAAAAC	10083660
RM 1038	3	TGGTTCGATTTCGGATTTT	AAGCTATTACAAAGCAGCTC	36773519
RM 1352	3	ACGAGTTGTACTCTGGTTGC	TCTCGGTTTTTATCTTGCTG	35433741
RM 232	3	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG	10095197
RM 514	3	AGATTGATCTCCCATTCCTCC	CACGAGCATATTACTAGTGG	38354880
RM 1373	3	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG	28316124
RM 571	3	GGAGGTGAAAGCGAATCATG	CCTGCTGCTCTTTTCATCAGC	36279079
RM 7000	3	CCCTTCTTTTCAACTGAATA	TTGTAACAATGAACTCGTTC	36915762
RM 3867	3	TTGACTGGAACATCGAGCTC	ATCCCCTCTACACCGTACCC	34856087
RM 218	3	TGGTCAAACCAAGGTCCTTC	GACATACATTCTACCCCCGG	8702745
RM 3719	3	GTAGGTCAACTTACACGCAGTG	GTACATACGACCAACGCTGC	35731912
RM 448	3	TCTGATCTTGATGCAGGCAC	TCTCCCGATTTGGACAGATC	34495121
RM 517	3	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC	6298762
RM 307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	16865934
RM 7563	4	CCACCGCTCGTAGAAAAAAC	GGGTTGAGATGCCTGTGC	22942121
RM 567	4	ATCAGGGAAATCCTGAAGGG	GGAAGGAGCAATCACCCTG	34983463
RM 335	4	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	763604
RM 280	4	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG	35462222
RM 255	4	TGTTGCGTGTGGAGATGTG	CGAAACCGCTCAGTTCAAC	31289565

RM 303	4	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAAGTTCGGT	28908370
RM 249	5	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC	11041719
RM 164	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	20464758
RM 13	5	TCCAACATGGCAAGAGAGAG	GGTGGCATTTCGATTCCAG	2076455
RM 163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	20457690
RM 8225	6	ATGCGTGTTTCAGAAATTAGG	TTGTTGTATACCTCATCGACAG	9239206
RM 225	6	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	3315378
RM 276	6	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	6075303
RM 253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC	5206122
RM 541	6	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG	20304998
RM 589	6	ATCATGGTTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	1259100
RM 340	6	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	30043709
RM 3827	6	GGACGGATTGTAGGTAGGAC	CCTTTCTTCAATCTGCATTC	23378465
RM 3300	7	GGCTACGCCTTCGTCGTAC	ACTAGTGCAGGGCAGCGTAG	19571420
RM 5499	7	TGGAGTACGACGTGATCGTG	CAGAAACGGGAGGGGATC	10134228
RM 5436	7	CAAAGGGGGTGTCTCTATG	GTTGCTCGTCCTACATGTGC	9171773
RM 5720	7	CCTGATAAATTGACAGTTAC	GAGAGTAGGAGTTGATAACA	29184213
RM 210	8	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	23883089
RM 152	8	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	705151
RM 25	8	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	4371944
RM 5432	8	GTTTCCCCACTTATCTCCCC	AAGCGAGGAGGGGTTTAGAG	4371988
RM 5556	8	ATCTCCCTCCCTCTCCTCAC	TCCACACCTTCACAGTTGAC	4531189
RM 5647	8	ACTCCGACTGCAGTTTTTGC	AACTTGGTCGTGGACAGTGC	2913740
RM 256	8	GACAGGGAGTGATTGAAGGC	GTTGATTTCCGCAAGGGC	25738966
RM 223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	22021511
RM 515	8	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC	21639843

RM 310	8	CCAAAACATTTTAAAATATCATG	GCTTGTTGGTCATTACCATTG	4964359
RM 447	8	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC	27941903
RM 44	8	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	11686201
RM 337	8	GTAGGAAAGGAAGGGCAGAG	CGATAGATAGCTAGATGTGGCC	184425
RM 547	8	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG	5461947
RM 38	8	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCAC	2038831
RM 1235	8	AGCAGAGGAGGAGATGATGG	GGACCAAAACGAAGCTATCC	1232681
RM 342	8	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTACCC	21350354
RM 434	9	TTTGTGGCTCACCAGTTGAG	TGGCGCATTGATGTCATC	11046418
RM 205	9	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTACGTTTCAGTG	24479344
RM 257	9	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	19184712
RM 242	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	20324402
RM 219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	9010430
RM 245	9	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG	23964362
RM 496	10	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC	24703987
RM 304	10	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG	20860234
RM 287	11	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	19188007
RM 206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	25045284
RM 229	11	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	21261527
RM 224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	30450718
RM 17	12	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA	25980202
RM 260	12	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	8229359
RM 3331	12	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC	22341707
RM 19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	3937986