SCREENING OF RICE (*Oryza sativa* L.) MAPPING POPULATION FOR SUBMERGENCE AND DROUGHT TOLERANT TRAITS

THESIS

SUBMITTED TO THE
ACHARYA NARENDRA DEVA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE (AGRICULTURE)

IN

AGRICULTURAL BIOTECHNOLOGY

BY

VISHWASH KUMAR MISHRA
Id. No. A-10051/17

DEPARTMENT OF PLANT MOLECULAR BIOLOGY & GENETIC ENGINEERING
ACHARYA NARENDRA DEVA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, NARENDRA NAGAR (KUMARGANJ),
Ayodhya-224229 (U.P.)
INDIA

2020


आचार्य नरेन्द्र देव कृषि एवं प्रौद्योगिक विश्वविद्यालय, कुमारगंज, अयोध्या
ACHARYA NARENDRA DEVA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, KUMARGANJ
• AYODHYA •
U.P. INDIA
उत्तर प्रदेश भारत
अन्नं हि जीवानां ज्येष्ठम्

Dr. Shambhoo Prasad
Associate Professor

DEPARTMENT OF PLANT MOLECULAR BIOLOGY & GENETIC ENGINEERING
A.N.D. University of Agriculture and Technology, Kumarganj,
Ayodhya-224 229 (U.P.) India

CERTIFICATE-I

This is to certify that the thesis entitled **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"** submitted for the degree of **'Master of Science'** in the subject of **Agricultural Biotechnology** to the Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.) is a bonafide research work carried out by **Vishwash Kumar Mishra, Id. No. A-10051/17** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation has been duly acknowledged.

Narendra Nagar
January, 2020

(Shambhoo Prasad)
Major Advisor and Chairman

CERTIFICATE-II

This is to certify that the thesis entitled **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"** submitted by **Mr. Vishwash Kumar Mishra, Id. No. A-10051/17** to the **Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.)** in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Agricultural Biotechnology** has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with an external examiner.

(External Examiner)

Major Advisor & Chairman — **(Shambhoo Prasad)**
Associate Professor
Department of Plant Molecular Biology & Genetic Engineering

Member (Major) — **(Adesh Kumar)**
Assistant Professor
Department of Plant Molecular Biology & Genetic Engineering

Member (Minor) — **(R.P. Singh)**
Assistant Professor
Department of Agri. Biochemistry

Member (Related Field) — **(Shiv Nath)**
Associate Professor
Department of Genetics & Plant Breeding

Dean's Nominee — **(Ved Prakash)**
Professor
Department of Soil Science

Dean, College of Agriculture — **Head of Department**


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ACKNOWLEDGEMENT

*First of all, Successful completion of my thesis is marked by the gracious presence and blessing of **"God"** in my life though these blessing are beyond my thanks but still I pray to mark presence always ahead in my life.*

At the end of my thesis I would like to thank all those people who made this thesis possible and an unforgettable experience for me.

*I would like to express my deepest sense of Gratitude to my Advisor **Dr. Shambhoo Prasad**, Associate Professor, Department of Plant Molecular Biology & Genetic Engineering, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, who offered his continuous advice and encouragement throughout the course of this thesis. I thank him for the systematic guidance and great effort he put into training me in the scientific field. Like a charm!*

*I feel profound delighted and prerogative to express my deep sense of gratefulness to **Dr. D. K. Dwivedi**, Professor & Head, Department of Plant Molecular Biology & Genetic Engineering, & Dean, College of Home Science, for his inspiring guidance, imperative and facile suggestion throughout the course of the present investigation and preparation of this manuscript. I shall always bestowed remain indebted to him for his parental care affection and generosity upon me. I am highly indebted to as without him it was not possible to complete the thesis work.*

*I have a deep sense of gratitude for the members of my advisory committee **Dr. Adesh kumar**, Assistant Professor, Department of Plant Molecular Biology & Genetic Engineering, & Dean, College of Home Science, **Dr. R.P. Singh** , Assistant Professor, Department of Agri. Biochemestry , **Dr. Shiv Nath**, Associate Professor, Department of Genetics and Plant Breeding**, Dr.Ved Prakash**, Professor, Department of soil Soil Science, as Dean Nominee for their constructive criticism, co-operation and pertinent suggestion at various stages of investigation.*

I pay my heartiest gratitude, obligations and sincere regards to ***Dr. Bijendra Singh****, Hon'ble Vice-Chancellor &* ***Dr. V. N. Rai****, Dean, College of Agriculture, for providing necessary facilities for carrying out the present investigation.*

I am immensely thankful to faculty members of the Department of Plant Molecular Biology and Genetic Engineering especially, ***Dr. N. A. Khan****,* ***Dr. Adesh Kumar*** *and all supporting staffs Mr. S. P. Singh, Mr. Keshav, Mr. Aditya, Mrs. Sita Pati, Mr. Ram Krishan, Mr. Ramadhar and Mr. Ashok for diligent guidance, timely help and providing all the assistance during my course investigation.*

I gratefully acknowledge most sincere and generous co-operation provided by my super seniors ***Dr. Tanvi Singh Chauhan, Dr. Bandana Jaiswal, Mrs. Shalini Srivastva, Dr. Gyanendra Kumar, Dr. Pradeep Kumar Bharti, Dr. Pratibha Yadav, Dr. Sonam Singh, Mrs. Sanghmitra, Dr. Divya Srivastava, Vaibhav Singh*** *and I am also thankful to my seniors* ***Praveen Tiwari, Nandan Singh, Pankaj Singh, Vishwajeet Singh*** *for their help, support and co-operation to complete this manuscript.*

I also bestow my heartiest thanks to my batchmates ***Ashish Kumar ,Rahul Kumar, Bhawna Mathur, Jaswant Singh Abhishek Verma, Anuj Kumar,and Pranci Tiwari*** *for their help, support and co-operation to complete this manuscript.*

I wish to record my sincere thanks to my lovely Juniors ***Meenakshi, Samridhi Singh, Ram Milan , Reshu, Shagun, Rahul and Niyaz*** *who always stood by my side to help me directly or indirectly during entire curriculum of my M.Sc. programme.*

I can't dare to forget my heartfelt and loving thanks to my special and lovely friends, ***Vikash, Prasant Mishra, Prabhat, Shiv Kishor and Raja***

Shukla *for their love, affection, care and encouragement. They are specific icon of my heart and always made me more efficient and energetic.*

I extend my heartiest thanks to my father Late. ***Mr. Anoop Kumar Mishra*** *. I also give my warmest thanks to my whole* ***"Family"*** *because the credit of my success goes to my family and my mother* ***Mrs. Uma Mishra*** *whose blessings are always with me that could never fall down to me. I special thanks to my sweetest brother & siste* ***Somya, Supriya, Vishesh (Raj), Vinay, Gyanendar, Pooja, Reeta and Puneet. I*** *am highly thankful to my lovely mama ji Mr.* ***R.K Tripathi , my lovely Fhufha ji Mr. O.P. bajpaiyi. my lovely elder Brother Mr. Dharmendra and my loving jija ji Mr. Mohlt*** *have always been providing me mental strength and courage for the successful completion of the work acknowIedging them for all they have done for me will be belittling them.*

My grandfather Iate. Ji Mr.Chandra bali for their constant encouragement and inspiration and my grandmother, Mrs. Sharweswari

Fmally my hearties, thanks to my special Akrati Dev, Ashish and jsawant for providing me necessary facilities and support to complete this manuscript.

Narendra Nagar, Kumarganj ***(Vishwash Kumar Mishra)***
January, 2020

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ABBREVIATIONS

ANOVA	Analysis of variance
bp	Base pair
CM	Centimorgan
C-TAB	Cetyl trimethyl ammonium bromide
ddH_2o	Double distilled water
DNA	Deoxy ribonucleic acid
d NTP	Deoxy nucleotide triphosphate
EC	Electrical conductivity
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium bromide
g	Gram
Kbp	Killobase pair
KD	Kilo Dalton
mA	Milli ampere
MAS	Marker assisted selection
Mg	Milligram
M	Molar
mM	Millimolar
MP	Mapping population
mV	Milli volt
OD	Optical Density
PCR	Polymerase chain reaction
PVP	Polyvinyl pyruvate
QTL	Quantitative trait loci
RILs	Recombinant inbred lines
RPM	Round per minute
RAPD	Random amplification polymorphic DNA
SSR	Simple Sequence Repeat
TAE	Tris-acetate EDTA
Taq pol	Thermus aquaticus polymerase
TE	Tris EDTA
Tm	Melting temperature

Chapter-I

INTRODUCTION

Rice is one of the most staple food crop of India as well as many parts of the world. It is the world's single most important food crops and primary food source for one third of the world population. Rice is grown in more than a hundred countries, with a total harvested area of approximately 158 million hectares, contributes about 40 to 43% of total food grain production and continues to play a key role in the national food and livelihood security system.

In addition, rice constitutes one of the main agricultural exports. Rice is cultivated worldwide over an area of about 158 million hectare with annual production of 650.19 million tones (Press Information Bureau *Government of India* 2017-18). India ranks first in area and second in production of rice after China. In India it is grown over an area of 43.19 million hectares having production of 110.15 million tones and average productivity of 2550 kg/ha. It contributes to 65% of the total population. Uttar Pradesh is an important rice growing state of the country. The area and production of rice in the state is about 5.95 million hectares and 13.27 million tones, respectively with productivity of 2230 kg per hectare (Ministry of Agriculture & Farmers Welfare Government of India, 2018).

Rice is currently grown in varied environmental conditions where it shows different levels of response to abiotic stress, depending on the environmental condition of origin and cultivation (Rananwake and Hewage, 2014). The climate change, such as drought, flooding, salinity and high temperature have detrimental impacts on rice production, especially in developing countries. Rice crops are prone to various types of

stresses, both biotic and abiotic. Biotic stresses include insect pests, fungus, bacteria, viruses, and herbicide toxicity. Among abiotic stresses, drought, cold, submergence and salinity are also well studied in rice (Ansari *et al*., 2015).

Under rainfed lowland condition, among number of abiotic stress, submergence and drought are major ones and it affects rice production a lot. Drought and submergence are the two major limiting factors that reduce rice production. These two abiotic stresses can completely destroy crop production in extreme conditions, and consequently both of stresses are considered as key determinants of global food security. Moreover, climate change is also projected to undermine global food security (Sarkar *et al.,* 2006).. Rice plantation is oldest agriculture in Asia and supplies more than 80% of calorie and 75% of protein consumed by people of these continents (Rabiey, 1996).

Water logging is defined as a water logging is a condition of land in which the soil profile is saturated with water either temporarily or permanently. It creates the damages to plants as consequences of slow rates of gas exchange, severe shading by turbid water, mechanical damages due to strong flow rates and solute carrying capacity of flooded water (Michael *et al*., 2001). Water logging is one of the most hazardous natural occurrences, which can also be called as flood, submergence, soil saturation, anoxia, and hypoxia, which are generally used to describe water logging conditions depending upon the moisture or water level on the field. (Mohanty *et al*.1985)

Though rice is a crop that requires flooded and irrigated condition for cultivation, most of the rice varieties are susceptible to flooding if the water stagnates keeping the plants submerged under water for more than

seven days causing leaf or stem elongation, leaf rotting, loss of dry mass and also lodging after the flood water recedes. Submergence caused by flash flood is a key factor limiting the yield of lowland rice (Goswami *et al.,* 2015).

Flash floods are highly unpredictable and can occur at any growth stage of the rice crop, resulting in yield loss of 10% to 100%, depending on water depth, duration of submergence, temperature, turbidity of water, light intensity, and age of the crop, etc (Setter *et al*. 1997).

Drought is one of the major abiotic stresses affecting plant growth and reducing crop productivity, which has been estimated to affect 70% of crop yield (Bray *et al*., 2000) Drought stress is a problem in approximately 45% of agricultural areas and the largest global constraint to productivity, becoming a major issue in scientific reports (Ahmadi *et al.* 2012; Ambavaram *et al.* 2014; Heinemann *et al.* 2015; Todaka *et al.* 2015). Drought is defined as the inadequacy of water availability including precipitation of soil moisture storage capacity in quality and distribution during the life of the crop. Plant responses to drought stress are very complex. Drought response at the cellular stage depends on the plant stage when drought occurs, duration of drought and plant species (Prasad *et al.,* 2012).

Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture. Rice varieties which cope with drought using their root systems to maintain their plant water status comes under drought avoidance category. Such varieties therefore minimize the yield losses caused by drought (Singh *et al*., 2012). Mechanisms for improving water uptake, storing it in plant cell and reducing water loss confer drought avoidance. Rice varieties which avoid

drought usually have deep, coarse roots with a high ability of branching and penetration, higher root to shoot ratio, elasticity in leaf rolling, early stomatal closure and high cuticular resistance (Wang *et al.,* 2006).

Molecular markers are the tools that can be used to detect the presence of desire character which is economically important. Marker assisted backcrossing (MABC) is an attractive tools for breeding vastly using in a large number of research aim at identifying genomic regions of interest. Molecular markers that are tightly linked with economically important traits have been identified and/or used for MABC in rice including resistance of bacterial blight, virus infections, and tolerance submergence, drought, grain quality and much more. (Hasan *et al.,* 2015).

Potential of backcrossing for foreground selection approach has been established by applying molecular markers named as simple sequence repeats (SSRs) and single nucleotide polymorphisms. SSR are more popular in rice because they are highly informative, mostly monolocud, codominant, easily analyzed and cost effective (Garcia *et al.,* 2004). SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice sub species (Ni *et al*., 2002).

So, developing dual resistant rice for submergence at vegetative stage and drought at reproductive stage is current need to rice growing farmers in rainfed low land areas. Nagina-22 is drought tolerance rice genotype. It is used as a donor perent and crossed with Swarna sub-1 for developing dual tolerance rice lines. Swarna sub1 already has a trait for flooding tolerance. Submergence and drought tolerant Swarna sub1 will fulfill the demand of the farmer of rainfed low land ecosystem by transferring drought tolerant trait for tested donor. Such variety will be

developed by RIL backcross method by identifying QTL as well as developing high yielding dual tolerance rice varieties by backcross method. Such variety will be developing by backcross method through following objectives:-

1. Screening of existing mapping population (Swarna Sub1 x N22) x Swarna Sub1, BC_2F_3 for submergence and drought traits by phenotyping and genotyping.
2. The Foreground Selection of existing mapping population by molecular markers.
3. Identify the lines for yield and yield attributing traits.

Chapter-II

REVIEW OF LITERATURE

Review of literature pertaining to the topic **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"** have been collected and reviewed systematically under the following heading.

Classification:

Oryza sativa contains two major subspecies: the sticky, short grained *japonica* or *sinica* variety, and the non sticky, long-grained *indica* variety. *Japonica* varieties are usually cultivated in dry fields, in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia, while *indica* varieties are mainly lowland rice, grown mostly submerged, throughout tropical Asia. Rice occurs in a variety of colors, including: white, brown, black, purple, and red rice. Black rice (also known as purple rice) is a range of rice types, some of which are glutinous rice. Varieties include Indonesian black rice and Thai jasmine black rice.

A third subspecies, which is broad-grained and thrives under tropical conditions, was identified based on morphology and initially called *javanica*, but is now known as *tropical japonica*.

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Monocots
(unranked) : Commelinids
Order : Poales

Family : Poaceae

Genus : *Oryza*

Species : ***Oryza sativa***

Binomial name: *Oryza sativa*

Rice as a model cereal crop:

As a cereal grain, it is the most important staple food for a large part of the worlds human population. Rice has now become a model plant among all the monocots. Rice has many types of genetic and molecular studies as parallel to Arabidopsis in dicots (McCouch and Doerge, 1995). Rice has smallest genome size among the known monocots (approximately 400 Mb, 250-300 Kb corresponding to one cM), and high density molecular map consisting of thousands of DNA markers for marker assisted selection (MAS). Rice has efficient transformation and regeneration capacity from the protoplast culture.

Submergence:

Physiological approaches for submergence tolerance:

Tamang *et al*., (2015) reported that flash floods occasionally result in complete submergence of plants in agricultural and natural ecosystems. When immersed in water, plants encounter multiple stresses including low oxygen, low light, nutrient deficiency, and high risk of infection. As flood waters subside, submerged plants are abruptly exposed to higher oxygen concentration and greater light intensity, which can induce post-submergence injury caused by oxidative stress, high light, and dehydration. Recent studies have emphasized the significance of multiple stress tolerance in the survival of submergence and prompt recovery following desubmergence. A mechanistic understanding of acclimation responses to

submergence at molecular and physiological levels can contribute to the deciphering of the regulatory networks governing tolerance to other environmental stresses that occur simultaneously or sequentially in the natural progress of a flood event.

Ray *et al*., (2013) studied on the genetic variability among different submergence tolerant genotypes. An experiment of Six submergence and medium stagnant water tolerant high yielding genotypes along with two standard check varieties having submergence tolerance were evaluated in the real submergence and/or medium stagnation prone environments of the farmers' field. Flash floods can result in yield loss up to 100% depending on different climatic & agronomic factors. Flash-flooding adversely affect at least 16% of the rice lands of the world (~22 m ha) and 1 billion USD annual loss in South & Southeast Asia. In Bangladesh, ~2.0 mha of rice lands are unfavorably affected by excess water and periodically suffer from flash-floods and reduces 5% average yield in Bangladesh. In this circumstance Improvement of germplasm is likely the best option to withstand submergence and stabilize productivity in these environments. Progress in germplasm improvement has been slow but can substantially be enhanced if the physiological and genetic bases of submergence tolerance are well understood. This review focuses on current physiological understanding of tolerance to submergence in rice with greater emphasis on floodwater environments new genetic resources. However, BRRI dhan52 could be disseminated in the single flash flood prone areas of Rangpur region in order to increase productivity.

Sarkar *et al*., (2009) conducted during rainy season of 2005–07 under favourable rainfed lowlands and controlled submergence at Cuttack, as well as under natural farmers' field. Under flash-flooding, genotypes

with Sub1 survived complete submergence stress with turbid water for up to 12 days, whereas genotypes without Sub1 did not survive. The submergence stress was not so severe in farmers' fields, yet 'Swarna' Sub1 gave higher grain yield than 'Swarna' at all sites with a yield advantage of up to 1.65 tonnes/ha (an average of 0.81 tonnes/ha over five sites). The results suggest that rice genotypes with Sub1 have great potential for improving the productivity of rainfed lowland rice prone to flash flooding.

Biswajit and Sabyasachi (2015) studied on 10 days old rice seedlings of three rice genotypes viz. FR13A, Mahananda and Swarna were sprayed with 10μMolar/Lt solution of gibberellic acid (GA_3) retardant paclobutrazol and stress hormone abscisic acid (ABA), gibberellic acid (GA_3) and ethylene inhibitor substances like STS solution of concentration 0.02 Molar, 20 μMolar, 0.6 mili Molar; 10 μMolar $CoCl_2$ and 240ppm $AgNO_3$ solution on the leaves prior to two days of submergence under drum and pot screening method in each separate set up in three replications. Gibberellin and ethylene inhibitors reduce the stem elongation and increases survival% after complete four days full submergence. The important genetic variability components viz. CV, GCV, PCV, h%, GA and GA% of mean were estimated for different agro morphological characters after treatment effect. Manipulation of gibberellic acid and ethylene action are very important for breeding submergence tolerance in rice.

Akinwale *et al.*, (2012) studied on the submergence tolerance of 20 rainfed lowland rice (*Oryza sativa* L.) cultivars consisting of six Nigerian rainfed lowland mega rice cultivars, five Asian submergence tolerant mega varieties, four landraces, two lowland NERICAs and three parents of Sub1 varieties were evaluated in a natural water pond that allows maintenance of

flood water depth of 1.5m for a period of 14 days. Thirty days old seedlings were submerged for 14 days under 100 cm of water followed by normal condition. Survival counts were taken visually 10 days after withdrawal of flood water. Data were also collected on plants for stem elongation, date at 50% flowering, plant height, number of tillers at maturity, number of panicles at maturity and grain weight. Plant survival recorded 10 days after de-submergence showed large cultivar differences. Percentage survival varied from 3.2 to 97.5%. All mega varieties with Sub1 gene had a significantly higher percentage survival and grain yield.

Setter and Laureles (1996) reported that adverse effects of elongation growth on tolerance to complete submergence for up to 14 d were evaluated in rice seedlings of cultivars which differed in submergence tolerance. There is a good negative correlation between per cent survival and elongation growth of genotypes during complete submergence (r= 0.81). When elongation growth underwater is minimized by application of a gibberellin biosynthesis inhibitor, per cent survival increases by as much as 50 times for one cultivar. These effects are likely related to elongation growth since (i) addition of gibberellin had the opposite effect by reducing survival, and (ii) when the elongation inhibitor and gibberellin were added together, there was no effect on elongation growth and the per cent survival did not change.

Kuanar *et al.*, (2017) reported that Stagnant flooding (SF) is an important constraint which prolonges partial submergence damages of rice plants and reduces grain yield. Due to the heterogeneity in flood-prone ecosystem, many different types of traditional rice varieties are being grown by the farmers. The local landraces adapted to extreme in water availability could be the sources of new gene(s) which would be utilized to

improve the adaptability of rice to SF with high yield. A total of 16 rice varieties were selected after initial screening from more than 400 rice varieties which were collected from eastern states of India. The reduction of root oxidase activity, leaf area, and leaf dry weight was higher in susceptible varieties under SF compared to control.

Biochemical approaches for submergence tolerance:

Panda and Sarkar (2013) reported that Nonstructural carbohydrate (NSC) accumulation in submergence tolerant rice cultivars (cv) was studied in six Indica rice [*Oryza sativa* (L.)] cv under control and simulated submerged conditions. Tolerant cultivars accumulated greater contents of NSC compared to the susceptible cultivars. Starch and total NSC content showed significant positive association with survival percentage. On the other hand, elongation due to submergence was significantly a negative association with survival. The CO2 photosynthetic rate, chlorophyll content, maximum photochemical efficiency of PS II (Fv/Fm), and activities of Rubisco were not significantly different between tolerant and susceptible cv under control condition. The ADP glucose pyrophosphorylase (AGPPase) activity was significantly higher in the tolerant cv and was a positive association with starch/NSC, whereas Fructose 1,6-diphosphatase (FDPase) activity was significantly higher in susceptible cv compared to tolerant cv and was a negative association with starch/NSC. Greater activities of AGPPase along with lower activities of FDPase might facilitate greater accumulation of NSC in tolerant rice cultivars.

Tsuji *et al.*, (2003) studied on Post-hypoxic injuries in plants are primarily caused by bursts of reactive oxygen species and acetaldehyde. In agreement with previous studies, we found accumulations of acetaldehyde

in rice during re-aeration following submergence. During re-aeration, acetaldehyde-oxidizing aldehyde dehydrogenase (ALDH) activity increased, thereby causing the acetaldehyde content to decrease in rice. Interestingly, re-aerated rice plants showed an intense mitochondrial ALDH2a protein induction, even though ALDH2a mRNA was submergence induced and declined upon re-aeration. This suggests that rice ALDH2a mRNA is accumulated in order to quickly metabolize acetaldehyde that is produced upon re-aeration.

Joshi and Rao (2009) said that **s**ubmergence tolerance is an important agronomic trait for rice grown in eastern India; where flash flooding occurs frequently and unpredictably during the monsoons. Generation of somaclones for the two submergence tolerant rice cultivar FR13A and FR43B through gamma irradiation and molecular analysis of the somaclones for the variation in the pyruvate decarboxylase (*pdc*) gene was investigated. FR43B showed a relatively higher frequency of callus induction than FR13A. However, the % regeneration of somaclones in both the genotypes gradually decreased with increase in the level of radiation dose. The somaclones of FR43B showed greater tolerance to submergence than FR13A. The doses/concentration of GR 20Kr and GR 25Kr irradiation increased the morphological and yield parameters over those in controls. All the somaclones with the pdc1 were tolerant to submergence irrespective of the gamma dose there by suggesting that pdc1 gene is directly linked to submergence tolerance in rice.

Banerjee *et al.*, (2015) conducted study on three rice varieties, significantly differed in their ability, when subjected to submergence have been studied in relation to physiological attributes A sharp increase in guaiacol peroxidase and glutathione reductase characterized the plants'

response to sub-mergence irrespective of varieties. The expression of Guaiacol peroxidase was increased in FR13A followed by Swarna Sub1A and Swarna. Glutathione reductase was measured in terms of oxidation of NADP (H) and both FR13A and Swarna Sub1A recorded maximum oxidation than Swarna under submergence.

Molecular approaches for submergence tolerance:

Septiningsih *et al.*, (2013) found that flooding stress is one of the most important abiotic stresses constraining rice production, especially in rain-fed lowland areas. The effect of this stress has in tensified in past decades and is predicted to increase in the years to come as a result of global climate change. At the International Rice Research Institute (IRRI), breeding for tolerance to submergence imposed by flash flooding during the vegetative stage has been one of the institute's priority objectives for more than three decades. Several tolerant breeding lines have been developed through conventional breeding; however none of those early varieties has been widely accepted by farmers. An important breakthrough was the identification of the major quantitative trait locus (QTL) SUB1 in the mid-1990s, which led to the identification of three ethylene responsive factors (ERFs), of which SUB1A is the primary contributor for tolerance.

Septiningsih *et al.*, (2014) Submergence is an escalating problem in many rice producing areas. A submergence tolerance gene, SUB1, derived from FR13A was previously introduced into six mega varieties through marker assisted backcrossing (MABC) with the final product selected at the BC2 or BC3 generation. Their phenotype was similar to the original varieties, but they could withstand complete inundation for up to 2 or 3 weeks. Several of these varieties have been released in South and Southeast Asia; nonetheless the development of additional submergence

tolerant varieties is indispensable to provide farmers with diverse choices of varieties that are preferable for the local needs and to avoid ecological vulnerability due to planting only one variety across vast areas. To accelerate this effort, the SUB1 gene has now been introgressed into two new popular varieties from Indonesia and the Philippines, i.e. Ciherang and PSB Rc18, respectively, through MABC using only one backcross (BC1) with the previously developed IR64-Sub1 as the donor. Since this new donor is closely related to both recurrent parents, a more rapid MABC approach can be pursued due to the similarity of genetic backgrounds. Using this strategy, new submergence tolerant varieties Ciherang-Sub1 and PSB Rc18-Sub1 were developed in less than 2 years, presenting a promising approach to convert additional popular varieties in the future.

Bishnu Pada Ray (2018) found that Submergence stress regularly affects 15 million hectares or more of rainfed low land rice areas in South and South-east Asia, molecular markers that were tightly linked with Sub1, flanking Sub1, and unlinked to Sub1 were used to apply foreground, recombinant, and background selection, respectively, in backcrosses between a submergence-tolerant donor and the widely grown recurrent parent. The highest grain yield (5.24t/ha) was obtained from BPR6 followed by BRRI dhan52 (5.03t/ha) and Survival % was also obtained as highest in BPR6 (92%). In case of BPR6, farmers choose this line due to higher yield than others & also attractive grain size. However, it is expected that the promising sub1 line BPR6 will be possible to develop high yielding submergence tolerant variety with three to four weeks tolerance to increase the rice production in the submergence prone areas of Bangladesh where arises single flash flood under different cropping patterns.

Jena and Mackill (2008) advocated that Marker-assisted backcross breeding has been used to effectively integrate major genes or quantitative trait loci with large effect into widely grown varieties. Pyramiding different resistance genes using MAS provides opportunities to breeders to develop broad-spectrum resistance for diseases and insects. The use of cost-effective DNA markers derived from the fi ne mapped position of the genes for important agronomic traits and MAS strategies will provide opportunities for breeders to develop high-yielding, stress-resistant, and better-quality rice cultivars.

Tiwari (2018 The Sub 1 QTL is a single gene that has the LOD score of 36 which explained phenotypic variance of 69% conferring tolerance to complete submergence of two weeks. The submergence -1 (Sub-1) locus representing a cluster of three ethylene responsive factor (ERF) genes: sub1A, sub1B and sub1C. Identification of the SUB1 gene was the entry point for enabled marker assisted selection (MAS) for submergence tolerance. Popular varieties also are known as mega varieties which possessed high yielding and good grain quality was used as the recurrent parent in marker assisted backcrossing (MABC). Beyond the Sub 1 varieties, the present day rice breeders paid attention to tolerance to stagnant flooding and tolerance to anaerobic germination under deep water and flash flood during seedling germination respectively. Quantitative Trait Loci (QTLs) have been identified for anaerobic germination also referred to as AG (AG1 and AG2) and stagnant flooding for deepwater rice (SNORKEL1 and SNORKEL2).

Sarkar *et al.*, (2006) studied on the environments of rainfed lowland rice are highly variable both over time and location. Flash-flooding and submergence adversely affect at least 16% of the rice lands of the world

(~22 m ha). In eastern India, ~13 m ha of rice lands are unfavourably affected by excess water and periodically suffer from flash-floods and complete submergence. Improvement of germplasm is likely the best option to withstand submergence and stabilize productivity in these environments. However, progress in germplasm improvement has been slow but can substantially be enhanced if the physiological and genetic bases of submergence tolerance are well understood and extent of damages over time and location is known. This review focuses on current physiological. understanding of tolerance to submergence in rice with greater emphasis on floodwater environments, new genetic resources and potential of DNA marker technology for incorporating multiple traits associated with tolerance, to enhance and speed progress through breeding. Research on the aspect has been further facilitated by the recent application of chlorophyll fluorescence spectrophotometry as a rapid and non-destructive technique to screen submergence-tolerant cultivars.

Samal *et al.*, (2014) studied on breeding for enhancement of tolerance to submergence in rice (*Oryza sativa* L.) is the major objective in development of varieties for flood prone, rainfed ecologies. A cluster of three ethylene response factor (ERF) like genes at the *Sub1* locus on chromosome 9 are known to confer tolerance to complete submergence for about two weeks. With an objective to identify superior alleles, haplotyping of *Sub1* locus in fifty selected rice accessions using a set of gene specific markers for *Sub1A* and *Sub1C* genes were conducted. In these accessions, ten haplotype patterns having different combinations of genes were identified. The results of the study revealed that *Sub1A* is not the only principal determinant for conferring tolerance to submergence, as highly tolerant genotypes like Auspachali and Palbeda, (H9 haplotype) and

moderately tolerant ones like Gitanjalipatnai, Lathipatnai, Gopalbhog, Katrangi and Darsal III do not possess the tolerant allele *Sub1A* of FR13A suggesting that novel genes other than *Sub1A*, present in these accessions, can also contribute to submergence tolerance in rice.

Fukao *et al*., (2006) reported that Submergence-1 (Sub1), a major quantitative trait locus affecting tolerance to complete submergence in lowland rice (*Oryza sativa*), contains two or three ethylene response factor (ERF)–like genes whose transcripts are regulated by submergence. In the submergence-intolerant japonica cultivar M202, this locus encodes two ERF genes, Sub1B and Sub1C. In the tolerant near-isogenic line containing the Sub1 locus from the indica FR13A, M202(Sub1), the locus additionally encodes the ERF gene Sub1A. During submergence, the tolerant M202(Sub1) displayed restrained leaf and internode elongation, chlorophyll degradation, and carbohydrate consumption, whereas the enzymatic activities of pyruvate decarboxylase and alcohol dehydrogenase were increased significantly compared with the intolerant M202.

Schmitz *et al*., (2013) found that Submergence 1A (SUB1A), is an ethylene response factor (ERF) that confers submergence tolerance in rice (*Oryza sativa*) via limiting shoot elongation during the inundation period. SUB1A has been proposed to restrict shoot growth by modulating gibberellic acid (GA) signaling. Our transcriptome analysis indicated that SUB1A differentially regulates genes associated with brassinosteroid (BR) synthesis during submergence. Consistent with the gene expression data, the SUB1A genotype had higher brassinosteroid levels after submergence compared to the intolerant genotype. Tolerance to submergence can be activated in the intolerant genotype by pretreatment with exogenous

brassinolide, which results in restricted shoot elongation during submergence.

Yasmin *et al*., (2014) many studies have examined the effects of ethylene on in vitro plant growth and development, often with controversial results. Ethylene accumulates in culture vessels due to both the release from the tissues and the physical entrapment due to the need for closed containers. This hormone has several effects on plant regeneration, depending on the plant species and even the cultivar. A prerequisite for ethylene use for in vitro culture is thus to formulate a specific protocol for the genotype of interest. In rice, ethylene is a key regulator of adaptation strategies to low oxygen environments. In particular, the SUBMERGENCE1A (SUB1A) gene, when present, drives the acclimation response which when activated by ethylene produced by submerged plants leads to adaptation through reduced plant growth and ethanolic fermentation enhancement. This gene is restricted to a limited number of rice for which a very specific response to ethylene is expected, whatever the source. This paper reports the regeneration differences between a SUB1A rice landrace (indica-aus, FR13A) and a non-SUB1A variety (japonica, Nipponbare). Our results suggest that regeneration protocols with exogenous ethylene precursors supply are required for the FR13A rice harbouring the SUB1A gene to overcome the problem of low regeneration efficiency.

Alpuerto *et al.*, (2016) found that the submergence-tolerance regulator, SUBMERGENCE1A (SUB1A) of rice (*Oryza sativa* L.) modulates gene regulation, metabolism and elongation growth during submergence. Its benefits continue during de-submergence through protection from reactive oxygen species and dehydration, but the

reislimited understanding of SUB1A's role in physiological recovery from the stress. Here, we investigated the contribution of SUB1A to de-submergence recovery using the two near isogenic lines, submergence-sensitive M202 and tolerant M202(Sub1). No visible damage was detected in the two genotypes after 3d of submergence, but the sub lethal stress differentially altered photosynthetic parameters and accumulation of energy reserves.

Azarin *et al.*, (2017) rice is a unique crop which is generally grown in submerged soil. However, not all rice varieties according to its genotype can tolerate to complete flooding. The selection using DNA markers is now frequently used in real breeding programs, including for the development of submergence tolerant rice. This review summarizes the researches devoted the problem of breeding of submergence tolerant rice. Data on mechanisms, adaptation strategies, loci associated with tolerance to this stress are given. We had placed great emphasis on investigations aimed at application of molecular-genetic markers in the breeding of complete flooding tolerant varieties.

Physiological approaches for submergence tolerance:

Fukai *et al.*, (1999) developed a screening method for drought resistance, either by selecting directly for yield under drought conditions, selecting indirectly using physiological or morphological characteristics associated with drought resistance or some combination of both of these selection strategies. They evaluated different methods that are available for development of drought resistant cultivars in rainfed lowland rice and suggested breeding methods that would be effective in producing cultivars that are adapted to the drought prone rainfed lowland ecosystem.

Pantuwan *et al.*, (2002) worked on field screening of rice for drought tolerance and reported that genotypic variation in maintaining internal plant water status at flowering was associated with grain yield under drought.

Venuprasad *et al.*, (2006) conducted a study to evaluate the effectiveness of direct selection for yield under drought stress in upland rice in populations derived from crosses between irrigated high-yielding cultivars and upland-adapted cultivars. Random $F_{2:4}$ lines from five populations were screened for grain yield in fully irrigated lowland fields under non stress conditions and in uplands under severe reproductive-stage drought stress. Stress caused mean yield reduction of 64% across populations. Broad-sense heritability for yield was not consistently lower in stress than in non-stress trials. Response to selection was evaluated in two crosses in subsequent seasons. Stress-selected lines had a yield advantage of 25 to 34% over random lines when evaluated at stress levels similar to those in which they were selected. Yield gains under very severe stress occurred only in a population derived from a highly tolerant parent.

Lafitte *et al.*, (2006) evaluated the cultivars in field experiments in the Philippines to assess their responses to drought in terms of plant height, heading date, and grain yield. The number of plants selected within a population was not associated with the per se drought response of the donors in the direct evaluation, indicating the wide presence of cryptic genetic variation for drought tolerance in the apparently drought-susceptible cultivars. The genetic background of the recurrent parent affected the number of plants selected, as did the selection environment (upland versus lowland nurseries). These drought-selected introgression lines represent a useful genetic resource to develop improved cultivars for

farmers in rainfed or water-scarce rice-growing regions, and also to improve our understanding of the genetic and molecular basis of drought tolerance in rice.

Fukai (2010) screened a large number of genotypes under drained conditions in the field in the wet season. The selection was mostly based on grain yield adjusted for flowering time and potential yield, but spikelet sterility, leaf water potential, and delay in flowering were also considered for the selection of drought-tolerant genotypes. A large number of putative drought-tolerant lines were selected and crossed with elite lines with high potential yield and grain quality.

Bunnag and Pongthai (2013) Water status is one of the critical factors affecting rice production. Rice cultivars tolerant to drought stress at the vegetative stage under field conditions were selected. Seven rice cultivars, namely, KDML 105, IR58821, CT9993, IR62266, IR57514, IR52561 and BT, were included in this study. The plant height, number of tillers per plant, leaf rolling, leaf death, leaf water potential, relative leaf water content and proline content in plants subjected to drought stress for 0, 20 and 60 days were recorded. Based upon the levels of water stress tolerance, three groups of rice cultivars were recog- nized, as follows: highly drought-tolerant, moderately drought-tolerant and drought-sensitive cultivars. The CT9993 rice cultivar was considered to be a highly drought-tolerant cultivar. The moderately drought-tolerant cultivars included KDML 105, IR58821, IR57514, IR52561 and BT. The IR62266 cultivar was considered sensitive to drought.

Reuben *et al.*, (1990) reported that water deficit at vegetative stage of growth significantly reduced tiller number in both susceptible and tolerant genotypes of rice but the reduction in tiller number was more in

susceptible than in tolerant genotypes. However, it has been reported that tiller number was slightly decreased due to water deficit at flowering stage in rice cultivars (Chaturvedi and Ingram, 1991).

Efisue *et al.*, (2010) drought stress delayed flowering and was more pronounced in early than late-flowering progenies. High-tillering progenies had larger reduction in tiller number than low-tillering progenies under stress. Drought tolerance (little leaf drying), taller plants and less leaf rolling were significantly associated with rapid ability to recover at 3 and 10 days after drought-stress relief. The putative traits identified could be used as indicators of drought stress tolerance in a breeding program.

Kumar *et al.*, (2015) in this study, we evaluated a mega rice variety, Swarna-Sub1 improved for drought tolerance under submergence and drought stress condition. In submergence evaluation, rice lines having DTY2.1 and DTY3.1 locus showed intolerance response alone or in the presence of Sub1 locus. But, Swarna-Sub1 showed 100% survival rate in the absence of DTY QTLs to submergence stress. In drought evaluation, we found that rice lines with DTY loci showed enhanced drought tolerance in the presence of Sub1 locus like delayed leaf rolling and the highest rate of seed setting as compared to rice lines having only Sub1 or DTY locus. Furthermore, in gene expression analysis, expression of drought-inducible genes (DREB1A, SalT, LIP9, LEA3, Rab16A) was found strongly in rice lines having both Sub1 and DTY combination rather than single locus. In protein profile, we found rice lines having both Sub1 and DTY locus showed an increased amount of protein level as compared to lines having only DTY locus. These results support that thus improved Swarna-Sub1 variety for drought tolerance can be used in rain-fed lowland and upland areas where incidence of submergence and drought stress occurs subsequently.

Liu *et al*., (2007) two upland rice varieties (IRAT109, IAPAR9) and one lowland rice variety (Zhenshan 97B) were planted in summer and treated with both normal (full water) and drought stress in the reproductive stage. Panicle water potential (PWP) and leaf water potential (LWP) were measured every 1.0–1.5h over 24h on sunny days. Both PWP and LWP of upland varieties started to decrease later, maintained a higher level and recovered more quickly than that of the lowland variety. The results show that PWP can be used as an indicator of plant water status based on the parallel daily changes, and the high correlation between PWP and LWP. Similar correlations were also observed between PWP, LWP and eight traits related to plant growth and grain yield formation. PWP seemed to be more effective for distinguishing the upland rice varieties with different drought-tolerant ability. Differences in PWP and LWP between upland and lowland rice varieties were also observed at noon even under normal water conditions, implying the incorporation of the drought-tolerant mechanism to improve the photosynthesis and yield of traditional paddy rice.

Jaleel *et al*., (2009) plant growth and productivity is adversely affected by nature's wrath in the form of various biotic and abiotic stress factors. Water deficit is one of the major abiotic stresses, which adversely affects crop growth and yield. These changes are mainly related to altered metabolic functions, one of those is either loss of or reduced synthesis of photosynthetic pigments. This results in declined light harvesting and generation of reducing powers, which are a source of energy for dark reactions of photosynthesis. These changes in the amounts of photosynthetic pigments are closely associated to plant biomass yield. This review describes some aspects of drought induced changes in morphological, physiological and pigments composition in higher plants.

Usman *et al.*, (2014) drought is the major problem for the rice. Drought affects directly the growth of rice, especially in low rainfall season. To confirm this effect, the experiments were conducted at the research lab of University of Gujrat during 2012. The rice variety which was used for the experiments was Rice-386.The design for the experiment was CRD (complete randomized design) with four replicates having four treatments. Drought affected the rate of photosynthesis, accumulation of total soluble sugars, root length, shoot length, shoot and root weight, chlorophyll "a" and "b" and also Carotenoids. Drought affected the growth and reduced the fresh shoot and root weights, root and shoot lengths and the photosynthetic pigments which ultimately reduced the photosynthetic rate and physiology and biochemical processes. It was concluded that water stress is very hazardous and interferes with the survival for rice.

Fathi and Tari (2016) [International Journal of life Science] Drought is the most important abiotic factor limiting growth, adversely affect growth and crop production. Stresses, resulting in the non-normal physiological processes that influence one or a combination of biological and environmental factors. Stress can damage which has occurred as a result of an abnormal metabolism and may reduce growth, plant death or the death of the plant develops. Production is limited by environmental stresses, according to different scholars estimates, only 10 percent of the world's arable land is free from Stress, in general, a major factor in the difference between yield and potential performance, environmental stresses. Drought and stress is the most common environmental stresses that almost 25 percent of agricultural lands for agricultural farm products in the world is limited. Drought risk to successful production of crops worldwide and occurs when a combination of physical and environmental factors causing stress in plants and thus reduce production.

Courtois *et al.*, (2000) stated that during vegetative stress leaf scoring score reduces transpiration. Leaf relative water content indicates maintenance of favourable plant water status.

Lafitte *et al.*, (2004b) reported that RWC (relative water content) correlation was strongly influenced by genetic differences in yield potential and maturity. Leaf relative water content (RWC) in the irrigated control was significantly correlated with % spikelet sterility (SS) and yield. No correlation was observed between leaf RWC at the end of 14-day stress period and 55% in either stress or control treatment leaf water status appears to be related to yield and 55% in some cases, but the relatively weak correlation indicated that other characteristic of the lines are also important in determining the integrated response to reproductive stage drought stress.

Boonjung and Fukai (1996) examined phenological development, shoot dry matter production, grain yield and yield components of rice in relation to drought occurring at various stages of growth. They found that when drought occurred during vegetative stages, it had only a small effect on yield. This decrease in grain yield was associated with low dry matter production during the drought period. They finally suggested that variation in yield components due to water availability is related to the variation in dry matter production at particular growth stages.

Pirdashti *et al.*, (2004) reported that water deficit during vegetative, flowering and grain filling stages reduced mean grain yield by 21%, 50% and 21% on average in comparison to control, respectively. The reduction in grain yield at flowering stage is due to reduction in fertile panicle and filled grain percentage

Biochemical approaches for submergence tolerance:

Vajrabhaya *et al*., (2001) showed that after 5 weeks in nutrient solution, the 6 week old drought tolerant seedling had ~ 4 fold increase in total soluble sugar content, while the original drought sensitive line had only 2.5 fold increase. After 5 weeks of drought treatment 9-15 fold increase in proline content while original line had 5-fold increase. These data suggested that the ability to accumulate the solute contributes to better performance in drought-tolerance (Mostajeran and Rahimi-Eichi, 2009).

Pirdashti *et al*., (2006) observed that drought stress in vegetative growth stage, increased days to flowering and leaf rolling in different cultivars. Drought stress in different growth stages, increased proline content and those cultivars with higher proline content had higher grain yield in drought stress. There were significant correlation between chlorophyll content, proline content, and relative water content and also between selected traits and grain yield.

Mackill *et al*., (2010b) observed that the three more QTLs (qtl3.1, qtl1.1, qtl 9.1) for drought tolerance have also been identified. These four QTLs are being pyramided with the hope that drought tolerance of rice varieties would increase significantly. Specifically, research is under way to incorporate these drought QTLs within the popular mega-varieties (i.e., IR64) and with the aim of having a yield advantage of more than 1 t/ha under drought.

Borah *et al*., (2012) studied that abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth, and development. The leaf growth and Chlorophyll content has significantly shown to vary from the control ones while the grain yield was not affected. While many plant species naturally

accumulate proline and protein as major organic osmolytes when subjected to different abiotic stresses.

Rao *et al.*, (2016) studied that cultivated and upland varieties need to be assessed and analyzed for drought tolerance traits which could beused in screening and breeding programs for drought tolerance. Accumulation of solutes, i.e., proline, total free aminoacids and sugars; biomass production, Relative Water Content (RWC) and the levels of antioxidant enzymes,viz., Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase(GR), and Superoxide Dismutase(SOD) were analyzed in response to water stress. Maximum proline accumulation was seen within 24hrs of stress, after 10 days TH decreased its proline to one-third, whereas in N22 doubled. Although amino acids doubled within 24hrs, gradually they depleted in N22. This may be due to conversion of aminoacids into proline which could be the most compatible soloute.

Samota *et al.*, (2017) studied that water stress is one of the major problems of crop production in most of the countries, particularly in rice growing areas. The scavenging system in drought tolerant variety nagina-22 exhibited higher CAT, POD and SOD activities, than in the drought susceptible variety (pusa sugandh-5) drought-susceptible variety, PS-5 was markedly affected even at the lowest drought level used. The activity of antioxidant enzymes CAT, POD and SOD in the drought tolerant and drought susceptible varieties increased markedly during drought stress. Drought tolerance of the rice varieties associated with build up of antioxidant enzymes and proline. Among the biotic elicitors, MJ was found to be the most effective priming reagent, followed by PBZ. Present findings could be explored further to mitigate drought stress in order to improve rice yield in dry land areas.

Molecular marker approaches for submergence tolerance:

Allah (2009) made crossing between three resistant and two susceptible parents to determine the genetic characteristics under drought conditions during 2002 and 2003 rice growing seasons. The resistant varieties were IET 1444, Moroberekan and Gaori, while the susceptible varieties were Sakha 101 and Sakha 102. Measurements on genetic variability, inbreeding depression, heritability, potence ratio, heterosis and genetic advance, were calculated for parents, F1 and F2 populations from the three studied crosses. The F2 and F3 lines derived from six crosses were grown in a RCBD experiment in three replications to study the genetic of leaf rolling. The total number of lines in F2 segregated 3:1 (rolled : unrolled) and F3 lines segregated in 1:2:1 (rolled : segregating : unrolled).

Ndjiondjop (2010) found that was a consistent negative effect of drought on plant height and grain yield across genotypes' drought-tolerance levels and also across genotype types. Plant height (up to 20.9 cm reduction) and grain yield (up to 1700.8 kg/ha reduction) were more reduced for sensitive genotypes than for moderately tolerant (maximum reductions of 14.9 cm and 1509.5 kg/ha) and tolerant genotypes (maximum reductions of 14.0 cm and 972.8 kg/ha). Flowering (start, 50%, and 100%) and maturity were consistently delayed across genotype types and tolerance levels. Mean delays of 6.5, 21.8, and 9.4 days were observed for start, 50%, and 100% flowering, respectively. Maturity was also delayed, with consistency across genotype types.

Ha *et al.*, (2009) in this study, the correlation coefficients among agro-morphological variation, genetic diversity, and drought tolerance in 44 rice cultivars were analyzed. The drought tolerance at seeding stage

(DTS) was significantly proportional to drought tolerance at vegetative stage (DTV) (r = 0.60). In addition, DTS and DTV had strong significant positive correlation to leaf roll (r = 0.87 and 0.54, respectively). Means of unfilled grains and tilling per panicle were proportionally correlated to DTS (r = 0.22 and 0.25, respectively), and DTV (r = 0.20 and 0.36, respectively).

Kuma (2018) with the change in the global scenario drought is becoming one of the major problem among other stress and its effect is more severe in rice whose life cycle completely depends on water. Whether it occurs during any stage (early, intermittent and late) it affects crop and its effect is more severe when this stress coincides with reproductive stage of the crop growth. However, rice respond to it by sending signals to shoot which generates signals in terms of physical, chemical and biological form. Hence, screening of plants at this stage is most effective for development of drought resistance. Varieties like IR-36, IR-64 has been released but through conventional breeding it requires a lot of time to release a variety. Hence molecular strategy has been adopted and focus was given on adopting qtl introgression. Qtls like qDTY1.1, qDTY12.1 has pronounced effect on yield potential during drought stress.

Sakai *el al.*, (2010) Protocols were established for screening rice genotypes for tolerance to water-limited conditions under field conditions. These protocols were successfully used to select the best genotypes for further experiments. Rice genotypes responded differently when subjected to water-limited conditions. Experiments conducted under field conditions indicated that rice genotypes Curinga and CT6241 performed much better in terms of grain yield under water-limited conditions than varieties Azucena, Nerica, CICA8 and Palmar. Curinga, CT6241, CICA8 and

Palmar were selected for further studies. The first two genotypes are tolerant and whereas the last two are susceptible to water stress.

Kanagaraj *et al.*, (2010) identify markers linked to drought resistance using 23 recombinant inbred (RI) lines of IR20/nootripathu two india ecotypes with extreme drought response. The parents were screened for polymorphism using 1206 rice microsatellite primer pairs. Out of 134 SSR polymorphic primers between parents, three primers showed polymorphism between bulks. These three primers co-segregated among the individual RI lines constituting the respective bulks. The genomic regions flanked by these markers have been reported to be associated with several drought resistance component traits and will be useful in marker assisted breeding for drought resistance in rice.

Biradar *et al.*, (2015) identified that of the genomic region containing few or more genes controlling these complex traits is a basic idea of QTL mapping. The large number of QTL mapping studies for diverse crop species has provided an abundance of DNA marker-trait associations. The information obtained on the QTL analysis can be utilized for the crop improvement through marker aided selection and molecular breeding. The basic knowledge about DNA markers, principle of QTL mapping, statistical tools and techniques used in QTL analysis and applications of QTL mapping has been reviewed. This paper will be a key reference for the beginners and research scholars who are involved in QTL mapping in crop plants.

Nahar *et al.*, (2016) found that oxidative stress is overcome by the inherent capacity of plants to produce antioxidant species which may be enzymatic or non-enzymatic in nature. If however antioxidant defence mechanism cannot overpower the ROS generated, they cause oxidative

damage to the plant tissues such as lipid peroxidation, protein oxidation, DNA damage, etc. resulting in cell death. Unlike other stresses, drought affects the physiology and biochemistry of the rice which adversely affects in the morphology and consequently delimits the yield of the plant. Therefore, understanding the morphological, biochemical and molecular mechanisms involved in rice against drought is utmost necessary for rice breeders to improve the rice for drought tolerant/resistance varieties for future green revolution. In this review, an attempt has been made to highlight the complex regulatory network involved in rice against drought with special emphasis on morphological, physiological and molecular mechanisms and to discuss the prospective and challenges for future plant breeders.

Chapter-III

MATERIALS AND METHODS

The present research work was undertaken for **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"** the investigation was carried out at Student Instructional Farm, submergence tank and net house of N.D. University of Agriculture and Technology, Kumarganj, Ayodhya. The geographical location of the experimental site is as follows.

Geography:

The geographical situation of Ayodhya district lies between a latitude range from 24º 47" north to 26 º 56" north and longitudes of 81º 12" east and 83º 98" east, an altitude of 113 meters above the mean sea level in the gangtic alluvium soil of Eastern Uttar Pradesh.

3.1 Experimental site:

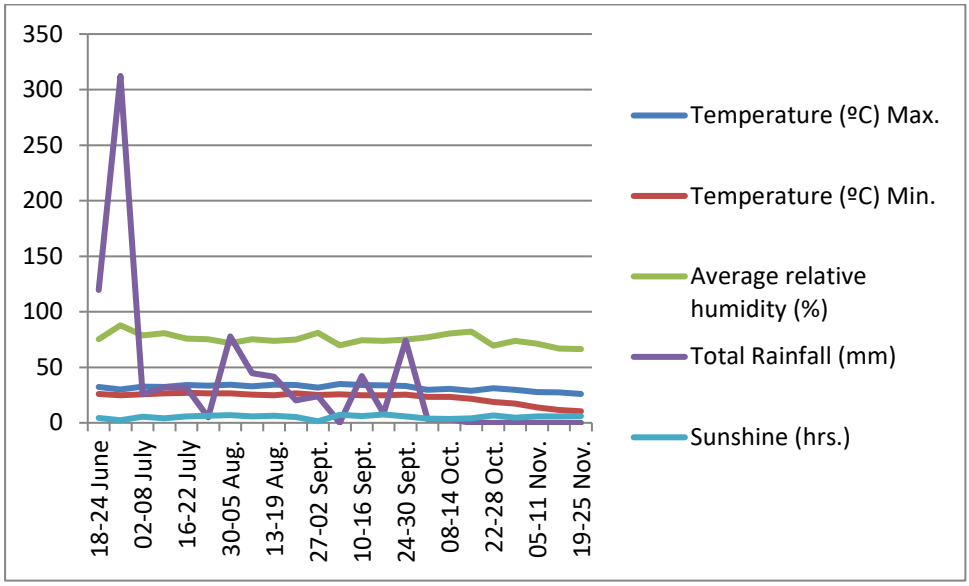
The experiment was conducted at "Student Instructional Farm" of A. N.D. University of Agriculture and Technology, Kumarganj, Ayodhya; Submergence tank department of Crop physiology A.N.D. University of Agriculture and Technology, Kumarganj, Ayodhya.

3.2 Climatic condition:

The Ayodhya district falls in semi-arid zone, receiving a mean annual rainfall of about 1200 mm, in which about 80% of the total precipitation is received during monsoon season, from July to end of September with few showers in winter. The winter months are cold and occasionally frost occurs in this period while summers are hot and dry.

Table 3.1: Weather condition during the crop season 2018-2019

Standard week	Temperature (^{0}C)		Average relative humidity (%)	Total Rainfall (mm)	Sunshine (hrs.)
	Max.	Min.			
18-24 June	32.4	26.0	75.2	119.6	4.4
25-01 June	30.0	24.7	87.8	312.3	2.2
02-08 July	32.5	25.7	78.6	25.6	5.6
09-15 July	32.3	26.5	80.6	32.0	4.2
16-22 July	34.1	27.0	75.7	31.8	5.7
23-29 July	33.5	26.5	75.1	05.0	6.5
30-05 Aug.	34.2	26.4	71.9	77.8	7.0
06-12 Aug.	32.9	25.4	75.2	44.6	5.7
13-19 Aug.	34.2	24.9	73.9	41.6	6.5
20-26 Aug.	34.0	26.4	75.0	20.1	5.3
27-02 Sept.	31.7	25.0	81.0	24.0	1.4
03-09 Sept.	35.0	25.7	69.9	00.0	7.2
10-16 Sept.	34.1	24.8	74.5	42.0	6.2
17-23 Sept.	33.8	24.9	73.7	08.4	7.5
24-30 Sept.	33.1	25.3	75.0	74.0	5.7
01-07 Oct.	29.8	23.5	77.0	03.6	3.8
08-14 Oct.	30.6	23.5	80.3	03.1	3.6
15-21 Oct.	28.9	21.6	82.3	00.0	4.2
22-28 Oct.	31.1	18.7	69.4	00.0	6.7
29-04 Nov.	29.6	17.3	73.9	00.0	4.6
05-11 Nov.	27.7	14.0	71.2	00.0	5.8
12-18 Nov.	27.3	11.5	67.0	00.0	5.7
19-25 Nov.	26.0	10.4	66.4	00.0	6.0



3.3 Experimental materials:

The material for this study consisted four rice genotypes/ lines (Nagina-22, Swarna Sub-1, NDR-97, NDR9830102 and NDR-70 for development of submergence and drought dual tolerance rice genotype. NDR9830102 is a dual stress (submergence and drought) tolerant. Thirty-five days old seedlings were exposed for 14 days complete submergence in submergence tank of crop physiology department.

Sowing:

Bold and healthy seeds of four rice genotypes (Nagina-22, Swarna Sub-1, NDR-97, NDR-9830102) and mapping population were sown in the pot each pot containing 12 kg clay loam soil and NPK 120:80:60 kg/hectare respectively. Thirty five days old seedling were exposed for 14 days completely submergence in submergence tank.

Table 3.2: The parentage of the rice genotype used for the study

Genotype	Parentage
NDR-97	Nagina-22 x Ratna

NDR-9830102	
Swarma sub-1	FR13A x IR49830
Nagina-22	Rajbhog
BC_3F_4	(Swarna*Sub-1*xNagina-22) x Swarna*Sub1*

The experiment was conducted in earthen pots at the experimental site of the Department of Plant molecular biology and genetic engineering, and submergence treatment in Department of Crop Physiology Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.).

Treatment : 2

1. Control
2. Submergence (14 days complete submergence)
3. Drought (15days at reproductive stage)

Replication : 5

Submergence Treatment:

Submergence treatment was given to 35 days old seedlings in submergence tank for 14 days. At the time of submergence, water level was maintained at 2m height. So that leaves cold submerged in the water. The level of tank water was maintained through water pipe up to 14 days during treatment. Control plant set was kept under aerobic condition.

Observations:

The observations were recorded before submergence, just after de-submergence.

- Before submergence: At the time treatment
- Just after de-submergence.

Observation:

Observations were taken at three stages of crop growth before submergence, just after de-submergence and after recovery 20 days.

3.4 Biochemical analysis for submergence and drought.

3.4.1 Submergence:

3.4.1. (a) Estimation of catalase activity:

A) Catalase activity was assayed colorimetrically according to method given in Analytical Biochemistry (Sinha, 1972). Catalase facilitates the dismutation of H_2O_2 to water and O_2 according to the reaction.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + 1/2\ O_2$$

The enzyme plays an important role in association with SOD as well as in photorespiration and glycolate pathway.

Reagents:

1. Phosphate buffer, (0.1 M), pH 7.0
2. Potassium dichromate acetic acid (5% $K_2Cr_2O_7$) + glacial acetic acid in 1:3 ratio.
3. H_2O_2 (0.2 M)

Procedure:

Took 100 mg plant sample and crushed in 10 ml of 0.1 M phosphate buffer (pH 7.0). Centrifuge the leaf extract at 10,000 rpm at 4^0C for 15 minutes. Collect the supernatant and stored at low temperature. Used supernatant for enzyme assay and estimate the enzyme activity as given below.

Test	Blank	Reagents
1.25	--	H_2O_2
0.50ml	0.50 ml	Enzyme extract
3.25 ml	4.50 ml	Phosphate buffer
5.00ml	5.00 ml	

Took 1 ml supernatant, 1ml H_2O_2, 3 ml phosphate buffer in test tube. Kept it at 37^0C for 3 min in water bath. Take 1ml algenat + 3mol potassium dichromate and mixed proper l kept it in water bath for 10 min. Took O.D at 570 nm. against blank. Result expressed as enzyme unit per g^{-1} fresh weight $mint^{-1}$.

3.4.1.1. (b) Estimation of peroxidase activity:

Peroxidase activity was assayed according to method of Hammerchmidt *et al.,* (1982). The enzyme catalyses the oxidation of a substrate by removal of hydrogen which combines with H_2O_2.

$$AH_2 + H_2O_2 \xrightarrow{\text{Peroxidase}} 2H_2O + A$$

Reagents:

1. Phosphate buffer (0.1M) pH 6.0
2. Pyrogallol (0.1N)
3. H_2O_2 (0.02%)

Procedure:

Took 200 mg of plant sample and homogenized in 10 ml of 0.1m phosphate buffer (pH 6.0) and centrifuged at 10,000 rpm for 15 minutes at 4^0C. Supernatant was collected and stored at low temperature. 1 ml of

enzyme extract was taken in a test tube in which 2 ml phosphate buffer (pH 6.0), 1.0 ml Pyrogallol and final volume 6 ml by H_2O_2 were made. Absorbance (OD) was recorded at 430 nm against blank with the help of spectrophotometer.

3.5 Drought treatment:

Four rice genotypes/ line along with parents and mapping population were exposed for 15 days drought stress at reproductive stage in rain out shelter by receding the water. Drought stress was released at the end of 15 days by watering the pots. Data related to drought parameters were recorded at the end of drought.

3.5.1 Leaf rolling

Table 3.3: Leaf rolling score chart (IRRI scale) for drought screening

S.N.	Score leaf rolling	Score
1	No leaf rolling	**0**
2	Very light (just start)	**1**
3	Leaf roll (high recovery half roll)	**3**
4	Medium (but recovery rolled)	**5**
5	High leaf rolling (not recovery)	**7**
6	Very high leaf rolling (died)	**9**

3.5.2 Relative water content (RWC):

The relative water content (RWC) was determined by the method of described by Weatherley (1965). Leaf discs were cut from the leaves, weight and saturated them by floating on distilled water in Petri dishes. After four hours, the discs were surface dried with tissue paper then the

leaves in oven at 80^0C for 20 hours after dried weight of the lives. RWC was calculated by the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Saturated weight} - \text{dry weight}} \times 100$$

3.5.3 Chlorophyll content:

The chlorophyll contents was measured with the help of Chlorophyll Tester TYS-A (Soil Plant Analysis Development, SPAD).

Measuring principle:

TYS-A Chlorophyll Tester measures the leaf of light transmittance in two wavelength range to determine the relative quantity of current leaf chlorophyll. The measurement was calculated by the amount of leaf transmitted light. Red light and infra redare emitted by light source. The extra ray filtered through, the projection of light were left and arrived in detector. Then transmitted light is converted into analog signals by the detector. The amplifier magnifies the image of analog signals which connected to microprocessor. At last LCD shows the relative amount of chlorophyll content which stored in memory. The stored data can be called or deleted and the average of the stored data can also be calculated.

3.5.4 Estimation of free proline content:

Proline content estimated content was estimated in rice leaf (Bates, 1973):

Reagent:

A. Glacial acetic acid- Analytical grade

B. Sulphosalycylic acid (3%) - 3gm of sulphosalycylic acid dissolved in 100ml, of Distilled water.

C. Orthophosphoric acid (6M) - Required volume of orthophosphoric acid (38.1ml) was taken and made to 100ml using distilled water to get 6N orthophosphoric acid.

D. Acid ninhydrin- Ninhydrin (1.25g) was dissolved in a blend of 30ml of glacial acetic acid and 20ml of 6M orthophosphoric acid.

Procedure:

1. 0.5g plant tissue was taken and homogenized in 5ml of 3% sulphosalycylic acid using pre washed mortor and pestle.
2. Filtered the homogenate through whatman No-1 filter paper and collected filtrate was used for the estimation of proline content.
3. Took 2ml of extract in test tube and 2ml of glacial acetic acid and 2ml of ninhydrin reagent in a test tube.
4. Reaction mixture was heated in boiling water bath at 100^0C for 1 hour. Pink color was developed.
5. After cooling the reaction mixture 4ml of toluene was added and then transferred to a separating funnel.
6. After thorough mixing, the chromospheres containing toluene was separated and its absorbance was read at 520nm in spectrophotometer against toluene blank was recorded.
7. Standard curve of proline was prepared by taking 5 to 100 ug/ml proline concentration.

3.6 Isolation of genomic DNA from rice leaves:

Total genomic DNA from fresh leaves of rice varieties were extracted using CTAB method given by **Murray and Thompson (1980).** CTAB method was used for isolation of DNA from rice leaves. CTAB was

used to precipitate the nucleic acid at low salt concentration and low temperature (4^0C).

Preparation of buffers and standard solution:

Stock Solution: **(100 ml):**

0. 1 M Tris (pH 8.0)	12.14 g
0.5 M EDTA (pH 8.0)	18.00 g
5 M NaCl	29.22 g

Extraction Buffer (pH 8.0):

C-TAB	3 g
1 M Tris	10 ml
0.5 M EDTA	4 ml
5 M NaCI	28 ml
PVP	2 g
2 β-mercaptoethanol	35 µl
Maintained final volume	100 ml.

Ethanol: 70%	**100 ml**
Absolute ethyl alcohol	70 ml
Distilled water	30 ml

Chloroform: Isoamyl alcohol

Chloroform	96 ml
Isoamylacohol	4 ml
Total volume	100 ml

TE Buffer:	**100 ml**
1 M Tris (pH-8	1 ml
0.5 M EDTA	200 µl

Made total volume 100 ml by distilled water. pH of TE buffer was adjusted to 8.0.

Ethidium Bromide:

Ethidium bromide	0.1 g
Distilled water	10 ml

It was stored in dark bottle at room temperature.

Loading dye (1X):

Glycerol	5% (w/v)
Xylene cyanol	0.041% (w/v)
Bromophenol blue	0.041% (w/v)

Made volume 10ml.

TAE Buffer (1 x TAE per litre):

0.1 M Tris (pH 8.0)	4.84 g
Glacial Acetic acid	1.14 ml
0.5 M EDTA	2 ml

Total volume was made up to 1000 ml. The pH of TAE buffer was adjusted to 8.0

The steps of DNA isolation are:

1. 100 mg fresh leaves of each sample of rice were taken and grind in liquid nitrogen with the help of mortar and pestle. Powdered leaves were transferred into centrifuge tube.

2. 4ml pre-heated (65^0c) extraction buffer added in the centrifuge tubes and kept it in water bath at 65 ^{0}C for 1hr.
3. Intermittingly, shake the tubes during incubation period of heating.
4. Cooled the tubes at room temperature after incubation periods.
5. Added 4 ml of chloroform: iso-amyl alcohol (24:1).
6. Mixed properly by inverting centrifuge tubes 25-30 times,
7. Centrifuge test tubes containing solution miture at 6000 rpm for 15 minutes at 4 ^{0}C.
8. Supernatant was transferred in fresh eppendrof tubes.
9. In supernatant, equal volume of isopropanol and half volume of 5 M NaCl were added and stored it at 4 ^{0}C for overnight.
10. Then, eppendrof tubes were centrifuged at 8000 rpm for 15 minutes. Supernatant was discarded and pellet was washed with 70% ethanol.
11. Pellet was re-suspended in 50 µl of TE buffer and stored at 4 ^{0}C.

Purification of genomic DNA

Following steps were used for purification of the DNA:

1. RNase solution (10 mg/ml) @ 50 µg/ml was added to DNA sample and incubated at 37 ^{0}C for one hour.
2. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed gently.
3. Mixture obtained in step 2 was spinned at 10000 rpm for 2 minute at room temperature; aqueous phase was taken out and transferred to a fresh microfuge tube. Extracted twice with equal volume of chloroform: isoamyl alcohol (24:1), centrifuged and taken out the aqueous phase.

4. Volume of 3M sodium acetate (pH 4.8) was added to above mixture and mixed properly. 2.5 times absolute alcohol was added and mixed by quick gentle inversion to precipitate the DNA
5. Mixture obtained in step 4 was centrifuged at 9000 rpm for 5 minute in a microfuge tube to obtain the pellet. Supernatant was removed carefully; pellet was washed with 70% cold ethanol. Pellets were dried in air and dissolved pellet (DNA) in 50 µl TE buffer.

Agarose Gel electrophoresis:

1. Agarose gel of 0.8% was casted in 1X TAE buffer containing ethidium bromide (2 µl).
2. After solidification of gel, 10 µl of genomic DNA with 5 µl of loading dye were properly mixed.
3. DNA sample were loaded in the well of gel properly.
4. Gel was run at constant voltage (40 V for three hours).
5. Gel was then visualized on U.V. using gel documentation system.

Dilution of DNA for PCR:

20 µl of autoclaved TE buffer was taken and 5 µl genomic DNA was added to make 50 to 100 mg per µl in each sample, based on their quantification volume.

Quantification of DNA:

1. Took 1 ml TE buffer in a cuvette and calibrated the spectrophotometer at 260 nm as well as 280 nm wave length.
2. Added 5 µl of DNA mixed properly and recorded the optical density (O.D.) at both 260 and 280 nm.
3. Estimated the DNA concentration according to the following formula.

$$\text{Amount of DNA (mg/µl)} = \frac{\text{(OD)260 x 50 x dilution factor}}{100}$$

Composition of master mix: 20 µl water mix were made in following composition

S.No.	Components	Amount (µl)
1.	DNA	1.00
2.	PCR buffer	3.00
3.	dNTP	1.00
4.	Mg Cl_2	1.00
5.	Primer Forward	0.50
6.	Primer revers	0.50
7.	Taq polymerase	0.25
8.	Water	12.75
	Total	20.00

Reaction cycles for SSR primer:

	Temperature (^{0}C)	Time (min.)	Cycle
Segment I	94	5	1
Segment II	94	0.30	35
	55	0.35	
	72	1	
Segment III	72	7	
Segment IV	4	Forever	Forever

SSR Primer use for PCR:

Name	Sequences Forward	Sequences Reverse	Chromosome no	Product size
RM 201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	1	158
RM 215	CCCATGCGTTTAACTAATTCT	CGTTCCATCGATCCGTATGG	1	147

RM 219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	2	202

3.7 Observations recorded for yield and yield components:

Observations were recorded on randomly selected five plants from each genotype in each replication. The data were recorded on following characters:

1. **Days to 50% flowering:** This was recorded as number of days from the date of sowing to the emergence of 50% panicles.
2. **Plant height (cm):** It was measured in centimeters from the ground level to the tip of main panicle excluding awn at the time of maturity.
3. **Tillers per plants:** The total number of panicle bearing tillers were counted from 5 randomly selected plants and average were taken for each variety.
4. **Number of grain per panicle:** It was determined by counting total number of filled and unfilled grains in each replication.
5. **Test weight (g):** Test weight was recorded by taking weight of 1000 matured dried seeds of each genotype in each replication with the help of electronic balance.
6. **Grain yield (g):** Grain yield per plant was recorded from five randomly selected plant and dried it to 12-14% moisture. It average out to owe as grain yield per plant.

Chapter-IV

EXPERIMENTAL FINDINGS

The present investigation entitled **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"** has been presented in this chapter . Overall findings have been categorized and presented under the following sub headings:

- Physio-biochemical approaches for screening of submergence and drought traits screening
- Molecular approaches
- Yield and yield attributing traits

4.1 Physio-biochemical approaches for screening submergence tolerance traits:

Shoot elongation (cm):

Submergence treatment affected shoot elongation and plant height of rice plant (Table 4.1). Highest plant height was recorded in Nagina-22 before and after the submergence while lowest in Swarna sub 1. High shoot elongation was recorded in Naginna-22 (42.03%) and NDR-97 (35.00%) while low in Swarna sub 1 (18.18%), NDR-70-2 (18.29%) and NDR-70-1 (21.43%).

Fig. 4.1: Parents and rice lines during submergence treatment

Fig. 4.2: De-submergence of parents and rice lines

Fig. 4.3: Morpho-physiological changes in rice lines at end of 15 days drought treatment

Table 4.1: Effect of submergence treatment on shoot elongation (cm) of rice genotypes and lines

S.No.	Genotypes	Plant height (cm)		Shoot elongation	Percent increase
		BS	AS		
1	Swarna sub1	57.20	67.60	10.40	18.18
2	Nagina-22	76.60	96.80	30.20	42.03
3	NDR-9830102	63.20	72.20	9.00	14.24
4	NDR-97	74.40	100.00	25.93	35.00
5	NDR-70-1	67.20	81.60	14.40	21.43
6	NDR-70-2	67.80	80.20	12.40	18.29
7	NDR-70-3	64.20	80.50	16.30	25.39
8	NDR-70-4	66.00	81.20	15.20	23.03
	CV%	3.30	4.20		
	CD at 5%	3.74	2.89		
	SE(m)±	1.29	0.99		

Catalase activity

The catalase activity abruptly increased in all rice genotypes after desubmergence (Table 4.2). The highest catalase activity after de-submergence was recorded in Swarna Sub1(283.08%) followed by NDR-70-3 (215.45%) and NDR-9830102 (204.71) while lowest in Nagina-22 (42.99%) and failed to survive few hours after desubmergence. The percent increase in catalase activity in rice lines varied from 179.30 to 215.45%.

Table 4.2: Effect of submergence treatment on catalase activity (unit* gm^{-1} min. fresh wt.) of rice genotypes and lines

S.No.	Genotypes	Control	Stress	Percent increase
1	Swarna sub 1	40.20	154.00	283.08
2	Nagina-22	44.20	63.20	42.99
3	NDR-9830102	55.20	168.20	204.71
4	NDR-97	41.80	77.60	85.65
5	NDR-70-1	54.20	151.40	179.34
6	NDR-70-2	49.80	153.60	208.43
7	NDR-70-3	49.20	155.20	215.45
8	NDR-70-4	51.80	157.20	203.47
	CV%	4.20	2.10	
	CD at 5%	2.61	3.74	
	SE(m)±	0.90	1.29	

***Unit:** one unit is defined as the amount of enzyme that catalase the conversion of one mol of substrate per minute under the specified condition.

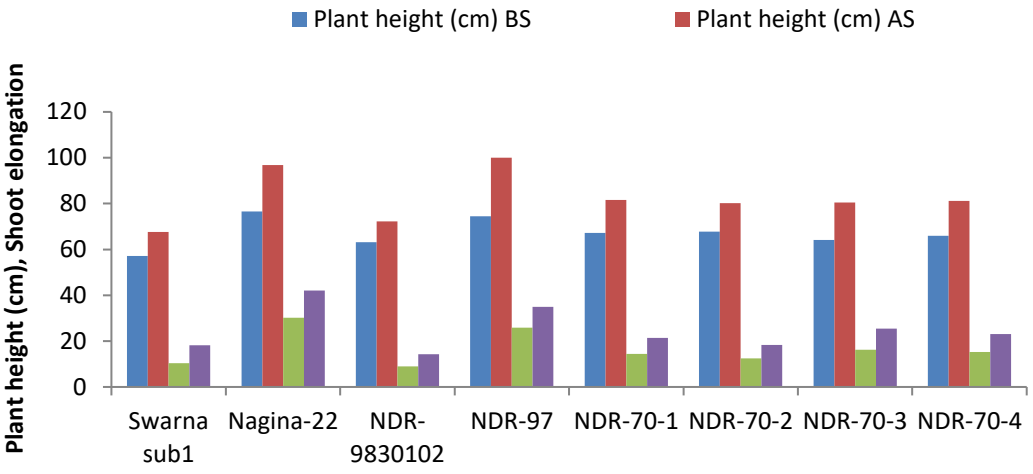


Fig. 4.4: Effect of submergence treatment on shoot elongation (cm) of rice genotypes and lines

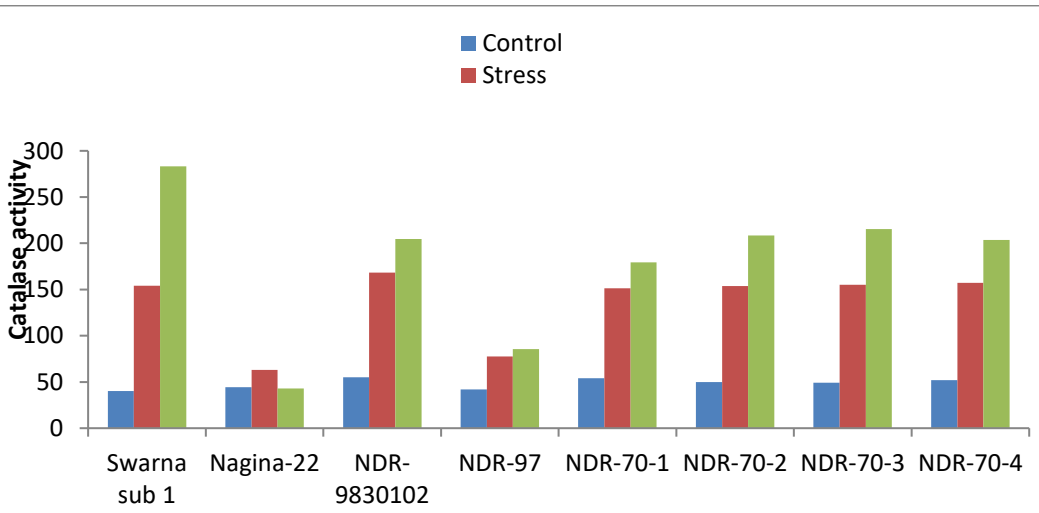


Fig. 4.5: Effect of submergence treatment on catalase activity (unit* gm-1 min. fresh wt.) of rice genotypes and lines

Peroxidase activity:

Peroxidase activity suddenly increased in rice genotypes after desubmergence (Table 4.3). The high percent increase peroxidase activity was estimated in NDR-70-3 (414.50%), NDR-9830102 (408.97%) and swarna Sub-1 (406.25%) while low in Nagina-22 (288.61%) NDR-70-1 (374.68%) and NDR-97 (383.95%) just after desubmergence.

Table 4.3: Effect of submergence treatment on peroxidase activity (unit* g^{-1} fresh wt. min^{-1}) of rice genotypes and lines

S.No.	Genotypes	Control	Stress	Percent Increase
1	Swarna sub1	16.00	81.00	406.25
2	Nagina-22	15.80	61.40	288.61
3	NDR-9830102	15.60	79.40	408.97
4	NDR-97	16.20	78.40	383.95
5	NDR-70-1	15.80	75.00	374.68
6	NDR-70-2	15.60	77.80	398.72
7	NDR-70-3	15.20	78.20	414.50
8	NDR70-4	16.20	79.80	392.39
	CV%	12.20	2.90	
	CD at 5%	2.49	2.91	
	SE(m)±	0.86	1.01	

Unit*: one unit is defined as the amount of enzyme that peroxidase the conversion of one mol of substrate per minute under the specified condition.

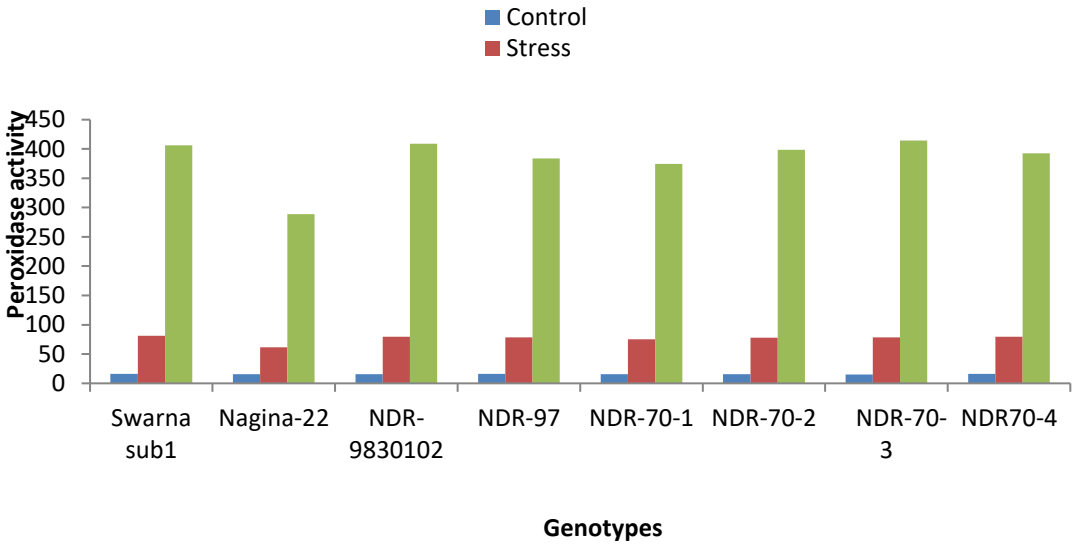


Fig. 4.6: Effect of submergence treatment on peroxidase activity (unit* g-1 fresh wt. min-1) of rice genotypes and lines

Leaf rolling:

Rice genotypes showed a genetic variability in leaf rolling at same label of drought stress (Table 4.4). The highest leaf rolling was noted on Swarna Sub-1 (5) while lowest in Nagina-22 (1) and NDR-70-1 (1). The leaf rolling in rice lines ranged from 1-3 scale.

Table 4.4: Screening of rice genotypes and lines on the basis of leaf rolling (IRRI scale) under drought stress condition at reproductive stage

Score leaf rolling (Standard IRRI scale)	Score	Genotypes	Score
No. of leaf rolling	0	Swarna *Sub1*	5
Very light (just start)	1	Nagina-22	1
Leaf roll (high recovery, half roll	3	NDR-9830102	1
Medium (but recovery rolled leaf)	5	NDR-97	3
High leaf rolling (not recovery)	7	NDR-70	
Very high leaf rolling (died)	9	NDR-70-1	1
		NDR-70-2	3
		NDR-70-3	3
		NDR-70-4	1

Relative water content (%):

Relative water content (RWC) is an indicator of water status in plant under stress condition. Drought stress significantly reduced the water content in rice genotypes (Table 4.5). High reduction in RWC was recorded in Swarna sub-1 (25%), NDR-9830102 (18%) minimum reduction was recorded in Nagina-22. In rice lines, percent reduction in RWC ranged from 9 to 11%.

Table 4.5: Effect of drought stress on relative water content (%) of rice genotypes and lines at reproductive stage

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	84.4	63.30	25.00
2	Nagina-22	87.2	75.80	13.07
3	NDR-9830102	85.4	70..03	18.00
4	NDR-97	87.2	77.80	10.78
5	NDR-70-1	84.2	74.60	11.40
6	NDR-70-2	86	77.60	9.77
7	NDR-70-3	85.4	74.20	13.11
8	NDR-70-4	84	76.44	9.00
	CV%	3.20	5.80	
	CD at 5%	3.57	5.64	
	SE(m)±	1.23	1.95	

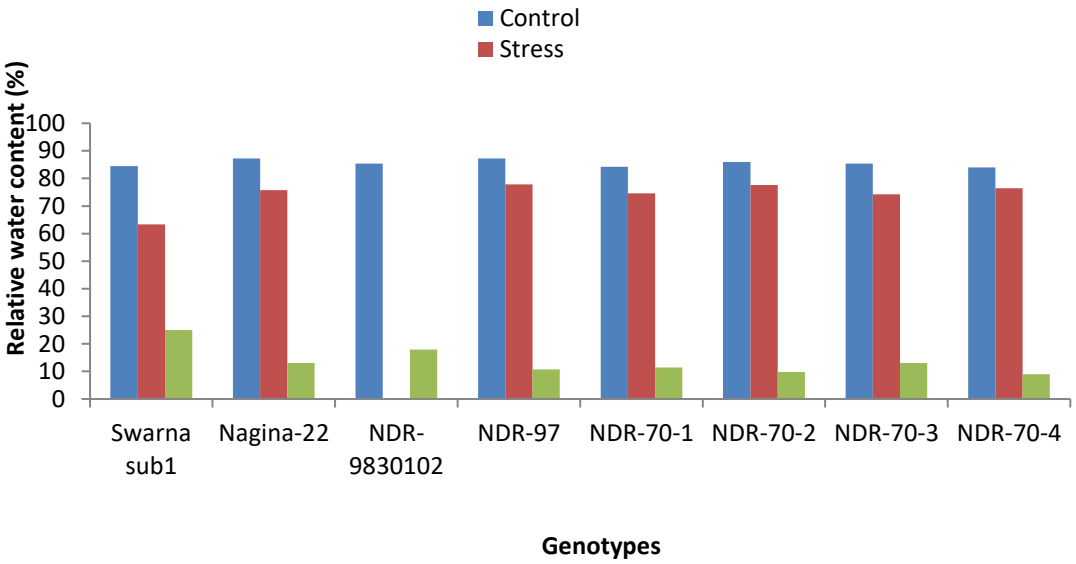


Fig. 4.7: Effect of drought stress on relative water content (%) of rice genotypes and lines at reproductive stage

Total proline content

Proline content abruptly increased in all rice genotypes after drought stress (Table 4.6). High proline content was estimated in NDR-70-3 (282.89) followed by Nagina-22 (275%), NDR-70-2 (271.79%) and minimum in Swarna sub-1 (225%) under drought regimes at reproductive stage.

Table 4.6: Effect of drought stress on proline content (µg g^{-1} Fresh wt.) of rice genotypes and rice lines at reproductive stage

S.No.	Genotypes	Control	Stress	Percent Increase
1	Swarna sub1	16.00	52.00	225.00
2	Nagina-22	16.00	60.00	275.00
3	NDR-9830102	16.20	59.40	266.67
4	NDR-97	15.60	58.40	247.35
5	NDR-70-1	15.80	55.20	249.36
6	NDR-70-2	15.60	58.00	271.79
7	NDR-70-3	15.20	58.20	282.89
8	NDR-70-4	16.20	59.60	267.90
	CV%	11.80	12.00	
	CD at 5%	2.40	11.80	
	SE(m)±	0.85	4.09	

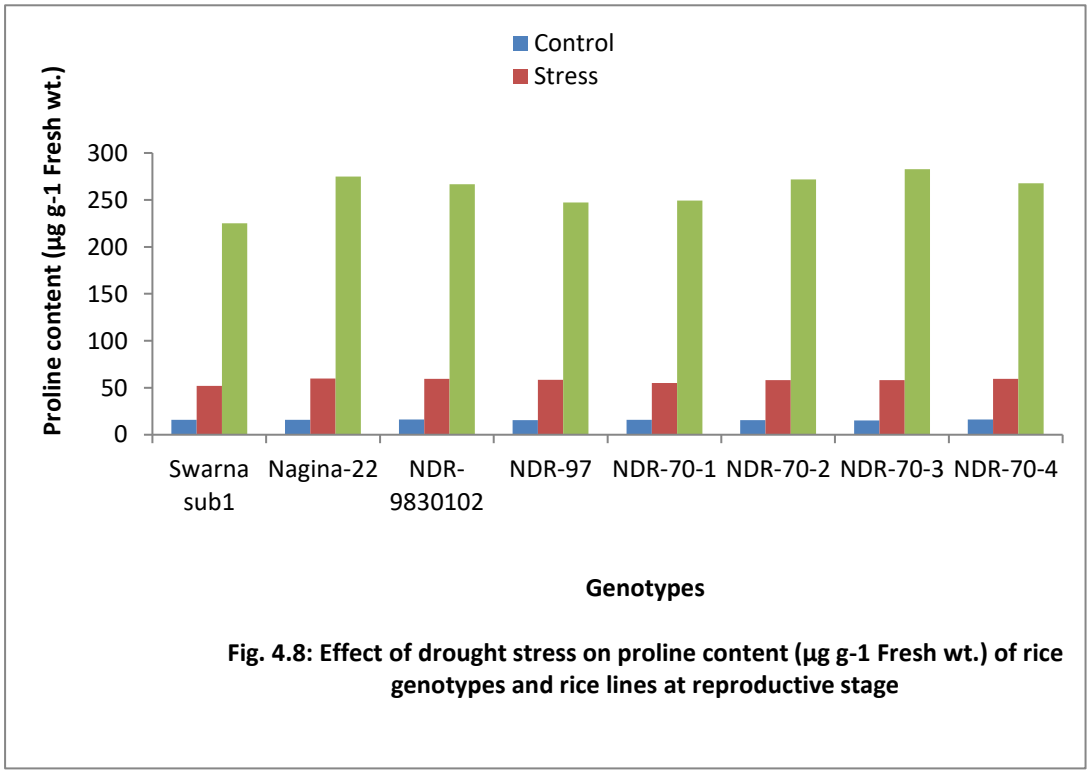


Fig. 4.8: Effect of drought stress on proline content (µg g-1 Fresh wt.) of rice genotypes and rice lines at reproductive stage

Molecular approaches

Genomic DNA was isolated from parent Swarna Sub1, Nagina 22, NDR-9830102 and rice lines NDR-70-1, NDR-70-2, NDR-70-3 and NDR-70-4. The quality of genomic DNA was checked in gel doc for proper quantification of PCR reaction. The drought related SSR primers RM201, 215 and RM219 were used for fore ground reaction of rice lines for drought related traits. RM201 showed monomorphism in rice lines and it parents while RM215 and RM219 showed polymorphism with various product size in parents Swarna sub 1, Nagina-22 and rice lines.

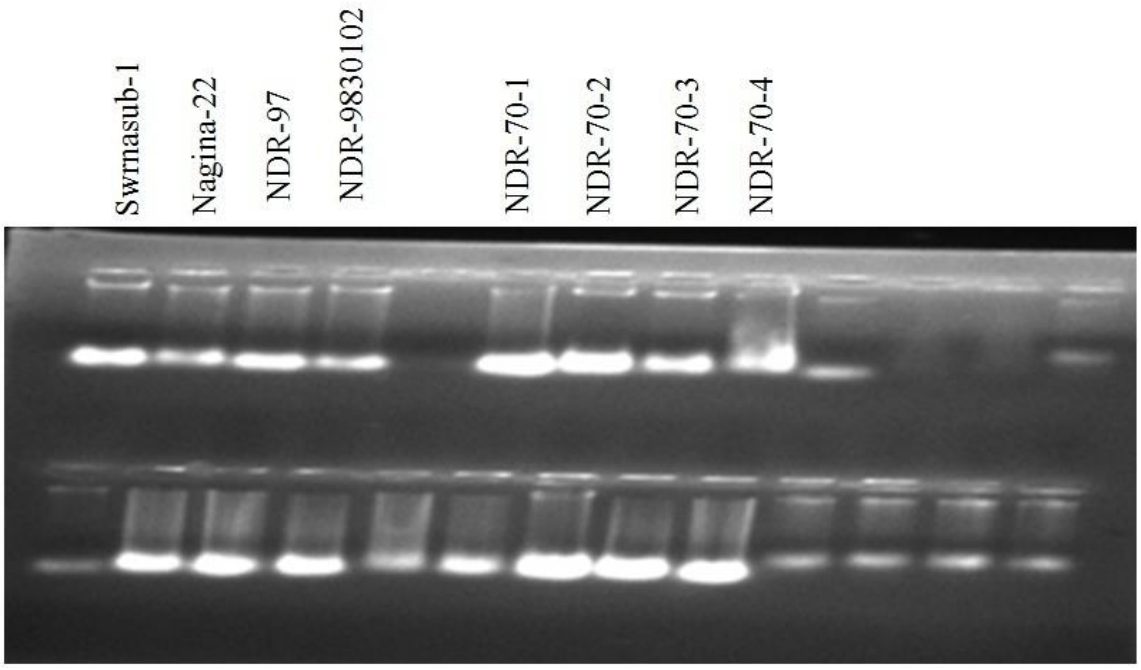


Fig. 4.9: Quantification of genomic DNA of rice lines

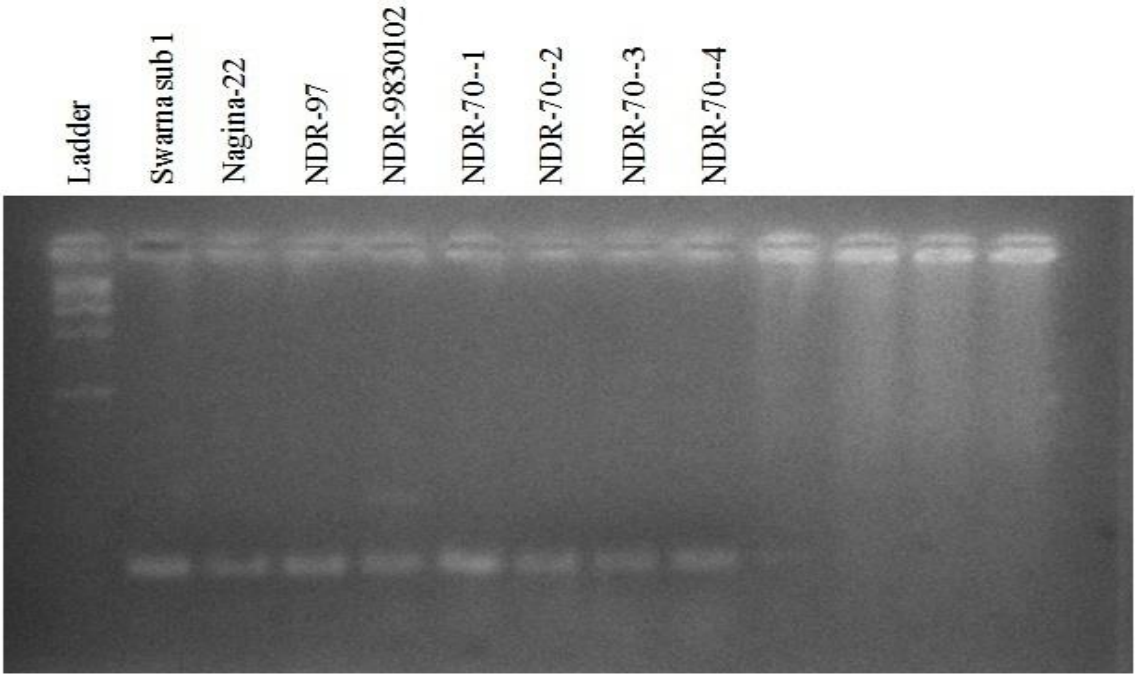


Fig. 4.10: Amplification RM 201 marker in parent and rice lines

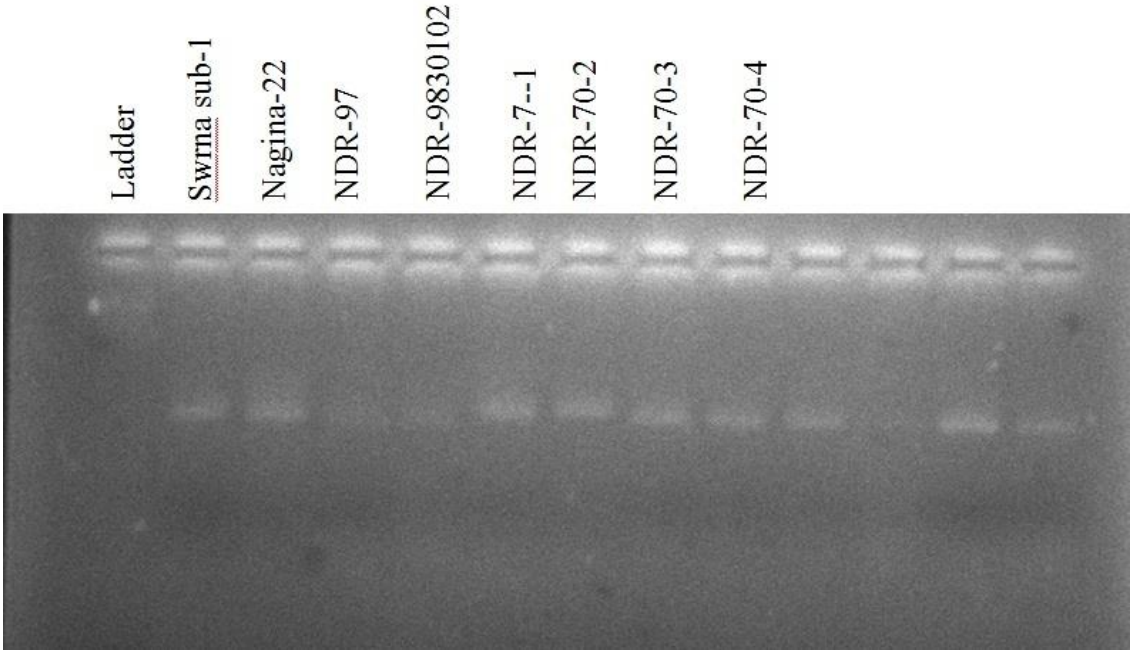


Fig. 4.11: Amplification RM 215 marker in parent and lines

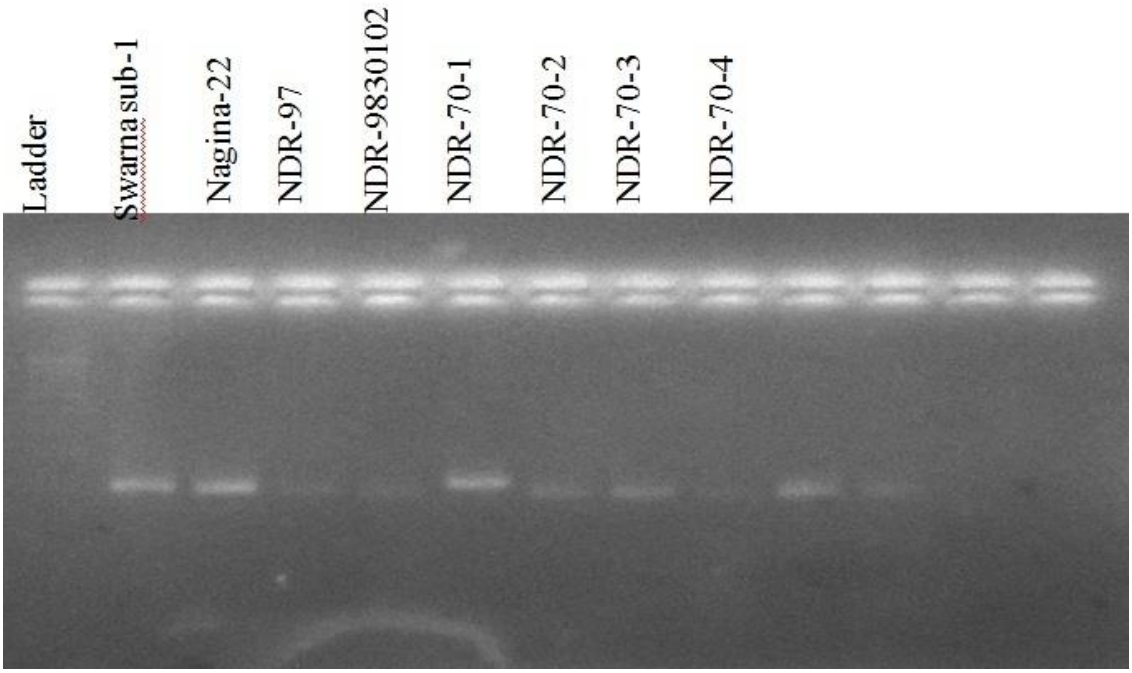


Fig. 4.12: Amplification RM 219 marker in parent and lines

Yield and yield attributing traits:

1. Days to 50% flowering

Generally submergence delayed flowering as well as maturity in rice genotypes (Table 4.7 and 4.8). The maximum delays in 50% flowering and maturity (100% flowering) was recorded in SN-180-70-2 (16.05%) and NDR-70-4 (14.05), NDR-70-3 (13.70%) and Swarna sub 1 (11.41) while minimum in NDR-9830102 (9.45%) in terms of percent increase over control condition.

Table 4.7: Effect of submergence and drought stress on days to 50% and 100% flowering duration of rice genotypes

S.No.	Genotypes	Control	Stress	Percent Increase
1	Swarna sub1	121.20	135.00	11.41
2	Nagina-22	83.6	0	00
3	NDR-9830102	65.6	71.8	9.45
4	NDR-97	74.8	0	00
5	NDR70-1	76.60	86,40	12.79
6	NDR-70-2	77.20	89.60	16.05
7	NDR-70-3	77.40	88.00	13.70
8	NDR-70-4	77.20	88.40	14.51
	CV%	2.60	9.20	
	CD at 5%	2.75	10.70	
	SE(m)±	0.95	3.70	

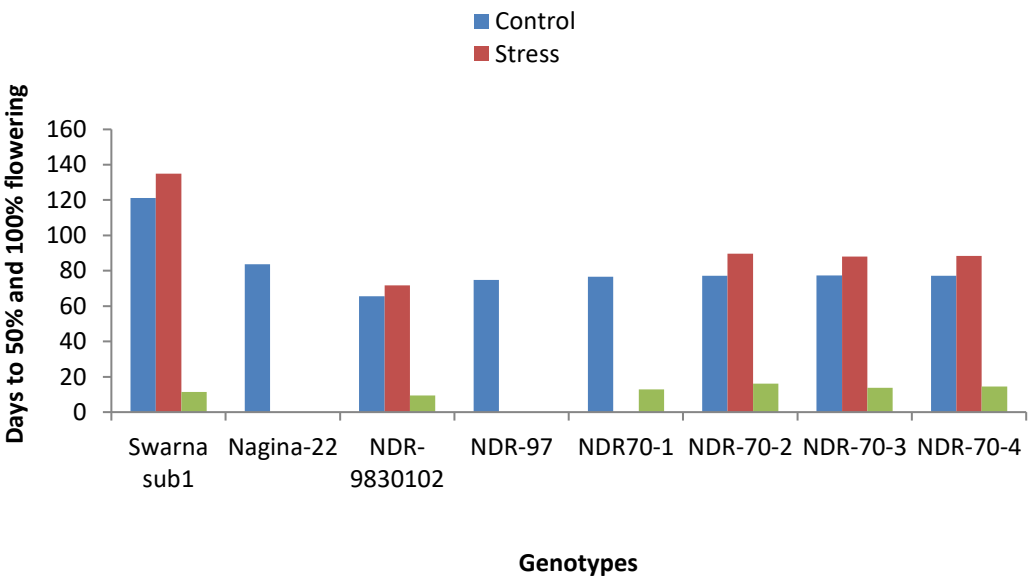


Fig. 4.13: Effect of submergence and drought stress on days to 50% and 100% flowering duration of rice genotypes

Table 4.8: Effect of submergence and drought stress on 100 % flowering of rice genotypes

S.No.	Genotypes	Control	Stress	Percent Increase
1	Swarna sub1	136.40	145.00	6.30
2	Nagina-22	79.60	82.40	3.52
3	NDR-9830102	71.8	76.6	6.68
4	NDR-97	71.80	76.60	6.69
5	NDR-70-1	82.80	91.20	10.14
6	NDR-70-2	85.00	92.00	8.24
7	NDR-70-3	82.20	93.00	13.14
8	NDR-70-4	83.60	93.00	11.24
	CV%	2.50	2.40	
	CD at 5%	2.90	2.94	
	SE(m)±	1.00	1.01	

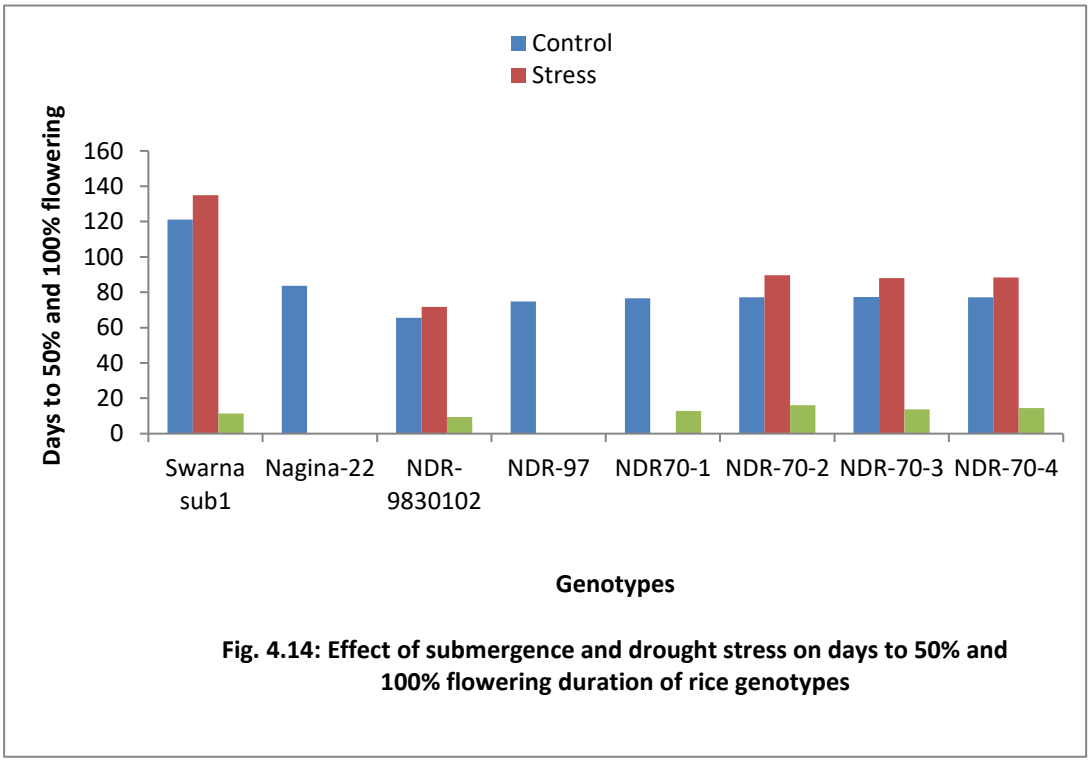


Fig. 4.14: Effect of submergence and drought stress on days to 50% and 100% flowering duration of rice genotypes

2. Plant height:

Submergence and drought treatment affected the plant height of rice genotypes/ lines (Table 4.9). Highest plant height was recorded in Nagina-22 (107.80cm) and lowest Swarna sub 1 (75.80cm) under control condition. Nagina could not survive after submergence treatment. The high plant height was noted in NDR-9830102 (84.80cm) at reproductive stage.

The highest reduction in height was noted in Nagina-22 (100%), while lowest in Swarna sub 1 (16.88%).

Table 4.9: Effect of submergence and drought stress on plant height (cm) of rice genotypes and mapping population.

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	75.80	63	16.88
2	Nagina-22	107.80	0	100.00
3	NDR-9830102	95.60	84.8	11.29
4	NDR-97	83.20	70.72	85.00
5	NDR-70-1	82.20	67.80	17.51
6	NDR-70-2	83.80	68.80	17.89
7	NDR-70-3	87.60	68.40	21.91
8	NDR-70-4	84.40	67.80	19.66
	CV%	3.20	4.20	
	CD at 5%	3.63	2.87	
	SE(m)±	1.25	0.99	

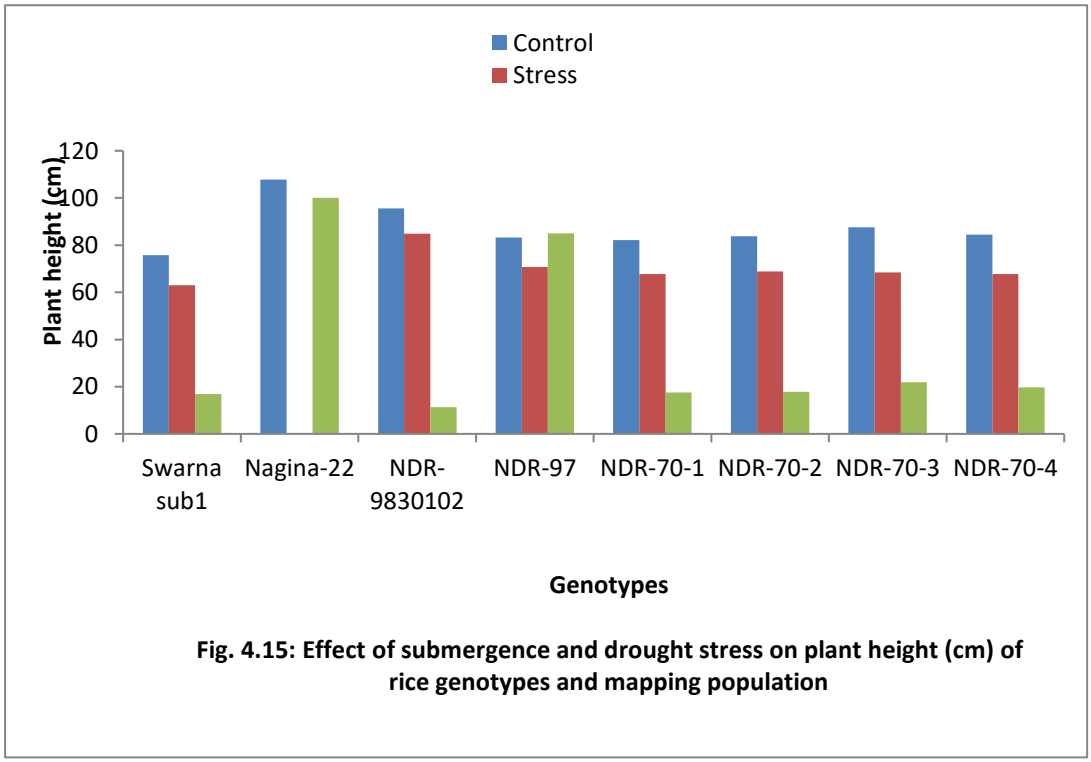


Fig. 4.15: Effect of submergence and drought stress on plant height (cm) of rice genotypes and mapping population

Number of tillers per plant:

Submergence and drought treatment significantly affected tiller number per plant (Table 4.10). High reduction in tiller number was recorded in Nagina-22 (100%) and NDR-97 (65%) and lowest in Swarna sub-1 (20.53%) and NDR-70-1 (31.25%).

Table 4.10: Effect of submergence and drought stress on number of tillers per plant of rice genotypes and mapping population

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	16.00	10	37.50
2	Nagina-22	15.60	0	100
3	NDR-9830102	11.80	9.4	20.33
4	NDR-97	13.60	5.51	65.00
5	NDR-70-1	16.00	11	31.25
6	NDR-70-2	16.00	11	31.25
7	NDR-70-3	15.60	10.8	30.76
8	NDR-70-4	15.80	10.8	31.64
CV%		8.50	11.80	
CD at 5%		1.67	1.21	
SE(m)±		0.58	0.42	

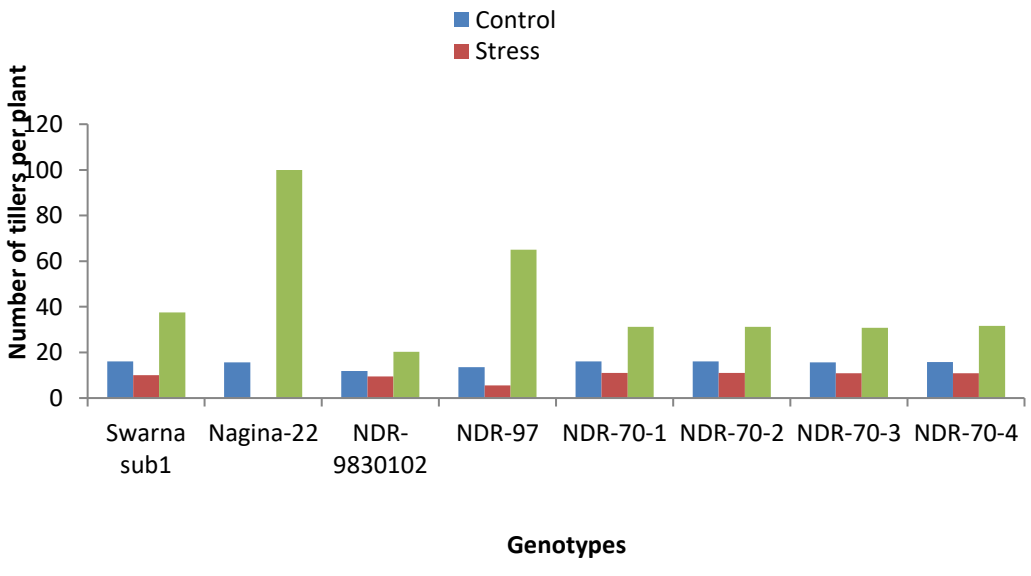


Fig. 4.16: Effect of submergence and drought stress on number of tillers per plant of rice genotypes and mapping population

1. Number of panicles per plant:

All genotypes showed variability in panicle bearing tillers per plant (Table 4.11) High reduction in panicle per plant recorded Nagina-22 (100%) NDR-97 (100%) and no any panicle were found and while less in NDR-70-1(20.89%), NDR-70-4 (21.21%) and NDR-9830102 (26.78%).

Table 4.11: Effect of submergence and drought stress on number of panicles per plant of rice genotypes and mapping population

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	14.40	9.00	37.50
2	Nagina-22	14.00	0.00	100
3	NDR-9830102	11.20	8.20	26.78
4	NDR-97	13.00	0	100
5	NDR-70-1	13.40	10.60	20.89
6	NDR-70-2	14.20	10.20	28.16
7	NDR-70-3	13.60	9.80	27.94
8	NDR-70-4	13.20	10.40	21.21
	CV%	830	10.20	
	CD at 5%	1.44	0.46	
	SE(m)±	050	0.33	

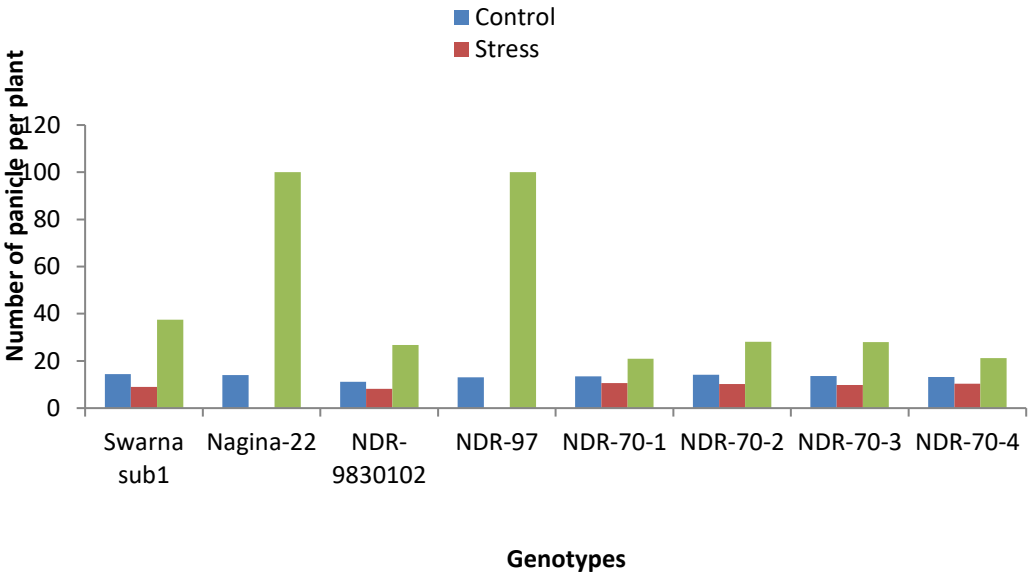


Fig. 4.17: Effect of submergence and drought stress on number of panicles per plant of rice genotypes and mapping population

Number of grains per panicles:

All rice genotypes showed the genetic variability in number of grain per panicle under submergence and drought stress condition (Table 4.12) Nagina-22 and NDR-97 showed the maximum reduction percent (100%) while minimum reduction was noted in NDR-70-1 followed by (28.04%) NDR-70-2 (30.32%) NDR-70-2 (30.32%) and NDR-9830102 (55.84%).

Table 4.12: Effect of submergence and drought stress on number of grains per panicle of rice genotypes

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	198.40	61.20	69.15
2	Nagina-22	209.60	0	100.00
3	NDR-9830102	214.80	94.51	55.84
4	NDR-97	125.60	0	100.00
5	NDR-70-1	156.40	112.61	28.04
6	NDR-70-2	165.60	115.92	30.32

7	NDR-70-3	166.60	114.96	34.98
8	NDR-70-4	167.20	108.68	58.49
	CV%	10.76	0.36	
	CD at 5%	31.17	1.04	
	SE(m)±	13.60	1.60	

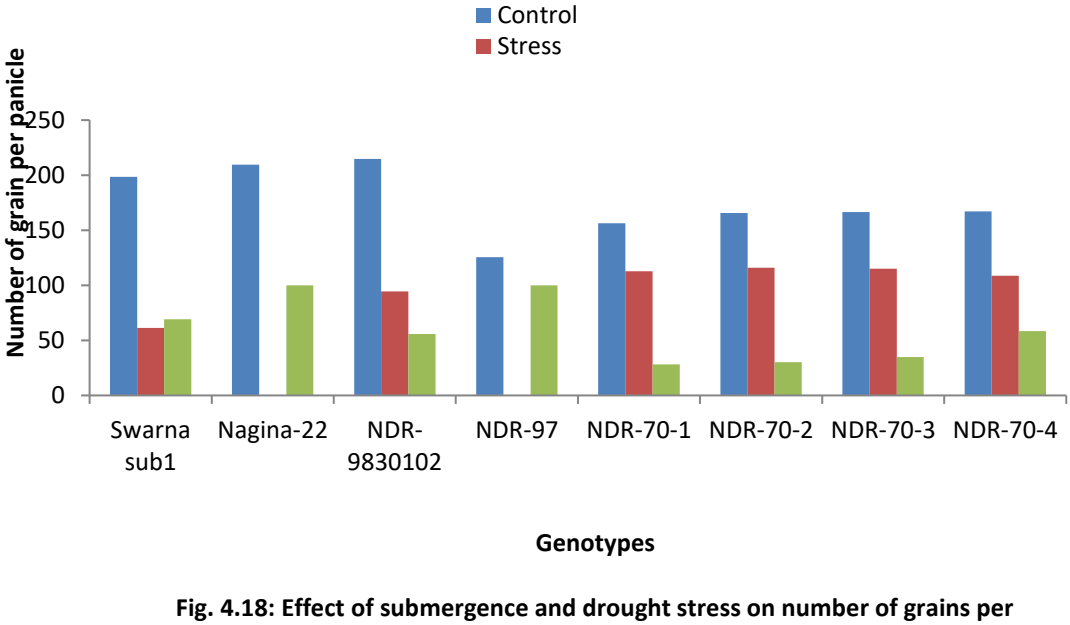


Fig. 4.18: Effect of submergence and drought stress on number of grains per panicle of rice genotypes

Test weight (g):

Rice genotypes showed genetic variability in test weight under submergence and drought stress (Table 4.13). In submergence condition NDR-97, Nagina-22 showed maximum reduction percent (100%) and could not produce any yield. Minimum reduction was recorded in NDR-70-4(22.35%) swarna sub-1 (24.17%) and NDR-70-1 (24.70%).

Table 4.13: Effect of submergence and drought stress on test weight (g) of rice genotypes and mapping population

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	18.20	13.80	24.17
2	Nag ina-22	22.58	0	100
3	NDR-9830102	18.80	13.40	28.72
4	NDR-97	23.30	0	100
5	NDR-70-1	17.60	12.80	27.27
6	NDR-70-2	17.00	12.80	24.70
7	NDR-70-3	17.20	12.80	25.58
8	NDR-70-4	17.00	13.20	22.35
	CV%	6.00	620	
	CD at 5%	1.2	1.10	
	SE(m)±	0.5	0.40	

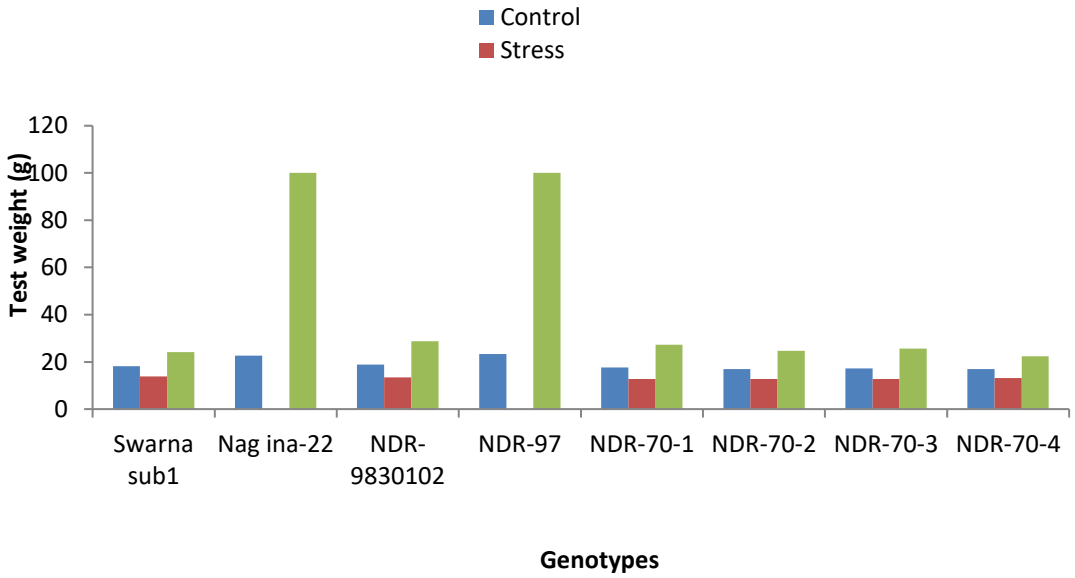


Fig. 4.19: Effect of submergence and drought stress on test weight (g) of rice genotypes and mapping population

Grain yield per plant (g):

All rice genotypes showed the genetic variability in grain yield per plant under submergence and drought condition (Table 4.14). High reduction in grain yield was recorded in Nagina-22 (100%) and NDR-97 (100%) and lowest in Swarna sub-1 (26.71%) and NDR-70-1 (27.11%).

Table 4.14: Effect of submergence and drought stress on grain yield per plant of rice genotypes and mapping population

S.No.	Genotypes	Control	Stress	Percent reduction
1	Swarna sub1	29.79	20.60	30.84
2	Nagina-22	30.79	0	100
3	NDR-9830102	41.74	29.45	29.44
4	NDR-97	39.40	0	100
5	NDR-70-1	27.00	29.68	27.11
6	NDR-70-2	29.10	20.69	29.10
7	NDR-70-3	27.40	18.72	26.71
8	NDR-70-4	28.00	20.32	28.57
	CV%	12.30	12.30	
	CD at 5%	5.04	0.97	
	SE(m)±	1.741	0.33	

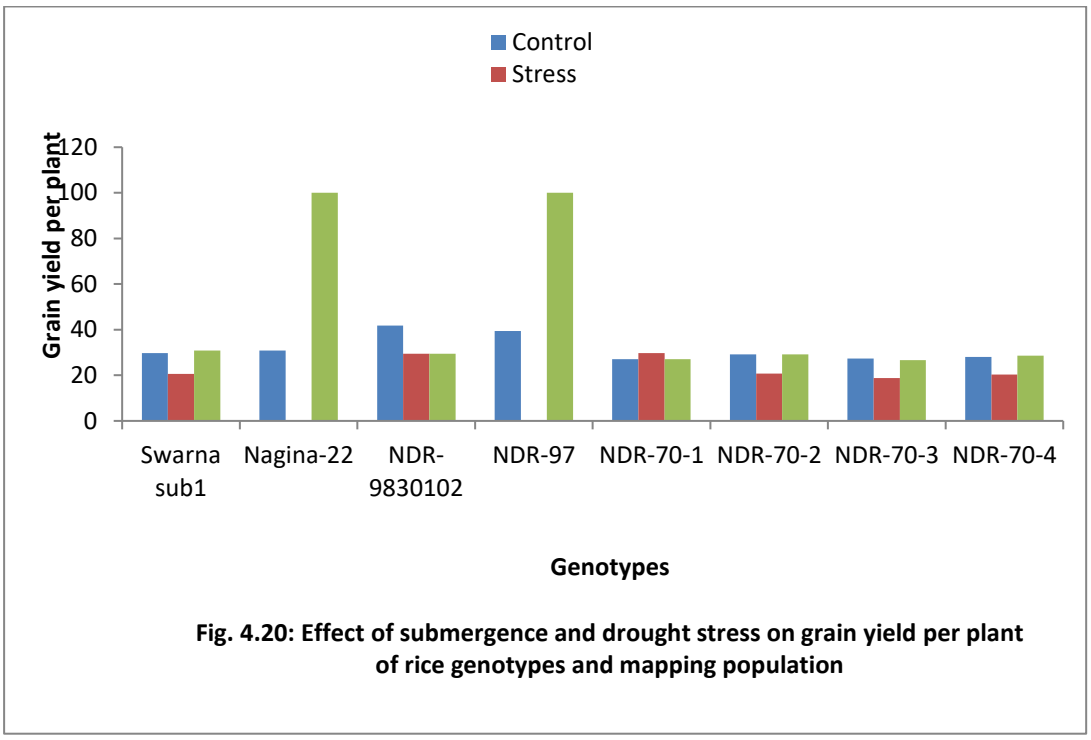


Fig. 4.20: Effect of submergence and drought stress on grain yield per plant of rice genotypes and mapping population

Chapter-V

DISCUSSION

In this chapter an attempt has been made to discuss the results obtained during the present studies in the light of recent advancement in the field of **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"** associated with tolerance and susceptibility for submergence and drought.

Rice is grown under diverse ecological conditions (irrigated, lowland, upland, coastal, drought and flood prone areas) in tropical Asian countries such as India. Rice crops are prone to various types of stresses, both biotic and abiotic. Under rainfed lowland condition, among number of abiotic stress, submergence and drought are major ones and it affects rice production a lot. Drought and submergence are the two major limiting factors that reduce rice production. These two abiotic stresses can completely destroy crop production in extreme conditions, and consequently both of stresses are considered as key determinants of global food security.

Submergence

Submergence is a type of flooding stress and is defined as a condition where the entire plant is fully immersed in water (complete submergence) or at least part of the shoot terminal is maintained above the water surface (partial submergence

Shoot elongation rate

Shoot elongation during submergence, particularly in short-term submergence such as flash-floods, is thought to have an adverse effect on

flash-flood tolerance due to wasted carbohydrates and lodging after desubmergence (Setter and Laureles, 1996; Kawano *et al.*, 2002; Ram *et al*., 2002). Rice cultivars Sabita and Hatipanjari recommended for cultivation in semi-deep to deep water condition (50-100 cm water depth at least for a month during crop growing period) exhibited the elongation of both leaf sheath and leaf lamina due to complete submergence (Sarkar *et al*., 1996).

Elongation under short-term submergence is a negative character, because when water recedes, taller plants tend to lodge and lead to total yield loss. Flood-tolerant genotypes under submergence can thrive by effective energy maintenance through lower leaf expansion, minimum elongation, less chlorosis (Setter and Laureles 1996), high DMP, high carbohydrate reserve, and a strong antioxidant enzyme system (Sarkar *et al*. 2001).

Shoot elongation during submergence is controlled by hormones such as ethylene; the gas interacting with other hormones, including abscisic acid (ABA), gibberellins (GA) and auxin (Jackson, 2008). A cascade model has been proposed based on the study of the stem elongation of deep-water rice stems (Kende *et al*., 1998). The proposed chain reaction probably applies also to leaf elongation of young rice seedlings during submergence since ABA is known to decline in submerged rice leaves (Ram *et al*., 2002),

Catalase and peroxidise

The catalase is one of the highest turnover rates for all enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and is indispensible for ROS detoxification in peroxisomes during stress

condition (Sairam and Srivastava, 2001). Anion free radicals converted to H_2O_2 by the SOD enzyme and detoxify to less toxic compound and the H_2O_2 can be eliminated by CAT and POD (Hasheminasab *et al*., 2012).

Antioxidant enzymes provide tolerance to rice genotypes to environmental stresses. For exm. drought tolerant species of pigeon pea (Cajanuscajan) (Kumar *et al*., 2011), wheat (*Triticum aestivum*) (Hasheminasab *et al*., 2012; Omar, 2012) and black gram (Phaseolus mungo) (Pratap and Sharma, 2010) had higher activities of SOD, POD and CAT than the drought-sensitive species.

Under water stress conditions, the proline content showed highest Catalases are involved in scavenging H_2O_2 generated during the photo-respiration and β-oxidation of fatty acids (Morita *et al* 1994). Catalase and peroxidase activity abruptly increased high in tolerant plant over control after desubmergence due to formation of the reactivity oxygen species (ROS) just after desubmergence. The ROS are chemically aggressive species and the attack of free radicals on the polyunsaturated fatty acid components of membrane lipids initiates lipid peroxidation, an autocatalytic process that changes membrane structure and function (Verma *et al* 2003).

Ushimaru *et al.* (1992) reported that the increased activity of these enzymes under submergence might be due to possible absorption of molecular oxygen from the surrounding water. The combined action of enzymes is important for detoxifying toxicity. SOD merely acts on the superoxide anion, converting it to another reactive intermediate (H_2O_2), and CAT acts on H_2O_2, converting it to water and oxygen (Mates 2000).

Flood tolerant genotypes under submergence can thrive by effective energy maintenance through lower leaf expansion, minimum elongation,

less chlorosis (Setter and Laureles 1996), high DMP, high carbohydrate reserve, and a strong antioxidant enzyme system (Sarkar *et al.* 2001) . Similarly, A. Anandana and P. Arunachalamb (2012) reported that the activities of all antioxidant enzymes were significantly greater in tolerant/avoidance genotypes than in the susceptible (C10) genotypes under both submergence and desubm ergence. Seedlings of tolerant genotypes had higher survival, and the roots were more viable with greater capacity to elongate. Moreover, they had higher peroxidase activity and lesser increases in both electrolyte leakage and malondialdehyde production during submergence.

Drought

Drought is one of the major environmental stresses causing growth retardation and yield loss of plants. Of all the abiotic stresses which grossly reduce the crop productivity in general, drought is the most threatening one. In the last few years, efforts have been made to identify drought resistant traditional rice varieties and subsequently to develop drought tolerant rice through breeding approaches. But due to poor understanding of the morphophysiological and molecular basis of the growth and yield of rice in drought conditions, breeding efforts to improve drought tolerance rice have been hampered (Tuberosa and Salvi, 2006). Most of the crops are sensitive to water deficits, particularly during flowering to seed development stage (Salter *et al.*, 1967).

Leaf rolling

Leaf rolling is one of the drought avoidance mechanisms to prevent water deficits during drought stress (O'Toole and Change, 1978). Loresto *et al.* (1976a,b), Loresto and Chang, (1981) and Chang and Loresto, (1986)

have suggested leaf rolling as a criterion for scoring drought tolerance in tall and semi-dwarf rice cultivars.

A previous examination of the relationship between leaf rolling and water uptake in the field did not detect differences in depletion of soil water among genotypes with different leaf rolling using a neutron probe (Turner *et al.*, 1986), but this may have been due to methodological limitations in the measurements or spatial variation in the field. Interestingly, root growth has been suggested to be related to stomatal conductance and not leaf rolling (Dingkuhn *et al.*, 1999). Rice molecular genetic studies using mutants have identified several genes involved in leaf rolling in rice; all of which appear to influence aspects of leaf morphology and anatomy, including bulliform cells (Hu *et al.*, 2010; Li *et al*., 2010; Zhang *et al.*, 2009).

Leaf rolling is one of the acclimation responses of rice and is used as a criterion for scoring drought tolerance. Leaf rolling is hydronasty that leads to reduced light interception, transpiration and leaf dehydration (Kadioglu and Terzi, 2007). It may help in maintaining internal plant water status (Gana, 2011; Ha, 2014). If cell turgor is maintained under drought stress, it will result in delayed leaf rolling.

Increased leaf rolling under severe stress has the advantage of preventing water loss and radiation damage. Variation in leaf rolling among genotypes has a genetic basis, and QTLs associated with leaf rolling have been reported in rice (Subashri et al, 2009; Salunkhe et al, 2011). Thus, leaf rolling is an adaptive response to water deficit in rice, and leaf angle is a character usually associated with plasticity in leaf rolling when internal water deficit occurs (Chutia and Borah, 2012).

Leaves plays a vital role in photosynthesis, respiration, and transpiration for plant growth and development (Govaerts *et al.,* 1996). Establishment of moderate leaf rolling is considered an important agronomic strategy in rice that can increase photosynthesis and reduce transpiration (Lang *et al.,* 2004; Zhang *et al.,* 2009; Zou *et al.,* 2011), Generally, leaf rolling is induced by water loss from bulliform cells on the leaf upper epidermis in rice (O'Toole J and Cruz, 1980), suggesting that the number and density of bulliform cells may affect the extent of leaf rolling.

RWC

RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration (Anjum *et al.,* 2011). A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006) that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. Exposure of plants to drought stress substantially decreased the leaf potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique *et al.,* 2001). When two poplar species were submitted to progressive drought stress, the decrease of RWC in the water-stressed cuttings was 23.3% in *Populus cathayana*, whereas it was 16% in *Populus kangdingensis*. RWC was affected by the interaction of severity, duration of the drought event and species (Yang and Miao, 2010).

The RWC is considered as the best integrated measurement of plant water status, and it represents the variations in water potential, turgor potential, and the osmotic adjustment (OA) of the plant (Bhushan *et al.,*

2007) (Choudhary *et al*. 2009) screened four-week sold rice seedlings against drought and all tested rice varieties showed a steady increase in RWC around 48-72 hours. Thereafter, a gradual decline was reported during the initial stages. Furthermore, Chaudhary *et al*.(2009) stated that the increase of RWC during the first 2-3 days would be the cause of OA as a result of increased of proline content during the first 24-48 h.

During drought stress the relative water content of plant decreases to an extent of 60–80% with increase in osmotic potential of the plant cells (Singh *et al*. 2015). This increases the level of osmolytes and ensures the plant to maintain its water content during drought enabling the plant to sustain its growth and yield (Blum 2005).

Proline

According to Umezawa *et al*. (2006) and Krasensky and Jonak (2012), plants have the ability to accumulate non-toxic compounds such as proline which protects cells damage due to low water potential of cells, which is a way of adaptation of plants to drought stress tolerance. When plants are faced by drought stress, the osmotic pressure of the plant cell regulates many process through the accumulation of non-toxic solutes inside the cell (Lisar *et al*., 2012; Lipiec *et al*., 2013). Osmolite accumulation is also due to increased biosynthesis without degradation (Lisar *et al*., 2012).

Osmotic potential of cell balanced by the proline accumulation in plants under water stress (Pireivatloum *et al.*, 2010). Tolerance to drought-stress in higher plants correlates to the levels of antioxidant systems and substrates (Athar *et al.*, 2008). Accumulation of proline content under water stress indicates accumulated proline might act as a compatible solute

regulating and reducing water loss from the plant cell during water deficit (Yokota *et al.*, 2006) and play important role in osmosis regulation (Fedina *et al*., 2002).

Proline accumulation provides energy for survival and growth of the plant under oxidative stress (Kumar *et al*., 2011). Thus, the proline content is a good indicator for screening drought tolerant varieties in water stress condition (Bayoumi *et al*., 2008; Kumar *et al*., 2011; Rahdari *et al.*, 2012). Proline was extracted from 100 mg of leaf sample by using ninhydrin reagent in 3% (w/v) aqueous sulfosalicylic acid (Bates *et al.*, 1973) The multiple roles of proline in protecting plants against abiotic stresses are very well documented. As an osmoticum it is used for osmotic adjustment under salt and drought stresses, protects cell structure and acts as a scavenger of damaging free radicals (Nanjo *et al.*, 1999).

High proline accumulation under drought condition have been also observed by a number of workers (Blum, 1989; Pandey and Srivastava, 1989; Hou *et al.*, 1990). It is also evident that tolerant genotypes accumulated more proline content in comparison to susceptible one. Osmotic potential of cell balanced by the proline accumulation in plants under water stress (Pireivatloum *et al*., 2010). Tolerance to drought-stress in higher plants correlates to the levels of antioxidant systems and substrates (Athar *et al*., 2008). The major reason for increase in the proline concentration during water stress was due to lesser incorporation of continuously synthesized proline amino acid during proline synthesis.

Proline accumulation is also responsible for the hydration of biopolymers, surviving as a readily utilizable energy source and serving as a nitrogen source during periods of inhibited growth. Other possible ways

of proline accumulation are through increased proteolysis or due to decreased protein synthesis. Clifford *et al.* (1998).

The mechanism that evolved in proline accumulation is, salinity stress causes ABA mediated stomatal closure that limits the fixation of CO2, resulting in decreasing carbon reduction by Calvin cycle (Lawlor & Cornic 2002) makes NADP+ non available for electrons acceptance during photosynthesis. In this circumstances, Photosynthetic reducing power NADPH2 donate election to glutamate for biosynthesis of proline and regenerate NADP+ for further election acceptance (Wang *et al*. 2007).

It was stated that drought stress leads to an increase in proline accumulation (Nakashima *et al*. 2014). Increase in proline content helped to maintain tissue water status and avoid a reduction in cell damages induced by water deficit or drought stress. Accordingly, cell damage attributed to the reduction in active and reactive oxygen species (Bray *et al*. 2000). The increase in proline under drought stress suggests that low water potential in plants had triggered proline accumulation. This result is similar to the findings by (Hirayma and Shinozaki, 2010).

Molecular marker

Molecular marker technology of serves as a tool for selecting complex traits and allow breeder to track genetic loci controlling submergence and drought resistance traits without having to measure the phenotype. Thus, reducing the need for extensive field testing over space and time (Nguyen *et al.*, 1997).

Several types of molecular markers have been developed. They are mainly RFLP, RAPD, Microsatellites, and SSR these markers, microsatellites have shown great promise in genetic diversity analysis,

genome mapping and gene tagging because of technically simple, time savings, highly informative and require small amount of DNA. The microsatellites are the regions of short tandemly repeats DNA sequences that exhibits repeated units of less than 6 bp in length. Babu *et al.*, (2003) documented the location of drought tolerant QTLs in rice in the regions of chromosome 1 (plant water status), chromosome 3 (biomass yield under stress), chromosome 4 (root morphological traits) and chromosome 9 (RWC and delayed flowering due to stress).

Yield

The reduction in grain yield under stagnant flooding conditions in rainfed lowlands is attributed to the alteration of several growths and yield attributes, such as plant height, tiller number, dry matter accumulation, number and weight of panicles, and harvest index (Sarkar and Das, 2003).

Chapter-VI

SUMMARY AND CONCLUSION

This chapter enumerates the complete synthesis and extraction of experimental findings of the research work entitled **"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits".** Experiment was conducted during wet season 2018-19 with following objectives:

1. Screening of existing mapping population (Swarna Sub1 x N22) x Swarna Sub1, BC_2F_3 for submergence and drought traits by phenotyping and genotyping.
2. The Foreground Selection of existing mapping population by molecular markers.
3. Identify the lines for yield and yield attributing traits.

Experiment was conducted under submergence condition in submergence tank department of crop physiology and drought condition in net house department of Plant Molecular Biology and Genetic Engineering and also at instructional farm Acharya Narendra Deva University Agriculture and Technology kumarganj, Ayodhya for screening purpose during Kahrif season 2018-19. The four mapping population lines NDR-70-1, NDR-70-2, NDR-70-3, NDR-70-4 along with parent Nangina-22 and Swarna Sub1 were evaluated in control, submergence and drought condition for various morpho-physio-biochemical and molecular traits.

5.1 Genotypic selection for submergence related traits:

- Three primers RM-201, RM-215 and RM-219 were used as foreground selection for validation of drought related traits in rice

lines and its parents. These primers in rice lines and its parent with various product size and conferred drought tolerant traits in rice lines.

5.2 Physio-biochemical indices for drought related traits:

- The rice lines NDR70-1, NDR-70-2, NDR-70-3, NDR-70-4 and genotypes Swarna Sub1 showed high catalase and peroxidase activity just after desubmergence comparatively Nagina-22 and NDR-97.
- High relative water content was recorded in Nagina-22, NDR-97, NDR-983010, rice lines NDR-70-1, NDR-70-2, NDR-70-3 and NDR-70-4 while low in Swarna Sub1 under drought stress condition.
- Less leaf rolling was observed in four rice lines NDR-70-1, NDR-70-2, NDR-70-3, NDR-70-4 and Nagina-22 fallowed by NDR-9830102 and NDR-97 showed better performance under drought stress with some variability.
- High proline content was recorded in four rice lines NDR-70-1, NDR-70-2, NDR-70-3, NDR-70-4 and parent Ngina-22 comparatively Swarna-Sub1 at reproductive stage under drought stress environment.

Yield and yield components

- Genotypes Nagina-22, NDR-9830102, NDR-97 and four rice lines NDR-70-1, NDR-70-2, NDR-70-3, NDR-70-4 population maintained stability and showed less percent reduction in plant height, number of tillers per plant under drought condition in comparison to Swarna Sub1.

- Genotypes Nagina-22, NDR-97, NDR-9830102 and rice lines NDR-70-1, NDR-70-2, NDR-70-3, NDR-70-4 showed less percent reduction in plant height, days to 50% and 100% flowering under drought stress condition.
- Less reduction in grain yield were noted in Nagina-22, NDR-70-1, NDR-70-2, NDR-70-3, NDR-70-4, NDR-97, NDR-9830102 at reproductive stage under stress drought stress.

Conclusion:

The four rice line NDR-70-1, NDR-70-2, NDR-70-3 and NDR-70-4 showed better performance under submergence and drought stress condition on the basis of physio-molecular indices.

Chapter-VII

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Department of Plant Molecular Biology and Genetic Engineering,
Acharya Narendra Deva University of Agriculture and Technology
Narendra Nagar (Kumarganj), Ayodhya-224 229 (U.P.)

Title-"Screening of rice (*Oryza sativa* L.) mapping population for submergence and drought tolerant traits"

Major Advisor and Chairman
Dr. Shambhoo Prasad
Associate Professor (PMB&GE)

Name of student
Vishwash Kumar Mishra
M.Sc. (Ag.) Biotechnology
I.D. No.: A-10051/17

ABSTRACT

An experiment was conducted with four rice genotypes / lines, NDR-9830102, NDR-97, Swarna Sub1, Swarna Sub1 and rice lines NDR-70-1, NDR-70-2, NDR-70-3 and NDR-70-4 to evaluate the submergence and drought related traits in pot and field condition. Thirty five days old rice seedlings were exposed to Submergence treatment. Plant height, days to 50% flowering, days to maturity was recorded at the end of submergence treatment. The rice genotypes / lines NDR-9830102 showed high increase in plant height while Swarna Sub1 and some plants of mapping population showed less increase in plant height. High catalase and peroxidase activity was recorded in Swarna sub1 and some mapping population plants and less in Nagina 22. Nagina 22 failed survives after 20 days of desumergence. Survived plant exposed to 15 days drought stress at reproductive stage. Swarna Sub1, NDR 9830102 and rice lines showed less percent reduction in days to 50% flowering and maturity over others. Relative water content (RWC), leaf rolling and proline content were recorded at the end of drought treatment. High proline content, RWC and less leaf rolling were recorded in NDR-9830102 and some plant of mapping population under drought stress environment. The NDR-9830102 and rice lines both submergence and drought resistance traits. Parental polymorphism of rice genotypes were done by 17 SSR primers tested for validation of submergence and drought related traits in parent Swarna Sub1, Nagina-22 and lines. Out of 3 markers, only RM 201, RM 215 and 219 confirmed the drought tolerant traits.RM 219 amplified in both submergence and drought tolerance rice plants. By the phenotyping of rice lines under stress condition it was observed that only four plants showed the submergence tolerance at vegetative stage and drought tolerance at reproductive stage.

(Shambhoo Prasad) **(Vishwash Kumar Mishra)**