Identification of molecular markers and Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice (*Oryza sativa* L.*)* using association genetic analysis.

NITHYA N.
(2016-21-008)

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522
KERALA, INDIA

2020

Identification of molecular markers and Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice (*Oryza sativa* L.*)* using association genetic analysis

by

NITHYA N.
(2016-21-008)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"**Identification of molecular markers and Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice (*Oryza sativa* L.*)* using association genetic analysis**"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani, **Nithya N.**

Date: (2016-21-008)

CERTIFICATE

Certified that this thesis entitled **"**Identification of molecular markers and Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice (*Oryza sativa* L.*)* using association genetic analysis**"** is a record of research work done independently by Mrs. Nithya N. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani,
Date:

Dr. Beena R.
(Major advisor)
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram

ACKNOWLEDGEMENT

Thanks to Almighty 'GOD' who is more benevolent and merciful made me capable to complete this task.

It is with great reverence I place on record, my deepest sense of gratitude and indebtedness to my major advisor ***Dr. Beena R.****, Assistant Professor, Department of Plant Physiology, College of Agriculture, Vellayani, for her meticulous supervision, soft and sincere suggestions, untiring help and constant encouragement throughout the progress of this study.*

With great pleasure I express my heartiest and esteem sense of gratitude to ***Dr. Viji.M. M****, Professor and Head, Department of Plant Physiology, for her worthy guidance, constant encouragement, inspiring help and parental support throughout the period of investigation during the period of endeavor.*

My heartfelt thanks are due to ***Dr. Roy Stephen****, Professor, Department of Plant Physiology, member of my advisory committee for his meticulous guidance, valuable suggestions, keen interest, wholehearted help and constructive criticism and also for the realization of the project.*

I am greatly indebted to ***Dr. Manju.R.V.*** *Professor, Department of Plant Physiology, for her support throughout the period of research work.*

I am extremely grateful to the members of advisory committee, ***Dr.Abida.P.S****, Professor, Department of Plant physiology, R.A.R.S, Pattambi ,* ***Dr.J.Sreekumar****, Scientist, ICAR-CTCRI and* ***Dr. V. G. Jayalakshmi****, Professor, Department of Plant*

Breeding and Genetics, for their valuable suggestions and cooperation during the course of present investigation.

I am thankful to my classmate ***Manasa*** *for her friendship and kind help in times of need. I acknowledge the boundless affection, unsolicited help, companionship and moral support rendered by my friends* ***Reshma, Gayathri chechi, Ammu chechi, Maheswari chechi, Arya chechi*** *whom I admire a lot. I warmly remember their role in making the period of my study here a memorable and cherished one.*

My special thanks goes to my entire friends and seniors from my department and from RARS , Pattambi whom I must name individually; ***Khadeeja itha, Yousuf sir, Athira, Sneha, Krishna, Rajani chechi, Anila chechi, Vipin , Yogesh bayya, and Beena chechi.***

Finally, I am thanking my juniors ***Stephen, Afna, Sudha, Sreevardhan, Amrita, Sreerag , Bhavana*** *and special thanks to* ***Neethu*** *without their lovely affection and kind help without which I may never have completed my research work.*

At this time of thesis submission, I remember with pleasure the sacrifice and support from my Husband, Achan, Amma, my in laws, and special mention to my daughter daksha which I couldn't replace at any cost. Also, the unflinging support given by my brother, grandmother and family members have been a precious source of strength throughout the course of my study here.

Once again I am thanking everyone who helped me during my research programme…… ***Nithya N.***

CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF PLATES

16	SSR profile of 81 rice genotypes using primer RM283	90-91
17	SSR profile of 81 rice genotypes using primer RM455	90-91
18	SSR profile of 81 rice genotypes using primer RM259	90-91
19	SSR profile of 81 rice genotypes using primer RM178	90-91
20	SSR profile of 81 rice genotypes using primer RM328	90-91
21	SSR profile of 81 rice genotypes using primer RM1018	90-91
22	SSR profile of 81 rice genotypes using primer RM246	90-91
23	SSR profile of 81 rice genotypes using primer RM151	90-91

LIST OF ABBREVIATIONS

DES	Directorate of Economics and Statistics
IMD	Indian Meteorological Department
DNA	Deoxyribo Nucleic Acid
FAO	Food and Agricultural Organization
Mha	Million hectares
IRRI	International Rice Reseach Institute
PVC	Poly Vinly Chloride
DAS	Days After Sowing
g/m^{-2}	$grams/meter^{-2}$
SSR	Simple Sequence Repeats
PCR	Polymerase Chain Reaction
cM	centimorgan
RARS	Regional Agricultural Research Station
SAS	Statistical Analysis Software
SDS	Sodium Lauryl Sulphate
TE buffer	Tris-EDTA buffer
UV-VIS	Ultraviolet-Visible
OD	Optical density
dNTP	Deoxynucleotide Triphosphates
TBE buffer	Tris-Borate-EDTA buffer
CD	Critical Difference
GLM	Generalized linear model
LD	Linkage disequilibrium
MLM	Mixed linear model
QTL	Quantitative Trait Loci
PTB	Pattambi released varieties

PCA	Principal component analysis
Q-Q plot	Quantile-quantile plots
%	per cent
^{0}C	Degree celsius
cm	centimeter
g	Gram
cm^{3}	cubic centimeter
r	Correlation coefficient
ml	Milliliter
µl	microlitre
ng/µl	nanogram/microlitre
mM	millimolar
pM	picomolar
nm	nanometer
bp	base pairs
U	Units
rpm	rotations per minute
et al.	and other co-workers
$Plant^{-1}$	per plant
i.e.	that is
FYM	Farm Yard Manure
Kg	kilo grams

1. INTRODUCTION

Rice is the main staple food for one-third of people throughout the world, supplying up to 80% of the daily calories (Ashkani *et al*., 2015). It plays vital role in the national food grain supply. Rice contributes 43 per cent of overall food grain production and 46 per cent of overall cereal output in the country. India has the largest area under rice cultivation between the rice-growing countries of the world, and ranks second in production next to China. Rice is grown worldwide in an area of 167 million hectares with an annual yield of 769.9 million tons. Globally, India ranks second in production after China (166.5 million tonnes) (FAO, 2018).

Rice belongs to the Oryza genus and the Poaceae family, has 22 known species and great economic value (Bajaj and Mohanty, 2005). Rice is cultivated in a more diverse environment than any other major food crop in the world and its production is water intensive. Rice uses nearly 80% of the total freshwater resources for irrigation (Bouman *et al*., 2007). Exploring ways of rising water usage for rice production is therefore of great strategic importance for sustainable crop production in the face of water scarcity in the world. Drought is the most destructive abiotic factor and over 50% of the world's arable land is expected to be impacted by drought in 2050 (Singhal *et al*., 2016).

Drought is a climatic factor that occurs due to low or lack of rainfall. Mostly, drought stresses arise when the soil has low water levels and a constant depletion through evaporation and transpiration of water. Drought is a major abiotic stress affecting rice growth, mostly in the rainfed climate, which ultimately affects crop production and yield. Rice needs to adapt a variety of physiological mechanisms with a complicated regulatory network to counter and cope with unfavorable conditions induced by drought stress (Nahar *et al*., 2016). The general severity of drought is often compounded by erratic and unpredictable rainfall and high temperatures, high levels of solar radiation, and poor soil characteristics under rainfed conditions in marginal areas.

In Kerala, the region's declining rainfall, moonsoon failure, and the monsoon's late onset leads to drought. In 1983, 1985, 1986 and 1987, 2003, 2009 and 2012, Kerala had severe dry spells and droughts despite the wet climate (Nathan, 2012). Similar to the rice farming

scenario in the country, Kerala's rainfed upland is a negligible part of the total rice region. Statistics indicate the presence of 97,069 ha, which is 2.5% of the total geographical area (DES, 2014). This is an example of the vast underutilized and unused potential of state-owned rice farming.

It is estimated that rice demand in 2025 will be 140 million tones. This increased demand for rice from irrigated areas alone can no longer be met. There is a need for greater efforts to increase the contribution of rainfed areas to overall agricultural production.

The development of drought-resistant cultivars in the rainfed region would help increase rice production. However, conventional breeding for resistance to drought is slow due to poor understanding of genetic control of drought resistance and difficulties in phenotyping breeding progeny for tolerance to drought. Phenotypic selection of secondary traits is labor intensive. Molecular marker technology serves as a method for selecting these complex features and enables breeders to track genetic loci controlling drought tolerance traits without having to measure the phenotype, thereby removing the need for substantial space and time for field research.

Molecular markers help recognize quantitative trait loci (QTLs) associated with drought resistance characteristics and their use in the breeding of high yielding rice varieties suitable for drought-prone areas through marker-assisted breeding, thereby reducing the need for extensive field tests over time and space. Although conventional QTL mapping is an important tool for QTL tagging, it is time-consuming and resource-intensive, by using association analysis, these limitations can be overcome (Vasant, 2012).

Association analysis is a powerful tool used to map loci with high resolution and quantitative characteristics such as drought tolerance. It takes advantage of cumulative historical recombination events in the natural population and aims to identify the causative polymorphisms of complex traits (Muthukumar *et al.,* 2015). Association mapping also helps to estimate the association between genotypes and phenotypes in a group of individuals with a disequilibrium population (Pradhan *et al*., 2016).

Thus, in the present study, a total of 81 rice genotypes from different geographic locations were evaluated for drought tolerance and plant production traits under irrigated and drought stress conditions. The rice genotypes were screened using a total of 100 SSR

primers. By linking genetic polymorphism to the phenotypic data, it is possible to identify QTLs and molecular markers to improve the tolerance of drought in rice. Therefore the present study was carried out with the following aim:

1. To identify molecular markers, Quantitative Trait Loci (QTLs) associated with drought tolerance and plant production traits in rice under drought condition.

2. REVIEW OF LITERATURE

Rice is a very important Indian crop, and millions of daily Indians find comfort in it. With its high carbohydrate content, it is known to provide fast energy, and is a staple food made by most Indians. Therefore the importance of rice in the country is ignored. India is not only the leading consumer of rice but also the second largest producer (166.5 million tons) after China (FAO, 2018). Rice costs 35 to 60 percent of the calories consumed by three billion people in Asia alone. Rapid population growth worldwide is growing in demand for a corresponding increase in grain yields (Liang *et al*., 2010) and the demand needs to 50% increased by 2025 (Khush, 2001). Among the rice-eating countries, 40% more rice needs to be improved by 2030 (Zhu *et a*l., 2010). Various types of rice with very high agricultural characteristics, such as high productivity, water stress tolerance, etc should be produced to achieve this goal.

2.1 EFFECT OF DROUGHT ON RICE

Drought is a major disruption factor that negatively affects rice development, especially in the rainy season which ultimately affects production and productivity. Rice is one of the most drought-tolerant crops, especially in the reproductive stage (Agarwal *et al*., 2016). Water shortages have been reported to have a major impact on rain-fed rice, especially in the flowering stage, where crops are most affected by severe drought, leading to low productivity (Pantuwan *et al*., 2002). Rice needs to adapt a series of physiological mechanisms to a complex network to control and meet adverse conditions caused by drought stress (Nahar *et al*., 2016).

Blum, 2011 reported that water stress causes a reduction in soil moisture content to meet plant water demand resulting in slower growth and rice development resulting in reduced crop yields. Rice is an original natural semiaquatic plant and is therefore more vulnerable to drought than any other crop (O 'Toole 2004). Drought affects all morpho-physiological processes (Lanceras *et al*., 2004). It results in a reduction in cell expansion and cell counts (Sokoto and Muhammad 2014). Drought also affects the root branch (Clark *et al*., 2008). Apart from this the drought causes a decrease in leaf numbers, tillers, plant height and severe drought causes the leaves to dry out and eventually plant to death (Ji *et al*., 2012).

Globally, rice grows on 154 million hectares (MH), and about 45 percent of this area is subject to low rainfall conditions with low yielding potential (Verulkar *et al*., 2010). Fischer *et al*. (2012) noted that rainfed rice has grown to 60 Mha of land area. In Asia, water stress is the biggest threat to low-grade rice production (46 Mha) and upland (10 MH), affecting crop yields (Pandey et al., 2007). Wassmann *et al*. (2009) reported that rice grows under very different environmental conditions than other major plants in the world and is also a semiaquatic plant and its product is highly waterlogged. Rice is the largest consumer of water about 5000 liters of water needed to produce one kilogram of rice (Singh *et al*., 2012). Bimpong *et al*. (2011) reported that water stress is the major abiotic stress limiting rice production in natural rainfall areas and high altitudes. Drought can only be defined as reduced yields due to water shortages (Bernier *et al*., 2008). Srividya *et al*. (2011) reported that drought stress is severe among abiotic stresses and reduced yields by 15-50 percent depending on the intensity and severity of the rice season. Global drought causes a decrease of 18 million tons of rice per year (Lakshmi *et al*., 2012). Climate change is affecting water resources and the frequency of drought and floods is expected to increase in the future. Ray *et al*. (2015) reported that climate variability affects yields at approximately 0.1 / t / ha per year for 54% of the world's rice production areas.

2.2 MECHANISMS OF DROUGHT TOLERANCE

Tolerance to drought is a complex trait and its manifestation depends on various morpho-physiological and biochemical characteristics (Mitra, 2001). Responses of various plants to the drought situation include the drought escape, drought avoidance, tolerance and the drought recovery (Singh *et al*., 2012). Drought escape is an adaptive mechanism that requires the rapid development of plants in order to complete the entire life cycle before a drought event (Turner, 1979). Through the development of a deep root system, cultivars are able to absorb water from deeper soils that is the mechanism of drought avoidance (Gowda *et al*., 2011). Root elongation, branching and growth indicators are caused by stress and are caused by other environmental factors, such as nutrient uptake and hormone status, especially auxin and ABA. The severity of the drought at planting and germination stages puts it at a level of avoiding crop compression and whether deep or productive roots will grow with increased dry matter

accumulation (Bhatnagar-Mathur *et al*., 2013). Beena *et al*. (2017) reported that the deep root system is the most consensual of the traits that contribute to avoidance to drought condition.

Drought tolerance mechanisms include cellular changes, morpho- physiological adaptations stiffness, that are controlled at various levels by genetic factors. Drought tolerance to cellular changes including, increased chlorophyll content, decreased osmotic potential and harvest index. Physiological acclimatization includes higher stomatal density and behavior; the decline and initial increase in mating between female and male flowers and maturation; further growth, accumulation, acquisition and separation of fruit and biomass yield. Reduction of osmotic potential in cytosol results in the accumulation of organic and inorganic compounds, leading to the maintenance of turgor pressure under water stress conditions. Osmotic adaptations occur by accumulation of proline, sucrose, glycine betaine, and other solutes in the cytoplasm, to improve water absorption. Proline is the most commonly studied osmotic adaptation because of its great ability to reduce stress under adverse conditions. Antioxidants are active antioxidants (ROS) that break down nutrients in plants, and drought tolerance is enhanced by expression. ROS includes hydroxyl-free radicals, superoxide radical, hydrogen peroxide and singlet oxygen, resulting in protein break down, lipid peroxidation, and destruction of damaged homeostasis, cellular oxidative damage, and DNA mutations. Enzymatic antioxidants include monodehydro ascorbate reductase (MDHAR), dehydro ascorbate reductase (DHAR), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APH).), guaiacol peroxidase (Guaiacol peroxidase).

Drought tolerance is a plant's ability to survive under insufficient supply of water or under intermittent water deficit condition. In rice drought tolerance is a complex trait regulated by polygenic effects involving complex physio-morphological mechanisms (Liu *et al*., 2006), such as increased elasticity in cell, osmotic adjustment, decreased cell size and desiccation tolerance by protoplasmic resistance (Sullivan and Ross, 1979). With the accumulation of proline, soluble sugars, glycine betaine and other stimuli in the cytoplasm, osmotic adjustment is obtained (Wei *et al*., 2014) by thus improving water absorption. The stomatal and dynamic function of mesophyll (Lauteri *et al*., 2014) and the biomass processing and separation (Xangsayasane *et al*., 2014)

allow rice plants to withstand the pressure of water scarcity. It has been shown that effective collection of abscisic acid (ABA) under drought stress significantly activates antioxidant enzymes and regulates stomatal movement (Ahmad *et al*., 2014) and carbon metabolism (Zhou *et al*., 2014) in addition many genes are involved in managing responses to drought. Biochemical reactions include increased antioxidant activity, which increases drought resistance by depleting reactive oxygen species. In addition, under drought pressure, unresponsive plants (resistant one) showed high levels of cysteine, Palmitoleic acid, oleic acid, arachidic acid, behemic acid, copper, potassium and magnesium, while high levels of Glycine, tyrosine, linoleic acid, linolenic acid, lignoceric acid and calcium were detected in sensitive plants compared to those grown under waterlogged conditions (Nam *et al*., 2014). The most important change is the accumulation of proline, as it behaves like an osmolyte. Proline chelates metals and thus plays an antioxidant role and signaling molecule (Fahramand *et al*., 2014).

2.3 EFFECT OF WATER STRESS ON PHYSIOLOGICAL PARAMETERS

Different studies showed early morphological changes in rice including reduced growth and development of rice.

Ahmadikhah and Marufinia (2016) reported that water stress had a significant impact on morphological properties, including plant height and crop yield, indicating that these symptoms were significantly affected by irrigation. Farooq *et al.* (2010) reported that the rice plant shows different physiological and morphological changes at different stages that includes reduced plant height, leaf rolling, leaf senescence, stomatal closure, decreased leaf elongation and lower drymatter production and in physiological changes which includes decreased chlorophyll content, transpiration and photosynthetic rate.

A comparative study of a drought tolerant (IRAT109) and a susceptible cultivar (Zhenshan 97B) showed significant differences in morpho-physiological changes due to drought conditions in leaf rolling and reduction of plant height. In the tolerant cultivar, the leaves and stem showed better elongation (Ji *et al*., 2012). During the reproductive stage drought can delay many developmental processes like panicle onset and anthesis (Rahman *et al*., 2002). The effect of drought stress on various physiological and morphological characters varies greatly between different rice genotypes (Kumar *et al.,* 2015). Therefore analyses of different shoot and root characters towards drought stress is important for establishing drought tolerant crop production systems.

There are various ways to detect drought tolerance in rice at different stages of growth (Todaka *et al.*, 2015). Reproductive stage is the most critical stage that affected by drought. Primary criteria for screening drought tolerant varieties are drought scoring (IRRI, 2014). Drought causes reduction in the photosynthetic rate and chlorophyll content which leads to reduction in the production of dry matter (Kadan *et al*., 2015). Pantuwan *et al*. (2002) reported drought stress that occurs during booting stage resulted in alteration of floret initiation. Zhu *et al*. (2004) reported that due to anther dehiscence failure and reduction in the starch accumulation in pollen grains causes increased amount of spikelet sterility observed at drought stress close to booting stage.

2.3.1 Leaf rolling & Leaf drying score

One of the rice genetically determined responses to water deficiency is leaf rolling. Leaf rolling results in a reduced area of the leaf exposed to light, water loss prevention through transpiration, and limited damage to radiation (Ha, 2014). In rice, rolling of leaves is an important feature (Xiang *et al*., 2012). Singh and Singh, 2000 reported that it is an adaptive response to the water deficit that helps to maintain the water balance in plant tissues under conditions of water scarcity and soil moisture depletion. Rolling of leaves is hydronasty that leads to reduced light interception, transpiration and dehydration of leaves (Kadioglu and Terzi, 2007). It can help maintain the internal condition of plant water (Ha, 2014). If cell turgor is stored under drought stress, it will cause delays in rolling of leaves. However, increased leaf rolling under severe stress has the advantage of preventing water loss and radiation damage (Salunkhe *et al*., 2011). Visual leaf rolling is an effective method for identifying drought resistance in rice, especially in vegetative stage (Salunkhe *et al*., 2011). Leaf rolling (outward) and folding (inward) are two morphological characters for which genetic variation exists among various rice genotypes (Xu, 2002). Plants response to drought can be determined by its degree of leaf rolling (Subashri *et al.*, 2008). Leaf rolling has a positive effect in maintaining high leaf water potential (Dingkuhn *et al*., 1989).

Rolled leaves of rice transpire 41 per cent less water than compared to the unrolled ones (Courtois *et al*., 2000). Singh *et al*. (2017) observed that the rice cultivars at 33 per cent field capacity and 66 per cent field capacity started to shows leaf rolling symptoms whereas cultivars at 100 per cent field capacity showed no rolling symptoms. In rice, leaf rolling factor under drought stress was studied as one of the best criteria in

estimating levels of drought tolerance in a large scale screening (Pandey and Shukla, 2015). Tiwari *et al.* (2017) reported that leaf rolling was varied among the genotypes at the end of the drought treatment. Highest leaf rolling was noticed in Swarna sub 1 and lowest in Nagina 22 (1). In control condition all genotypes showed no leaf rolling symptoms. Low or temporary rolling of the leaf is an adaptive feature of the tolerant plant to reduce its water loss (Chaturvedi *et al.,* 2012). Leaf drying rate can be used to determine drought resistance in all stages of rice (Laffite *et al*., 2003). A cut-off rate of 0 = no visible leaf symptoms and 5 = more than 40% of the leaf drying area is recommended for rice (Laffite H. R. 2002). Musila (2015) conducted a study on genetic analysis for drought tolerance and yield stability in interspecific rice germplasm. For this study they have used drought scoring procedure to identify the drought tolerant varieties and from this study they observed that all the genotypes showed signs of leaf drying from slight (score of 2) to severe (score of 4). The tolerant check showed the lowest leaf rolling and leaf drying scores of 3 and 2 respectively followed by the moderately drought tolerant check. Pushpam *et al*. (2017) screened rice genotypes for drought tolerance using wax petrolatum layer method and from this study they have noticed that the all genotypes recorded drying score as an average of 4.04 with a range of 0.0 to 9.00 under stress. Drought score is treated as an alternative approach to determine plant drought tolerance (Fen *et al*., 2015). Swapna and Shylaja (2017) conducted a study on screening for osmotic responses in rice varieties under drought condition and to conduct this study they have used 42 high yielding rice varieties. Drought score varied widely in rice varieties. Swarnaprabha, Kattamodan, Vaisakh, Samyuktha, Onam, Krishnannjana, Prathyasa, Remanika and Rokwazi have shown a droughts score of 3 in line with the prevailing drought tolerant check variety. Swarnaprabha and Katamodan rice varieties had a higher recovery potential than other varieties. Barik *et al.* (2019) reported that drought tolerant donor plants showed vey less SES score. Variation in leaf rolling score was observed a range of 0 to 9 with a mean of 2.89. Similarly leaf drying also showed wide variation with a mean value of 1.57 under drought stress condition.

2.3.2 Relative water content

Relative water content is probably the most accurate measure of a plant's water status in terms of physiological consequence of deficit of water. The water potential of the leaves are closely related to the leaf relative water content (RWC), but it undermines the osmotic adjustment. In flowering strong correlation may be observed between the crop yield and the RWC but it is not sufficient to ensure a good yield (Lafitte, 2002). Drought-resistant species showed higher water potential of leaves in their tissues compared to species found under soil moisture deficits. The highest RWC is reported to be associated with tolerant varieties of wheat (Martin et al., 1997). Akram *et al*. (2013) have observed significant reductions in related water content, transpiration rate, leaf water potential and increased leaf temperature when rice crops are exposed to drought stress. Dien *et al*. (2019) reported that relative water content of the leaves decreased significantly under water stress compared to control. The intermediate water content of leaves of all species has dropped significantly from 78.59% in the pre-drought situation to 41.19% in moderate drought stress and 20.16% in severe drought stress.

After conducting experiment in the reproductive phase of the four indica rice genotypes under water deficit stress, Cha-um *et al*. (2010) noted that the relative water content (RWC) in the PT1 flag group (groundwater subdivision) and IR20 (negative check) rice fields (drought victim) were significantly reduced when crops were exposed to deficit of water with 25 percent of the soil water content (SWC) and RWC recovery were delayed during rewatering. The RWC in both KDML105 (drought tolerant) and NSG19 (positive check) was kept at low water stress, but significantly reduced when plants were exposed to severe water shortages (7% SWC) and grew rapidly after re-irrigation. At the onset of panicles , Beena *et al*. (2012) observed a dramatic decrease in relative water content about 53.1% in selected inbred inbred lines (RIL's) for IR20 x Nootripathu and their parents when exposed to water stress. The reduction was more in IR20 (48.9%) compared to Nootripathu (65.2%). RWC has been identified as an important parameter for selecting plants that are tolerant to drought stress (Bunnag and Pongthai, 2013). RWC is considered to be the best combined measure of plant water status, and represents variability in water intensity, turgor pressue, and osmotic

adjustment (OA) of the plant (Bhushan *et al*., 2007). Choudhary *et al.* (2009) tested four-week rice seedlings for drought tolerance, and all tested rice varieties showed a strong increase in RWC by approximately 48-72 h (2-3 days). After that, a gradual decrease was registered during the early stages. Kumar *et al.* (2014) reported that under conditions of water stress, higher RWC values were recorded in drought tolerant varieties of rice compared to susceptible genotypes. Jayaweera *et al.* (2016) reported that the RWC of rice leaves fluctuates in the vegetation phase in both Godaheenati (4049) and Pokkali due to drought stress. Larkunthod *et al.* (2018) conducted a study of physiological responses under the stress of improved drought-tolerant lines with their parents in Thailand. In this study they found that RWC and LWP decreased with the increase in drought period imposed by 20% PEG supplement in all lines / rice.

2.3.3 Cell membrane stability (% leakage)

According to Blum and Ebercon (1981) Cell membrane stability (CMS) is a physiological index widely used in drought testing and temperature tolerance. Sullivan (1972) observed electrolyte leakage from leaf segments. Lower stability of membranes or increased injury indicates membrane lipid peroxidation, which results in high oxidative stress due to various environmental stressors including drought (Liebler *et al*., 1986). Tyagi et al. (1999) reported that MSI was superior in tolerant genotypes under water stress. Tolerant genotypes CR 143-2-2 and N22 under water stress showed a higher membrane stability index than susceptible genotypes PR 110 and PR 169. Tripathy *et al*. (2000) conducted a study on double haploid (DH) lines obtained from a cross between CT9993–5-10–1-M and IR62266-42–6-2. They observed a reduction in cell membrane stability between the parents and double haploids (DH). The mean CMS value for CT9993 was 91.9% and for IR62266 was 78.9%. There was significant difference for CMS among the 104 DH lines and the mean values ranged from 72.0% to 96.0% with a continuous variation. Swapna and Shylaja (2017) reported that the cell membrane stability index of the root (r = 0.2143, P =0.046) and shoot (r = 0.3656, P = 0.006) were positively correlated with the observed drought score. Ibrahim *et al.* (2019) evaluated six Egyptian rice cultivars differing in their drought stress tolerance and from this study they observed that membrane stability index decrease significantly in rice seedlings exposed to drought compared with control.

2.3.4 Leaf temperature

Under conditions of water stress plants are subjected to high temperatures which increase their risk to stress (Carpentier, 1996). At lower panicle temperature and canopy temperature a strong physiological activity was observed (Xu *et al.*, 2005). Blum *et al*. (1989) found an increase in leaf temperature associated with decline in rate of transpiration reflecting the degree of water stress in sorghum. Jones and Corlett (1992) observed that leaf temperature is associated with the plant stress. Garrity and O'Toole (1995) observed that the spikelet fertility and grain yield and were highly correlated with midday canopy temperature on the day of flowering. Babu *et al.* (2003) reported that rolling of leaves and drying was positively correlated with canopy temperature and negatively correlated with root thickness in rice. Chen *et al.* (2010) reported under severe water stress there was a significant increase in leaf temperature and decreased photosynthesis and transpiration rate. Through this observation they have concluded that the reduced transpiration may cause increase in leaf temperature. Halder and Burrage (2003) conducted a study on effect of drought stress on rice grown in nutrient film technique. From this they have observed that the increase in leaf temperature with increases of drought stress and this increase may be due to decrease in transpiration rate. Hirayama *et al*. (2006) reported with increase in leaf temperature under drought stress cause decrease in photosynthetic and transpiration rate.

2.3.5 Chlorophyll stability index

An indication of chlorophyll stability index (CSI) is an indication of plant tolerance potential. A higher CSI value means that the stress did not significantly affect the plant chlorophyll content. High CSI helps plants to withstand stress with better availability of chlorophyll. This leads to increased photosynthetic rate, more dry matter production, and higher productivity. This shows how chlorophyll can perform well under water stress (Madhan Mohan, 2000). Nahakpm (2017) evaluated eight rice genotypes. The genotypes were evaluated in terms of chlorophyll contents (Chl a, Chl b and Total Chl), Chl a/b ratio, Chl stability, expression of antioxidant enzymes, generation of reactive oxygen species and grain yield. Significant differences in chlorophyll a / b ratio were observed to indicate differences in the activity of the

chlorophyll synthesizing mechanism between varieties when exposure to drought stress. Among the varieties studied, significant differences in chlorophyll solid index (CSI) were noted between control plants and stressed plants. BRR-0028 showed high CSI with high yields despite of low chlorophyll content. Sharifi *et al.* (2012) found that the effect of drought stress on the chlorophyll a, b and total chlorophyll was significant. In all lines, the amount of chlorophyll a, b and total chlorophyll was reduced in the prescribed treatment. Results showed that in different lines, the stability index of chlorophyll b and total chlorophyll were significantly statistically different, but in the case of chlorophyll a stability index, there was no significant difference between lines. Behera *et al.* (2017) reported a decrease in chlorophyll and chlorpophyll stability index of rice genotypes under drought stress conditions and the decrease was 50 to 60%. Similar results were also reported by Agarie *et al*. (1995).

2.4 IMPACT OF DROUGHT ON MORPHOLOGICAL AND YIELD PARAMETERS

2.4.1 Plant height

Babu *et al*. (2003) used double haploid lines (DH) of CT9993-5-10-1-M / IR62266-42-6-2 to identity of quantitative trait loci (QTL) associated with drought tolerance in rice. They determined that the mean plant height had decreased by 3.8 cm below the strain in DHs. Some parents, CT9993, had shown no widespread reduction in plant height at the same time as IR62266 had a 4.2 cm reduction in plant height below the strain. Seven rice cultivars under study showed a moderate decrease in the growth rate of the stems under moderate drought stress (after 20 days of treatment) at the vegetative level (42 DAS). The decrease in growth rates has also become more dramatic under intense pressure (after 60 days of treatment) (Bunnag and Pongthai, 2013). Drought strain at the vegetative stage in rice led to a marked reduction in plant height. The lower plant height was found to be 12 % in Zhenshan97B and 3 % in IRAT109. The decrease was much lower in IRAT109, which also indicates its tolerance to water stress (Ji *et al*., 2012). Sokoto and Muhammad (2014) reported that the water stress at some stage of the vegetative level reduces plant height, number of tillers and leaf area. Beena *et al*. (2012) stated that the height of the plant was

reduced 10.4% across the RIL's of IR20 × Nootripathu as compared to irrigated control throughout drought stress. Genotypes with extra plant height remain larger in the size of the plants as a whole, reduce high light intensity and use water more rapidly, leading to lower plant water conditions (Kamoshita et al., 2004). Singh *et al.* (2017) noticed significant reduction in plant height of rice cultivars at 66% FC and 33% FC as compared to 100% FC. Singh *et al.* (2018) reported that drought affects both elongation as well as expansion growth (Shao *et al*., 2008), and inhibits cell enlargement more than cell division (Jaleel *et al.*, 2009). Drought stress induces reduction in plant growth and development of rice (Manikavelu *et al.*, 2006). Ahmed *et al*. (2017) reported that plant height under drought conditions vary significantly in both control and stress condition and among the varieties highest plant height was recorded for the variety Ganga (122.80 cm) under control condition and the variety BRRI dhan 57 got lowest plant height (68.03 cm) under drought condition. It was observed that individual variety had significant different plant height between control and drought stress condition. Each variety achieved lower height in drought stress condition compared to control condition.

2.4.2 Tiller number

Singh *et al*. (2018) reported reduction in number of tillers during drought stress period and the reduction was more in Swarna Sub1 variety (25.82%) and minimum in Nagina 22 (8.76%). The number of tillers decreases due to reduced growth and photosynthesis of the plant (Quampah *et al*., 2011). Singh *et al*. (2017) observed a significant reduction in the number of tiller under drought stress. The highest and lowest decline in tiller numbers under drought stress was observed in Chenier (65%) and RU1104122 (40%), respectively. Tiller number reductions is reported to be the main cause of reduced yields under water scarcity conditions during crop growth (Rahman *et al*., 2002; Sarvestani *et al*., 2008). Sikuku *et al.* (2012) reported that tiller numbers tended to decrease with increasing water deficit. Purbajanti *et al.* (2017) reported reduced number of tillers of rice under drought stress condition compared to saturated control condition. The number of tillers was reduced 39-58% under drought stress. The number of tillers/hill decreased with decreased soil moisture content. The reduction in number of tillers may be due to under drought stress plant cant able to produce enough assimilates (Mostajeran and Eichi, 2009). Behera *et al*. (2017) observed reduction in

number of tillers under drought stress applied to rice plants at different growth stages and the reduction was more when the stress was given during flowering stage. Zain *et al*. (2014) reported reduced number of tillers, plant height, and grain yield of rice with increased duration of water stress.

2.4.3 Days to 50% flowering

In rice, water stress during flowering can reduce the yield index by 60 percent, mainly due to reduced grain set. Five panicles in stressed plants fail to fully exercise (exit) from the flag leaf sheath due to the flow of flowers is not timely, and the percentage of spikelet opened in anthesis decreases (Deshmukh *et al*., 2007). Failure of panicles alone causes approximately 25 to 30 percent of sterility due to the fact that an unadulterated spikelet cannot eradicate anthesis and break the pollen, even if production is normal. Decreased flowering under drought conditions is linked to low water conditions and has become an indication of drought. Delay in flowering has also been associated with impoved spikelet sterility (Verulkar and Shrivastava 2009). Water stress during flowering and inflorescence production leads to inhibition of flowering (anthesis) or even complete inhibition as apical morphogenesis is prone to dehydration (Woperesis *et al.*, 1996). Ahmed *et al*. (2017) reported that days required to flowering vary significantly among the conditions as well as cultivars. Among the varieties BRRI dhan57 required less number of days for 50 % flowering in control condition (69.33 days) and also in stress condition (66.67 days). Sikuku *et al*. (2012) reported that control plants took the least days to reach 50% flowering whereas plants watered every six days took more days to reach 50% flowering. Haque *et al.* (2016) evaluated effect of drought stress on phenology of aus type rice. They observed that vegetative stage drought stress delayed phenological events of the genotypes. On an average, the stressed genotypes took 87, 93 and 123 days to first flowering, 50 percent flowering and maturity, respectively. Singh *et al*. (2018) reported that days to 50% flowering was significantly affected by drought stress and the reduction was more in Swarna sub 1 variety (18.56%) and minimum in Nagaina 22 variety (9.67%).

2.4.4 Yield per plant

Lafitte *et al*. (2003) reported that the number grains per panicle had no effect on grain yield under water stress condition. Basnayake *et al*. (2004) reported 9 to 51% and Ouk *et al*. (2006) reported a 12 to 46% reduction in yield due to drought in rice. Sah and Zamora (2005) noted that, in addition to reproductive levels, water scarcity at vegetative phase has significantly reduced grain yields per maize crop compared to waterlogged crops. The discount changed to 19.5 percent and 48.5 percent due to lack of water in the vegetative and reproductive phases, respectively, compared to waterlogged plants. Mild stress occur during grain filling which resulted in a yield reduction of 11.6% to 14.7% (Cai *et al*., 2006). Ahadiyat *et al*. (2014) reported that rice grain yields were significantly reduced under drought stress. Sikuku *et al*. (2010) conducted a study on the effects of water scarcity on the days of maturation and production of three American varieties and reported that there was a significant reduction in grain yield of NERICA varieties with an increase in water scarcity. Singh *et al*. (2010) examined six genotypes of rice under drought and controlled conditions. They observed a reduction in a few parameters including grain yield under drought conditions. Drought stress during flowering has reduced the yield of large quantities of grain compared to other stresses (Haque *et al*., 2016). Moisture stress can inhibit photosynthetic activity and facilitate grain transport resulting in reduced grain yield (Liu *et al*., 2008). Singh *et al*. (2018) noted a decrease in grain yield of rice genotypes under water stress compared to control. Reductions were higher in the Swarna Sub1 range (46.07%) and smaller reductions occurred in the Nagaina-22 variety (19.71%). Drought stress caused a decrease in yield and biomass of the rice crop by 25.4% and 25.2% and wheat yields by 27.5% and 25% respectively (Zhang *et al*., 2018).

2.4.5 Spikelet fertility percentage

During the reproductive period of rice, abiotic stresses such as drought showed a negative impact on different processes, depending on the developmental stage of their occurrence (Shi *et al*., 2015). While water-deficit stress is primarily responsible for decreasing spikelet fertility during the flowering stage (Rang *et al*., 2011). The impact of stress during the very early reproductive stage, which affects active panicle morphogenesis and growth, could also affect yield loss by changing the characteristics

of adult panicles (Wang *et al*., 2012). Studies in indica rice cultivars have shown that water-deficit stress during pre-flowering mainly affects the number of secondary branches, post-developmental branch and spikelet abortion (Kato and Kastura, 2010). Drought stress affects spikelet fertility and sustainable pollen growth, panicle exertion, pollen shedding and germination and the production of embryos involved in fertilization and initiation of grain filling. Grain yield loss occurs due to spikelet fertility reduction and the dry weight of fertile spikelets (Rang *et al*., 2011). Permanent loss of vegetative component is caused by drought, and during the developmental stage of flowering, flower anthesis and seed setting are severely affected, resulting in higher spikelet sterility, which reduces final yield (Shrestha, 2019). Grain yield showed strong associations at reproductive stage with spikelet fertility under drought stress. Upon reducing spikelet fertility, grain yield also decreases. The formation of pollen in rice plants is highly sensitive to drought stress. During meiotic stage, abiotic stress results in pollination failure, pollen sterility leading to zygotic abortion and eventually spikelet death, but only under extreme stress is female fertility affected (Barik *et al*., 2019). Serraj *et al.* (2009) reported the strong effects of drought on rice grain yield are in large part because of discount in spikelet fertility and panicle exertion. Yield loss of rice under the drought stress was associated with the reduction in spikelet fertility and grain weight (Kondhia *et al*., 2015). Kang *et al.* (2019) reported that drought stress had negative effects on spikelet fertility percentage (average, 9.8%), compared with control.

2.4.6 1000 grain weight

Ji *et al*. (2012) reported that under drought stress in Zhenshan97B and IRAT109, the rate of filled grain and 1000-grain weight decreased. The modification in the amount of stuffed grain in both cultivars is consistent with one thousand grain weight. Liu *et al.* (2006) reported that reduction in the 22% of number of spikelets per panicle and 15% for 1000-grain weight were observed when drought was applied at 7 days before heading and 10 days after heading. Kang *et al.* (2019) reported that yield components such as 1000 grain weight was decreased when exposed to water stress. Moonmon and Islam , 2017 observed that drought stress during grain filling stage was extremely destructive followed by panicle initiation stage regarding effective tillers/hill, total

spikelets panicle^{-1},1000 grain weight and grain yield hill^{-1}. This may be due to the significant decrease in photosynthetic rate resulting in reduced demand of assimilates for panicle growth and grain filling of rice. The most common parameters used to define tolerance of drought in rice breeding programs are yield parameters such as active tillers hill-1, maximum spikelets panicle^{-1}, filled grain panicle^{-1}, 1000-grain weight, percent sterility and grain yield hill^{-1} (Wang *et al*., 2012). Mehraban *et al.* (2018) observed significant reduction in thousand grain weight under stress compared to control. The predicted explanation for decreasing grain weight under drought stress may be due to drought affecting the emerging florets and decreasing the carpel weight at pollination.

2.4.7 Drought susceptibility index

Garg and Bhattacharya (2017) conducted a study on drought tolerance indices for screening some of rice genotypes in this they have used different indices to identify the intensity of drought on rice crop and they have reported that in drought suceptability index Ys and Yp are the mean yield of genotypes under stress and non-stress conditions and the genotypes with lowest value of DSI are more resistant to drought conditions. Result indicated that the genotype GAUTAM, RAU-1471- 10, RAU-1428-6-7-3-6, RAU-1415-3-5-76-9-5-3, RAU- 1397-25-8-1-2-5-4 had the lowest DSI followed by IR-36, RAU-1451-35-7-6-9-5-1. The drought-resistant type had good drought-tolerant performance, a small drought susceptibility index and a slight reduction in grain yields due to moisture stress (Vasant, 2012). Khan and Dhurve (2016) reported that Ys and Yp are the mean yield of genotypes under stress and non-stress conditions and the genotypes with drought susceptibility index < 1 are more resistant to drought conditions. Result indicated that the genotype IET-22743 had the lowest drought susceptibility index followed by IET-24061, IET-24062 and IET-23383 exhibited tolerance to drought while, genotypes IET-24061 followed by IET-24064, IET-24063 and IET-24069 exhibited susceptibility and all other genotypes were intermediate in nature.

2.5 QTL MAPPING FOR DROUGHT TOLERANCE

The method of building linkage maps and performing a quantitative trait loci (QTL) to classify trait-related genomic regions is known as QTL mapping **(**Collard *et al*., 2005). The discovery of genomic regions associated with quantitative traits such as

plant yield and stress on plants was largely obtained by QTL mapping (Borba *et al*., 2010). DNA markers and genome mapping techniques have become a powerful tool for genetic analysis of QTLs that control complex factors. QTL map enables the evaluation of positions, numbers, and magnitude of phenotypic effects and genetic pattern (Ashraf, 2010). Drought requires an analytical method for filtering and analysis of the impact of genetic factors using the QTL model. QTL mapping was developed in an effort to determine the genetic basis for a number of factors associated with drought tolerance, including osmotic adaptation, cell membrane stability (Tripathy *et al*., 2000) and leaf water status (Yue *et al*., 2006).

Hemamalini *et al.* (2000) detected 15 QTLs for morpho-physiological traits related to drought tolerance from DHL population of IR64 X Azucena. Venuprasad *et al.* (2002) identified QTLs for ten traits and these QTLs are distributed over chromosomes 1, 3,4,5,6 and 7 from a DH mapping population of IR 64 X Azucena. Hittalmani *et al.* (2002) reported 34 QTLs for various traits and among this one QTL is identified for grain yield under drought stress. From DH mapping population of CT9993 and IR 62266 Babu *et al.* (2003) reported a total of 47 QTLs for leaf rolling, drying score, days to 50% flowering, plant height, grain yield and spikelet fertility percentage respectively. Hittalmani *et al.* (2003) reported a QTL on chromosome 1 related to drought resistant traits such as leaf rolling, drying score, spikelet fertility percentage and grain yield from DH mapping population of IR 64 X Azucena. Lanceras *et al.* (2004) identified 77 QTLs for yield, yield components, panicle steritlity from RIL population of a cross between CT9993 and IR62266. From an introgression indica lines of rice Xu *et al.* (2005) reported 36 QTLs for yield related components under drought stress. Yue *et al.* (2006) identified QTLs *nlr2, nlr3 and nlr8* for leaf rolling on chromosome 2, 3 and 8 from a population of cross between Zhenshan 97 and IRAT109. Bernier *et al*. (2007) worked on a majority of 436 F3 individuals from the line between Vandana and Way Rarem and identified QTL on chromosome 12 which has a significant impact on yield under drought stress. Srinivasan *et al*. (2008) reported 19 Quantitative Trait Locis for grain yield, 50% flowering, spikelet fertility percentage and plant height. Kamoshitha *et al*. (2008) applied to the CT9993 / IR62266 population and identified four genomic regions on chromosomes 1, 4, 8 and 9 corresponding to QTLs

for yield traits under water stress. In cross-reference from Apo / IR64 Venuprasad *et al*. (2009a) reported QTL DTY3.1 with a strong effect on grain production under water stress. Xing *et al.* (2010) reported Quantitative Trait Locis for grain production on chromosomes 1, 5, 6 and 7 and grain weight 1, 3, 5 and 7 from Zhenshan97 / Minghui 63. Henry *et al*. (2014) reported on yield related QTL QDTY12.1 associated with lateral root growth under drought conditions.

2.6 MARKER ASSISTED SELECTION FOR DROUGHT RESISTANCE

Marker Assisted Selection (MAS) is a method by which DNA markers are used to help choose plant breeding materials (Collard *et al*., 2008). The Marker Assisted Selection is a DNA-based marker used by breeders for three main purposes: (a) to accumulate fine alleles according to beneficial alleles which is having power to replicating from generation to generation; (b) classifying desirable individuals into segregated breeding lines based on the entire genome or part of the genetic make-up; and (c) investigate reasonable alleles by distinguishing undesirable linkage loci. In modern breeding methods, common terms used include marker based selection, marker assisted pedigree selection, genomic selection or genome-wide selection, marker assisted recurrent selection and marker assisted backcrossing. Marker assisted backcrossing is the most effective and widely used method of the above-mentioned methods. Selective selection made by marker is a technology that helps to make conventional breeding more effective but not instead of conventional breeding (Chukwu *et al*., 2019). This includes genetic resources for the collection of target genes from the current germplasm used in breeding activities but does not include genetic engineering involving the transfer of isolated gene sequences (Oladosu *et al.*, 2018). Serraj *et al.* (2011) reported the discovery of major grain yield QTLs under drought has made it possible to use MAS to boost resistance to drought. MAS could make the development of drought-resistant varieties more efficient in introgressing QTL alleles, giving increased drought resistance through backcrossing to the genome of widely used cultivars (Bernier *et al.*, 2007). Serraj *et al*. (2011) reported drought have been difficult to manage with general phenotypic selection and is one of the most effective improvement with Marker Assisted Selection. Steele *et al*. (2005) observed QTLs that control root features from the Indian upland genus Kalinga III using selective markers.

Kumar *et al.* (2018) identified QTLs for yield related parameters under drought stress condition through MAS. In marker assisted back cross breeding markers can be applied in foreground, recombinant and background selections. Markers are used for selecting the target trait at the first stage of foreground selection. The second stage of recombinant selection includes the selection of a backcross progeny with a specific gene and tightly linked flanking markers so that the linkage drag can be minimized. The third stage is referred to as background selection and includes using background markers to identify backcross progeny (Kumar *et al*., 2014). The effectiveness of a marker assisted backcrossing program depends on a number of factors, including the size and reliability of the target QTL effect, the accuracy of the target gene , the rate of polymorphism in the background markers and the cost. Thomson *et al.* (2010) reported success of MABC in improving biotic and abiotic stress tolerance has been achieved with QTLs that have demonstrated high tolerance levels in many different genetic backgrounds and environments.

2.7 ASSOCIATION MAPPING:-

Association mapping (AM) is a relatively new and effective genetic tool for the dissection of complex traits and is a popular technique used to classify genes that control important traits (Borba *et al*., 2010). Association mapping has the promise of higher mapping resolution by exploitation of historical population-level recombination events that may enable gene level mapping of non-model organisms where linkage-based approaches are not feasible (Nordborg and Tavare, 2002). Natural diversity is used for the identification and utilization of useful allelic variants for crop improvement (Zhu *et al*., 2008). Association mapping includes the following steps (Ersoz *et al.,* 2009)

1. Germplasm set with a wide range of genetic diversity
2. Determination of population structure and effect on the sample
3. Population samples are phenotyped for trait of interest in different environments
4. Genotyping of mapping population with molecular markers
5. LD quantification using molecular marker data for the selected population genome
6. Assessment of the population structure and kinship
7. Phenotypical and genotypical data are associated with statistical approach

2.7.1 Concepts in AM

The main purpose of association mapping is to disseminate complex features and to identify QTLs (Zhu *et al*., 2008). QTLs obtained using association mapping are usually represented using 'Manhattan plots' indicating the interaction of markers on the chromosome. Y-axis displays - log10 (P value) of the organization for SNPs next to each chromosome on the x-axis, so all markers map position must be known.

2.7.2 Linkage disequilibrium and Population structure

The basic premise by which QTLs can be defined using association maping is due to linkage disequilibrium. LD is a cosegregation of alleles with two or more different loci (Slatkin, 2008). It checks the degree of marker association caused by their shared history. Often the LD and AM map names are used interchangeably. Association mapinpg depends on the extend of the LD in the genome. The severity of LD is therefore to be understood before AM is executed. When LD decreases within a short distance, high-resolution mapping is predicted, but a large number of markers are required (Rafalski, 2002). When LD extended long distance, mapping resolution is low, but a small amount of markers are required. Association analysis has two major advantages over the linkage map: (1) broad genetic variability and (2) higher mapping resolution (Remington *et al.,* 2001). Association genetic analysis has been widely used as genome wide association studies (GWAS) and candidate gene association studies (CGAS). GWAS tends to focus on the link between single nucleotide polymorphisms and major factors, while candidate gene association mapping analyzes the variability of a particular type gene often chosen on the basis of a biological hypothesis (Zhang *et al*., 2016).

The concept of LD was first described by the Jennings in 1917, and its quantification was invented by Lewtonin in 1964. Explanation of the most commonly used LD, D or D_ (standard D type), the difference between the observed gamet frequency of haplotypes and the expected gametic haplotype frequency under the equilibrium (D = PAB - PA PB = PAB Pab-PabPaB). In addition to D, various LD pathways (D ', r^2, D^2, D ∗, F, X (2), and δ) were designed to measure LD. Choosing the right LD methods actually depends on the purpose of the research, and one performs better than the other in certain cases and conditions; however, D 'and r^2 are the most commonly used LD levels. D 'teaches the comparison of different allele frequencies

across the loci and rises sharply with a small sample size and low allele frequencies; considering the objective, the appropriate LD correction rate required for map acquisition direction is r2 and that is an indication of marking compatibility. The r^2 value varies from 0 to 1, and is equal to 1 when there are only two haplotypes. A value of r^2 equal to 0.1 (10%) or higher considered a significant limitation of the LD's hard-to-express relationship to midnight loci.

Population structure is an important component of association mapping analysis since it can minimize both type I and type II errors between molecular markers and trait of interest in autogamous species such as rice (*Oryza sativa* L.) and barley (*Hordeum vulgare*) (Yu *et al.,* 2006). In kinship and population studies, SSR markers are primarily molecular markers because they are multiallic, reproducible, PCR-based and typically selectively neutral. Predominantly SSRs were primarily used to describe the rice population structure (Agrama *et al.,* 2007). Many statistical methods, such as structural association (SA), have been proposed to account for population structure and family relatedness, mixed model approach, principal component analysis (Zhu *et al.,* 2008).

2.7.3 Analysis methods for association mapping

Relatively simple statistical correlation tests (e.g. general linear models for normal distributed traits or non-parametric tests) were performed in early Association mapping (AM). Subsequently, more sophisticated methods were created for association mapping, which were routinely used in crops (Lipka *et al*., 2015). It is important to account for the impact of population structure (referred to as ' Q ') when conducting association mapping. The most common approach to population structure evaluation is to use marker information to identify subgroups within the experimental population. This is sometimes referred to as ' structured association ' and pieces of information on population structure are known as fixed effects and used in the study as cofactors. Using this approach, a collection of random markers is used to infer both the population structure and the panel's ancestry. Popular population structure estimation methods include: (1) using a computer program called STRUCTURE (Pritchard *et al*., 2000a) or (2) Principal component analysis (PCA) (Price *et al*., 2006). One of the main advantages of PCA is that the computational analysis is considerably simpler. It was subsequently determined that taking into account the level of genetic relatedness (called 'kinship';

referred to as 'K') improved the accuracy of AM (Yu *et al*., 2006). Mixed linear models (MLM) were used to include details on population structure and kinship (i.e. ' Q + K ' mixed model) which was superior in terms of reducing the false positive rate while retaining statistical power (Zhao *et al*., 2007). The most commonly used approaches in rice include efficient mixed model association (Kang *et al*., 2008), EMMA expediated, compressed MLM and population parameters previously determined (Zhang *et al.,* 2012). Recently, even more advanced methods have been developed.

2.7.4 Association genetic analysis studies

A range of AM studies have been reported for rice in the last decade. From 234 rice accessions Garris *et al*. (2005) identified five major group included *indica*, *aus*, tropical *japonica*, temperate *japonica* and aromatic. Borba *et al*. (2010) conducted a study on association mapping for yield and grain quality in rice and from this study they identified a total of 1,066 alleles with the set of 86 SSR markers on a panel of 242 accessions. Feng *et al.* (2016) conducted a study on Genome wide association mapping for grain shape traits in indica rice and in this study, association mapping based on 5291 single nucleotide polymorphisms (SNPs) was conducted to identify significant loci associated with grain shape traits in a global collection of 469 diverse rice accessions. A total of 47 SNPs were located in 27 significant loci for four grain traits, and explained 44.93 – 65.90 % of the phenotypic variation for each trait. Swamy *et al* .(2017) analysed association mapping of yield and yield related traits under reproductive stage drought stress in rice and they identified Linkage Disequilibrium (LD) analysis revealed that LD decreased with an increase in distance between marker pairs and the LD decay varied from 5– 20 cM. Fujino *et al.* (2015) conducted a study on genome wide association mapping focusing on a rice population derived from rice breeding programms in a region and from this study they have identified six QTLs identified for the heading date and Seventeen QTLs were identified for low temperature germinability. The largest allelic difference was detected at *qLTG12a*, 37.4 between 51.9 of allele A and 14.5 of allele B in an arc-sine transformation value of the germination rate. A significant association was detected at the marker S103 for *qLTG3-1, qLTG3a*.

Fei *et al*. (2014) conducted a study on genotype and environment interactions for agronomic traits after conducting association analysis and from this study they were identified ten traits out of 14 agronomic traits were tested to identify QTLs by genome-

wide association mapping. A total of 23 QTLs were identified for the 10 traits. After conducting experiment for association mapping of yield and yield related traits under reproductive stage drought stress Swamy *et al.* (2017) reported Out of the 125 SSR markers genotyped in the 75 Malaysian genotypes, 119 (95.2%) were found to be polymorphic. The number of SSR markers differed according to different chromosomes: with the highest number of 21 chromosome markers 2, followed by 17 chromosome markers 1. After performing the structural analysis the 75 genotypes were grouped into three subpopulation. POP1 consisted of 23 genotypes; POP2 consisted of 18 genotypes and POP3 consisted of 34 genotypes among the three subpopulations. In POP1, POP2 and POP3, the fixation index (Fst) was 0.277, 0.270 and 0.194 respectively. Study of LD in the entire population revealed 7072 LD pairs, 2264 (32%) of which were significant pairs (P>0.05). The result of the association analysis revealed that overall there were 198 significant marker trait associations.

From 100 accessions of wheat Mathew *et al*. (2019) identified total of 75 marker trait association with a linkage disequilibrium threshold of 0.38 at 5 cM. Thirty-seven of the MTAs were found in drought-stressed condition and 48% were the B genome, where the majority of the quantitative trait loci (QTLs) for Root Biomass, Shoot Biomass and GY were previously identified. Norton *et al.* (2018) analysed total of 298 rice cultivars for association analysis for grain and biomass traits in rice and observed that there were five distinct subpopulation after structural analysis and the average LD decayed across the entire rice genome for these cultivars at 243 kbp, but it's not uniform across the chromosome. Association analysis resulted a total of 2720 SNPs significantly associated with grain yield, 8399 SNPs associated with straw biomass and 1853 SNPs associated with harvest index. Fei *et al.* (2016) investigated 416 rice accessions with total of 143 SSR markers for association analysis. Among the seven subpopulations detected in the two years, a total of 27 QTLs were found to be strongly correlated with nine traits. Among them, 12 were identified in both years. Two QTL clusters influencing more than three traits were localized to chromosome 7 and 9. Deshmukh *et al.* (2019) conducted association analysis on 49 diverse rice accessions using 599 SSR primers. The 49 accessions were divided into three sub-populations by STRUCTURE analysis. The POP1 subpopulation consisted mainly of landraces, while the POP3 subpopulation consisted of advanced breeding lines and accessions of all classes to

POP2. Genome-wide association mapping found 61 markers consistently correlated with phenology, plant development and root characteristics in TPE (rainfed target population of environment) during drought in two or more trials.

3. MATERIALS AND METHODS

The study entitled "Identification of molecular markers and Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice (*Oryza sativa* L.*)* using association genetic analysis." was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani and RARS Pattambi during 2016-18 with the objective to identify molecular markers, Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice under drought condition. The details of the materials used and methods adopted for the field experiment as well as association analysis and procedures followed for laboratory analysis during the course of experimentation are described in this chapter.

3.1 EVALUATION OF SELECTED 81 RICE GENOTYPES FOR MORPHO-PHYSIOLOGICAL AND PLANT PRODUCTION TRAITS

3.1.1 Plant materials

A total of 81 germplasm rice accessions were utilized for marker trait association study (Table 1). These materials includes indigenous land races and improved varieties from R.A.R.S., Pattambi, Kerala Agricultural University and National Rice Research Institute, Cuttack (ICAR-NRRI), Odisha. A total of 81 rice genotypes composed of 36 improved varieties and 45 landraces were used for this experiment. These were short and medium duration genotypes having red or white bold type grains. Land races were reported for tolerance against various pests and water deficit. Land races having an average yield of 2-3t/ha, but improved varieties have 5-6t/ha. Improved varieties having moderate level tolerance or susceptible to various abiotic stresses.

3.1.2 Location

The study was conducted in open field condition at RARS, Pattambi (Plate 1)

3.1.3 Experimental details

The details of the field experiment are given in the table 2.

Table 1: Details of rice genotypes used in this study

Sl No	Varieties/Landraces	Origin	Ecotype	Group
35	Aswathy (Ptb 37)	India	*Indica*	Improved variety
36	Triveni (Ptb 38)	India	*Indica*	Improved variety
37	Jyoti (Ptb 39)	India	*Indica*	Improved variety
38	Sabari (Ptb 40)	India	*Indica*	Improved variety
39	Bharathi (Ptb 41)	India	*Indica*	Improved variety
40	SwarnaPrabha (Ptb 43)	India	*Indica*	Improved variety
41	MattaTriveni (Ptb 45)	India	*Indica*	Improved variety
42	Jayathi (Ptb 46)	India	*Indica*	Improved variety
43	Kairali (Ptb 490	India	*Indica*	Improved variety
44	Kanchana (Ptb 50)	India	*Indica*	Improved variety
45	Aathira (Ptb 51)	India	*Indica*	Improved variety
46	Aiswarya(Ptb 52)	India	*Indica*	Improved variety
47	Harsha (Ptb 55)	India	*Indica*	Improved variety
48	Varsha (Ptb 56)	India	*Indica*	Improved variety
49	Swetha (Ptb 57)	India	*Indica*	Improved variety
50	Anashwara(Ptb 58)	India	*Indica*	Improved variety
51	Samyuktha(Ptb 59)	India	*Indica*	Improved variety
52	Vaisakh (Ptb 60)	India	*Indica*	Improved variety
53	Sampada	India	*Indica*	Improved variety
54	Kunjukunjuvarna	India	*Indica*	Improved variety
55	N-22	India	*Aus*	Improved variety
56	ASD 16	India	*Indica*	Improved variety
57	ADT 37	India	*Indica*	Improved variety
58	Kazhiama	India	*Indica*	Traditional land race
59	Pandichempan	India	*Indica*	Traditional land race
60	Gandhakashala	India	*Indica*	Traditional land race
61	Kuttithekkan	India	*Indica*	Traditional land race
62	Jaya	India	*Indica*	Traditional land race
63	Jeerakashala	India	*Indica*	Traditional land race
64	Ponmani	India	*Indica*	Improved variety
65	Shreyas	India	*Indica*	Improved variety
66	Prathyasha	India	*Indica*	Improved variety
67	VelluthataryanSel	India	*Indica*	Traditional land race

Sl No	Varieties/Landraces	Origin	Ecotype	Group
1	Aryan (Ptb1)	India	*Indica*	Selection from Traditional land race
2	Ponnaryan (Ptb2)	India	*Indica*	Selection from Traditional land race
3	Eravapandy (Ptb3)	India	*Indica*	Selection from Traditional land race
4	Vellari (Ptb4)	India	*Indica*	Selection from Traditional land race
5	Velutharikayama (Ptb5)	India	*Indica*	Selection from Traditional land race
6	Athikkiraya (Ptb6)	India	*Indica*	Selection from Traditional land race
7	Parambuvattan (Ptb7)	India	*Indica*	Selection from Traditional land race
8	Thavalakkannan (Ptb8)	India	*Indica*	Selection from Traditional land race
9	Thavalakkannan (Ptb9)	India	*Indica*	Selection from Traditional land race
10	Thekkancheera (Ptb10)	India	*Indica*	Selection from Traditional land race
11	ThekkanChitteni (Ptb12)	India	*Indica*	Selection from Traditional land race
12	Kayama (Ptb13)	India	*Indica*	Selection from Traditional land race
13	Maskathi (Ptb14)	India	*Indica*	Selection from Traditional land race
14	Kavunginpoothala (Ptb15)	India	*Indica*	Selection from Traditional land race
15	Jeddu Halliga (Ptb17)	India	*Indica*	Selection from Traditional land race
16	Eravapandy (Ptb18)	India	*Indica*	Selection from Traditional land race
17	Athikkiraya (Ptb19)	India	*Indica*	Selection from Traditional land race
18	Vadakkan Chitteni (Ptb20)	India	*Indica*	Selection from Traditional land race
19	Thekkan (Ptb21)	India	*Indica*	Selection from Traditional land race
20	VeluthaVattan (Ptb22)	India	*Indica*	Selection from Traditional land race
21	Cheriya Aryan (Ptb23)	India	*Indica*	Selection from Traditional land race
22	ChuvannaVattan (Ptb24)	India	*Indica*	Selection from Traditional land race
23	Thonnooran (Ptb25)	India	*Indica*	Selection fromTraditional land race
24	Chenkayama (Ptb26)	India	*Indica*	Selection fromTraditional land race
25	Kodiyan (Ptb27)	India	*Indica*	Selection fromTraditional land race
26	Kattamodan (Ptb28)	India	*Indica*	Selection from Traditional land race
27	KaruthaModan (Ptb29)	India	*Indica*	Selection from Traditional land race
28	ChuvannaModan (Ptb30)	India	*Indica*	Selection from Traditional land race
29	Elappapoochampan (Ptb31)	India	*Indica*	Selection from Traditional land race
30	Aruvakkari (Ptb32)	India	*Indica*	Selection from Traditional land race
31	Arikkirai (Ptb33)	India	*Indica*	Selection from Traditional land race
32	ValiyaChampan (Ptb34)	India	*Indica*	Selection from Traditional land race
33	Annapoorna (Ptb 35)	India	*Indica*	Improved variety
34	Rohini (Ptb 36)	India	*Indica*	Improved variety

Sl No	Varieties/Landraces	Origin	Ecotype	Group
68	Gopika	India	*Indica*	Improved variety
69	Mahamaya	India	*Indica*	Improved variety
70	IGKVR-1	India	*Indica*	Improved variety
71	CUL-6	India	*Indica*	Improved variety
72	CUL-7	India	*Indica*	Improved variety
73	CUL14	India	*Indica*	Improved variety
74	CR DHAN 202	India	*Indica*	Aerobic
75	CR DHAN 305	India	*Indica*	Irrigated
76	CR DHAN 204	India	*Indica*	Aerobic
77	CR DHAN 205	India	*Indica*	Aerobic
78	Chomala	India	*Indica*	Traditional land race
79	CR DHAN 101	India	*Indica*	Upland
80	Uma	India	*Indica*	Improved variety
81	JS-4	India	*Indica*	Improved variety

Table 2. Particulars of field experiment

1. Crop	Rice : 81 genotypes
2. Design	9×9 lattice design
3. Number of treatments	Two 1. Water stress from panicle initiation to 25 consecutive days (by withholding irrigation) 2. Control
4. Replication	Two

3.1.4 Methodology

In this study, eighty one genotypes were evaluated under field condition. For the experiment, 10 grams of seeds of each genotype obtained from RARS, Pattambi, ICAR-NRRI, Cuttack were sown in pots filled with soil, sand and cowdung. Twenty one days old seedlings were transplanted to the open field at the rate of two seedlings per hill. Gap filling was done on 8^{th} day after transplanting and one healthy seedling was maintained in each hill. Each genotype was raised in four rows of 2m length. Spacing between two rows was 0.2m. Management practices were followed as per Package of practices recommendation. The cultural operations including weeding and plant protection measures were carried out as per package of practise of Kerala Agricultural University, Thrissur. General view of the experimental plot in experiment 1& 2 are given in plate 2 & 3. At the time of panicle initiation, irrigation was withdrawn for 25 consecutive days. Observations were taken on morphological and physiological parameters after induction of stress (Plate 4 & 5). After 25^{th} day, re-watering was done and plants were kept upto maturity. At the time of harvest, plant production traits were taken from each genotype under control and water stress condition.

3.1.5 Observations

3.1.5.1 Leaf rolling score

Leaf rolling was observed under field condition after imposing water stress at panicle initiation stage. The scoring of leaf rolling was done according to the Standard Evaluation System for Rice (SES) of IRRI (1996), Philippines. Leaf rolling was noted from the 10^{th} day of drought imposition during the time between 12pm and 1pm. Leaf rolling was scored on a scale from 1 to 9 as given below:

1 - Unrolled, turgid

3 - Leaf rim starts to roll

5 - Leaf folded into 'V' shape

7 - Rolled leaf covers part of leaf blade

9 - Leaf is rolled like an onion leaf

3.1.5.2 Leaf drying score

Leaf drying was observed under field condition after imposing water stress at panicle initiation stage. The scoring of leaf drying was done according to the Standard Evaluation System for Rice (SES) of IRRI (1996), Philippines. Leaf drying was scored on a scale from 0 to 9 as given below;

0 - Highly resistant: No symptoms
1- Resistant: Light tip drying
3- Moderately resistant: Tip drying to ¼ length in most leaves
5 - Moderately susceptible: ¼ to ½ of leaves fully dried
7 - Susceptible: More than 2 /3 of all leaves fully dried
9 - Highly susceptible: All plants apparently dead

3.1.5.3 Leaf temperature

Leaf temperature was measured at morning time between 9 am and 11 am using infrared thermometer.

3.1.5.4 Cell membrane stability index

Cell membrane stability index was estimated as per the procedure described by Blum and Ebercon (1981). Samples collected from both control and stress imposed plants were washed three times in deionised water to remove electrolytes adhered on the surface. Samples were kept in a capped vial (20ml) containing 10ml of deionised water and incubated in the dark for 24 hours at room temperature. The conductance was measured with a conductivity meter. After the first measurement, the vials were autoclaved for 15 minutes to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These two measurements were carried out individually for both control and stress treated plants. Cell membrane stability index was calculated by using following formula and expressed as per cent.

$$CMS\ (\%) = [1-(T_1/T_2)/1-(C_1/C_2)] \times 100$$

Where, T and C refer to the stress and control samples respectively. The subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

3.1.5.5 Chlorophyll stability index

Total chlorophyll content was estimated by the method of Hiscox and Israelstam (1979) and from that chlorophyll stability index was calculated using formula

Chlorophyll stability index (%) = (Total chlorophyll content at stress condition / Total chlorophyll at controlled condition)*100

3.1.5.6 Relative Water Content (RWC)

The relative leaf water content was measured based on the method described by Turner (1981). The relative leaf water content was determined in the fully expanded leaf. The fresh weights of the samples were recorded, and the leaves were immersed in distilled water in a Petri dish. After 2 hours, the leaves were removed, the surface water was blotted off and the turgid weight was recorded. The samples were then dried in an oven at 70°C for 48 hours. Then the dry weight was recorded. The relative leaf water content was calculated using the following formula and expressed as per cent.

$$RWC\ (\%) = [(FW - DW) / (TW - DW)) \times 100$$

Where, FW is the fresh weight; DW is the dry weight; and TW is the turgid weight.

3.1.5.7 Drought Susceptibility Index

This index is an improvement over the simple expression of yield under stress as percent of yield under non-stress conditions. Here, DSI is ratio of yield reduction due to stress in a given genotype as compared to the mean reduction over all genotypes in a given test. Originally they proposed a drought susceptibility index. Breeders prefer to use the calculation as a Drought Resistance Index (Fisher and Maurer, 1978).

$$DSI = (Ys/Yn)/ (Ms/Mn),$$

Where, Ys and Yn are the genotype yields (or biomass) under stress and non-stress respectively and Ms and Mn are the mean yields (or biomass) over all genotypes in the given test under stress and non-stress respectively.

3.1.5.8 Percentage Relative yield reduction

Percentage relative yield reduction (RYR) under stress was computed as (Saikumar *et al*., 2016):

$$RYR = 1 - (\text{grainyield stress} / \text{grainyield control}) \times 100$$

3.1.5.9 Plant height

Plant height was measured from the base of the plant to the tip of the plant and expressed in centimeters.

3.1.5.10 Tiller number

In each replication, total number of tillers at the time of flowering was counted and recorded.

3.1.5.11 Days to 50% flowering

Total number of days taken from sowing to exertion of 50% of panicles in each replication was taken.

3.1.5.12 Soil moisture content

The soil moisture content was estimated according to Black (1965) and may be expressed by weight as the ratio of the mass of water present to the dry to the dry weight of the soil sample, or by volume as ratio of volume of water to the total volume of the soil sample.

Procedure

1. Weigh aluminum tin, and record this weight (tare").

2. Place a soil sample of about 10g in the tin and record this weight as (wet soil).

3. Place the sample in the oven 105^{o} C, and dry for 24 hours or overnight.

4. Weigh the sample, and record this weight as weight of (dry soil + tare).

5. Return the sample to the oven and dry for several hours, and determine the weight of (dry soil +tare).

6. Repeat step 5 until there is no difference between any two consecutive measurements of the weight of (dry soil + tare).

$$\text{Soil moisture (\%)} = \frac{\textit{(Wt of wet soil)} - \textit{(Wt of dry soil)}}{\textit{(Wt of dry soil)}}$$

3.1.5.13 Yield per plant

The grain yield per plant was derived by taking the weight of filled grains in each panicle and expressed in grams.

3.1.5.14 Spikelet fertility percentage

The total numbers of filled and unfilled spikelets of three randomily selected primary tillers of the target plants in each treatment were counted. Then,

Spikelet fertility (%) was calculated by using the formula

$$\text{Spikelet fertility (\%)} = \frac{\text{No. of fertile spikelets}}{\text{Total number of spikelets}} \times 100$$

3.1.5.15 1000 grain weight

One thousand seeds were taken randomly from each replication, weighed and expressed in grams

3.1.6 **Statistical analysis**

Statistical analysis was carried out using the SAS program (SAS institute Inc., 1990). Principal component analysis and cluster analysis were done using R environment of statistical computing (R core Team, 2013).

3.2 IDENTIFICATION OF MICROSATELLITE MARKERS ASSOCIATED WITH DROUGHT TOLERANT AND PLANT PRODUCTION TRAITS IN RICE

3.2.1 Genomic DNA isolation

Genomic DNA from the selected 81 rice accessions was extracted using the method described by Dellaporta *et al*. (1983). Leaf samples were collected from 25 days old seedlings separately in labeled cover. 0.5 – 1 gram of leaf bits were transferred into prechilled mortar, frozen using liquid nitrogen and ground to a fine powder. The powdered samples were transferred to 20ml centrifuge tubes and mixed with 15ml of extraction buffer containing 20µl of ß-mercaptoethanol and 50mg of PVP (Polyvinyl pyrollidine) and kept at 4^0C. To the mixture 1ml of 20% SDS was added, mixed thoroughly and incubated at 65^0C for 1 hour in a water bath (Beston). 5ml of 5M potassium acetate was then added to it and kept on ice for 20 minutes. Centrifugation (Centrifuge 5430R Eppendorf) was performed at 12,000 rpm for 20 minutes and the clear aqueous phase was transferred to a fresh sterile tube. Equal volumes of ice cold isopropanol was added and mixed gently by inversion and kept in a -20^0C freezer until DNA was precipitated out. Centrifugation was performed at 12,000 rpm for 10 minutes and then the pellet obtained was dissolved in 500µl sterile double distilled water. To this, 3µl of RNase was added and incubated at 37^0C for 1 hour. To the mixture 500µl of chloroform: isoamyl alcohol mixture was added and mixed well for 15 minutes. Centrifuged at 12,000 rpm for 15 minutes and aqueous phase was transferred to another micro centrifuge tube without disturbing the inter phase. Two ml of ice cold absolute alcohol and 1/10 volume of sodium acetate were added and kept overnight incubation in -20^0C. Then it was centrifuged at 12,000rpm for 5 minutes and the supernatant was discarded. DNA pellet was washed with 500µl of 70% ethanol and air-dried completely. Then the DNA pellet was dissolved at 100µl of TE buffer and stored at -20^0C for further use.

3.2.2 Quantification and quality assessment of DNA samples

The quantity of DNA present in each sample was determined by reading the absorbance at 260nm and 280nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer). Purity was measured the ratio between the readings at 260nm and

280nm (OD 260/OD 280). Pure DNA samples having 260 nm/ 280 nm OD ratio between 1.7 and 1.8 (Sambrook and Russell, 2000). Quality was assessed by using gel electrophoresis with 5µl of crude DNA sample on agarose gel (0.8%) and stained with ethidium bromide.

3.2.3 Dilution of DNA samples

The stock DNA samples after quantification were diluted to 50ng/µl of working solutions for bulking and PCR. DNA dilutions were prepared by using the formula as follows:

$$M_1V_1 = M_2V_2$$

Where M_1 is the stock DNA concentration, V_1 is the volume of stock, M_2 is the working solution concentration and V_2 is the working solution volume to be prepared. Then the appropriate volume from the stock was transferred to 0.5 ml micro-centrifuge tube, and the volume was made to 100µl using TE buffer. The DNA working solutions were stored at -20^{o}C till further use.

3.2.4 PCR amplification using SSR primers

3.2.4.1 PCR reaction

PCR reaction was performed in a 20µl reaction mixture which consisted of,

a) Genomic DNA (25ng/µl) - 2.0µl
b) 10X Taq assay buffer A - 2.0µl
c) dNTPs mix (10mm each) - 1.5µl
d) Taq DNA polymerase (1µ) - 0.3µl
e) Forward primer (10µM) - 0.75µl
f) Reverse primer (10µM) - 0.75µl
g) Autoclaved distilled water - 12.7µl

Total volume 20µl

PCR reaction was carried out using Master Cycler gradient 5331-Eppendorf version 2.30.31-09, Germany. The thermal cycling was carried out with the following programme

Initial denaturation - 94^0C for 3 minutes

Denaturation - 94^0C for 1 minute

Primer annealing - 53^0C to 55^0C for 1 minute } 35 cycles

Primer extension - 72^0C for 1 minute

Final extention - 72^0C for 5 minutes

Incubation - 4^0C for infinity to hold the sample

3.3.5 Detection of polymorphism between the genotypes using SSR primers

One hundred primers were screened by PCR and their sequences are listed in Table 3. The amplified products were run along with marker (100bp ladder) on 2% agarose gel using 1X TBE buffer and stained with ethidium bromide. The profile was visualized under UV (312nm) transilluminator and documented in gel documentation system (Syngene G box documentation system). The documented SSR profiles were carefully examined for the polymorphism in banding pattern between the genotypes.

Table 3. List of SSR primers used for Association genetic analysis

S. No.	Marker	Primer F	Primer R
1.	RM1067	CGATGGAGAGAGAATGTCTAGC	TAATACGCAAGGCAGAAGGG
2.	RM246	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT
3.	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
4.	RM1032	TGGCACTTCACGTAGCAAAC	TGGTTCTGTTCTTGTGGCTG
5.	RM3825	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
6.	RM283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
7.	RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
8.	RM462	ACGGCCCATATAAAAGCCTC	AAGATGGCGGAGTAGCTCAG
9.	RM513	TCTAGTGGCCTCAAAAAGGG	GCAACGAAATCATCCCTAGC
10.	RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
11.	RM5735	AGGCTTGTCCAATACGATCG	TTCTGTTGCTGTAGTTGCCG
12.	RM222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG
13.	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
14.	RM86	TACACCTCATCGATCAATCG	CTTTCGAATCTGAAGATC
15.	RM104	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC
16.	RM151	GGCTGCTCATCAGCTGCATGCG	TCGGCAGTGGTAGAGTTTGATCTGC
17.	RM521	TTCCCTTATTCCTGCTCTCC	GGGATTTGCAGTGAGCTAGC
18.	RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
19.	RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC
20.	RM236	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC
21.	RM48	TGTCCCACTGCTTTCAAGC	CGAGAATGAGGGACAAATAACC
22.	RM1178	CAGTGGGCGAGCATAGGAG	ATCCTTTTCTCCCTCTCTCG
23.	RM520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG
24.	RM1022	CATGGGATGAGGGAGTAATG	CTTTGATAGCGGCTTTGTCC

25.	RM5628	CCGGATAAAGAGGGAGGAAG	TGTCGACCTCCAATATGCAG
26.	RM6484	GGGTTTCTTCGATCCACTTG	CTTTGGGGGAGAAAGGTAGC
27.	RM81	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC
28.	RM85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
29.	RM514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
30.	RM5633	GTGTAGCTGCTAGGCCGAAC	TTCCTTTCGCTACGTTGGAC
31.	RM470	TCCTCATCGGCTTCTTCTTC	AGAACCCGTTCTACGTCACG
32.	RM518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
33.	RM349	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG
34.	RM1113	GGGCGCATGTGTATTTCTTC	TGGGGAAAAACCACAAGCC
35.	RM5688	GCAGTGTCCAACCATCTGTG	ATCTGGTCACCCTTTGCTTG
36.	RM537	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC
37.	RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG
38.	RM3042	CAAAAAGGAATCAATGTGAA	GGCTGTTGAGAGGTAGAGAA
39.	RM1018	ATCTTGTCCCACTGCACCAC	TGTGACTGCTTTTCTGTCGC
40.	RM3351	ATGGAAGGAATGGAGGTGAG	TACCCCTACGTCGATCGATC
41.	RM1090	GTTATAGCGCACCCTGGATG	GAACCGAAGGGACATGTGTG
42.	RM507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
43.	RM538	GGTCGTTGAAGCTTACCAGC	ACAAGCTCTCAAAACTCGCC
44.	RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
45.	RM1054	TGCATATGTACCGCAACCTC	TTTCTGCATGATCCCCTCTG
46.	RM31	GATCACGATCCACTGGAGCT	AAGTCCATTACTCTCCTCCC
47.	RM178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC
48.	RM249	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC
49.	RM5642	AAAAACCGGCTAATCCCTCC	TTCGATGGGATTGATCGC
50.	RM1130	AGATCGGATTGGGATGGC	ACCCAACCAATTAGTGCCAC
51.	RM1031	GTGAAGGCACACCAACCG	GACGAGGATCGAATTCGAAG

52.	RM136	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC
53.	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
54.	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
55.	RM540	GCCTTCTGGCTCATTTATGC	CTAGGCCTGCCAGATTGAAC
56.	RM5745	ATGCCAAGTGGACGATGTAC	ACATGTGGGTAGTGGGATGG
57.	RM5753	AACATGCTCAACTTCTGGGC	GCTAGGTACGATCCAGCTGC
58.	RM314	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC
59.	RM225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
60.	RM461	GAGACCGGAGAGACAACTGC	TGATGCGGTTTGACTGCTAC
61.	RM5720	CCTGATAAATTGACAGTTAC	GAGAGTAGGAGTTGATAACA
62.	RM1132	ATCACCTGAGAAACATCCGG	CTCCTCCCACGTCAAGGTC
63.	RM1048	CAAGCCTATAATGTGAATTG	AATTTTTAGTTTGGGGTAGA
64.	RM455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
65.	RM474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
66.	RM1085	GGGGAAAAAGGAACACCTTC	ACAGGACAGACGACAATTGG
67.	RM47	ACTCCACTCCACTCCCCAC	GTCAGCAGGTCGGACGTC
68.	RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
69.	RM298	CTGATCACTGGATCGATCATG	CATGCCAAGATGCAACAG
70.	RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
71.	RM6925	TGAGAGGACGCTTGAAGAGG	GCACCTAGTGACTGAAGGTTG
72.	RM433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
73.	RM1109	TCAAAATCACGTGTATGTAAGC	TTTACAAAGGACAGAGGGC
74.	RM5637	CAACTCCAACGACGATGAAC	TGGTGAAGTGGAGTGGAGTG
75.	RM149	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG
76.	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
77.	RM32	AGTCTACGTGGTGTACACGTGG	TGCGGCCTGCCGTTTGTGAG
78.	RM1019	GTTTGAACAGTAGGACTTGT	AGAACATCTCACACTTCTCT

79.	RM1026	GCCTCTGGCAGAATAGCATC	TATCACTTTGCTGCCTAGGC
80.	RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
81.	RM5654	TGCAACTCGCGTATACAATA	CCAAGTTCGTTACAGCAGAG
82.	RM278	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
83.	RM328	CATAGTGGAGTATGCAGCTGC	CCTTCTCCCAGTCGTATCTG
84.	RM6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
85.	RM5629	AGCTCAACTCGACAACTCCC	CCATCTCCTCTTTCACCTCG
86.	RM5666	ACTTTCTCTCCATCGTTGCC	AACAGAGTTGTTTCGCTGCC
87.	RM5707	GACGTGGCACCCTAGTAAGC	GAAAGAGGAGATATGGGGCC
88.	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
89.	RM1083	CCTTGATTGCAGCATCCG	TTGAGCCTTTTACGAGACGG
90.	RM5926	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC
91.	RM5961	GTATGCTCCTCCTCACCTGC	ACATGCGACGTGATGTGAAC
92.	RM536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
93.	RM1124	AAGCTATCCCCCTTTTTGGC	AGGGATCGGTAGACCCAATC
94.	RM5923	ATAGTTCGGGGGGTAATTCG	GTCGATCGAGATAGTTGGGG
95.	RM5715	CTAGAGCCACCTCAAGCTCC	TGACCGTGGTCTTGTCTACG
96.	RM6615	GTCGACATGCGGATGCTG	ACCTCCATCTTGGCCTTCTC
97	RM17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
98.	RM1036	CTCATTTGTCGATTGCCGTC	ATGGGAGGAGTGATCAAACG
99.	RM1081	CACCTGCACATCATCATCAC	CGTTGGATTTGAATCTGGGG
100.	RM313	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC

3. 3.6 Association mapping

A total of 100 primers were used for genotyping the 81 rice accessions as described earlier. The basis of selection of 100 primers in this study was, the primers which distributed over 12 chromosomes also some of the primers which were reported in previous drought related study and also some new markers. The phenotypic data from the field trials and

genome wide polymorphic SSR marker data were used to study the marker-trait association. Analysis of variance (ANOVA) was performed using SAS. The genetic structure (Q) prediction and clustering of the population was done with the program STRUCTURE version 2.2 (Pritchard *et al*., 2000). The association analysis was performed using TASSEL v2.0.1 using 50000 time permutations for the correction of multiple testing (Pritchard *et al*., 2000). Markers with the adjusted *P* value <0.05 and r^2 value>0.1 were regarded as significant.

4. RESULT

The experiment was conducted to identify the molecular markers and Quanitative Trait Loci (QTLs) for drought tolerance and plant production traits in selected 81 rice genotypes using association genetic analysis. Phenotyping of genotypes was done at Regional Agriculture Research Station, Pattambi during Mundakan 2017 and Puncha 2018 and genotypic analysis was done at the Department of Plant Physiology, College of Agriculture, Vellayani. The rice plants were exposed to water stress condition at panicle initiation stage for a period of 25 consecutive days by withdrawing water along with an irrigated control and replicated twice. The physio-morphological and plant production traits were recorded after stress imposition from both treatments. Association analysis was carried out using 81 genotypes and 100 SSR primers to identify the microsatellite markers/QTLs linked to drought tolerant and plant production traits in rice. The data were statistically analysed and the results are presented in this chapter with suitable tables.

4.1 VARIATION IN PHYSIO-MORPHOLOGICAL AND PLANT PRODUCTION TRAITS UNDER WATER STRESS AND IRRIGATED CONTROL CONDITIONS IN MUNDAKAN 2017.

Trait mean values of all morpho-physiological and plant production traits of 81 rice genotypes under water stress and irrigated control conditions in experiment I are presented in Table 4 & 5. Over all mean values, range values and standard deviations of 81 rice genotypes under water stress and irrigated control conditions in experiment I are presented in Table 6.

4.1.1 Leaf rolling score

There was a significant variation in leaf rolling score among the genotypes under water stress condition. Average leaf rolling score recorded across the genotypes was 4.80, and it ranged from 1.78 to 7.78. Highest leaf rolling (7.78) was observed in Prathyasha and PTB55 (1.78) showed least leaf rolling symptoms among 81 genotypes.

4.1.2 Leaf drying score

Average leaf drying score recorded across the genotypes was 4.66 under water stress condition and it ranged from 1.56 to 9.00. Highest drying score (9.00) was observed in Prathyasha and Chomala (1.56) showed least leaf drying symptoms among 81 genotypes

4.1.3 Leaf temperature

Average leaf temperature recorded under water stress condition was 28.26°C and 27.50°C under irrigated condition. It ranged from 26.95°C to 30.30°C under water stress condition and 26.00°C to 29.35°C under irrigated control condition. Under water stress condition, maximum leaf temperature (30.30°C) was observed in PTB 1 and Prathyasha (26.95°C) recorded minimum leaf temperature. Under irrigated control condition maximum leaf temperature was observed in PTB1 (29.35°C) and Gandhakashala (26°C) recorded minimum leaf temperature.

4.1.4 Cell membrane stability index

Average cell membrane stability index across the 81 genotypes was 84.95% and it ranged from 80.28 to 94.36% in experiment I. Highest membrane stability index (94.36%) was observed in PTB 29 and Pandichempan (80.28%) showed lowest membrane stability index.

4.1.5 Chlorophyll stability index

Average chlorophyll stability index across the 81 genotypes was 86.14% and it ranged from 80.36 to 95.59% in experiment I. Highest chlorophyll stability index (95.59%) was observed in PTB 27 and Pandichempan (80.36%) showed lowest chlorophyll stability index.

4.1.6 Relative water content

Average relative water content across the 81 genotypes was 65.19% under water stress and 84.13% under irrigated conditions. It ranged from 57.34 -78.72% under water stress and 75.70 to 89.29% under irrigated conditions. Under water stress highest relative water content (78.72%) was observed in PTB15 and Prathyasha (57.34%) recorded lowest relative water content. Under irrigated control condition highest relative water content

(89.29%) was observed in PTB 28 and PTB13 (78.74%) recorded lowest relative water content.

4.1.7 Drought susceptibility index

Drought susceptibility index across the rice genotypes ranged from 0.86 to 1.14 in experiment I. Highest drought susceptibility index (1.14) was observed in Gopika and PTB 25 (0.86) showed lowest drought susceptibility index.

4.1.8 Percentage relative yield reduction

Percentage relative yield reduction across the rice genotypes ranged from 14.84% to 43.91% in experiment I. Highest Percentage relative yield reduction (43.91%) was observed in PTB25 and Gopika (14.84 %) showed lowest relative yield reduction.

4.1.9 Plant height

There was a significant variation in plant height among the genotypes under water stress and irrigated control conditions. Average plant height across the genotypes was 110.74 cm under water stress and 116.05 cm under irrigated conditions. It ranged from 82.50 cm to 135.00 cm under water stress and 87.50 cm to 144.0 cm under irrigated conditions respectively. Under water stress maximum plant height (135.00 cm) was observed in PTB 1 and ASD-16 (82.50) recorded minimum plant height. Under irrigated control condition maximum plant height (144.00 cm) was observed in PTB1 and Jaya recorded (87.50 cm) minimum plant height.

4.1.10 Tiller number

Average number of tiller per plant was 7.53 under water stress and 10.25 under irrigated conditions across the genotypes. It ranged from 5 to 11 under water stress and 7 to 14 under irrigated conditions respectively. Under water stress maximum number of tillers (10.5 $plant^{-1}$) was observed in PTB7 and Kuttithekkan (6.00 $plant^{-1}$) showed minimum number of tillers. Under irrigated control condition maximum (14 $plant^{-1}$) number of tillers was observed in PTB35 and Kuttithekkan showed (7.5 $plant^{-1}$) minimum number of tillers.

4.1.11 Days to 50% flowering

Average number of days to 50 % flowering was 95 under water stress and 89 under irrigated condition across the genotypes. It ranged from 68 days to 119 days under water

stress condition and 62 days to 114 days under irrigated condition. Delayed flowering was observed for genotypes under water stress condition.

4.1.12 Soil moisture percentage

The soil moisture percentage in field condition was estimated by gravimetric method. Under water stress condition the moisture percentage was 12.4% while under irrigated control condition it was 30.96%.

4.1.13 Grain yield/ plant

Average grain yield was 9.55 g in water stress condition and 12.94 g under irrigated control condition. Grain yield per plant ranged from 6.55- 14.47 g in water stress condition and 8.33 to 19.64 in irrigated control condition. Under water stress highest grain yield (14.47 g) was observed in PTB35 and Pandichempan (6.55 g) recorded lowest grain yield. Under irrigated control condition highest grain yield (19.64 g) was observed in PTB35 and Prathyasha (8.33 g) recorded lowest grain yield.

4.1.14 Spikelet fertility percentage

Spikelet fertility across the rice genotypes ranged from 54.19% to 78.22% under water stress and 64.96 to 86.91% under irrigated conditions with a mean of 65.26 and 75.43 %, respectively. Under water stress highest spikelet fertility percentage (78.22%) was observed in PTB35 and Ponmani (54.19%) recorded lowest spikelet fertility percentage. Under irrigated control condition highest spikelet fertility percentage (86.91%) was observed in PTB35 and Pandichempan (64.96%) recorded lowest spikelet fertility percentage.

4.1.15 Thousand grain weight

Thousand grain weight across the rice genotypes ranged from 17.55 g-25.35 g under water stress condition and 18.70 to 25.90 g under irrigated control condition with a mean of 22.71g and 23.55 g respectively. Under water stress highest thousand grain weight (25.35 g) was observed in PTB35 and Velluthataryan Selection (17.55 g) recorded lowest thousand grain weight. Under irrigated control condition highest thousand grain weight (25.95 g) was observed in PTB35 and Velluthataryan Selection (18.70 g) recorded lowest grain weight.

Table 4: Effect of water stress on morpho-physiological and plant production traits in experiment I (Mundakan 2017)

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
1	PTB 1	135.00	7.00	108.00	30.30	4.13	4.59	74.80	90.68	92.95	12.50	24.20	67.78	24.92	1.01
2	PTB 2	131.50	6.50	101.00	28.70	5.22	5.06	63.81	82.66	87.64	9.13	23.55	61.80	28.17	0.96
3	PTB 3	109.50	7.50	98.50	28.80	4.11	3.00	64.69	82.63	88.82	9.68	23.30	61.14	24.06	1.02
4	PTB 4	114.00	7.50	109.00	28.10	3.33	3.84	69.60	85.06	91.55	11.75	24.15	64.54	23.15	1.03
5	PTB5	124.00	7.50	99.50	28.25	5.22	5.06	63.87	82.78	87.88	9.30	21.30	61.58	25.30	1.00
6	PTB6	110.50	6.00	111.00	28.35	3.44	3.28	59.44	80.69	85.05	7.75	23.45	62.71	29.96	0.94
7	PTB7	110.50	10.50	89.00	29.30	2.56	1.73	71.16	91.49	95.61	11.38	24.85	74.43	40.10	0.81
8	PTB8	104.50	6.50	101.00	28.45	5.44	6.17	64.18	85.38	90.10	10.26	23.10	66.07	26.27	0.99
9	PTB9	120.50	6.50	104.00	28.80	4.44	4.06	68.24	84.70	87.18	10.21	23.30	61.50	24.77	1.01
10	PTB10	107.70	8.50	71.50	28.10	3.78	2.06	68.74	87.16	88.40	10.45	24.30	66.24	26.04	0.99
11	PTB12	111.00	6.50	103.50	28.30	4.11	4.11	67.15	86.70	90.22	11.75	23.10	68.30	19.72	1.08
12	PTB13	122.10	8.00	107.50	28.15	7.00	5.87	53.62	82.53	83.53	7.94	22.15	62.74	24.39	1.01

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
13	PTB14	122.80	7.50	100.50	28.10	6.22	6.22	53.35	80.53	82.25	7.44	22.15	54.74	29.21	0.95
14	PTB15	131.00	9.50	94.50	29.50	3.11	3.11	78.72	93.39	92.99	12.00	24.20	73.64	36.84	0.85
15	PTB 17	120.50	6.50	92.50	28.20	6.33	6.33	71.28	86.41	88.95	10.06	23.45	68.29	24.96	1.01
16	PTB18	132.70	6.00	101.00	28.60	6.44	6.78	64.23	82.23	87.83	7.30	23.40	64.54	28.29	0.96
17	PTB19	110.50	7.00	111.00	28.30	5.33	4.22	69.57	84.19	88.92	10.60	23.70	65.21	19.33	1.08
18	PTB20	112.65	9.50	93.00	27.65	7.33	7.11	67.49	84.18	86.64	9.21	22.95	67.44	29.64	0.94
19	PTB21	121.60	9.50	99.00	27.70	5.67	5.95	59.18	83.88	84.40	9.71	23.50	65.07	21.04	1.06
20	PTB22	119.45	7.50	83.50	28.20	7.00	5.76	63.71	82.86	84.51	7.91	22.25	62.07	35.27	0.87
21	PTB23	121.80	7.00	88.00	28.35	3.89	3.76	63.15	82.58	87.19	8.57	21.35	63.75	21.81	1.05
22	PTB24	117.45	6.50	85.50	28.50	7.11	9.11	63.78	85.59	89.00	8.58	23.35	63.09	31.77	0.92
23	PTB25	119.45	7.50	90.00	28.15	3.00	1.00	63.30	84.37	87.17	8.40	23.70	62.47	43.91	0.75

Sl No	Geno types	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
24	PTB26	124.80	8.50	93.00	29.60	4.22	4.22	74.24	92.95	82.63	11.47	22.45	71.51	39.28	0.82
25	PTB27	123.45	7.00	96.50	28.50	2.33	2.67	76.89	94.36	92.23	10.61	25.25	71.33	40.02	0.81
26	PTB28	121.65	8.50	85.50	29.00	2.22	1.11	71.69	91.52	94.38	11.49	24.65	72.54	38.13	0.83
27	PTB29	128.60	8.00	81.00	29.30	3.22	1.70	73.86	94.27	95.59	12.18	25.25	75.43	35.62	0.87
28	PTB30	117.95	8.50	82.00	28.65	3.56	2.17	73.19	92.70	88.78	12.10	25.35	73.97	20.87	0.89
29	PTB31	117.00	7.00	79.50	28.30	4.89	4.89	62.63	84.04	85.88	10.34	23.95	64.32	21.02	1.06
30	PTB32	110.00	6.00	91.00	28.15	5.78	7.65	62.61	82.79	84.25	9.32	23.15	63.20	24.94	1.01
31	PTB33	109.00	6.00	103.50	27.40	5.00	5.00	62.80	84.58	87.05	8.37	22.40	63.29	21.92	1.05
32	PTB34	105.00	11.00	76.50	28.30	4.89	3.89	68.86	93.07	87.64	14.47	24.70	78.22	17.95	1.10
33	PTB35	101.00	9.00	84.00	28.30	4.11	2.11	65.39	88.79	87.09	9.24	22.50	67.03	26.41	0.99
34	PTB36	101.00	8.00	77.50	28.40	4.22	6.56	63.95	84.48	85.22	10.24	23.30	65.44	22.17	1.04
35	PTB37	98.60	7.50	97.50	28.60	5.11	6.00	64.43	84.39	85.12	10.11	23.45	65.16	25.63	1.00

Sl No	Geno types	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
36	PTB38	95.85	7.50	76.00	28.20	4.11	5.89	66.08	84.99	84.17	9.45	22.65	63.06	27.84	0.97
37	PTB39	123.50	7.00	84.50	28.00	3.22	2.73	67.30	84.92	82.29	8.11	21.00	62.37	23.86	1.02
38	PTB40	121.70	9.50	109.00	28.40	4.56	3.78	69.37	89.19	88.05	12.47	24.40	69.04	23.85	1.02
39	PTB41	121.00	8.00	106.00	28.20	6.44	6.54	69.44	89.19	86.53	10.14	23.70	67.40	28.15	0.96
40	PTB43	104.35	7.00	79.00	27.30	4.67	3.89	71.12	91.56	88.20	13.24	24.70	68.70	23.69	1.02
41	PTB45	91.50	9.50	78.50	28.65	3.56	2.78	68.46	87.20	85.22	12.08	23.90	69.07	20.47	1.07
42	PTB46	96.00	9.00	89.00	28.40	5.78	5.00	62.20	82.01	84.91	9.21	22.10	62.72	27.88	0.97
43	PTB49	110.40	8.00	86.00	28.50	3.89	3.22	63.60	85.32	87.25	9.88	23.10	64.60	30.03	0.94
44	PTB50	118.00	6.00	85.00	28.55	3.78	2.89	64.20	83.39	83.82	8.81	21.90	64.17	30.71	0.93
45	PTB51	123.20	7.00	96.50	28.10	5.78	6.78	64.30	83.63	86.12	10.22	23.10	67.00	19.56	1.08
46	PTB52	112.40	6.00	94.00	28.85	7.44	7.51	65.73	83.47	87.07	12.45	23.95	67.85	20.29	1.07
47	PTB55	102.50	7.50	83.00	28.70	1.78	1.56	68.49	88.41	88.26	11.23	24.25	68.95	36.22	0.85
48	PTB56	101.00	7.00	87.50	27.95	4.67	3.32	61.39	81.41	83.25	9.48	21.15	61.83	25.15	1.00

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
49	PTB 57	126.00	7.50	104.00	27.80	6.89	7.67	63.89	83.89	84.56	9.56	22.85	65.14	21.74	1.05
50	PTB 58	130.50	9.00	100.00	28.65	3.67	3.22	64.90	84.69	85.22	10.27	23.45	66.97	29.00	0.95
51	PTB 59	131.50	7.50	87.00	28.70	5.00	3.78	72.52	82.51	94.22	12.15	24.35	77.94	25.98	0.99
52	PTB 60	100.50	9.50	81.00	29.70	3.11	1.44	71.34	92.15	92.58	12.30	23.10	73.50	35.19	0.87
53	Sampada	111.50	7.00	107.50	27.05	3.00	1.67	59.09	81.40	83.47	7.70	21.20	61.21	29.85	0.94
54	Kunjukunjuvarna	109.50	6.00	103.00	28.30	5.00	3.56	70.02	88.57	89.14	7.96	23.70	72.58	23.13	1.03
55	N-22	119.00	7.50	67.50	28.60	2.78	3.95	61.58	88.14	85.46	12.05	21.75	62.52	24.76	0.86
56	ASD-16	122.00	8.50	94.50	27.60	5.00	5.00	63.97	82.64	81.89	8.09	21.25	62.14	26.19	0.99
57	ADT-37	128.50	8.00	102.50	27.40	4.89	9.00	62.73	83.45	81.19	8.10	21.80	62.84	26.21	0.99
58	Kazhiama	122.00	5.00	108.50	27.70	6.11	7.11	56.84	82.34	80.36	6.55	20.75	58.70	22.04	1.05
59	Pandichempan	112.50	6.00	96.50	28.30	6.00	4.00	59.97	80.28	82.50	7.59	22.10	62.14	26.42	0.99
60	Gandhakashala	110.00	7.00	117.50	27.05	6.22	6.22	62.91	81.46	83.59	8.31	22.65	61.67	28.34	0.96
61	Kuttithekkan	91.50	5.00	93.00	27.60	5.33	6.67	62.44	81.58	83.63	7.39	22.15	61.56	29.47	0.95
62	Jaya	99.50	6.50	99.00	27.55	4.22	5.11	62.14	83.10	84.47	8.92	22.75	63.30	30.04	0.94

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
63	Jeerakashala	89.00	6.00	119.00	27.95	4.22	5.00	61.58	80.57	81.74	7.60	21.70	61.29	18.16	1.10
64	Ponmani	107.90	8.00	109.50	27.90	4.56	6.73	60.73	80.46	81.00	7.43	21.85	54.19	29.92	0.94
65	Shreyas	101.00	8.00	90.50	28.70	6.89	5.78	64.61	84.51	84.76	10.15	22.55	67.67	18.90	1.09
66	Prathyasha	102.00	6.00	82.50	26.95	7.78	6.54	57.34	80.46	80.87	7.09	19.35	60.06	25.58	1.00
67	Velluthataryan Sel	90.00	7.50	93.50	27.60	6.00	5.89	62.29	83.70	85.02	8.09	17.55	63.23	25.30	1.00
68	Gopika	93.00	7.00	94.00	28.20	5.89	5.78	62.88	84.32	84.13	8.38	20.50	63.80	14.84	1.14
69	Mahamaya	91.00	6.50	95.00	28.30	6.11	6.00	61.87	82.53	83.47	7.24	20.10	66.61	63.29	0.96
70	IGKVR-1	88.00	6.50	101.50	27.60	5.22	3.44	64.15	81.40	81.71	8.66	23.50	65.36	66.61	1.07
71	Cul-6	82.50	6.50	107.50	27.80	4.11	2.89	65.14	81.92	84.96	8.27	22.95	65.08	65.36	0.98
72	Cul-7	92.65	7.50	108.50	27.65	3.11	2.78	63.47	84.14	84.48	9.47	22.35	54.19	65.08	0.99
73	Cul-14	107.00	7.50	107.50	28.45	5.56	5.95	61.10	81.29	81.35	6.65	20.85	62.75	62.54	0.89
74	CR DHAN202	113.00	6.50	86.50	27.75	4.89	5.00	63.43	83.01	83.67	8.64	21.20	62.04	62.75	1.00
75	CR DHAN305	115.00	8.50	101.00	27.80	2.78	2.76	61.76	81.88	83.20	7.31	19.90	59.30	62.04	1.01

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SPF	%RYR	DSI
76	CRDHAN204	95.00	8.00	86.50	27.75	7.00	7.11	60.11	82.18	80.41	6.56	19.50	59.30	21.31	1.06
77	CRDHAN205	94.50	8.00	89.00	28.60	5.89	7.00	64.84	81.71	82.16	7.89	20.55	62.83	24.93	1.01
78	Chomala	95.50	8.00	100.50	28.55	3.11	1.56	67.61	88.78	88.40	11.53	20.50	69.23	35.07	0.87
79	CR DHAN 101	93.50	8.50	91.50	28.10	6.22	7.67	63.34	84.98	84.67	9.51	23.55	63.37	22.68	1.04
80	Uma	98.50	10.50	97.00	28.65	4.11	3.11	69.05	81.89	85.18	11.23	24.25	69.79	17.01	1.11
81	JS-4	96.50	8.50	101.00	27.80	5.11	6.00	61.47	81.12	84.17	7.52	22.15	64.00	27.84	0.97
	mean	110.74	7.53	88.47	28.26	4.80	4.66	65.19	84.95	86.14	9.55	22.71	65.26	25.48	1.00
	CD(0.05)	3.79	2.42	4.67	0.413	2.92	1.99	3.36	2.65	2.72	0.941	0.560	2.15	1.23	0.56

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), Leaf rolling score- LR, LD- Leaf drying score, RWC- Relative water content (%), CMS- Cell membrane stability index (%), CSI- Chorophyll stability index (%), YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%), %RYR- Percentage relative yield reduction, DSI- Drought susceptibility index.

Table 5: Variation in morpho-physiological and plant production traits in rice under irrigated control condition in experiment I (Mundakan 2017)

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
1	PTB 1	144.10	10.00	105.00	29.35	87.96	16.65	25.20	74.31
2	PTB 2	134.15	9.50	96.00	27.20	82.91	12.71	24.35	70.18
3	PTB 3	118.50	9.00	89.50	27.40	86.96	12.74	23.90	71.57
4	PTB 4	118.50	10.50	103.50	27.10	83.68	15.29	24.80	76.84
5	PTB 5	126.95	10.00	96.50	27.35	85.24	12.45	21.85	71.24
6	PTB 6	121.75	9.00	100.00	27.45	82.96	11.07	23.95	74.40
7	PTB 7	120.40	13.50	81.00	28.15	89.74	19.00	25.75	85.52
8	PTB 8	109.50	8.50	96.00	27.80	85.18	13.92	23.70	73.26
9	PTB 9	127.00	9.00	98.00	27.20	84.08	13.57	24.10	72.75
10	PTB 10	118.00	12.00	66.50	27.55	83.37	14.13	25.20	73.69
11	PTB12	116.50	9.50	97.00	27.15	85.56	14.63	24.00	78.74
12	PTB13	129.50	11.00	100.00	27.60	78.74	10.50	23.00	73.11
13	PTB14	129.90	10.50	94.50	27.80	75.70	10.51	22.95	65.61
14	PTB15	132.50	12.50	91.00	29.00	89.38	19.00	25.10	83.66
15	PTB 17	129.00	10.00	86.50	27.40	86.40	13.40	24.45	80.82
16	PTB18	137.50	8.50	98.50	28.30	81.72	10.18	24.10	74.76
17	PTB19	117.10	10.50	91.00	27.05	87.96	13.14	24.45	77.42
18	PTB20	119.50	11.50	86.00	27.50	86.12	13.09	24.05	80.54
19	PTB21	124.50	13.00	93.50	27.10	81.79	14.96	24.70	76.10
20	PTB22	123.80	10.50	78.50	27.45	82.34	12.33	23.70	73.34
21	PTB23	126.75	11.00	77.00	27.25	83.56	10.96	22.70	75.47
22	PTB24	125.00	9.50	79.00	27.70	85.45	12.58	24.70	72.03
23	PTB25	124.50	11.00	83.00	27.45	81.84	14.98	24.50	71.00
24	PTB26	133.10	11.50	86.50	28.10	90.17	18.89	23.10	81.88
25	PTB27	130.00	10.00	90.00	27.70	89.65	17.69	25.85	82.78

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
26	PTB28	128.50	12.00	75.00	27.20	89.29	17.78	25.35	82.41
27	PTB29	132.85	11.00	75.50	27.10	88.86	18.82	25.95	85.72
28	PTB30	126.00	11.50	74.00	28.10	88.21	18.30	25.75	73.89
29	PTB31	124.50	10.00	74.50	27.30	82.26	13.09	24.65	75.30
30	PTB32	114.50	9.00	86.50	27.15	81.72	12.41	23.80	71.21
31	PTB33	112.00	9.00	83.50	26.95	83.91	10.72	23.40	73.78
32	PTB34	113.50	14.00	75.00	27.95	87.14	12.55	25.15	76.53
33	PTB35	109.50	12.00	80.50	27.90	84.90	19.64	22.90	86.91
34	PTB36	109.00	11.00	70.00	28.00	85.83	18.21	24.30	75.00
35	PTB37	106.00	10.50	91.50	28.20	84.87	13.60	24.10	76.03
36	PTB38	100.50	10.00	76.50	28.10	85.01	13.10	23.50	71.15
37	PTB39	129.00	10.50	99.50	27.10	84.50	10.65	21.55	75.77
38	PTB40	126.50	12.00	97.50	28.00	87.14	16.37	25.45	79.95
39	PTB41	125.00	10.50	78.00	27.60	87.23	14.11	24.55	75.51
40	PTB43	111.50	10.00	76.50	26.90	88.16	17.35	25.30	78.68
41	PTB45	100.00	12.00	82.00	27.70	88.29	15.19	24.65	82.58
42	PTB46	100.50	12.00	83.00	27.95	79.92	12.77	23.00	74.99
43	PTB49	117.50	10.00	84.00	27.00	83.54	14.12	23.90	77.92
44	PTB50	122.00	9.00	92.00	28.00	85.28	12.72	22.50	75.15
45	PTB51	127.00	9.00	92.00	27.20	86.29	12.71	23.85	79.68
46	PTB52	113.00	9.00	75.50	27.80	85.37	15.62	24.50	78.12
47	PTB55	107.00	11.00	83.50	27.75	88.13	17.61	24.95	80.36
48	PTB56	105.50	9.50	91.50	27.60	82.75	12.67	21.90	71.65
49	PTB57	131.00	11.00	91.50	27.20	84.97	12.21	23.40	74.56
50	PTB58	133.30	11.50	81.50	27.15	86.37	14.47	24.20	77.10

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
51	PTB 59	135.00	10.00	76.00	27.45	86.89	16.42	24.85	81.21
52	PTB 60	106.00	12.50	101.00	28.25	88.23	18.98	24.05	84.90
53	Sampada	114.50	9.50	95.50	26.60	81.28	10.97	22.20	74.10
54	Kunjukunjuvarna	113.00	8.50	61.50	27.40	87.34	10.36	24.35	77.00
55	N-22	121.50	9.50	88.50	27.85	82.04	18.81	22.70	70.62
56	ASD-16	127.00	10.50	93.50	27.10	83.66	10.96	22.20	71.00
57	ADT-37	131.50	10.00	104.00	27.05	83.51	10.97	22.30	70.24
58	Kazhiama	126.50	7.50	89.50	26.45	77.06	8.40	21.50	71.30
59	Pandichempan	115.50	8.00	112.50	27.40	80.99	10.32	23.15	64.96
60	Gandhakashala	113.50	9.50	114.00	26.00	81.95	11.59	23.30	71.72
61	Kuttithekkan	97.00	7.50	92.50	26.90	81.21	10.47	23.45	73.65
62	Jaya	106.50	9.50	86.00	27.25	82.89	12.75	23.70	71.59
63	Jeerakashala	91.50	8.50	107.00	27.10	82.19	9.28	22.85	73.89
64	Ponmani	112.00	11.00	84.50	27.70	79.11	10.60	22.85	74.85
65	Shreyas	107.00	10.50	78.00	27.65	79.65	12.51	23.20	79.87
66	Prathyasha	107.00	9.50	88.00	26.10	78.27	9.52	20.70	70.73
67	VelluthataryanSel	93.50	9.50	90.00	27.05	79.91	10.83	18.70	72.08
68	Gopika	100.00	9.00	91.00	27.50	80.26	9.84	21.70	71.52
69	Mahamaya	97.50	9.00	97.50	27.80	77.78	10.14	21.10	72.31
70	IGKVR-1	91.00	10.00	101.00	27.10	80.63	10.86	24.30	81.99
71	Cul-6	87.50	8.00	100.50	27.25	81.35	11.28	22.20	69.77
72	Cul-7	97.50	9.50	90.00	27.20	80.96	12.83	22.50	71.58
73	Cul-14	111.00	9.50	87.00	28.10	80.69	10.06	21.80	66.91
74	CR DHAN202	116.50	7.00	85.00	27.35	84.59	11.65	22.30	73.23
75	CR DHAN305	118.50	10.50	80.50	27.45	82.80	9.70	21.80	73.39
76	CRDHAN204	100.00	10.50	88.00	27.25	84.47	8.33	20.30	69.66
77	CRDHAN205	98.00	10.00	90.00	27.95	83.14	10.51	21.55	70.89
78	Chomala	99.50	11.00	89.50	28.10	85.69	17.79	21.40	70.22
79	CR DHAN 101	96.00	11.00	89.50	27.65	84.43	12.30	24.70	82.62

80	Uma	103.50	13.50	93.50	28.30	88.97	13.53	25.55	81.42
81	JS-4	101.00	11.50	101.00	27.20	84.01	10.42	23.40	79.58
	MEAN	116.05	10.25	94.65	27.50	84.13	12.94	23.55	75.43
	CD(0.05)	5.09	2.50	7.86	0.271	2.92	1.01	0.70	2.39

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), RWC- Relative water content (%),YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%).

Table 6: Over all mean values, range and standard deviation of rice genotypes under water stress & irrigated control in experiment 1 (Mundakan, 2017)

Sl No	Traits	Mean		Range		SD	
		Water stress	Control	Water stress	Control	Water stress	Control
1	Plant height (Cm)	106.28	112.29	80.15-130	86.50-140.50	12.56	12.28
2	Tiller number	6.34	8.88	5.00-8.50	6.50-13.00	0.845	1.23
3	Days to 50% flowering	91.26	85.78	65.50-115.50	63.00-112.00	10.86	10.36
4	Leaf temperature (◦C)	29.56	27.94	27.30-30.55	27.10-28.70	0.548	0.385
5	Leaf rolling score	4.05		1.11-7.84		1.39	
6	Leaf drying score	4.00		1.17-7.67		1.37	
7	Relative water content (%)	61.07	78.89	50.35-72.98	70.36-86.26	4.79	3.89
8	Cell membrane stability index (%)	81.83		75.53-92.24		3.70	
9	Chlorophyll stability index (%)	82.11		75.52-92.85		3.85	
10	Yield/plant (g)	8.40	11.55	6.06-12.17	7.94-18.62	1.57	2.15
11	Thousand grain weight (g)	21.78	22.66	17.15-24.00	17.45-24.95	1.33	1.35
12	Spikelet fertility percentage (%)	61.04	70.91	50.47-73.79	61.12-81.93	4.39	4.58
13	Percentage relative yield reduction	29.46		17.91-46.94		4.96	
14	Drought susceptability index	1.00		0.75-1.17		0.070	
15	Soil moisture percentage (%)			10.75-24.96			

4.2 VARIATION IN PHYSIO-MORPHOLOGICAL AND PLANT PRODUCTION TRAITS UNDER WATER STRESS AND IRRIGATED CONTROL CONDITIONS IN PUNCHA 2018.

Trait mean values of all morpho-physiological and plant production traits of 81 rice genotypes under water stress and irrigated control conditions in experiment II are presented in Table 7 & 8. Over all mean values, range values and standard deviations of 81 rice genotypes under water stress and irrigated control conditions in experiment II are presented in Table 9.

4.2.1 Leaf rolling score

There was a significant variation in leaf rolling score among the genotypes under water stress condition. Average leaf rolling score recorded across the genotypes was 4.05, and it ranged from 1.11 to 7.84. Highest leaf rolling (7.84) was observed in Prathyasha and PTB29 (1.11) showed least leaf rolling symptoms among 81 genotypes**.**

4.2.2 Leaf drying score

Average leaf drying score recorded across the genotypes was 4.00 under water stress condition and it ranged from 1.17 to 7.67. Highest drying score (7.67) was observed in PTB 18 and PTB 27 (1.17) showed least leaf drying symptoms among 81 genotypes.

4.2.3 Leaf temperature

Average leaf temperature recorded under water stress condition was 29.26°C and 27.94°C under irrigated condition. It ranged from 26.95°C to 30.55°C under water stress condition and 27.10°C to 28.70°C under irrigated control condition. Under water stress condition, maximum leaf temperature (30.55°C) was observed in PTB 1 and Prathyasha (27.30°C) recorded minimum leaf temperature. Under irrigated control condition maximum leaf temperature was observed in PTB1 (28.70°C) and Velluthataryan Selection (27.10°C) recorded minimum leaf temperature.

4.2.4 Cell membrane stability index

Average cell membrane stability index across the 81 genotypes was 81.83% and it ranged from 75.53 to 92.24% in experiment II. Highest membrane stability index (92.24%) was observed in PTB 27 and Pandichempan (75.53 %) showed lowest membrane stability index.

4.2.5 Chlorophyll stability index

Average chlorophyll stability index across the 81 genotypes was 82.11% and it ranged from 75.52 to 92.85%. Highest chlorophyll stability index (92.85 %) was observed in PTB7 and Ponmani (75.52%) showed lowest chlorophyll stability index.

4.2.6 Relative water content (%)

Average relative water content across the 81 genotypes was 61.07% under water stress and 78.89% under irrigated conditions. It ranged from 50.35-72.98 % under water stress and 70.36-86.26% under irrigated conditions. Under water stress highest relative water content (72.98%) was observed in PTB27 and ADT 37 (50.35%) recorded lowest relative water content. Under irrigated control condition highest relative water content (86.26%) was observed in PTB 28 and PTB13 (70.36%) recorded lowest relative water content.

4.2.7 Drought susceptibility index

Drought susceptibility index across the rice genotypes ranged from 0.75 to 1.17 in experiment II. Highest drought susceptibility index (1.17) was observed in Shreyas and PTB 25 (0.75) showed lowest drought susceptibility index.

4.2.8 Percentage relative yield reduction

Percentage relative yield reduction across the rice genotypes ranged from 17.91% to 46.94% in experiment II. Highest Percentage relative yield reduction (46.94%) was observed in PTB25 and Shreyas (17.91 %) showed lowest relative yield reduction.

4.2.9 Plant height

There was a significant variation in plant height among the genotypes under water stress and irrigated control conditions. Average plant height across the genotypes was 106.28 cm under water stress and 112.29 cm under irrigated conditions. It ranged from 80.15 cm to 130.00 cm under water stress and 86.50 cm to 140.50 cm under irrigated conditions

respectively. Under water stress maximum plant height (130.00 cm) was observed in PTB 1 and ASD-16 (80.15 cm) recorded minimum plant height. Under irrigated control condition maximum plant height (140.50 cm) was observed in PTB1 and Jaya recorded (86.50 cm) minimum plant height.

4.2.10 Tiller number

Average number of tiller plant $^{-1}$ was 6.34 under water stress and 8.88 under irrigated conditions across the genotypes. It ranged from 5 to 8.50 under water stress and 6.50 to 13.00 under irrigated conditions respectively. Under water stress maximum number of tillers (8.5 plant^{-1}) was observed in PTB7 and Kuttithekkan (5.00 plant^{-1}) showed minimum number of tillers. Under irrigated control condition maximum (13 plant^{-1}) number of tillers was observed in PTB35 and Pandichempan showed (7 plant^{-1}) minimum number of tillers.

4.2.11 Days to 50% flowering

Average number of days to 50 % flowering was 92 under water stress and 86 under irrigated condition across the genotypes. It ranged from 65 days to 115 days under water stress condition and 63 days to 112 days under irrigated condition. Delayed flowering was observed for genotypes under water stress condition.

4.2.12 Soil moisture percentage

The soil moisture percentage in field condition was estimated by gravimetric method. Under water stress condition the moisture percentage was 10.75% while under irrigated control condition it was 24.96%.

4.2.13 Grain yield/ plant

Average grain yield was 8.40 g in water stress condition and 11.55 g under irrigated control condition. Grain yield per plant ranged from 6.06- 12.17 g in water stress condition and 7.94 to 18.62g in irrigated control condition. Under water stress highest grain yield (12.17 g) was observed in PTB35 and Ponmani (6.06 g) recorded lowest grain yield. Under irrigated control condition highest grain yield (18.62 g) was observed in PTB35 and Pandichempan (8.11 g) recorded lowest grain yield.

4.2.14 Spikelet fertility percentage

Spikelet fertility across the rice genotypes ranged from 50.47 to 73.79% under water stress and 61.12 to 81.93% under irrigated conditions with a mean of 61.04% and 70.91 % respectively. Under water stress highest spikelet fertility percentage (73.79%) was observed in PTB35 and Velluthatarya Selection (50.47%) recorded lowest spikelet fertility percentage. Under irrigated control condition highest spikelet fertility percentage (81.93%) was observed in PTB35 and Velluthataryan Selection (61.12%) recorded lowest spikelet fertility percentage.

4.2.15 Thousand grain weight

Thousand grain weight across the rice genotypes ranged from 17.15 to 24.00 g under water stress condition and 17.45 to 24.95 g under irrigated control condition with a mean of 21.78 g and 22.66 g respectively. Under water stress highest thousand grain weight (24.00 g) was observed in PTB35 and Velluthataryan Selection (17.15 g) recorded lowest thousand grain weight. Under irrigated control condition highest thousand grain weight (24.95 g) was observed in PTB35 and Velluthataryan Selection (17.45 g) recorded lowest grain weight.

Table 7: Effect of water stress on morpho-physiological and plant production traits in experiment II (Puncha 2018)

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
1	PTB 1	130	6.00	102.50	30.55	3.89	3.50	71.03	85.18	87.53	11.50	23.25	60.42	28.57	1.01
2	PTB 2	126.5	6.00	96.50	29.55	5.02	4.54	59.73	79.50	84.22	8.39	22.70	56.69	31.37	0.97
3	PTB 3	106	6.00	94.00	28.95	2.49	3.19	58.86	79.19	85.28	8.56	22.65	57.06	29.70	1.00
4	PTB 4	110	5.50	98.00	28.60	3.24	3.43	64.47	81.46	87.52	10.06	22.80	59.65	31.42	0.97
5	PTB 5	120	7.00	92.50	28.75	5.13	4.21	59.03	79.13	76.76	9.00	20.10	57.17	31.39	0.97
6	PTB 6	103	6.00	105.00	29.05	3.35	5.54	54.14	77.73	80.86	6.85	22.40	57.44	35.47	0.92
7	PTB 7	107	8.50	84.50	29.50	2.35	1.76	67.07	88.29	92.85	10.56	23.65	67.79	41.64	0.79
8	PTB 8	102	5.50	94.50	28.95	5.35	6.32	59.46	83.00	86.73	8.40	22.25	61.21	31.55	0.97
9	PTB 9	113.5	6.00	98.50	29.35	4.35	5.76	62.04	81.34	84.58	8.12	22.90	57.89	32.06	0.96
10	PTB 10	105	7.00	70.00	28.60	3.81	3.54	63.95	82.68	83.38	8.09	22.20	60.54	33.26	0.95
11	PTB 12	105.45	7.00	98.50	28.65	4.00	4.44	62.06	83.34	85.31	9.51	22.40	62.40	24.29	1.07

Sl No	Genotypes	PH	NOT	DFF	LT-2	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
12	PTB 13	117.5	5.50	102.00	28.55	6.95	5.81	59.43	80.34	76.82	6.61	21.55	57.33	27.44	1.03
13	PTB 14	117.5	7.50	96.50	28.65	6.22	7.33	51.05	77.68	75.70	6.56	21.90	50.73	35.91	0.91
14	PTB 15	126.3	7.00	90.00	29.85	3.11	3.11	72.93	90.60	90.25	10.09	22.90	71.27	43.94	0.75
15	PTB 17	115.95	6.00	91.00	28.50	6.33	5.44	67.10	81.67	85.30	9.45	22.70	63.64	26.94	1.04
16	PTB 18	129.6	5.50	98.00	29.10	6.33	7.67	59.04	79.88	83.66	6.49	22.70	61.50	28.76	1.01
17	PTB 19	104.65	6.50	108.00	28.55	5.33	4.22	62.82	80.39	84.70	9.00	23.20	62.81	26.09	1.05
18	PTB20	106.8	7.00	84.50	28.45	7.33	6.67	63.07	81.39	82.74	8.23	22.10	62.89	33.88	0.94
19	PTB 21	116.4	7.00	94.50	27.70	5.81	6.54	56.47	81.19	80.65	9.14	21.75	61.05	35.50	0.92
20	PTB 22	116.45	8.00	86.50	28.35	7.00	5.44	57.62	80.73	79.31	6.95	21.55	58.34	41.79	0.83
21	PTB 23	115.75	5.50	85.50	28.55	3.95	4.81	59.30	79.23	82.91	6.17	20.65	59.84	37.96	0.88
22	PTB 24	113.5	7.00	82.50	28.80	7.22	7.33	59.43	82.05	86.60	7.10	22.60	57.89	35.10	0.92
23	PTB 25	113.75	6.50	87.00	28.80	3.11	3.11	60.19	81.73	82.26	7.11	23.15	57.67	46.94	0.75
24	PTB 26	121.3	6.00	93.50	27.95	4.33	4.44	69.03	89.47	79.56	10.11	22.00	67.53	41.28	0.79
25	PTB 27	119	8.00	91.50	27.75	2.78	1.67	72.98	91.33	87.98	9.51	23.55	66.74	41.50	0.79

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
26	PTB 28	115.5	6.00	85.00	28.75	2.78	2.67	72.90	90.06	91.88	10.00	21.25	69.08	39.86	0.81
27	PTB 29	126.4	8.50	78.50	28.50	2.11	2.78	70.83	89.74	91.12	11.00	23.50	71.45	37.57	0.84
28	PTB 30	114	6.00	78.50	29.05	3.11	2.43	70.38	89.67	84.96	10.62	24.00	70.18	37.89	0.84
29	PTB 31	115	5.50	77.00	28.45	4.78	5.33	58.93	81.48	81.82	8.55	22.90	60.18	29.75	1.00
30	PTB 32	106.25	5.50	88.50	28.60	5.72	5.70	59.12	81.31	80.79	9.06	21.45	59.56	26.16	1.05
31	PTB 33	101.5	6.00	100.00	27.80	5.00	6.22	59.06	81.51	82.27	8.11	22.15	57.89	24.91	1.07
32	PTB 34	100	5.50	73.50	28.70	4.89	5.00	62.81	92.24	83.35	12.17	24.00	73.79	26.75	1.04
33	PTB 35	95.7	5.50	81.00	29.15	3.33	3.33	62.43	87.23	83.69	12.17	21.10	62.55	31.55	0.97
34	PTB 36	97	7.50	72.50	28.85	4.11	4.56	60.51	80.89	81.98	9.13	22.10	62.01	26.68	1.04
35	PTB 37	92.5	8.00	95.00	28.90	5.11	4.11	59.98	83.07	80.57	8.95	23.15	62.77	27.35	1.03
36	PTB 38	91.665	6.00	72.50	28.80	4.11	5.56	63.07	80.84	81.90	8.11	22.00	58.39	33.06	0.95
37	PTB 39	118.5	6.50	79.00	28.10	3.26	3.21	64.09	81.23	79.36	7.06	20.40	57.34	30.22	0.99
38	PTB 40	118.5	6.00	104.50	28.75	4.44	6.11	65.26	84.19	84.44	10.05	23.85	64.56	30.62	0.99
39	PTB 41	116.5	6.00	103.50	28.50	6.39	5.48	63.95	84.55	82.29	8.40	22.45	62.85	35.15	0.92

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
40	PTB 43	100	5.50	77.50	28.35	4.67	6.00	65.15	87.68	83.73	10.28	22.44	62.84	29.93	1.00
41	PTB 45	89.4	6.50	76.00	28.50	3.56	3.78	64.64	83.76	80.98	10.73	22.30	63.69	24.26	1.08
42	PTB 46	90	6.50	85.50	28.80	5.78	4.11	66.05	79.14	81.37	8.77	21.25	57.95	27.61	1.03
43	PTB 49	103.5	8.00	84.50	27.95	3.33	3.33	60.53	82.23	82.46	8.96	22.55	60.44	31.12	0.98
44	PTB 50	113.5	5.50	81.00	28.75	3.33	3.89	60.22	79.63	80.37	7.13	21.25	57.74	37.67	0.88
45	PTB 51	121	6.00	93.00	27.70	5.78	6.33	59.64	81.22	81.29	9.39	22.40	62.19	20.29	1.13
46	PTB 52	102.5	6.00	91.00	28.60	7.59	6.10	62.04	81.06	81.74	10.17	23.20	61.95	28.18	1.02
47	PTB 55	99	6.00	79.50	27.95	2.78	3.00	64.92	84.18	84.01	10.50	24.00	63.68	37.97	0.84
48	PTB 56	95.5	5.50	85.00	28.90	4.72	4.37	58.43	78.73	80.05	9.05	20.25	57.12	26.96	1.04
49	PTB 57	122	6.50	100.00	27.95	7.00	5.89	60.09	79.31	80.31	8.56	20.60	60.57	25.58	1.06
50	PTB 58	126.5	6.50	98.00	28.80	3.59	3.67	61.28	81.18	81.44	8.12	21.60	60.79	36.50	0.90
51	PTB 59	126	5.50	84.50	28.85	5.11	6.00	68.55	89.63	84.05	10.17	22.55	72.95	28.24	1.02
52	PTB 60	96	6.00	77.00	28.90	2.89	2.22	68.43	78.02	89.70	11.18	21.45	68.69	36.54	0.85
53	Sampada	105.5	5.50	104.50	27.30	3.70	3.22	56.33	78.02	80.81	7.49	21.30	58.73	24.61	1.07

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
54	Kunjukunjuvarna	102.5	6.00	100.50	28.40	5.11	5.22	66.44	83.66	82.39	7.28	23.25	66.23	29.56	1.00
55	N-22	116.5	6.00	65.50	28.95	3.11	2.78	68.31	84.73	91.68	10.17	21.10	59.38	39.92	0.81
56	ASD-16	118.25	6.50	92.00	28.10	4.89	6.33	59.32	79.20	78.58	7.22	20.10	57.05	28.28	1.02
57	ADT-37	123.5	8.50	96.00	27.55	4.84	3.70	59.43	80.67	77.29	6.39	20.75	57.90	32.74	0.96
58	Kazhiama	118	7.00	107.00	27.70	6.11	6.22	53.47	75.73	75.62	6.06	20.20	56.62	25.28	1.06
59	Pandichempan	109	5.50	94.50	28.05	6.00	6.00	56.88	79.19	77.80	6.39	22.05	57.83	31.60	0.97
60	Gandhakashala	104	6.50	111.50	28.65	6.22	5.33	59.26	79.38	80.44	7.13	22.10	58.23	29.51	1.00
61	Kuttithekkan	88.6	7.00	88.50	27.35	5.22	6.56	56.71	77.84	79.64	6.79	22.25	57.55	31.71	0.97
62	Jaya	95.85	5.50	97.00	28.30	4.22	4.11	55.79	79.73	81.41	7.51	21.30	56.78	33.79	0.94
63	Jeerakashala	84.5	5.50	115.50	27.85	4.22	6.56	56.00	76.79	77.32	6.29	21.00	57.73	25.52	1.06
64	Ponmani	102.5	5.50	108.50	30.55	5.70	4.65	56.18	77.00	75.52	6.07	20.40	59.88	36.53	0.90
65	Shreyas	97	6.50	89.00	29.55	6.89	4.56	61.51	81.11	81.39	9.12	22.10	62.18	17.91	1.17
66	Prathyasha	100	6.00	79.00	28.95	7.84	4.92	53.68	78.36	77.22	6.06	19.25	55.55	24.88	1.07
67	VelluthataryanSel	84	6.50	90.00	28.60	6.11	4.44	57.64	81.56	80.71	7.51	17.15	57.95	20.46	1.13

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
68	Gopika	89.5	6.00	91.00	28.75	6.00	4.22	63.12	81.18	79.83	7.72	19.36	60.29	23.69	1.08
69	Mahamaya	91	6.00	92.00	29.05	6.22	4.56	58.41	79.49	79.56	6.11	20.10	58.23	31.27	0.98
70	IGKVR-1	84.5	6.00	98.50	29.50	5.22	4.78	61.60	81.17	78.83	8.05	22.51	64.45	20.42	1.13
71	Cul-6	80.15	6.00	104.00	28.95	4.22	4.33	60.61	81.67	81.65	8.00	21.25	60.55	26.87	1.04
72	Cul-7	89	6.00	106.50	29.35	3.22	3.78	55.88	79.72	79.23	9.00	20.15	58.00	27.69	1.03
73	Cul-14	101	5.00	103.00	28.60	5.59	4.10	56.17	77.83	77.43	6.16	19.60	50.47	35.55	0.92
74	CR DHAN202	107.5	7.00	83.50	28.65	4.78	6.00	59.14	79.64	79.33	8.03	20.80	60.50	27.05	1.04
75	CR DHAN305	110	6.00	98.50	28.55	3.11	3.00	58.55	78.55	78.41	7.05	19.35	58.73	24.80	1.07
76	CRDHAN204	90.35	7.50	84.00	28.65	5.00	4.89	54.00	78.17	75.77	6.06	19.00	60.73	25.09	1.06
77	CRDHAN205	92	6.00	86.50	29.85	5.89	5.67	55.18	79.34	78.37	7.01	20.35	62.23	25.40	1.06
78	Chomala	91.5	6.00	98.50	28.50	2.72	2.37	65.26	86.13	84.98	10.18	19.85	63.89	36.73	0.85
79	CR DHAN 101	91.3	7.50	88.00	29.10	6.11	5.22	57.93	81.60	80.56	8.17	22.50	59.84	23.43	1.09

Sl No	Genotypes	PH	NOT	DFF	LT	LR	LD	RWC	CMS	CSI	YPP	TGW	SFP	%RYR	DSI
80	Uma	93.5	7.50	93.50	28.55	3.11	3.43	64.80	79.85	82.48	11.00	23.70	65.95	19.80	1.14
81	JS-4	91	7.00	98.50	28.45	5.11	6.22	57.68	78.56	81.28	6.29	21.25	60.18	29.25	1.00
	Mean	106.28	6.34	85.78	29.56	4.05	4.00	61.07	81.83	82.11	8.40	21.78	61.04	29.46	1.00
	CD (0.05)	3.50	1.46	3.63	0.635	1.99	1.97	3.08	2.56	3.33	0.770	0.612	2.28	1.87	0.82

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), Leaf rolling score- LR, LD- Leaf drying score, RWC- Relative water content (%), CMS- Cell membrane stability index (%), CSI- Chorophyll stability index (%), YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%), %RYR- Percentage relative yield reduction, DSI- Drought susceptibility index.

Table 8: Variation in morpho-physiological and plant production traits in rice under irrigated control condition in expriment II (Puncha 2018)

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
1	PTB 1	140.50	8.50	101.00	27.50	83.21	14.10	24.00	71.55
2	PTB 2	129.00	9.50	92.50	27.80	76.11	10.23	23.65	67.13
3	PTB 3	115.50	7.00	88.50	27.20	80.74	10.17	23.65	66.79
4	PTB 4	112.50	9.00	100.00	27.50	80.06	12.67	23.80	72.38
5	PTB 5	121.50	8.50	92.50	27.30	79.75	10.11	20.90	68.11
6	PTB 6	117.23	6.50	100.00	27.85	80.43	9.62	23.35	70.17
7	PTB 7	116.00	11.00	80.00	28.05	85.78	18.13	24.75	81.29
8	PTB 8	106.00	7.50	92.50	28.10	79.88	12.27	23.10	68.94
9	PTB 9	123.50	8.00	94.00	28.15	76.99	11.95	23.75	69.06
10	PTB 10	114.00	9.50	63.50	27.60	79.71	12.12	24.65	70.28
11	PTB 12	113.50	9.50	94.00	27.45	80.00	12.56	23.25	74.34
12	PTB 13	125.50	9.00	99.00	27.50	70.36	9.11	22.35	69.62
13	PTB 14	126.00	9.00	91.00	27.25	70.84	9.24	22.10	62.06
14	PTB 15	128.50	10.50	88.50	27.75	83.30	18.00	24.15	79.90
15	PTB 17	124.00	8.50	84.50	27.50	83.85	11.94	23.70	77.55
16	PTB 18	132.50	8.00	93.50	28.15	76.53	9.11	23.45	71.18
17	PTB 19	114.00	9.00	87.50	28.70	79.48	12.17	24.30	72.94
18	PTB20	116.50	9.50	80.00	28.40	79.32	12.44	23.00	73.51
19	PTB 21	120.50	11.00	91.00	27.40	76.85	11.17	22.70	71.50
20	PTB 22	119.50	9.50	71.00	27.15	75.34	11.93	22.35	67.78
21	PTB 23	123.50	10.00	71.00	28.15	77.99	9.95	21.20	71.23
22	PTB 24	122.00	8.00	74.00	27.70	79.49	10.94	23.20	67.67
23	PTB 25	121.00	9.00	78.50	28.50	74.00	13.39	24.00	66.72

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
24	PTB 26	130.00	11.00	81.50	28.50	84.55	17.22	22.50	77.68
25	PTB 27	126.00	9.50	85.50	27.50	85.80	16.72	23.55	75.51
26	PTB 28	124.00	11.00	71.50	28.15	86.26	16.63	22.35	77.89
27	PTB 29	128.50	9.50	70.00	28.55	85.91	17.62	21.40	80.65
28	PTB 30	121.00	10.50	71.00	27.80	84.75	17.10	23.40	71.80
29	PTB 31	120.00	9.00	71.50	28.30	75.75	12.17	23.85	71.35
30	PTB 32	109.00	7.50	81.00	27.65	74.36	12.27	22.40	66.55
31	PTB 33	105.50	7.00	73.50	28.20	77.01	9.80	22.85	67.08
32	PTB 34	110.00	13.00	70.00	28.20	81.23	16.62	24.35	81.93
33	PTB 35	106.50	10.00	77.00	28.40	81.77	18.62	22.10	70.19
34	PTB 36	103.50	9.50	63.50	28.00	81.90	12.45	23.65	71.52
35	PTB 37	100.50	9.50	86.00	28.20	78.76	12.32	23.70	70.07
36	PTB 38	102.50	9.00	70.50	27.65	77.76	12.12	22.60	68.29
37	PTB 39	124.00	9.50	94.00	28.15	79.77	10.11	20.95	71.10
38	PTB 40	121.50	10.00	95.00	27.75	82.54	14.49	24.95	74.69
39	PTB 41	121.50	8.50	73.50	27.75	84.02	12.95	23.70	71.28
40	PTB 43	109.00	9.00	71.00	27.55	85.09	14.67	24.10	74.95
41	PTB 45	100.50	9.00	76.00	27.85	83.71	14.16	23.80	79.13
42	PTB 46	96.50	10.00	79.50	28.10	76.17	12.12	22.70	70.63
43	PTB 49	110.00	7.00	79.00	27.95	79.39	13.00	23.30	71.22
44	PTB 50	117.50	7.00	87.50	27.85	81.19	11.44	22.00	70.18
45	PTB 51	125.00	7.50	89.50	27.95	81.60	11.78	23.10	75.92
46	PTB 52	109.50	8.50	71.00	27.55	78.89	14.16	24.00	71.94
47	PTB 55	102.50	10.50	77.50	28.05	84.44	16.92	24.60	73.05

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
48	PTB 56	100.00	6.50	90.00	27.10	77.71	12.39	21.20	65.63
49	PTB 57	126.50	9.50	88.50	27.45	79.70	11.50	22.75	70.12
50	PTB 58	130.00	10.00	78.00	27.40	79.72	12.78	23.30	71.09
51	PTB 59	130.00	9.50	72.50	27.55	82.45	14.17	24.10	76.54
52	PTB 60	101.50	11.00	95.50	27.70	85.23	17.62	23.10	79.22
53	Sampada	109.50	9.00	91.00	27.35	77.16	9.94	22.00	67.68
54	Kunjukunjuvarna	105.00	7.50	63.00	27.80	83.13	12.34	24.00	71.77
55	N-22	116.50	8.00	84.00	27.90	77.28	16.93	22.40	65.31
56	ASD-16	122.50	9.00	91.00	27.90	76.27	10.06	21.20	67.90
57	ADT-37	130.50	8.50	101.00	28.35	77.94	9.50	21.70	65.18
58	Kazhiama	123.00	8.00	85.00	27.80	71.53	8.11	20.85	61.12
59	Pandichempan	111.50	7.00	107.50	27.70	77.60	9.34	22.70	67.51
60	Gandhakashala	110.00	7.50	81.00	27.65	73.39	10.12	23.00	66.39
61	Kuttithekkan	95.00	7.50	89.00	28.00	76.64	9.94	23.15	69.73
62	Jaya	101.00	7.50	94.00	27.75	75.19	11.34	22.45	67.61
63	Jeerakashala	90.00	7.50	87.50	27.60	78.99	8.45	22.00	68.78
64	Ponmani	108.00	9.50	104.00	27.60	73.30	9.56	21.80	68.68
65	Shreyas	102.50	8.50	93.00	28.15	79.53	11.11	22.75	72.22
66	Prathyasha	104.00	8.00	99.50	27.45	74.16	8.06	19.85	67.55
67	VelluthataryanSel	94.00	9.00	84.00	27.40	72.22	9.44	17.45	66.72
68	Gopika	98.00	9.00	88.50	27.75	79.96	10.11	20.25	71.28
69	Mahamaya	96.00	8.00	87.50	28.00	80.05	8.89	20.90	69.39
70	IGKVR-1	89.00	9.00	82.00	28.45	76.50	10.12	23.20	78.75
71	Cul-6	86.50	8.00	78.50	28.10	72.40	7.94	21.90	64.27

Sl No	Genotypes	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
72	Cul-7	93.50	7.50	89.50	27.80	72.83	11.44	21.20	67.51
73	Cul-14	107.50	8.50	100.50	27.15	77.01	8.55	21.15	62.75
74	CR DHAN202	113.50	8.00	108.00	28.65	75.64	10.00	21.85	69.05
75	CR DHAN305	115.50	10.00	112.00	27.75	74.17	9.38	20.00	70.16
76	CRDHAN204	96.50	9.00	90.00	28.30	73.40	8.39	19.75	65.22
77	CRDHAN205	94.00	8.50	90.50	27.90	75.71	9.39	21.00	66.56
78	Chomala	96.10	9.50	94.00	27.10	79.94	16.09	20.80	65.90
79	CR DHAN 101	93.50	8.50	88.50	27.80	78.99	10.67	23.30	77.78
80	Uma	100.50	11.50	94.50	28.55	83.70	13.72	24.40	77.97
81	JS-4	97.50	8.00	91.00	28.15	78.04	8.89	22.60	74.55
	Mean	112.29	8.88	91.26	27.94	78.89	11.55	22.66	70.91
	CD (0.05)	3.74	2.04	4.25	1.08	2.44	0.760	1.34	3.08

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), RWC- Relative water content (%),YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%).

Table 9: Over all mean values, range and standard deviation of rice genotypes under water stress & irrigated control in experiment II (Puncha, 2018).

Sl No	Traits	Mean		Range		SD	
		Water stress	Control	Water stress	Control	Water stress	Control
1	Plant height (Cm)	106.28	112.29	80.15-130	86.50-140.50	12.56	12.28
2	Tiller number	6.34	8.88	5.00-8.50	6.50-13.00	0.845	1.23
3	Days to 50% flowering	91.26	85.78	65.50-115.50	63.00-112.00	10.86	10.36
4	Leaf temperature (◦C)	29.56	27.94	27.30-30.55	27.10-28.70	0.548	0.385
5	Leaf rolling score	4.05		1.11-7.84		1.39	
6	Leaf drying score	4.00		1.17-7.67		1.37	
7	Relative water content (%)	61.07	78.89	50.35-72.98	70.36-86.26	4.79	3.89
8	Cell membrane stability index (%)	81.83		75.53-92.24		3.70	
9	Chlorophyll stability index (%)	82.11		75.52-92.85		3.85	
10	Yield/plant (g)	8.40	11.55	6.06-12.17	7.94-18.62	1.57	2.15
11	Thousand grain weight (g)	21.78	22.66	17.15-24.00	17.45-24.95	1.33	1.35
12	Spikelet fertility percentage (%)	61.04	70.91	50.47-73.79	61.12-81.93	4.39	4.58
13	Percentage relative yield reduction	29.46		17.91-46.94		4.96	
14	Drought susceptability index	1.00		0.75-1.17		0.070	
15	Soil moisture percentage (%)			10.75-24.96			

4.3 CORRELATION STUDIES

The data on various parameters which were recorded under the irrigated control and water stress conditions in rice genotypes were subjected to correlation analysis. The results of correlation among phenotypic traits under irrigated control are given in table 13, 14, 15 and 10, 11, 12 (water stress) respectively.

4.3.1 Correlation between drought resistant traits and yield under water stress condition in Mundakan 2017 and Puncha 2018.

In experiment I (Mundakan 2017) under water stress condition grain yield per plant showed significant & positive correlation with number of tillers (0.394^{**}), days to 50% flowering (0.535^{**}), relative water content (0.743^{**}), cell membrane stability index (0.693^{**}), chlorophyll stability index (0.688^{**}), thousand grain weight (0.717^{**}), spikelet fertility percentage (0.790^{**}) and drought susceptibility index (0.371**). Grain yield showed significant negative correlation with leaf temperature (-0.227^{**}), leaf rolling score (-0.289^{**}), leaf drying score (-0.313^{**}) and percentage relative yield reduction (-0.377^{**}). In experiment II (Puncha 2018) the yield per plant showed significant & positive correlation with relative water content (0.632^{**}), cell membrane stability index (0.576^{**}), cholorophyll stability index (0.568^{**}), thousand grain weight (0.511^{**}), spikelet fertility percentage (0.635^{**}) and drought susceptibility index (0.356^{**}). Grain yield showed significant and negative correlation with leaf temperature (-0.356^{**}), leaf rolling score (-0.266^{**}), leaf drying score (-0.237^{**}) and percentage relative yield reduction (-0.356^{**}). Under combined correlation analysis in water stress condition also grain yield per plant showed significant and positive correlation with tiller number, days to 50% flowering, relative water content, cell membrane stability index, chlorophyll stability index, thousand grain weight, spikelet fertility percentage and drought susceptibility index. Whereas it was significantly and negatively correlated with the traits leaf temperature, leaf rolling score, leaf drying score and percentage relative yield reduction.

Table 10: Correlation of traits with yield under water stress condition in experiment I (Mundakan 2017)

	PH	NOT	DFF	LT	RWC	CMS	CSI	YPP	TGW	SPF	LR	LD	RYR	DSI
PH	1	0.004^{NS}	-0.037^{NS}	0.294**	0.226**	0.296**	0.317**	0.187*	0.305**	0.171*	0.085^{NS}	-0.017^{NS}	0.022^{NS}	-0.025^{NS}
NOT	0.004^{NS}	1	-0.200*	0.237**	0.265**	0.304**	0.182*	0.394**	0.223**	0.367**	-0.205**	-0.228**	-0.042^{NS}	0.041^{NS}
DFF	-0.037^{NS}	-0.200*	1	-0.079^{NS}	-0.233**	-0.367**	-0.237**	-0.227**	-0.230**	-0.335**	0.189*	0.191*	-0.058^{NS}	0.058^{NS}
LT	0.294**	0.237**	-0.079^{NS}	1	0.556**	0.457**	0.575**	0.535**	0.427**	0.459**	-0.202*	-0.262**	-0.130^{NS}	0.129^{NS}
RWC	0.226**	0.265**	-0.233**	0.556**	1	0.775**	0.710**	0.743**	0.597**	0.755**	-0.384**	-0.423**	-0.117^{NS}	0.114^{NS}
CMS	0.296**	0.304**	-0.367**	0.457**	0.775**	1	0.664**	0.693**	0.562**	0.742**	-0.316**	-0.353**	-0.139^{NS}	0.136^{NS}
CSI	0.317**	0.182*	-0.237**	0.575**	0.710**	0.664**	1	0.688**	0.639**	0.672**	-0.283**	-0.374**	-0.086^{NS}	0.081^{NS}
YPP	0.187*	0.394**	0.535**	-0.227**	0.743**	0.693**	0.688**	1	0.717**	0.790**	-0.289**	-0.313**	-0.377**	0.371**
TGW	0.305**	0.223**	-0.230**	0.427**	0.597**	0.562**	0.639**	0.717**	1	0.660**	-0.210**	-0.225**	-0.072^{NS}	0.066^{NS}
SPF	0.171*	0.367**	-0.335**	0.459**	0.755**	0.742**	0.672**	0.790**	0.660**	1	-0.337**	-0.351**	-0.162*	0.158*
LR	0.085^{NS}	-0.205**	0.189*	-0.202*	-0.384**	-0.316**	-0.283**	-0.289**	-0.210**	-0.337**	1	0.756**	-0.097^{NS}	0.099^{NS}
LD	-0.017^{NS}	-0.228**	0.191*	-0.262**	-0.423**	-0.353**	-0.374**	-0.313**	-0.225**	-0.351**	0.756**	1	-0.063^{NS}	0.066^{NS}
RYR	0.022^{NS}	-0.042^{NS}	-0.058^{NS}	-0.130^{NS}	-0.117^{NS}	-0.139^{NS}	-0.086^{NS}	-0.377**	-0.072^{NS}	-0.162*	-0.097^{NS}	-0.063^{NS}	1	-1.000**
DSI	-0.025^{NS}	0.041^{NS}	0.058^{NS}	0.129^{NS}	0.114^{NS}	0.136^{NS}	0.081^{NS}	0.371**	0.066^{NS}	0.158*	0.099^{NS}	0.066^{NS}	-1.000**	1

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), Leaf rolling score- LR, LD- Leaf drying score, RWC- Relative water content (%), CMS- Cell membrane stability index (%), CSI- Chorophyll stability index (%), YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%), % RYR- Percentage relative yield reduction, DSI- Drought susceptibility index.

Table 11: Correlation of traits with yield under water stress condition in experiment II (Puncha 2018)

	PH	NOT	DFF	LT	RWC	CMS	CSI	TGW	SPF	YPP	LR	LD	RYR	DSI
PH	1	0.044^{NS}	0.047^{NS}	0.089^{NS}	0.245**	0.231**	0.251**	0.267**	0.118^{NS}	0.174*	-0.027^{NS}	-0.015^{NS}	0.122^{NS}	-0.121^{NS}
NOT	0.044^{NS}	1	-0.100^{NS}	-0.129^{NS}	0.107^{NS}	0.122^{NS}	0.014^{NS}	0.116^{NS}	0.104^{NS}	0.026^{NS}	-0.029^{NS}	-0.015^{NS}	0.133^{NS}	-0.133^{NS}
DFF	0.047^{NS}	-0.100^{NS}	1	-0.008^{NS}	-0.428**	-0.333**	-0.301**	-0.329**	-0.374**	-0.236**	0.099^{NS}	0.112^{NS}	-0.234**	0.234**
LT	0.089^{NS}	-0.129^{NS}	-0.008^{NS}	1	0.178*	0.243**	0.373**	0.222**	0.141^{NS}	0.253**	-0.207**	-0.126^{NS}	-0.092^{NS}	0.094^{NS}
CMS	0.245**	0.107^{NS}	-0.428**	0.178*	1	0.723**	0.628**	0.449**	0.748**	0.576**	-0.265**	-0.237**	0.138^{NS}	-0.138^{NS}
RWC	0.231**	0.122^{NS}	-0.333**	0.243**	0.723**	1	0.701**	0.478**	0.723**	0.632**	-0.374**	-0.359**	0.148^{NS}	-0.147^{NS}
CSI	0.251**	0.014^{NS}	-0.301**	0.373**	0.628**	0.701**	1	0.504**	0.619**	0.568**	-0.347**	-0.318**	0.083^{NS}	-0.083^{NS}
TGW	0.267**	0.116^{NS}	-0.329**	0.222**	0.449**	0.478**	0.504**	1	0.472**	0.511**	-0.216**	-0.218**	0.189*	-0.191*
SPF	0.118^{NS}	0.104^{NS}	-0.374**	0.141^{NS}	0.748**	0.723**	0.619**	0.472**	1	0.635**	-0.256**	-0.260**	0.086^{NS}	-0.087^{NS}
YPP	0.174*	0.026^{NS}	0.253**	-0.236**	0.576**	0.632**	0.568**	0.511**	0.635**	1	-0.266**	-0.237**	-0.356**	0.356**
LR	-0.027^{NS}	-0.029^{NS}	0.099^{NS}	-0.207**	-0.265**	-0.374**	-0.347**	-0.216**	-0.256**	-0.266**	1	0.840**	-0.030^{NS}	0.032^{NS}
LD	-0.015^{NS}	-0.015^{NS}	0.112^{NS}	-0.126^{NS}	-0.237**	-0.359**	-0.318**	-0.218**	-0.260**	-0.237**	0.840**	1	-0.051^{NS}	0.053^{NS}
RYR	0.122^{NS}	0.133^{NS}	-0.234**	-0.092^{NS}	0.138^{NS}	0.148^{NS}	0.083^{NS}	0.189*	0.086^{NS}	-0.356**	-0.030^{NS}	-0.051^{NS}	1	-1.000**
DSI	-0.121^{NS}	-0.133^{NS}	0.234**	0.094^{NS}	-0.138^{NS}	-0.147^{NS}	-0.083^{NS}	-0.191*	-0.087^{NS}	0.356**	0.032^{NS}	0.053^{NS}	-1.000**	

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (^{o}C), Leaf rolling score- LR, LD- Leaf drying score, RWC- Relative water content (%), CMS- Cell membrane stability index (%), CSI- Chorophyll stability index (%), YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%), % RYR- Percentage relative yield reduction, DSI- Drought susceptibility index

Table 12: Correlation of traits with yield under water stress (Mundakan 2017 & Puncha 2018)

	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF	CMS	CSI	LR	LD	RYR	DSI
PH	1.000	0.024^{NS}	0.011^{NS}	0.223**	0.234**	0.190*	0.296**	0.148^{NS}	0.275**	0.289**	0.026^{NS}	-0.019^{NS}	0.100^{NS}	-0.102^{N}
NOT	0.024^{NS}	1.000	-0.134^{NS}	0.197*	0.234**	0.328**	0.163*	0.352**	0.276**	0.152^{NS}	-0.181*	-0.216**	0.045^{NS}	-0.048^{NS}
DFF	0.011^{NS}	-0.134^{NS}	1.000	-0.060^{NS}	-0.317**	-0.272**	-0.316**	-0.407**	-0.424**	-0.289**	0.133^{NS}	0.140^{NS}	-0.205**	0.208**
LT	0.223**	0.197*	-0.060^{NS}	1.000	0.483**	0.461**	0.388**	0.357**	0.369**	0.552**	-0.218**	-0.190*	-0.030^{NS}	0.026^{NS}
RWC	0.234**	0.234**	-0.317**	0.483**	1.000	0.735**	0.566**	0.770**	0.770**	0.733**	-0.409**	-0.429**	0.032^{NS}	-0.039^{NS}
YPP	0.190*	0.328**	0.272**	-0.461**	0.735**	1.000	0.662**	0.762**	0.675**	0.673**	-0.295**	-0.313**	-0.273**	0.267**
TGW	0.296**	0.163*	-0.316**	0.388**	0.566**	0.662**	1.000	0.594**	0.530**	0.601**	-0.227**	-0.250**	0.110^{NS}	-0.116^{NS}
SPF	0.148^{NS}	0.352**	-0.407**	0.357**	0.770**	0.762**	0.594**	1.000	0.767**	0.668**	-0.314**	-0.348**	-0.010^{NS}	0.006^{NS}
CMS	0.275**	0.276**	-0.424**	0.369**	0.770**	0.675**	0.530**	0.767**	1.000	0.666**	-0.307**	-0.317**	0.034^{NS}	-0.038^{NS}
CSI	0.289**	0.152^{NS}	-0.289**	0.552**	0.733**	0.673**	0.601**	0.668**	0.666**	1.000	-0.342**	-0.372**	0.008^{NS}	-0.016^{NS}
LR	0.026^{NS}	-0.181*	0.133^{NS}	-0.218**	-0.409**	-0.295**	-0.227**	-0.314**	-0.307**	-0.342**	1.000	0.897**	-0.101^{NS}	0.101^{NS}
LD	-0.019^{NS}	-0.216**	0.140^{NS}	-0.190*	-0.429**	-0.313**	-0.250**	-0.348**	-0.317**	-0.372**	0.897**	1.000	-0.092^{NS}	0.092^{NS}
RYR	0.100^{NS}	0.045^{NS}	-0.205**	-0.030^{NS}	0.032^{NS}	-0.273**	0.110^{NS}	-0.010^{NS}	0.034^{NS}	0.008^{NS}	-0.101^{NS}	-0.092^{NS}	1.000	-0.999**
DSI	-0.102^{NS}	-0.048^{NS}	0.208**	0.026^{NS}	-0.039^{NS}	0.267**	-0.116^{NS}	0.006^{NS}	-0.038^{NS}	-0.016^{NS}	0.101^{NS}	0.092^{NS}	-0.999**	1.000

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), Leaf rolling score- LR, LD- Leaf drying score, RWC- Relative water content (%), CMS- Cell membrane stability index (%), CSI- Chorophyll stability index (%), YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%), % RYR- Percentage relative yield reduction, DSI- Drought susceptibility index.

4.3.2 Correlation between morpho-physiological traits and yield under irrigated control condition in Mundakan 2017 and Puncha 2018

In experiment I (Mundakan 2017) under irrigated control condition grain yield showed significant and positive correlation with plant height (0.269^{**}), tiller number (0.457^{**}), days to 50% flowering (0.382^{**}), relative water content (0.726^{**}), thousand grain weight (0.734^{**}) and spikelet fertility percentage (0.685^{**}). Grain yield showed significant and negative correlation with leaf temperature (-0.267^{**}). In experiment two (Puncha 2018) all the traits were significantly and positively correlated with grain yield except the leaf temperature (0.075^{NS}). In combined correlation analysis under water stress, yield per plant was significantly and positively correlated with the traits tiller number, days to 50% flowering, relative water content, thousand grain weight and spikelet fertility percentage. Whereas, it was significantly negatively correlated with the trait leaf temperature.

Table 13: **Correlation of traits with yield under irrigated control condition in experiment I (Mundakan 2017)**

	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
PH	1	0.109^{NS}	0.027^{NS}	0.099^{NS}	0.267^{**}	0.269^{**}	0.362^{**}	0.088^{NS}
NOT	0.109^{NS}	1	-0.228^{**}	0.312^{**}	0.375^{**}	0.457^{**}	0.349^{**}	0.451^{**}
DFF	0.027^{NS}	-0.228^{**}	1	-0.184^{*}	-0.222^{**}	-0.267^{**}	-0.127^{NS}	-0.233^{**}
LT	0.099^{NS}	0.312^{**}	-0.184^{*}	1	0.341^{**}	0.382^{**}	0.276^{**}	0.238^{**}
RWC	0.267^{**}	0.375^{**}	-0.222^{**}	0.341^{**}	1	0.726^{**}	0.582^{**}	0.622^{**}
YPP	0.269^{**}	0.457^{**}	0.382^{**}	-0.267^{**}	0.726^{**}	1	0.734^{**}	0.685^{**}
TGW	0.362^{**}	0.349^{**}	-0.127^{NS}	0.276^{**}	0.582^{**}	0.734^{**}	1	0.612^{**}
SPF	0.088^{NS}	0.451^{**}	-0.233^{**}	0.238^{**}	0.622^{**}	0.685^{**}	0.612^{**}	1

Table 14: Correlation of traits with yield under irrigated condition in experiment II (Puncha 2018)

	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
PH	1	0.155^{*}	0.008^{NS}	-0.039^{NS}	0.214^{**}	0.261^{**}	0.241^{**}	0.115^{NS}
NOT	0.155^{*}	1	-0.231^{**}	0.116^{NS}	0.312^{**}	0.446^{**}	0.135^{NS}	0.446^{**}
DFF	0.008^{NS}	-0.231^{**}	1	-0.117^{NS}	-0.284^{**}	-0.357^{**}	-0.057^{NS}	-0.259^{**}
LT	-0.039^{NS}	0.116^{NS}	-0.117^{NS}	1	0.071^{NS}	0.075^{NS}	0.089^{NS}	0.202^{*}
RWC	0.214^{**}	0.312^{**}	-0.284^{**}	0.071^{NS}	1	0.696^{**}	0.458^{**}	0.681^{**}
YPP	0.261^{**}	0.446^{**}	0.357^{**}	0.075^{NS}	0.696^{**}	1	0.578^{**}	0.678^{**}
TGW	0.241^{**}	0.135^{NS}	-0.057^{NS}	0.089^{NS}	0.458^{**}	0.578^{**}	1	0.465^{**}
SPF	0.115^{NS}	0.446^{**}	-0.259^{**}	0.202^{*}	0.681^{**}	0.678^{**}	0.465^{**}	1

Table 15: Correlation of traits with yield under irrigated control (Mundakan 2017 & Puncha 2018)

	PH	NOT	DFF	LT	RWC	YPP	TGW	SPF
PH	1.000	0.135^{NS}	0.019^{NS}	0.040^{NS}	0.253**	0.271**	0.316**	0.101^{NS}
NOT	0.135^{NS}	1.000	-0.250**	0.295**	0.389**	0.487**	0.280**	0.481**
DFF	0.019^{NS}	-0.250**	1.000	-0.221**	-0.268**	-0.320**	-0.096^{NS}	-0.252**
LT	0.040^{NS}	0.295**	-0.221**	1.000	0.352**	0.314**	0.285**	0.328**
RWC	0.253**	0.389**	-0.268**	0.352**	1.000	0.746**	0.570**	0.693**
YPP	0.271**	0.487**	0.320**	-0.314**	0.746**	1.000	0.704**	0.708**
TGW	0.316**	0.280**	-0.096^{NS}	0.285**	0.570**	0.704**	1.000	0.574**
SPF	0.101^{NS}	0.481**	-0.252**	0.328**	0.693**	0.708**	0.574**	1.000

PH- Plant height (cm), NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C), RWC- Relative water content (%),YPP- Yield per plant (g) , TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%).

4.4 PRINCIPAL COMPONENT ANALYSIS

Phenotypic data of 81 rice genotypes for 14 morpho-physiological and plant production traits under drought stress were utilized to generate genotype by trait biplot graph (Figure:1) for analysis with first two principal components. Table 16 presents the details of the first 2 principal components under water stress and Table 17 presents the details of proportion of variance of 14 principal components. Among the 14 principal components under water stress the first principal component explained 41.77% of variation, while second component explained 16.57% of variation. Among the 14 morpho-physiological and plant production traits relative water content, cell membrane stability index and chlorophyll stability index, yield and spikelet fertility percentage contributed towards maximum for diversity. The 1st (top left) and 4th (bottom left) quadrant contained 31 genotypes , out of which 13 genotypes from 1st and 4th quadrant were identified as drought tolerant varieties and these varieties exhibited high leaf temperature, relative water content, cell membrane stability index, chlorophyll stability index, yield and spikelet fertility percentage. Under drought stress condition important parameters like relative water content, cell membrane stability index, chlorophyll stability index and spikelet fertility percentage showed higher values for genotypes in 1st and 4th quadrant. The 2nd and 3rd quarter contains almost all the local varieties that includes moderately tolerant and susceptible varieties exhibiting higher leaf rolling score and drying score. The encircled area in the figure; depicted five highly drought tolerant genotypes *viz*., PTB 7, PTB 15, PTB27, PTB28, PTB29, whereas other highly drought tolerant varieties including PTB55, PTB60, N-22 and chomala were farther from the circle in the 1st (top left) and 4th (bottom left) quadrant respectively.

Under irrigated control condition total of eight morpho-physiological and plant production traits were used to generate the biplot graph (Figure: 2). Details of the components are given in table 18 and proportion of variance of each principal components are given in table 19. Under irrigated control condition the first principal component explained 48.9 per cent of variation, while second component explained 16.57 per cent of variation. Among the 8 morpho-physiological and plant production traits relative water content, spikelet fertility percentage contributed towards maximum diversity. The 1st (top left) and 4th (bottom left) quadrant contained 36 genotypes , out of which 13 genotypes from 1st and 4th quadrant were identified as drought tolerant varieties and these varieties exhibited high leaf temperature, relative water content, tiller number, yield and spikelet fertility

percentage. Under irrigated control condition important parameters like relative water content, thousand grain weight and spikelet fertility percentage showed higher values for genotypes in 1st and 4th quadrant. The 2nd and 3rd quarter contains almost all the local varieties that include moderately tolerant and susceptible varieties. The encircled area in the figure; depicted ten drought tolerant and high yielding genotypes *viz*., PTB 7, PTB 15, PTB 25, PTB27, PTB28, PTB29, PTB 35,PTB 55, PTB 60 and Uma.

Table 16: Principal component analysis under water stress

Characters	PC1	PC2
Proportion of Variance	0.4177	0.165
Plant height	-0.1118	0.2988
Tiller number	-0.1810	0.0089
Days to 50 % Flowering	0.1690	0.0054
Leaf temperature	-0.2506	0.0881
Relative water content	-0.3725	-0.0374
Cell membrane stability index	-0.3648	-0.0011
Chlorophyll stability index	-0.3542	0.0742
Yield	-0.3599	-0.1511
1000 grain weight	-0.2979	0.0864
Spikelet fertility percentage	-0.3579	-0.1607
Leaf rolling score	0.2364	-0.1286
Leaf drying score	0.2490	-0.0918
Percentage relative yield reduction	0.0282	0.6380
Drought susceptibility index	-0.0270	-0.6384

Table 17: Proportion of variance of different principal components under water stress

Sl No	Principal components	Proportion of variance
1	PC1	0.417
2	PC2	0.165
3	PC3	0.104
4	PC4	0.079
5	PC5	0.058
6	PC6	0.046
7	PC7	0.037
8	PC8	0.028
9	PC9	0.016
10	PC10	0.012
11	PC11	0.011
12	PC12	0.010
13	PC13	0.008
14	PC14	0.000

Table 18: Principal component analysis under irrigated control

Characters	PC1	PC2
Proportion of Variance	0.489	0.145
Plant height	-0.1654	0.6495
Tiller number	-0.3490	-0.2160
Days to 50 % flowering	0.2068	0.5611
Leaf temperature	-0.2860	-0.3006
Relative water content	-0.4306	0.0707
Yield	-0.4554	0.0937
1000 grain weight	-0.3862	0.3278
Spikelet fertility percentage	-0.4290	-0.0694

Table 19: Proportion of variance of different principal components under control

Sl No	Principal components	Proportion of variance
1	PC1	0.489
2	PC2	0.145
3	PC3	0.100
4	PC4	0.088
5	PC5	0.078
6	PC6	0.048
7	PC7	0.030
8	PC8	0.019

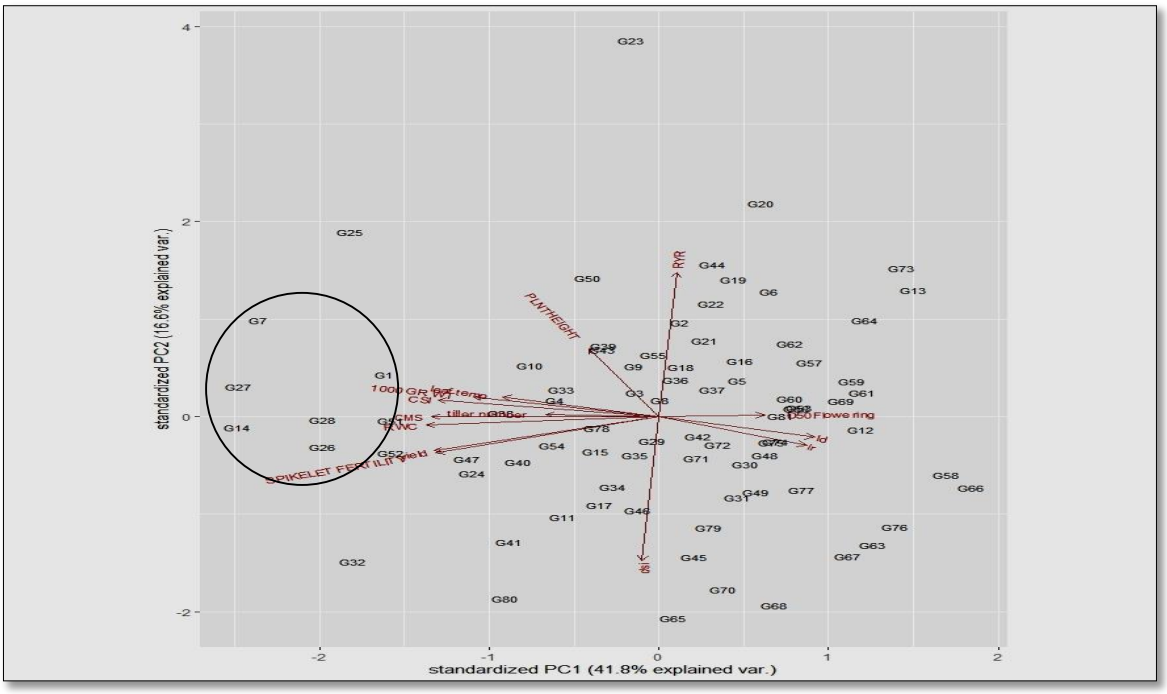

Figure: 1 Genotype-by-trait-biplot analysis of 81 rice genotypes for two principal components under water stress

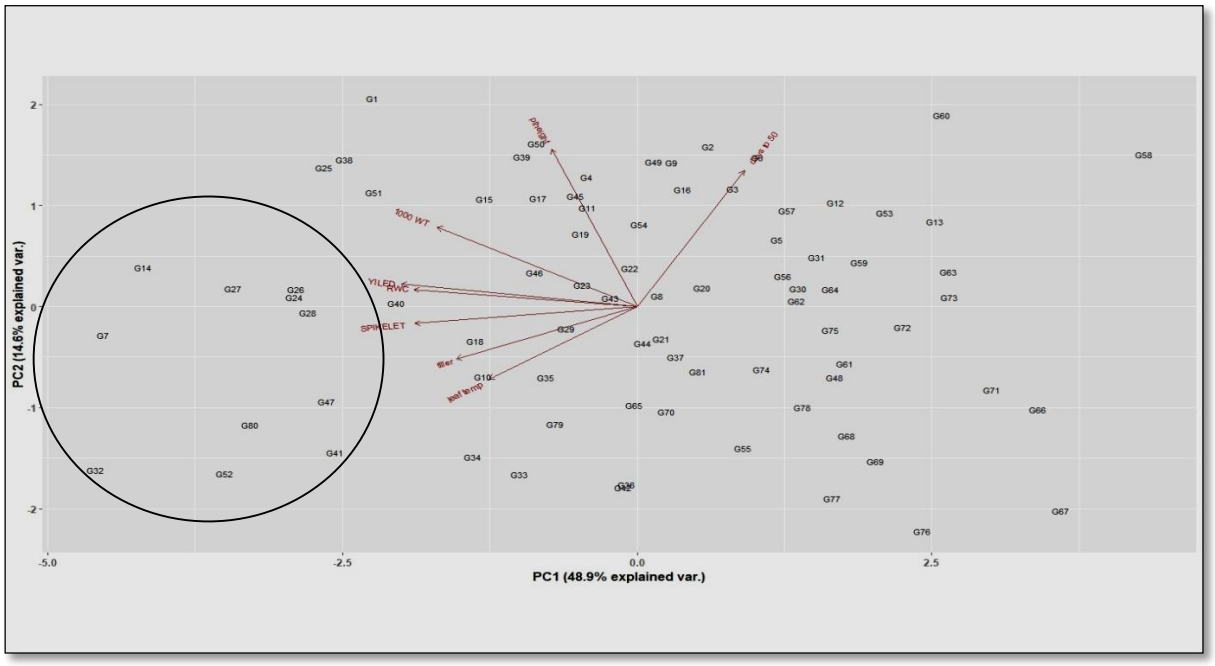

Figure: 2 Genotype-by-trait-biplot analysis of 81 rice genotypes for two principal components under irrigated control

4.5 CLUSTER ANALYSIS

Clustering by Ward method was done to establish a relationship between the 81 rice genotypes. Similar types of genotypes were clustered together based on the phenotypic data. All the genotypes were clustered into mainly 5 clusters under water stress as well as irrigated control condition. Under water stress condition the maximum number of genotypes (25) was clustered in cluster IV, whereas minimum numbers of genotypes were grouped in cluster II (10) cluster I, III and V consisted of 14, 19 and 12 genotypes respectively (Figure 3). Cluster I comprised of most of the identified drought tolerant and high yielding genotypes. Some drought tolerant varieties are grouped in cluster V also. Cluster mean and general mean for each of the traits under water stress are shown in table 20. Under irrigated control condition the maximum number of genotypes were grouped in cluster II (24) and minimum in cluster III (11). Cluster I, IV and V comprised of 14, 15 and 17 genotypes (Figure: 4). In which most of the drought tolerant and high yielding varieties are grouped in cluster I. Table 21 represents cluster mean and general mean of the traits under irrigated control condition.

Table 20: Cluster mean and general mean for morpho-physiological and plant production traits under water stress in rice.

TRAITS	CLUSTER1	CLUSTER2	CLUSTER3	CLUSTER4	CLUSTER5	GEN MEAN
PH	111.614	99.348	108.229	107.119	112.390	107.740
NOT	7.696	6.475	6.763	6.610	7.292	6.967
DFF	80.911	93.625	93.842	85.270	78.979	86.525
LT	28.768	28.110	28.082	28.433	28.490	28.376
RWC	69.998	57.997	59.851	63.407	63.248	62.900
CMS	88.997	80.536	80.748	82.873	84.393	83.509
CSI	88.499	80.158	81.296	84.752	84.955	83.932
YPP	11.558	7.035	7.853	9.259	8.295	8.800
TGW	23.662	20.213	21.388	22.833	22.242	22.068
SPF	70.521	60.090	59.737	63.068	62.493	63.182
LR	3.425	6.117	6.564	5.269	4.626	5.200
LD	3.051	5.680	6.722	5.355	4.220	5.006
RYR	29.706	24.609	26.034	25.408	32.289	27.609
DSI	0.970	1.041	1.021	1.029	0.934	0.999

PH- Plant height (cm) , NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (°C) , Leaf rolling score- LR, LD- Leaf drying score, RWC- Relative water content (%), CMS- Cell membrane stability index (%) , CSI- Chorophyll stability index (%), YPP- Yield per plant (g), TGW- Thousand grain weight (g) , SPF- Spikelet fertility percentage (g), % RYR- Percentage relative yield reduction, DSI- Drought susceptibility index.

Table 21: Cluster mean and general mean for morpho-physiological and plant production traits under irrigated control condition in rice

TRAITS	CLUSTER1	CLUSTER2	CLUSTER3	CLUSTER4	CLUSTER5	GEN MEAN
PH	118.13	105.70	103.65	124.48	120.54	114.50
NOT	11.12	8.82	10.13	9.16	9.30	9.71
DFF	84.51	98.70	86.72	88.45	99.79	91.63
LT	27.90	27.46	27.89	27.72	27.58	27.71
RWC	86.54	78.13	81.23	80.43	83.23	81.91
YPP	15.61	10.13	12.02	11.31	13.09	12.43
TGW	24.35	21.76	23.49	22.82	23.95	23.27
SPF	79.97	69.08	74.98	70.69	74.33	73.81

PH- Pant height , NOT- no. of tillers, DFF- Days to 50% flowering, LT- Leaf temperature (ºC) RWC- Relative water content (%), YPP- Yield per plant (g), TGW- Thousand grain weight (g), SPF- Spikelet fertility percentage (%).

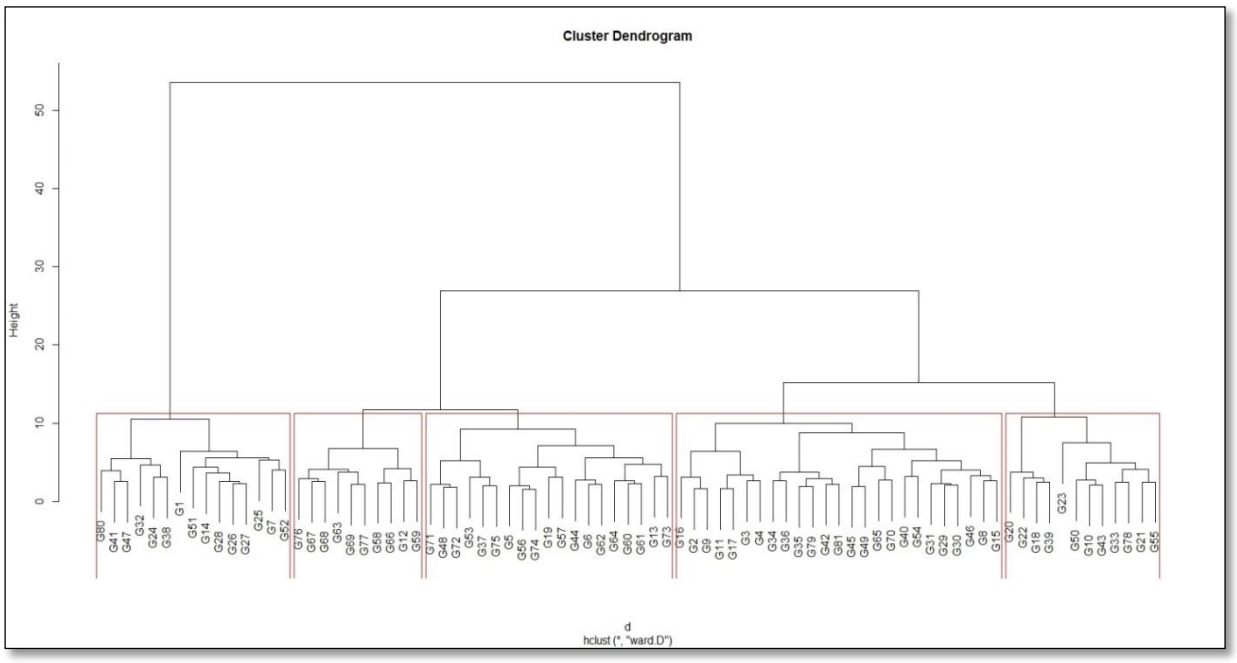

Figure 3: Cluster analysis under water stress based on morpho-physiological and plant production traits

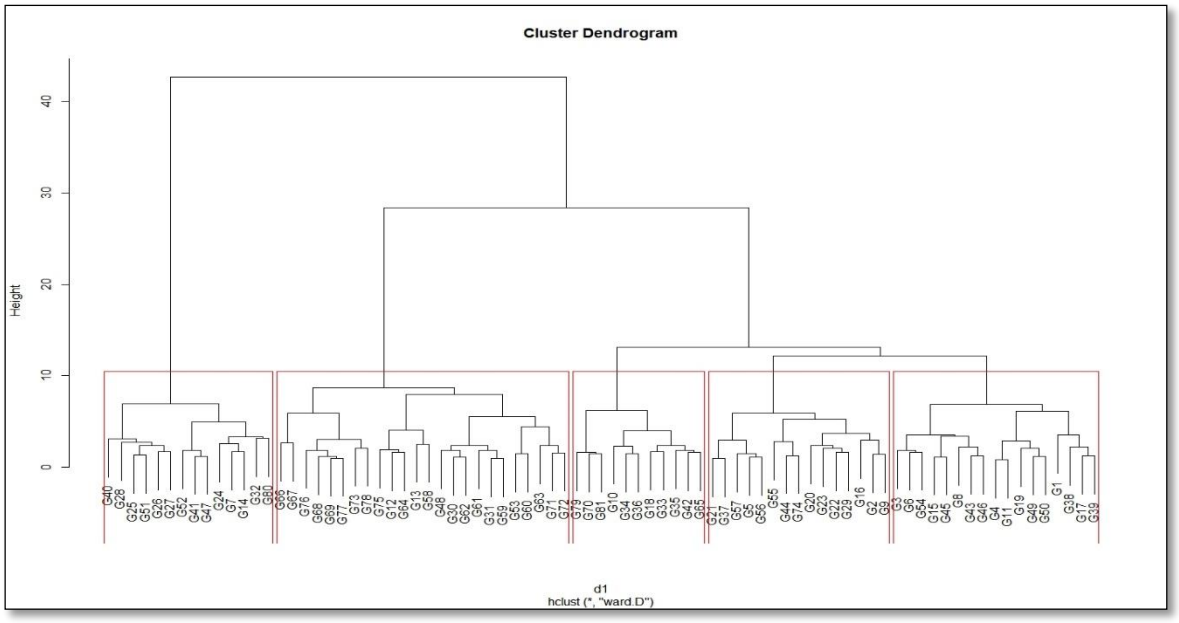

Figure 4: Cluster analysis under irrigated control based on morpho-physiological and plant production traits.

4.6 QUALITY AND QUANTITY ASSESSMENT OF DNA SAMPLES

Purity of DNA samples obtained for selected 81 genotypes for association genetic analysis are presented in the table 22.

4.7 IDENTIFICATION OF SSR MARKERS ASSOCIATED WITH DROUGHT TOLERANT AND PLANT PRODUCTION TRAITS IN RICE USING ASSOCIATION GENETIC ANALYSIS

A total of 100 SSR markers distributed in different chromosomes of rice were selected randomly and used to amplify the DNA samples. The amplified products were run along the agarose gel stained using ethidium bromide. The amplified products were visualized and documented in gel documentation system. The bands developed were scored as per the standard procedure. Out of the 100 SSR markers screened 40 markers showed polymorphism (Plates: 6-23). The genotypic score value of 100 SSR markers were used in the software STRUCTURE to get the population structure value. Genotypic score, phenotypic data and population structure value were used in the software TASSEL to identify the molecular markers/ QTLs linked to drought tolerant and plant production traits.

Table 22: Quality of DNA samples of rice genotypes used for association genetic analysis

Sl No	Varieties	A_{260}/A_{280} value	Sl No	Varieties	A_{260}/A_{280} value	Sl No	Varieties	A_{260}/A_{280} value
1	PTB1	1.79	31	PTB33	1.72	61	Kuttithekkan	1.73
2	PTB2	1.80	32	PTB34	1.84	62	Jaya	1.89
3	PTB3	1.81	33	PTB35	1.80	63	Jeerakashala	1.78
4	PTB4	1.82	34	PTB36	1.85	64	Ponmani	1.77
5	PTB5	1.77	35	PTB37	1.76	65	Shreyas	1.71
6	PTB6	1.79	36	PTB38	1.86	66	Prathyasha	1.79
7	PTB7	1.83	37	PTB39	1.88	67	Velluthataryan sel	1.74
8	PTB8	1.80	38	PTB40	1.77	68	Gopika	1.74
9	PTB9	1.76	39	PTB41	1.86	69	Mahamaya	1.82
10	PTB10	1.82	40	PTB43	1.73	70	IGKVR-1	1.86
11	PTB12	1.84	41	PTB45	1.84	71	Cul-6	1.79
12	PTB13	1.82	42	PTB46	1.88	72	Cul-7	1.80
13	PTB14	1.79	43	PTB49	1.79	73	Cul-14	1.87
14	PTB15	1.80	44	PTB50	1.75	74	CR DHAN 202	1.83
15	PTB17	1.80	45	PTB51	1.82	75	CR DHAN 305	1.82
16	PTB18	1.81	46	PTB52	1.80	76	CR DHAN 204	1.82
17	PTB19	1.81	47	PTB55	1.75	77	CR DHAN 205	1.84
18	PTB20	1.81	48	PTB56	1.81	78	Chomala	1.80
19	PTB21	1.79	49	PTB57	1.78	79	CR DHAN 101	1.73
20	PTB22	1.80	50	PTB58	1.65	80	Uma	1.88
21	PTB23	1.83	51	PTB59	1.90	81	JS-4	1.76
22	PTB24	1.81	52	PTB60	1.75			
23	PTB25	1.81	53	Sampada	1.87			
24	PTB26	1.74	54	Kunju Kunju varna	1.75			
25	PTB27	1.90	55	N-22	1.76			
26	PTB28	1.73	56	ASD-16	1.77			
27	PTB29	1.68	57	ADT37	1.73			
28	PTB30	1.78	58	Kazhiama	1.76			
29	PTB31	1.78	59	Pandichempan	1.78			
30	PTB32	1.86	60	Gandhakashala	1.80			

4.8 POPULATION STRUCTURE

For analyzing the populations structure a set of 100 SSR markers were selected for 81 genotypes. To analyse the data set, parameters of the population admixture model and correlated frequency of alleles were considered. The hypothetical subpopulations were considered as K= 2– 8 and, the package was run with 5 independent runs for each K. Length of burn-in period were set as 50,000. By inferring on Delta K of Evano *et al.* (2005) identified the most the suitable K value for determining genetic clusters as K = 2 (Figure: 5). Summary plot of Q matrix is showed in Figure 6. The number of populations were visualized using STRUCTURE 2.3.4, where the genotypes that scored >0.80 were considered as pure and <0.80 as admixture. When K= 2 all 81 genotypes were grouped into two major groups. First group was comprised of 65 genotypes and the second group comprised of 16 genotypes with 6 admixtures, drought tolerant genotypes formed the second cluster and all other genotypes comprised the first cluster.

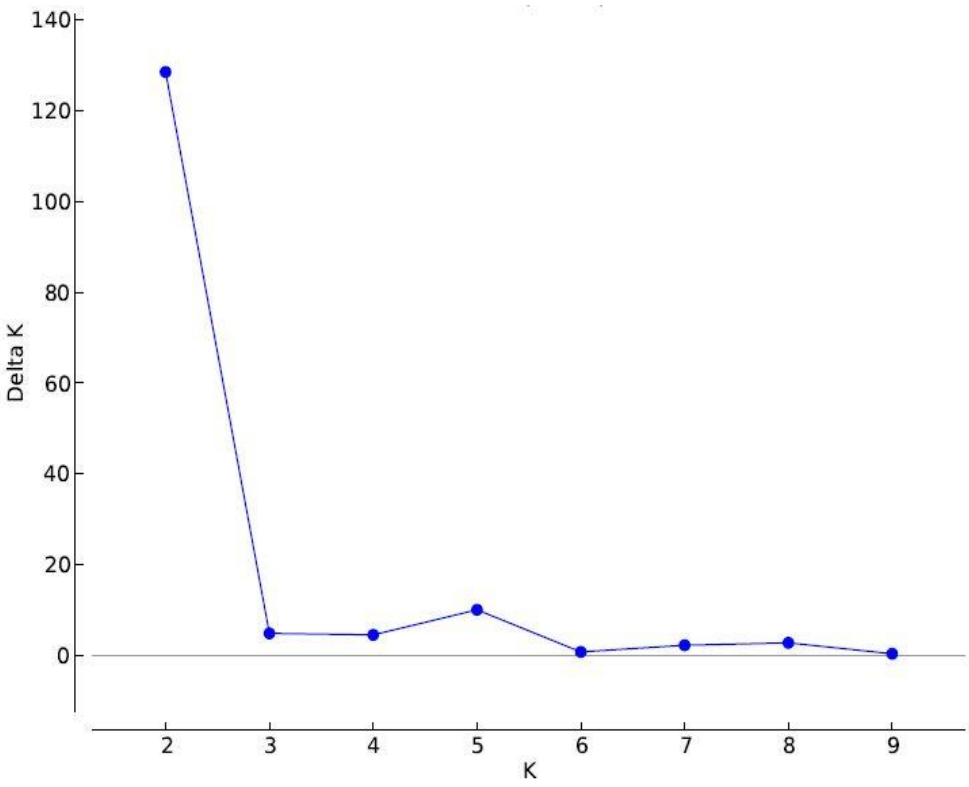

Figure 5: Estimates of subpopulations using delta K-values

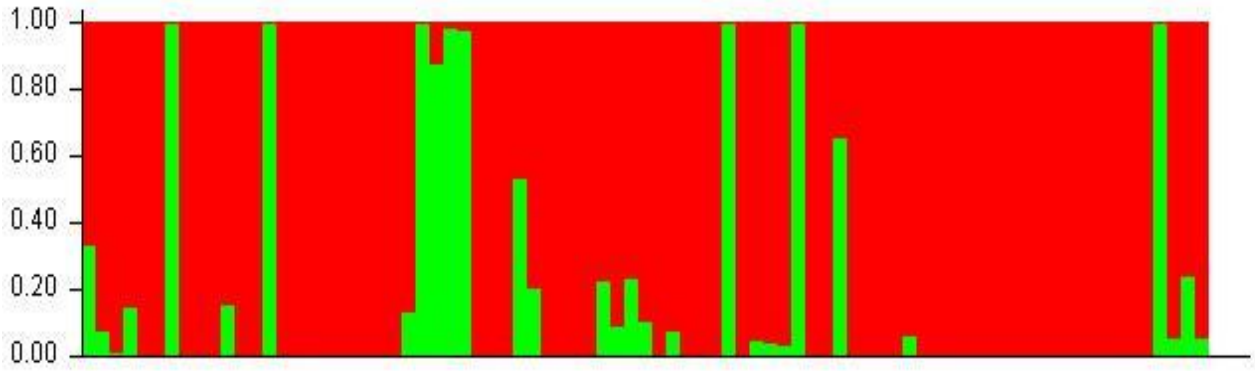

Figure 6: The summary plot of Q matrix estimates

4.9 LINKAGE DISEQUILIBRIUM

In the two hydrological treatments, a large phenotypic and genotypic variation was observed among the 81 rice genotypes for all characters. This result showed that this germplasm can generate considerable phenotypic variation for association mapping. A total of 100 SSR markers were used and 40 were polymorphic among the accessions. In this study, we used the association genetic analysis to map QTLs for the 14 morpho-physiological and plant production traits assessed under the two hydrological treatments. Using genotypic data from 100 SSR markers, the linkage disequilibrium pattern of 81 rice accessions was generated. A total of 136 associations were observed in GLM analysis and MLM analysis resulted in 29 marker trait association based on the *P* and r^2values in water stress as well as irrigated control condition. LD was distributed unequally on each chromosome was more concentrated on chromosomes 1 and 5. LD analysis in the whole population showed there were significant LD pairs (P<0.05). Total 52 LD pairs were observed under water stress and irrigated control condition and out of these there were 46 inter chromosomal LD pairs and 6 intra chromosomal LD pairs. The LD scatter plot showed a reduction in the number of significant LD pairs as the interval distances between marker pairs increased. There was a sharp decline in LD decay for the linked markers at 250 Cm. Triangle plots for pair wise LD between SSR markers demonstrated significant LD blocks in the association genetic analysis in water stress condition (Figure:7) and irrigated control condition (Figure: 8).

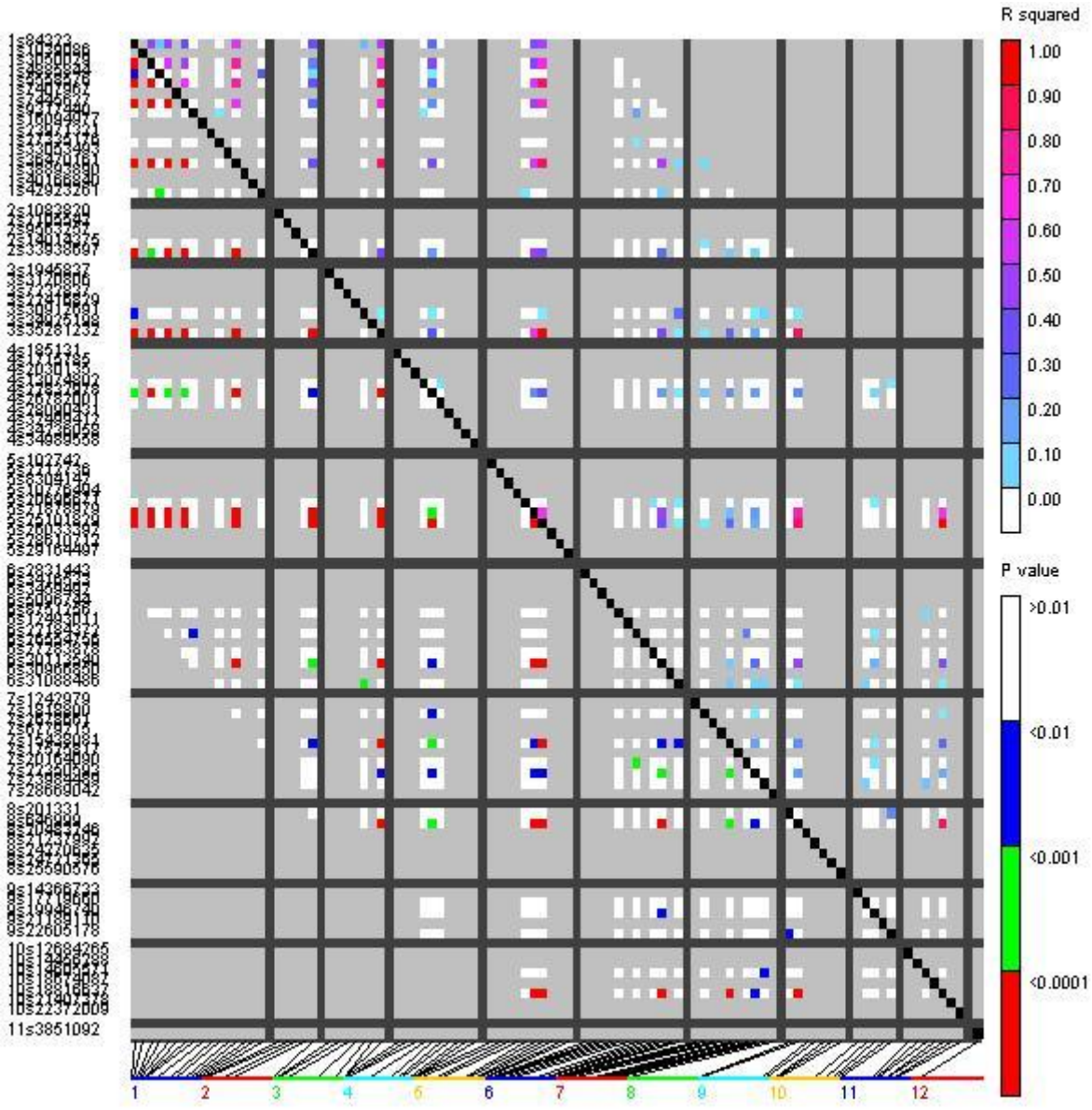

Figure 7: The triangle LD plot for a pair wise SSR marker under water stress condition: Polymorphic SSR markers were plotted as different colour codes. Each pixel above the diagonal represents the r^2 value of corresponding markers and each pixel below the diagonal represents the *P* value.

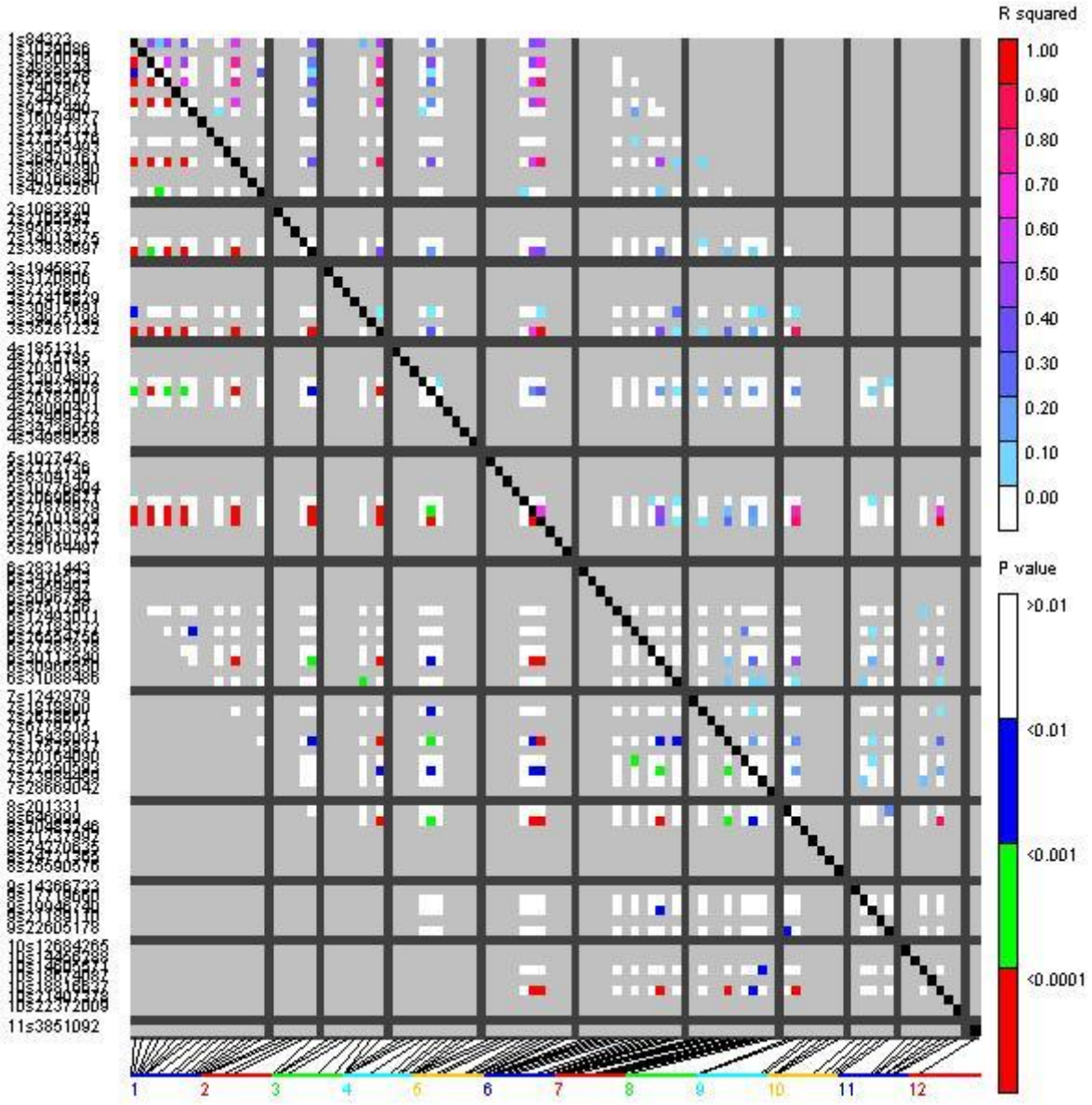

Figure 8: The triangle LD plot for a pair wise SSR marker under irrigated control condition: Polymorphic SSR markers were plotted as different colour codes. Each pixel above the diagonal represents the r^2 value of corresponding markers and each pixel below the diagonal represents the *P* value.

4.10 MARKER TRAIT ASSOCIATION

The marker trait association for all the traits under water stress and irrigated control condition were calculated using GLM and MLM model of TASSEL 5 software. In GLM method, we are considering only population structure value. While, in MLM analysis we are considering both population structure and kinship value. The results of association analysis under water stress and irrigated control condition by GLM analysis are presented in table 23 and 24. Marker trait association by MLM analysis under two hydrological treatments are presented in table 25 & 26. The squared allelic frequency correlation values in GLM analysis ranges from 0.0506-0.5113. The GLM analysis revealed that there were 136 marker trait associations including water stress and irrigated control condition, and MLM analysis resulted 29 marker trait associations.

4.10.1 Marker trait association under water stress by GLM analysis

Under water stress condition a total of 5 markers (RM1026, RM1019, RM5923, RM5633 and RM1032) were associated with plant height were identified. The associated markers were presented on chromosome 1, 4, 9, 8 and 11. Significant association for tiller numbers were observed for markers RM48, RM461, RM455, RM151 and RM1178 which were mapped on chromosomes 6, 7, 1 and 2. A total of 3 markers were identified for the trait days to 50% flowering and these are RM1018, RM455 and RM5633 and which were mapped on chromosomes 4 and 7.

Strong associations for the leaf temperature were observed with RM6100, RM3825, RM6925, RM5715, RM1090, RM1178, RM514 and RM178. These markers were mapped on chromosomes 10, 1, 8, 12, 5, 2 and 3. Among the markers RM 3825 wad having higher *P* value (4.98E-06) and RM6100 was having higher r^2value (0.244). In total seven SSR markers were detected to be associated with the trait relative water content and these are RM6100, RM3825, RM6925, RM1090, RM1031, RM5642 and RM259. These markers were mapped on chromosomes 1, 5, 6 and 8. Among the identified markers *P* value was lower for RM1090 (7.83E-08) and RM6925 was having higher r^2value (0.388). A total of 13 markers were associated with cell membrane stability index and those markers are RM6100, RM3825, RM6925,RM 490, RM462, RM455, RM1031, RM259, RM474, RM72, RM17, RM151 and RM520. The associated markers are presented on chromosomes 1, 3, 6, 7, 8, 10 and 12. Among the associated markers the higher r^2 (0.511) and lower *P* value (6.43E-14) were observed for marker RM3825. There were total 9 SSR markers associated

with the trait chlorophyll stability index and those markers are RM6100, RM3825, RM6925, RM490, RM1090, RM48, RM462, RM1067 and RM259. The associated markers were presented on chromosomes 1, 2, 5, 8 and 10. Among the identified markers RM6925 was having higher r^2value (0.406) and *P* value was lower for RM6100 (7.69E-10). A total 5 SSR markers were associated with the traits leaf rolling score and leaf drying score. The identified markers are RM6100, RM514, RM178, RM17 and RM259; these markers are identified on chromosomes 1, 3, 5, 10 and 12.

Strong associations for the yield per plant were observed with RM3825, RM3042, RM6925, RM462, RM461, RM283, RM328, RM455, RM48 and RM1067. The associated markers were presented on chromosomes 1, 2, 4, 7, 6, 8 and 9. Among the associated markers RM461 was having higher r^2value (0.292) and *P* value was lower for RM328 (9.08E-06). The markers associated with thousand grain weight are RM3825, RM3042, RM6925, RM462 and RM3351. The associated markers were presented on chromosomes 1, 3, 5 and 12. Among the identified markers higher r^2 (0.208) and lower *P* value (1.87E-05) were observed for the marker RM3042. Spikelet fertility percentage was associated with the markers RM3825, RM462, RM461, RM259, RM105 and RM455. The associated markers are located on chromosomes 1, 6, 7 and 9. Among the identified markers higher r^2 value was observed for the SSR marker RM461 (0.329) and *P* value was lower for RM455 (8.95E-07). Two same SSR markers were associated with traits percentage relative yield reduction and drought susceptibility index and those are RM1132 and RM1019 which were identified on chromosomes 7 & 8.

4.10.2 Marker trait association under irrigated control

There were total 50 marker trait association were observed under irrigated control condition. Considering the GLM statistics, RM1026, RM1019, RM5923, RM5715, RM5633, RM1032, RM1031 and RM520 was associated with the trait plant height. The associated markers are located on chromosomes 1, 4, 6, 8, 9, 11 and 12. Among the identified markers the RM1019 was having lower *P* value and higher r^2value thus indicating the most significant marker for plant height. Significant association for the trait tiller number was observed with SSR markers RM490, RM461, RM455, RM3825, RM3042 and RM462. These associated markers are located on chromosomes 1, 4, 6 and 7. Among the associated markers RM461 was having lower *P* value and higher r^2value (0.241). Lowest *P* value was observed for the marker RM3042 (9.79E-05). A total of 5 SSR markers were associated with

days to 50% flowering. These markers are RM490, RM514, RM178, RM17 and RM474. Among the identified markers the strong associations was observed for marker RM490 with higher r^2value (0.117) and lower *P* value (0.00175).

A significant association were observed with the trait leaf temperature on SSR markers RM3825, RM3042, RM474, RM259 and RM1083. These associated markers are presented on chromosomes 1, 4, 7 and 10. Among the identified markers strong associations was observed with markers RM259 with higher r^2value (0.236) and RM3042 with lower *P* value (6.09E-04). Total 9 SSR markers were linked to relative water content. The associated markers are RM6100, RM3825, RM6925, RM462, RM455, RM259, RM514, RM178 and RM5642. The markers are located on chromosomes 1, 3, 5, 6, 7, 8 and 10. Among the identified markers the strong association observed with markers RM462 with higher r^2value (0.308) and RM3825 with lower *P* value (8.55E-07). The common markers observed for leaf temperature and relative water content are RM3825 and RM259.

Yield per plant was associated with the markers RM3825, RM3042, RM6925, RM48, RM461, RM462 and RM1090. The identified markers are located on chromosomes 1, 2, 4, 5, 6 and 8. Among the markers higher r^2value was observed for RM462 (0.316) and *P* value was lower for RM1090 (6.36E-06) thus indicating the strong association. Two markers were associated with the trait thousand grain weight were identified on chromosomes 1 and 4. The identified markers are RM3042 and RM283. Spikelet fertility percentage was associated with the SSR markers RM462, RM461, RM455, RM151, RM48, RM6925, RM3825 and RM1067. The associated markers are presented on chromosomes 1, 2, 6, 7 and 8. Among the associated marker strong association was observed with RM 462 (0.250). The common markers observed for yield and spikelet fertility percentage are RM3825 and RM462.

The markers RM3825, RM6925, RM3042, RM462 and RM455 showed significant associations with many phenotypic traits in both water stress as well as irrigated control conditions.

Table 23: Marker trait association under water stress by GLM analysis

Sl No	Traits associated	Markers	*P* Value	r^2 value	Chromosome No
1	Plant height	RM1026	0.00332	0.10403	9
		RM1019	8.47E-04	0.13223	8
		RM5923	0.03381	0.05576	11
		RM5633	0.01736	0.06956	4
		RM1032	0.00738	0.08739	1
2	Tiller Number	RM48	0.00112	0.12658	2
		RM 461	0.00164	0.11869	6
		RM455	2.29E-04	0.15881	7
		RM151	4.38E-05	0.1916	1
		RM1178	0.00329	0.10421	2
3	Days to 50% flowering	RM1018	0.02669	0.06064	4
		RM455	0.01724	0.0697	7
		RM5633	0.04161	0.0515	4
4	Leaf temperature	RM6100	2.69E-06	0.24461	10
		RM3825	4.98E-06	0.23317	1
		RM6925	3.83E-06	0.23808	8
		RM5715	0.03265	0.05648	12
		RM1090	1.34E-04	0.16953	5
		RM1178	0.00159	0.1193	2
		RM514	7.83E-04	0.13386	3
		RM178	7.83E-04	0.13386	5
5	Relative water content	RM6100	2.04E-08	0.3301	10
		RM3825	2.13E-08	0.32942	1
		RM6925	4.99E-10	0.38898	8
		RM1090	7.83E-08	0.30747	5
		RM1031	2.02E-06	0.24986	6
		RM5642	2.46E-05	0.20283	6
		RM259	1.09E-06	0.26107	1
6	Leaf rolling score	RM6100	1.47E-07	0.29659	10
		RM514	1.33E-07	0.29833	3
		RM178	1.33E-07	0.29833	5
		RM17	1.33E-07	0.29833	12
		RM259	1.74E-05	0.20951	1
7	Leaf drying score	RM6100	2.02E-08	0.33026	10
		RM514	1.09E-08	0.34035	3
		RM178	1.09E-08	0.34035	5
		RM17	1.09E-08	0.34035	12
		RM151	5.05E-06	0.23291	1
		RM259	2.21E-06	0.24824	1

Sl No	Traits associated	Markers	*P* Value	r^2 value	Chromosome No
8	Cell membrane stability index	RM6100	1.01E-10	0.41279	10
		RM3825	6.43E-14	0.51133	1
		RM6925	1.58E-10	0.4062	8
		RM490	1.72E-11	0.43809	1
		RM462	1.83E-12	0.46868	1
		RM455	1.08E-10	0.41176	7
		RM1031	6.02E-04	0.13923	6
		RM259	5.13E-08	0.31467	1
		RM474	3.88E-07	0.27963	7
		RM72	6.19E-07	0.27134	7
		RM17	5.90E-10	0.38643	12
		RM151	2.12E-06	0.24905	1
		RM520	0.03916	0.05275	3
9	Chlorophyll stability index	RM6100	7.69E-10	0.38238	10
		RM3825	1.57E-09	0.37133	1
		RM6925	1.55E-10	0.40648	8
		RM490	2.85E-06	0.24353	1
		RM1090	5.26E-07	0.27425	5
		RM48	2.07E-07	0.29068	2
		RM462	5.66E-08	0.31301	1
		RM1067	1.27E-08	0.33796	1
		RM259	1.48E-08	0.33542	1
10	Yield/plant	RM3825	1.33E-06	0.25747	1
		RM3042	2.57E-07	0.28687	4
		RM6925	2.15E-06	0.24876	8
		RM462	8.81E-07	0.26498	1
		RM461	1.81E-07	0.29299	6
		RM283	1.94E-05	0.20743	1
		RM328	9.08E-06	0.22187	9
		RM455	1.75E-05	0.20931	7
		RM48	4.72E-05	0.19015	2
		RM1067	5.54E-04	0.14092	1
11	Thousand grain weight	RM 3825	0.00217	0.1128	1
		RM 3042	1.87E-05	0.20814	4
		RM6925	0.00282	0.10741	8
		RM462	0.0032	0.10476	1
		RM3351	0.04347	0.05061	5

Sl No	Traits associated	Markers	P Value	r^2 value	Chromosome No
12	Spikelet fertility percentage	RM3825	5.09E-08	0.31478	1
		RM462	6.83E-08	0.3098	1
		RM461	2.09E-08	0.32968	6
		RM259	2.71E-07	0.28596	1
		RM105	3.77E-06	0.23839	9
		RM455	8.95E-07	0.26469	7
13	Relative yield reduction	RM1132	0.00225	0.11213	7
		RM1019	0.01015	0.08073	8
14	Drought susceptibility index	RM1132	0.00184	0.11621	7
		RM1019	0.01103	0.07901	8

Table 24: Marker trait association under irrigated control by GLM analysis

Sl No	Traits associated	Markers	*P* Value	r^2 value	Chromosome No
1	Plant height	RM1026	0.00203	0.11423	9
		RM1019	2.65E-04	0.15585	8
		RM5923	0.02164	0.06498	11
		RM5715	0.01436	0.0735	12
		RM5633	0.01169	0.07779	4
		RM1032	0.00719	0.08792	1
		RM1031	0.02035	0.06625	6
		RM520	0.04139	0.05161	3
2	Tiller Number	RM490	4.67E-06	0.23439	1
		RM461	3.24E-06	0.24119	6
		RM455	6.75E-06	0.22746	7
		RM3825	4.47E-05	0.1912	1
		RM3042	9.79E-05	0.17577	4
		RM462	3.80E-05	0.19438	1
3	Days to 50% flowering	RM490	0.00175	0.11727	1
		RM514	0.00605	0.09153	3
		RM178	0.00605	0.09153	5
		RM17	0.00605	0.09153	12
		RM474	0.00684	0.08896	7

Sl No	Traits associated	Markers	*P* Value	r^2 value	Chromosome No
4	Relative water content	RM6100	1.43E-06	0.25618	10
		RM3825	8.55E-07	0.26553	1
		RM6925	1.23E-07	0.29966	8
		RM462	7.16E-08	0.30899	1
		RM455	5.88E-07	0.27224	7
		RM259	4.08E-06	0.23689	1
		RM514	4.08E-06	0.23689	3
		RM178	4.08E-06	0.23689	5
		RM5642	8.15E-06	0.22392	6
5	Leaf temperature	RM3825	0.00153	0.1201	1
		RM3042	6.09E-04	0.13899	4
		RM474	0.00257	0.10934	7
		RM259	4.08E-06	0.23689	1
		RM1083	0.03166	0.05712	10
6	Yield/plant	RM3825	3.96E-07	0.27926	1
		RM3042	1.73E-07	0.29382	4
		RM6925	2.90E-07	0.28478	8
		RM48	3.34E-06	0.24061	2
		RM461	3.85E-07	0.27978	6
		RM462	4.62E-08	0.31642	1
		RM1090	6.36E-06	0.22858	5
7	Thousand grain weight	RM3042	3.40E-05	0.19652	4
		RM283	0.00313	0.10522	1
8	Spikelet fertility percentage	RM462	1.99E-06	0.25018	1
		RM461	1.36E-05	0.2142	6
		RM455	3.72E-05	0.19481	7
		RM151	3.37E-05	0.19671	1
		RM48	2.31E-04	0.15866	2
		RM6925	2.77E-04	0.155	8
		RM3825	1.44E-04	0.16809	1
		RM1067	0.00121	0.12494	1

4.10.3 Marker trait association under water stress by MLM analysis

Under water stress condition a total of 2 markers were associated with leaf temperature were identified. The associated markers were presented on chromosome 1. The associated markers are RM 490 and RM259. Two markers were associated with the trait chlorophyll stability index and these are RM490 and RM259. These markers are mapped on chromosome 1. Leaf rolling was associated with the marker RM1026 mapped on chromosome 9, while leaf drying score was associated with the markers RM1026 and RM259 mapped on chromosome 1 and 9. The Q-Q plot also confirmed the association of

the markers RM 490 with chlorophyll stability index, RM259 with chlorophyll stability index and leaf drying score then RM1026 with leaf rolling score (Figure 9).

Yield per plant were associated with the markers RM259 and RM3825 mapped on chromosome 1. Among these two markers strong association was observed with marker RM3825 based on the lower *P* value (0.02105). Marker RM5961 was associated with the trait thousand grain weight mapped on chromosome 11. Spikelet fertility percentage was associated with markers RM259 and RM1031. Among these markers strong association was observed with RM259 on the basis of lower *P* value (0.0098). Percentage relative yield reduction and drought susceptibility index was associated with markers RM5633 and RM1130 mapped chromosome 4 and 6.

Marker RM259 was linked with the traits leaf temperature, chlorophyll stability index, leaf drying score, yield and spikelet fertility percentage. Marker RM1026 was also linked with traits relative water content, cell membrane stability index, leaf rolling score and drying score, so this marker can be used for drought related studies since it was associated with the most of the drought related traits.

4.10.4 Marker trait association under irrigated control condition by MLM analysis

There were a total of 13 marker trait association were observed under irrigated control condition. Considering the MLM statistics, RM1026, RM1032 and RM5923 were associated with the trait plant height. The associated markers are located on chromosomes 1, 9 and 11. Among the identified markers the RM1032 was having lower *P* value (0.02061) and higher r^2value (0.07069) thus indicating the most significant marker for plant height. Significant association for the trait tiller number were observed with SSR markers RM259, RM455 and RM105. These associated markers are located on chromosomes 1, 7 and 9. Among the associated markers RM455 was having lower *P* value (0.00797) and higher r^2value (0.09389). One marker (RM105) was associated with the trait days to 50 % flowering, which is mapped on chromosome 9. Leaf temperature was associated with the markers RM455 and RM1083, mapped on chromosomes 7 and 10. The Q-Q plot also confirmed the association of marker RM455 with tiller number and leaf temperature (Figure 10). Marker RM455 was associated with the traits relative water content and spikelet fertility percentage, and this marker is located on chromosome 7. The diagrammatic representation

of putative QTLs of morpho-physiological and plant production traits are given in Figure: 11.

Table 25: Marker trait association under water stress by MLM analysis

Traits associated	Marker	Chromosome	*P* value	r^2 value
Leaf temperature	RM490	1	0.04996	0.05192
	RM259	1	0.02398	0.06945
Chlorophyll stability index	RM490	1	0.00736	0.07886
	RM259	1	0.00775	0.07778
Yield per plant	RM259	1	0.02936	0.06402
	RM3825	1	0.02105	0.07209
Thousand grain weight	RM5961	11	0.0312	0.05483
Spikelet fertility percentage	RM259	1	0.0098	0.07782
	RM1031	6	0.04376	0.04657
Leaf rolling score	RM1026	9	0.00386	0.10547
Leaf drying score	RM259	1	0.03024	0.05067
	RM1026	9	0.01671	0.06224
Percentage relative yield reduction	RM5633	4	0.04017	0.05729
	RM1130	6	0.01309	0.08492
Drought susceptibility index	RM5633	4	0.04094	0.05682
	RM1130	6	0.01345	0.08419

Table 26: Marker trait association under irrigated control condition by MLM analysis

Traits associated	Marker	Chromosome	*P* value	r^2 value
Plant height	RM1032	1	0.02061	0.07069
	RM1026	9	0.02744	0.06393
	RM5923	11	0.03843	0.05614
Tiller number	RM259	1	0.04674	0.05169
	RM455	7	0.00797	0.09389
	RM105	9	0.04999	0.05018
Days to 50% flowering	RM105	9	0.01254	0.08268
Leaf temperature	RM455	7	0.00233	0.1254
	RM1083	10	0.02162	0.06955
Relative water content	RM455	7	0.02576	0.06541
Yield per plant	RM259	1	0.01332	0.08121
	RM455	7	0.04964	0.05033
Spikelet fertility percentage	RM455	7	0.01943	0.0721

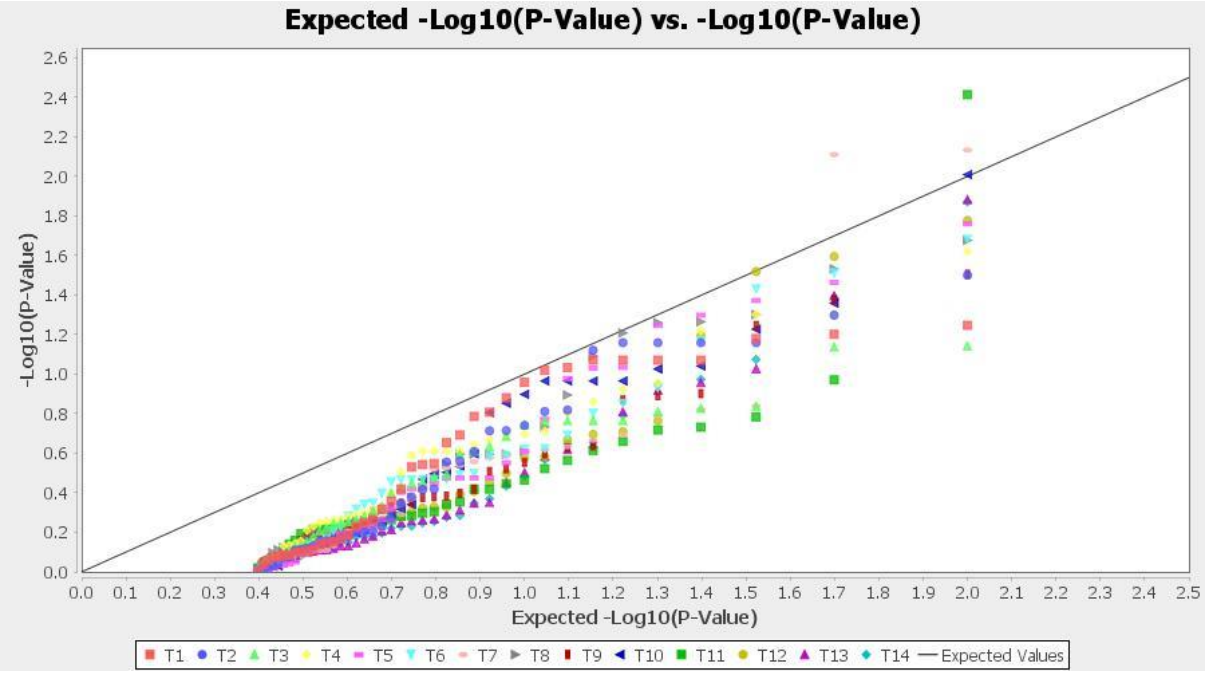

Figure 9: Quantile–Quantile (Q-Q) plot and distribution of marker-trait association from Mixed Linear Model analysis under water stress

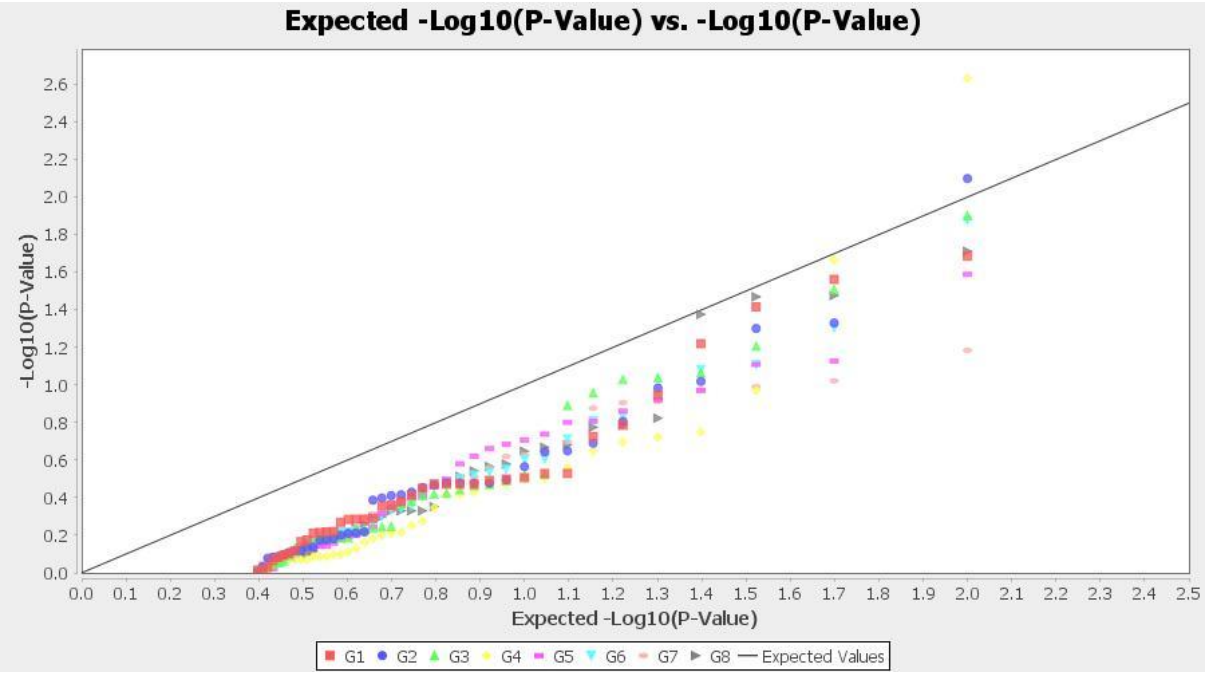

Figure 10: Quantile–Quantile (Q-Q) plot and distribution of marker-trait association from Mixed Linear Model analysis under irrigated control condition

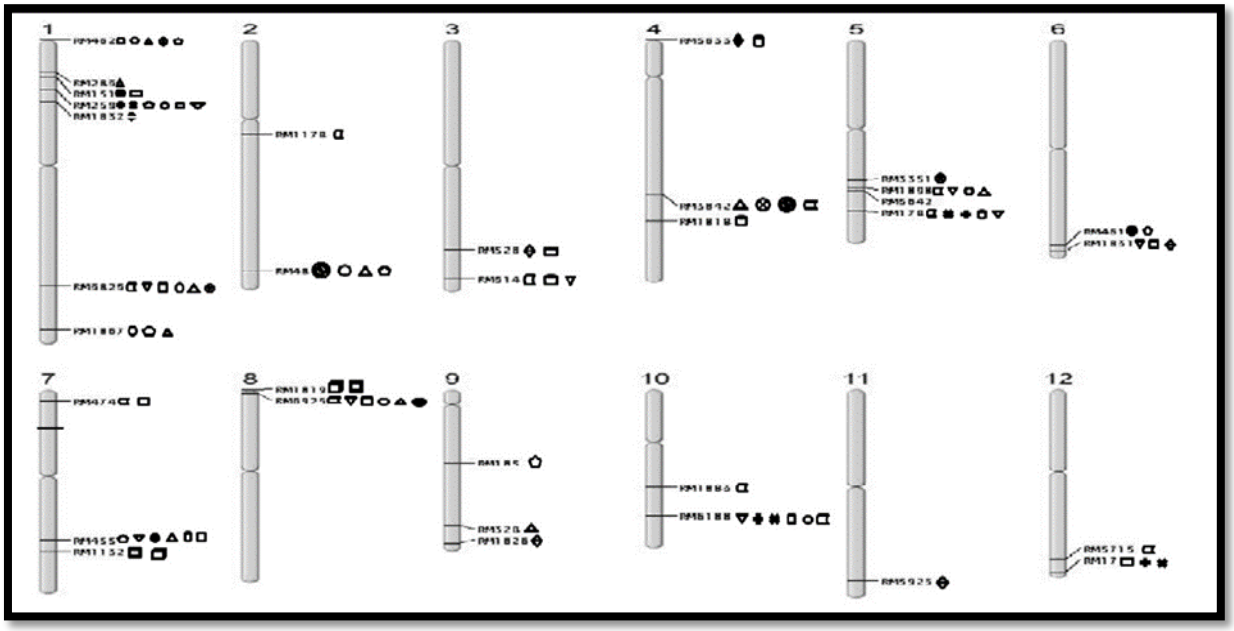

Figure 11: Identified QTLs for morpho-physiological and plant production traits

Plant height- ⟠, Tiller number- ⦸, Days to 50% flowering- ⌭, Leaf temperature- ⌶

Leaf rolling score- ⛝, Leaf drying score- ✠, Relative water content- ▽, Chlorophyll stability Index- ○ , Cell membrane stability index- □ , Yield- △, Thousand grain weight- ⊗

Relative yield reduction- ⧈ , Drought susceptibility index- ⍁

5. DISCUSSION

Rice (*Oryza sativa* L.) is an important food crop which needs a greater amount of water compared to other crops throughout its lifetime. It is cultivated on about 167 million hectares worldwide with an annual production of 769.9 million tons (FAO, 2018). More rice production is expected because of the rapid growth in the population (Khush, 2005). Water stress is the serious environmental issue in both rainfed and irrigated environments leading to reduced rice productivity. Under rainfed conditions, about 45 percent of the world's total area rice is grown (Pandey *et al*., 2005).

Drought represents a major challenge to restrict the production of rice. It affects morphological parameters in rice (reduced germination, plant height, plant biomass, number of tillers, specific features of the roots and leaves), physiological parameters (reduced photosynthesis, transpiration rate, stomatal conductance, water use efficiency, relative water content, chlorophyll content, photosystem II activity, membrane stability, carbon isotope discrimination and abscisic acid content), biochemical (accumulation of osmoprotectants like proline, sugars, polyamines and antioxidants) and molecular (altered expression of genes which encode transcription factors and defense related proteins) levels and thereby affects its yield (Pandey and Shukla 2015). Development of rice cultivars with inherent capacity to withstand drought will help to stabilize rice production, especially in water stress condition. In order to gain a full understanding of the mechanism of drought response in rice and to produce rice with improved drought tolerance, it is important to study on how different characteristics that affect rice productivity under water stress condition. Several putative traits have been proposed which contribute to drought resistance (Lafitte, 2003). The selection and use of these characteristics in plant breeding programs may lead to improved production under stress condition (Nguyen *et al*., 1997). Nevertheless, it is difficult, costly and labor-intensive to phenotypically select the drought-resistance traits (Kanbar and Shashidhar, 2010).

Mapping of genomic regions for drought tolerance and use of marker -assisted breeding is considered to accelerate the development of high-yielding rice in water-scarce environments. While conventional QTL mapping is an important tool in QTL tagging, it is time-consuming and resource-intensive. The key drawbacks of linkage mapping are that only two alleles at any given locus can be studied in bi-parental crosses and a low mapping resolution (Flint - Garcia *et al*., 2003). These limitations can be solved by using association genetic analysis. This is a powerful tool used to map loci with high resolution underlying quantitative traits such as drought tolerance. This takes advantage of cumulative historical recombination events in the natural population and aims to identify the causative polymorphisms of complex traits (Muthukumar *et al*., 2015). Association mapping also helps to assess the associations between genotypes and phenotypes in the study of individuals in a disequilibrium population (Pradhan *et al*., 2016). The association mapping is carried out in various crops such as maize, barley, wheat, rice, lettuce, and sorghum. Because rice is an entirely sequenced crop, it is well suited for association genetic analysis studies.

In the present study, 81 rice genotypes were used for phenotyping of morpho-physiological and plant production traits. Then, Association analysis was carried out by using 100 SSR primers. Significant variations were observed for all the parameters studied and the results obtained are discussed in this chapter with appropriate support from previous studies.

5.1 EFFECT OF WATER STRESS ON PHYSIOLOGICAL PARAMETERS

Drought score is viewed as an alternative approach for the assessment of drought tolerance (Fen *et al*., 2015). Visual score is a reliable measure of tolerance for the assessment of oxidative damage in plants and represents the dehydration of plant tissue associated with RWC (Cabuslay *et al*., 2002). As water stress grows, the plants naturally have developed a defensive mechanism to reduce the energy load on the leaf (Chaturvedi *et al*., 2012). In rice, leaf rolling factor under drought stress was considered to be one of the best criteria for estimating drought tolerance levels in large-scale screening (Pandey and Shukla, 2015). In this analysis, the degree of leaf rolling among rice genotypes under drought stress demonstrated the degree of tolerance to drought. Least leaf rolling and drying observed in

rice genotypes, Ptb-55 and Chomala, may be due to their failure to maintain the leaf water content under stress. Genotypes showing a leaf rolling score of 1 may have the capacity to sustain turgor pressure under stress. Similar findings were reported by Abd (2009) and Swapna and Shylaraj (2017) who reported that drought tolerant varieties showed a score between 0-3 and susceptible varieties recorded score of 7. Leaf rolling also reduces the photosynthetic surface and the area of light absorption and therefore reduces assimilate levels.

Plants that were exposed to stress resulted in higher value of leaf temperature than plants under control condition. A significant difference was observed among the genotypes and treatments for leaf temperature. The mean leaf temperature value was 28.26^0C and 27.50^0C under stress and control conditions in season one and 29.56^0C and 27.94^0C. PTB1 recorded the highest leaf temperature in both conditions in both experiments. These findings were supported by Jones and Corlett (1992) who reported that leaf temperature is associated with the plant stress level. Leaf temperature can be regarded as an indicator of the efficiency of plant water use, plant water status and potential indicator for drought avoidance mechanisms (Serraj *et al*., 2009). Decline in leaf water content and decreased transpiration rate can be the reason for the increment of leaf temperature (Rejeth, 2017). This result was in line with findings of Yang *et al.* (2011), they reported that canopy temperature increased with the increase in water stress. The temperature difference was up to 3^oC. Thus, the severe the drought stress, the higher the canopy temperature. Maintenance of the canopy temperature by means of transpiration cooling system would be insufficient with the occurrence of combined water deficit with heat stress conditions.

Plant cell membrane is one of the first sites of stress damage and plants ' ability to maintain membrane integrity dictates their resistance. It is one of the sub-traits used to study drought (Ozturk *et al.*, 2016). During stress period, the tolerant varieties maintained significantly higher membrane stability index compared to susceptible ones. In experiment I it ranged from 80.28-94.36% and in experiment II it ranged from 75.53-92.24 %. The rise in electrolyte leakage refers to the degree of cell membrane injury caused by water stress (Swapna and Shylaraj, 2017). Drought stress damage the selectively permeability of plasma

membrane thus change the internal composition of the membrane (Barnabas *et al*., 2007).The increase in membrane stability may be due to the presence of more saturated fatty acid in their membrane or to the retention of relatively high levels of leaf water content. Such results were followed by Savchenko *et al*. (2002), who reported that drought stress affects cell membrane fluidity either through protein denaturation or through increased unsaturated fatty acids.

The chlorophyll stability index (CSI) is an indication of the stress tolerance capacity of plants. A high CSI value means the drought had little impact on plant chlorophyll content. A lower CSI helps plants to endure stress by enhancing chlorophyll supply. This results in an improved photosynthetic efficiency, higher production of dry matter and higher productivity (Madhan Mohan, 2000). In experiment I it ranged from 80.36-95.59% and in experiment II it ranged from 75.52-92.85. The varieties that maintains higher CSI having drought tolerance mechanism compared to other varieties. These findings were supported by Ananthi *et al.* (2013) who reported that drought stress given for cotton genotypes KC2×MCU13 recorded higher values of CSI indicating that this combination possess drought tolerance characteristics. Similar results were obtained by Nahakpm (2018), where significant differences in chlorophyll stability index were observed between control and drought stressed plants, and in this condition they observed increased activities of antioxidant enzymes like peroxidise (POD), catalase (CAT) and superoxide dismutase (SOD) scavenging reactive oxygen species *viz*, hydrogen peroxide and superoxide anion. These findings can be described as one of the most tolerance potential of genotypes from destruction of chloroplast under stress condition thus scavenging ROS production. Chandola (2015) reported reduction in chlorophyll stability index under all the treatment without induction of stress.

Relative water content is considered to be the best integrated plant water status measurement and represents the plant's variations in water potential, turgor potential, and osmotic adjustment (OA**)** (Bhushan *et al.,* 2007). Rice varieties under drought stress condition showed significant reduction in the RWC in two experimets. The mean relative water content was 65.19 and 84.13 percent at flowering stage under water stress and irrigated conditions in experiment I and 61.07 and 78.89 per cent in experiment II respectively.

Relative water content of leaves indicates the actual water content to its maximum turgidity. Under circumstances of drought stress, varieties capable of sustaining greater RWC would probably retain protoplast hydration for a longer period, thus ensuring efficiency (Sikuku *et al*., 2012). Kumar *et al*. (2014) stated that higher RWC values were observed in drought stress tolerant genotypes of rice compared to susceptible genotypes under water stress conditions. Genotypes showed better maintenance of higher RWC ensuring better hydration and better internal tissue water relations with potentially higher pressure and showed better drought tolerance (Kardile *et al*., 2018). Therefore, it could be concluded that varieties which maintained higher RWC expressing drought tolerance than susceptible ones.

5.2 EFFECT OF WATER STRESS ON MORPHOLOGICAL PARAMETERS

Water stress given at panicle initiation stage significantly affected morphological traits such as plant height, tiller number and days to 50% flowering, indicating that the irrigation regime had highly affected these characteristics. The mean plant height of rice genotypes was measured 110.74cm and 116.05cm under stress and control condition respectively in experiment I and in experiment II the mean plant height was 106.28cm and 112.29cm under water stress and control conditions. These findings were supported by Ji *et al*. (2012) who reported that drought stress at vegetative stage in rice caused a prominent reduction in plant height. They observed a decrease in plant height of 12%. The decrease was less in some varieties which further indicates its tolerance to water stress. Jaleel *et al*. (2009) reported that rice cultivars shoot growth (Plant height and Leaf Area) is reduced by drought, which may be due to reduced cell expansion. The result of the present study are in agreement with Singh *et al*. (2017) and Lafitte *et al.* (2003) they found reduction in plant height of cultivars under drought stress. The results of this study were consistent with previous studies that also found significant reduction in plant height under water stress conditions (Manivannan *et al*., 2007). Singh *et al.* (2017) have suggested that the main drought-related decrease in plant height was due to the limited cell length and division of cells and decreased green leaves which serve as a source of carbon assimilation. This reduction in plant height may be because of the cell enlargement and division is affected by drought stress. This impediment to plant growth affects numerous biochemical and physiological processes,

including ion absorption, respiration, photosynthesis, growth promoters, carbohydrates, source-sink relationship and nutrient metabolism (Anjum *et al.*, 2017).

A significant difference was observed among the genotypes and treatments for the number of tillers per plant. Among the genotypes, mean tiller number in experiment I was 7.5 under stress condition and 10.25 under well watered conditions. In experiment II under water stress condition the mean tiller number was 6.34 and under irrigated control condition it was 8.88. There was a significant reduction in the number of tillers among the rice genotypes due to water stress when compared to irrigated conditions. The result of the study is in harmony with Moonmoon and Islam, 2017 who reported that the number of tillers/hill was decreased with drought (40% FC). Reduction of the effective production of tillers under low soil moisture may be due to limited supply of assimilate under water stress. It could also occur for less water to prepare enough food and inhibition of the cell division of meristematic tissue (Zubayer *et al*., 2007).

Water stress at panicle initiation stage can cause delay in flowering or early flowering in rice depending upon the nature of drought adaptation. In the present study, delayed flowering was noticed in most of the varieties which can be attributed to their mechanism of drought escape. The result of the study in agreement with study of Kang and Futakuchi, 2019 who reported that drought stress was associated with a substantial delay in flowering time. Flowering was delayed by 1.7–10.7 (average = 4.5) days under drought conditions compared with that under wet control conditions. Similar to this study, another study also reported that delays in plant flowering during 21 days of drought stress on an interspecific backcross population of *O. sativa* var. WAB56-104 × *O. glaberrima* var. CG14 (Ndjiondjop *et al*., 2010). Saikumar *et al.* (2016) also reported delay in flowering under drought stress condition. Lafitte *et al*. (2004) reported that flowering delay under drought-stress conditions is associated with an apparent delay in floral development when stress is imposed between panicle initiation and pollen meiosis. Late flowering lines suffered higher yield reduction than early maturing ones on an average. Anthesis and fertilization are particularly sensitive to drought in rice (Saikumar *et al.*, 2016). Therefore, flowering time is an important

determinant of grain yield under prolonged or severe drought stress conditions (Pantuwan *et al*., 2002a).

5.3 EFFECT OF WATER STRESS ON PLANT PRODUCTION TRAITS

Drought is the major environmental threat to the productivity of rice under rainfed condition. It is the greatest stress at all stages of rice growth and development, but it has the greatest impact during flowering, where the formation of grain is suppressed (Serraj *et al*., 2009). During the reproductive stage, rice vulnerability to drought stress is more pronounced; even mild stress can lead to drastic reduction of grain yield (Venuprasad *et al*., 2009a). In the present study drought stress at panicle initiation caused reduction of yield, thousand grain weight and spikelet fertility percentage compared to control condition. These findings are in line with the findings of Saikumar *et al.* (2016), Singh *et al.* (2010), Yang *et al.* (2019) and Alghabari and Ihsan, 2018. The impact of drought stress depends on intensity, duration and timing. The reduction in grain yield is primarily due to reduced tiller number, delayed initiation of panicles and spikelet sterility (Samarah, 2005). Plant water status and photosynthesis are the two essential physiological features that are influenced by moisture stress to a greater extent, leading to nutrient imbalances and source or sink limitations for successful production (Saikumar *et al.,* 2016). Anthesis and fertilization in rice are especially sensitive. When soil drying progresses, water supply to plant slowly declines, leading to ultimate loss in grain yield. Therefore, genotypes that can retain the potential for better water at the level of leaves, panicles and whole plants can survive and generate better grain yield. Drought stress at flowering is most extreme and destructive because it has a diverse pollination impact and causes flower abortion, grain abscission and a rise in the percentage of unfilled grain (Singh *et al*., 2012). It was found that the percentage of unfilled grains in sites affected by drought at the reproductive stage was significantly higher. This may be because the movement of assimilates to reproductive organs is reduced under drought stress (Rahman *et al*., 2002).

5.4 CORRELATION STUDY

Correlation gives an idea on nature and depth of relationship among various morpho-physiological traits under water stress and irrigated conditions. Under water stress condition correlation study gives an idea about which trait can improve the grain yield. In this study correlation analysis revealed that the grain yield was positively and significantly correlated with the traits relative water content, tiller number, days to 50% flowering, cell membrane stability index, chlorophyll stability index, thousand grain weight and spikelet fertility percentage. Similar results were reported in previous studies by Shinde *et al.* (2015) and Kumar *et al*. (2008). Negatively correlated with the traits leaf temperature, leaf rolling score, leaf drying score and percentage relative yield reduction. Similar observations were reported by Boopathi *et al*. (2013). Positive correlation between spikelet fertility percentage and grain yield was previously reported by Pradhan *et al*. (2015). Physiological parameters such as relative water content, cell membrane stability index, chlorophyll stability index were positively correlated with grain yield. Similar studies were reported by Nahakpam (2018), Dubey *et al.* (2018). The plant production traits like thousand grain weight and spikelet fertility percentage were positively correlated with grain yield. These results are in resonance with the findings of Dubey *et al.* (2018), Manickavelu *et al.* (2006), Zaman *et al*. (2018).

5.5 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) is a technique for reducing the dimensionality of large datasets, increasing interpretability but at the same time minimizing information loss (Jolliffe and Cadmia, 2016). In this study, there was a significant variation was observed among the selected rice genotypes (Figure 1). The tolerance among rice genotypes was not associated with the state origin. Under water stress condition principal component analysis further explained that the three representative variables from each of the main components (relative water content, cell membrane stability index, and chlorophyll stability index) and under irrigated control condition two main components (relative water content and spikelet fertility percentage) were sufficient to capture most of the data variation. So under drought stress these three variables and under irrigated control condition these two variables could be

used for screening purpose. In addition, the genotypes were classified according to their degree of drought resistance. Tolerant genotypes have higher relative water content, cell membrane stability index, chlorophyll stability index, thousand grain weight, spikelet fertility percentage and yield per plant compared to those of the susceptible ones. Genotypes, PTB7, PTB28, PTB29, PTB15, Chomala and one variety from other state (Nagina-22) were grouped under drought tolerant category. This study was agreed with earlier studies of Bhattarai and Subudhi (2019), Pradhan *et al.* (2016). The cultivars that have high PCA1 are suitable for drought stress and non stress condition (stable genotypes) and cultivars with lower PCA1 gave lower yield (unstable genotypes) Hosseini *et al.* (2012) and Rahimi *et al.* (2013). The rice genotypes showing tolerance for drought can be attributed to similar physiological responses and expression of genes under stress condition (Nounjan *et al*., 2018).

5.6 CLUSTER ANALYSIS

Cluster analysis is a group of multivariate techniques whose primary purpose is to group objects based upon attributes that make them similar. Genotypes with similar traits were grouped into one cluster. A total 14 genotypes (Ptb1, Ptb7, Ptb15, Ptb26, Ptb 27, Ptb28, Ptb29, Ptb30, Ptb60, Ptb45, Ptb41, Ptb35, Ptb55 and Ptb59) i.e., most of the drought tolerant and high yielding varieties were clustered in cluster I based on the tiller number, leaf temperature, relative water content, cell membrane stability index, cholorophyll stability index, thousand grain weight, spikelet fertility percentage and yield per plant. The average trait value of all these parameters relatively higher than the general mean, representing that these genotypes retain desirable agronomic traits (Iqbal and Rahman 2017). The parameters, percentage relative yield reduction and drought susceptibility index was high for genotypes under cluster V. Cluster V also include some of the drought tolerant varieties (N-22 and Chomala). The difference between cluster I and cluster V is, in cluster I it included traditional low yielding drought tolerant varieties. Whereas cluster V included improved high yielding drought tolerant varieties. After cluster I the mean value of all the traits was higher in Cluster V. Maximum numbers of genotypes were grouped in Cluster IV which includes most of the varieties released from Pattambi. Cluster II and III contains most of the susceptable varieties.

The trait mean value for all the parameters was lower in these clusters compared with other 3 clusters. Gilavaei *et al.* (2018) also reported similar results in which in their study most of the drought tolerant varieties were clustered in one group.

Under irrigated control condition, the maximum number of genotypes were grouped in cluster II that includes most of the traditional varieties. In contrast to first group, this one had minimum value regarding many of the traits, so that genotypes of this group had lowest average for traits such as tiller number, leaf temperature, relative water content, thousand grain weight, spikelet fertility percentage and yield per plant. Cluster I comprised of most of the drought tolerant and high yielding varieties. The trait mean values of all the parameters were higher in this cluster followed by the cluster V. A total of 15 genotypes were grouped in cluster IV, and the genotypes under this group possess maximum plant height compared with the other clusters. The third group comprised of 11 genotypes that include some of the pattambi released varieties and some improved rice varieties. With respect to leaf temperature this group had the highest average among all groups. The results of the cluster analysis showed that the mean value of most of the important traits were higher in cluster I. Thus, while selecting representive of drought tolerant and high yielding genotype from cluster I and hybridize between them that increase the choice of desirable genotypes under drought stress.

5.7 ASSOCIATION GENETIC ANALYSIS

The association mapping (AM) method provides opportunities to identify genetic variation in natural populations by mapping complex traits with high resolution (Zhu *et al*., 2008). It relies on the LD, which is sustained over generations between loci that are genetically linked to each other (Neumann *et al*., 2010). If LD occurs between a marker and a locus associated with a trait, then unique marker alleles of haplotypes may be correlated with highly statistically significant phenotypes (Cardon and Bell 2001). Population structure and genetic similarity between individuals can lead to spurious associations. Therefore, it is important to study the population structure with regard to the membership of individuals in the population and their genetic relationship among pairs of all the individuals used in the

study (Pritchard *et al*., 2000). Association analysis can be performed by two methods i.e., General linear model (GLM) and Mixed linear model (MLM). Compared to the general linear model (GLM), the Mixed Linear Model (MLM) method effectively eliminates false positives by incorporating population stratification and cryptic relationship (Yu *et al*., 2006). The population stratification is fit as a fixed effect by means of population structure or the principal component (Zhao *et al*., 2007). The cryptic relationship among individuals is defined as the variance and covariance of the random genetic effects from individuals.

5.7.1 POPULATION STRUCTURE

Association or linkage of disequilibrium mapping has become a common method for genetically dissecting complex traits in plants (Hall *et al.*, 2010). In association analysis population structure is an important criterion. Population structure is a consequence of deviations from random mating in the sampling population resulting in some people becoming closer to each other than others. The existence of a population structure can lead to "spurious associations" that is, associations between a phenotype and markers not associated with any causative loci. Structured association first scans a population for closely related clusters / subdivisions using Bayesian method, then uses clustering matrices (Q) in association mapping (by technical regression) to correct false associations. Population structure is an important component in the mapping of associations because it can be a source of Type I error in an autogamous species such as barley and rice (Agrama *et al*., 2007). The population structure of rice varieties under drought stress was previously reported by Swamy *et al.*(2017), Bhattarai and Subudhi (2019), Verma *et al*. (2019) in their study they have used 114 rice genotypes from north east india and 65 SSR primers for variability assessment for root and drought tolerance traits. In the present study population structure was analyzed for 81 rice accessions and has been clustered using STRUCTURE software with K= 2-8. In population structure analysis while doing analysis we don't know the real value of K, but when we are selecting the K value we should select minimum value of K that should give full structure of the population. In this experiment we have selected the K value as 2 i.e., when the delta K value was maximum. All the 81 genotypes used in this experiment were from the *indica* group except one variety from *Aus* subgroup but the structure analysis

grouped them into two main subgroups. Nearly 65 genotypes were grouped into first subgroup, while 16 genotypes in second subgroup with 6 admixtures. This admixture indicates the genotypes that showing some allelic reshuffling. This allelic exchange between different genotypes was due to the aggregation of several spontaneous mutations between genotypes from different geographical areas (Agrama *et al*., 2007).

5.7.2 LINKAGE DISEQUILIBRIUM

LD analysis was carried out for all the marker pairs. The number of significant intra- and inter-chromosomal LD pairs varied on different chromosomes. Chromosomes 1 had the highest number of significant intra- and inter chromosomal LD pairs, whereas chromosome 11 had the lowest number. There was a reduction in number of significant LD pairs as the interval distances between marker pairs increased. There was a sharp decline in LD decay for the linked markers at 250 cM. The significant LD blocks within a germplasm collection are highly useful in association mapping. There are numerous reports of LD patterns in rice. Olsen *et al.* (2006) and Mather *et al*. (2007) reported LD decay occurring at about 1 cM distance, whereas others reported LD decay at 20–30 cM distances using SSR markers (Vannirajan *et al*., 2012). Lu *et al.* (2015) reported LD decay at ~109.37 kb. Many factors, such as pollination itself, geographic isolation, pattern of evolution, mutation, selection pressure and genetic drift, affect the size and number of LD blocks (Gupta *et al*., 2005). In a predominantly self-pollinated crop species, such as rice, larger LD blocks are usually expected to stretch over several cM (Abdurakhmonov and Abdukarimov, 2008); Likewise, LD differed between different rice subspecies. In *indica* subspecies, the magnitude of LD was smaller than in temperate *japonica* or tropical *japonica* (Khush, 1997; Garris *et al*., 2005; Mather *et al*., 2007).

5.7.3 ASSOCIATION MAPPING FOR MORPHO-PHYSIOLOGICAL AND PLANT PRODUCTION TRAITS

In the present study a total of 100 SSR markers have been used for genotyping 81 rice genotypes. Among them 40 SSR markers showed polymorphism among the genotypes. Marker trait associations were detected using TASSEL software. A total of 136 marker trait

association were identified in this study with $P<0.05$ and $r^2>0.1$ and $r^2>0.05$ by GLM analysis and 48 marker trait association based on the *P* value in MLM analysis. The marker RM17 on chromosome 12 was linked to leaf drying score, leaf rolling score and cell membrane stability index under water stress condition. Lin *et al.* (2007) reported RM17 marker linked to thousand grain weights in F_2 mapping population under water stress condition. Similar in another study conducted by Mishra *et al.* (2018) reported RM17 marker for relative water content from landraces under water stress condition. Selvaraj *et al*. (2016) identified RM17 marker for photosynthetic rate, transpiration rate, stomata conductance and relative water content from RILs.

Under water stress condition, RM72 marker located on chromosome 7 was linked to the trait cell membrane stability index. Lin *et al.* (2007) reported this marker for leaf rolling score from F_2 mapping population. Mishra *et al*. (2018) also reported this marker for leaf rolling trait under water stress condition from RIL population. Barik *et al.* (2019) and Price *et al.* (2002) also reported this marker for leaf rolling trait in RIL population.

Marker RM328 was linked to the yield trait under water stress condition. Lang and Buu (2008) identified RM328 for many drought tolerant traits with *P* value <0.0001 mainly for drought recovery score. Using the advanced backcross lines at seedling stage, a QTL for increased root length and drought tolerance was mapped in the segment between RM201 and RM328 of chromosome 9 (Lang *et al*., 2013), which was close to the Dro1-KP allele for deep rooting (Uga *et al*., 2013).

The marker RM455 on chromosome 7 was associated with the traits such as spikelet fertility percentage, relative water content, number of tillers and yield per plant. This marker is associated with cluster V in cluster analysis, since it was associated with plant production traits in which cluster V included improved drought tolerant high yielding varieties. Tripathi *et al.* (2018) reported that RM455 marker was associated with the drought tolerant traits.

Marker RM461 located on chromosome 6 linked to the traits number of tillers, spikelet fertility percentage and yield per plant. Bhattarai and Subudhi (2019) found RM461 to be associated with the trait shoot dry weight under drought stress.

In this study SSR marker RM462 was associated with many traits such as cell membrane stability index, cholorophyll stability index, number of tillers, thousand grain weight, and spikelet fertility percentage. Zhu *et al.* (2018) reported RM462 and RM283 for heading date under water stress.

Marker RM151 was associated with the traits tiller number, cell membrane stability index and leaf drying score. Beena, R (2005) reported RM 151 for stress recovery.

The marker RM1067 located on chromosome 1 was associated with the traits cholorophyll stability index, yield and spikelet fertility percentage. Beena, R (2005) reported RM1067 for plant height.

The marker RM474 on chromosome 7 associated with the traits leaf temperature and cell membrane stability index. Verma *et al.* (2019) reported that RM474 was associated with root and drought tolerance traits. Rejeth (2017) also reported marker RM 474 which is identified in this study can be used for marker assisted selection for drought tolerance in rice. Sheeba *et al.* (2009) reported RM474 marker was associated with fertility restorer gene.

RM 520 located on chromosome 3 was linked with the traits plant height and cell membrane stability index. RM 520 was previously reported by Anupam *et al.* (2017) as a peak marker associated with QTLs, *qDTY2.1* and *qDTY3.1*, respectively. Suh *et al.* (2014) identified RM520 in NIL population at DTY3.1 region, a QTL related to yield under drought stress. The peak of DTY3.1 was located at10.0cM and flanked by RM520 (9.1cM) (Venuprasad *et al.*, 2009a). Shamsudhin *et al.* (2016) identified RM520 marker in BC_1F_1 population associated with the QTL qDTY2.2 and qDTY3.1, QTL related to yield under drought stress.

The marker RM1132 on chromosome 6 was associated with the traits drought susceptibility index and percentage relative yield reduction. Donde *et al.* (2020) reported RM1132 for grain yield. The marker RM6100 on chromosome 10 linked with the traits related to relative water content, leaf drying score, leaf drying rolling score and chlorophyll stability index. This RM6100 marker is linked with a major quantitative trait locus (QTL) on chromosome 10 for heat stress tolerance at flowering stage. Bharathkumar *et al.* (2014) reported RM6100 marker

which is associated with major QTL for heat tolerance at flowering stage. Donde *et al.* (2020) reported this marker linked to the grain yield.

Marker RM6925 on chromosome 8 linked with many traits including leaf temperature, relative water content, cell membrane stability index, chlorophyll stability index, thousand grain weight and spikelet fertility percentage. Prince *et al.* (2015) identified this marker related to the traits relative water content and grain yield.

The marker RM3825 was associated with the traits leaf temperature, relative water content, cell membrane stability index, chlorophyll stability index, yield per plant and thousand grain weight. Aswathi and Lal (2015) reported RM3825 for yield QTL *qDTY3.2*. Kanagaraj *et al.* (2010) reported RM3825 for drought resistant traits. Oraibi *et al.* (2014) reported RM3825 located on chromosome 1 of the rice between 135.8 and 143.7 cM, this region has been found to be linked with several drought tolerance traits such as plant height, biomass, deep root mass, basal root thickness, tiller number and deep root to shoot ratio. Chungada *et al*. (2015) reported drought tolerance linked alleles *Dr* (135.8 bp) and *Dr* (147 bp) for RM-302 and RM3825. In RIL population Verma *et al.* (2014) reported the marker RM3825 on chromosome 1 with drought tolerant traits. Beena, R (2005) reported RM3825 marker was associated with the traits plant height, panicle length, grain yield and straw yield.
Marker RM490 was linked with the traits Cell membrane stability index, chlorophyll stability index, number of tillers and days to 50% flowering. Mallikarjuna *et al.* (2013) reported this marker for associated with fertility restorer gene on *Rf* 3 locus.

Marker RM259 was linked with the traits leaf temperature, leaf drying score, chlorophyll stability index, yield and spikelet fertility percentage. This marker is associated with cluster I in cluster analysis, since it was associated with drought related traits in which cluster I included traditional low yielding drought tolerant varieties. Tripathy *et al.* (2018) also used this marker for parental polymorphism survey for drought tolerance. Donde *et al.* (2020) reported RM259 for drought tolerant traits. Sahoo *et al*. (2019) observed high level of

significance with the marker RM259 while used for linkage analysis for drought tolerance in kharif rice varieties.

The QQ plot is a graphical representation of the deviation from null hypothesis of observed *P* values. For each SSR, the observed *P* values are sorted from the largest to the smallest, and plotted against the expected values. If the observed values correspond to the predicted values, all points between the x-axis and the y-axis are on or near the middle line. If certain observed *P* values are obviously more important than expected, points will shift towards the y-axis as shown in the figure 9 &10. If there is an early distinction between the predicted and the observed .this means that many moderately significant *P* values are greater than expected. This finding is due to thousands of true positive; more often it is due to population stratification: systematic variations in allelic frequencies between subpopulations of the individuals being examined, so that a large number of *P* values are smaller than expected (Ehret, 2010).

Thus, Association genetic analysis can be effectively used for identifying DNA markers /QTLs linked to drought tolerant and plant production traits. The primers RM 259, RM1026, RM490 and RM455 which are identified in this study can be used for marker assisted selection for drought tolerance and improvement of plant production traits in rice. Specific markers that were found to be related to drought tolerance in rice were distributed in different chromosomes of rice. Thus, fine mapping of loci harboring these markers can be done to find out which genes in rice confer drought tolerance and yield improvement.

6. SUMMARY

The salient findings of the present study to identify the QTLs/molecular markers associated with the drought tolerant and plant production traits in rice using 81 rice genotypes are summarized below:

- In the first and second experiments, 81 rice genotypes consisting of improved varieties and local varieties were tested in two seasons (Mundakan 2017 and Puncha 2018) for their morpho- physiological characteristics under water stress.
- Significant variation was observed for all the traits under water stress compared to irrigated control condition.
- Significant positive correlation were found between grain yield with number of tillers, days to 50% flowering, relative water content, chlorophyll stability index, cell membrane stability index, thousand grain weight, spikelet fertility percentage and drought susceptibility index, while negative correlation were observed grain yield with leaf temperature, leaf rolling score, leaf drying score and percentage relative yield reduction.
- Under water stress the first principal component showed 41.77 per cent variation, while second component explained 16.57 per cent variation and third component with 10.46 per cent variation. Among the 14 morpho-physiological and plant production traits relative water content, cell membrane stability index and chlorophyll stability index,yield and spikelet fertility percentage contributed towards maximum diversity.
- Under irrigated control condition the first principal component explained 48.9 per cent variation, while second component explained 16.57 per cent variation and third component with 10 per cent variation. Among the eight morpho-physiological and plant production traits relative water content, spikelet fertility percentage contributed towards maximum diversity.
- Clustering by Ward method was done to establish a relationship among the 81 rice genotypes. Similar types of genotypes were clustered together. All the genotypes were clustered into mainly 5 clusters under water stress as well as irrigated control condition.

- Genotyping of 81 rice genotypes were done using 100 SSR markers. Among them, 40 primers demonstrated polymorphism among the genotypes used to classify the genomic regions (QTLs) associated with drought tolerant and plant production traits through association genetic analysis.
- The marker trait association for all the traits under water stress and irrigated control condition was calculated using GLM and MLM model of TASSEL5 software.
- A total of 136 associations were observed while performed GLM analysis and MLM analysis resulted in 29 marker trait association based on the *P* and r^2values in water stress as well as irrigated control condition.
- LD was distributed unequally on each chromosome and more concentrated on chromosomes 1 and 5. LD analysis in the whole population showed there were significant LD pairs (P>0.05). A total 52 LD pairs were observed under water stress and irrigated control condition and out of these there were 46 inter chromosomal LD pairs and 6 intra chromosomal LD pairs.
- The identified most significant markers and their corresponding QTLs are, RM455 (Chromosome7) was linked to tiller number, leaf temperature, relative water content, yield per plant and spikelet fertility percentage. RM490 (Chromosome1) was associated with leaf temperature and chlorophyll stability index. Marker RM259 (Chromosome1) was associated with leaf temperature, chlorophyll stability index, leaf drying score, yield per plant and spikelet fertility percentage. RM1026 (Chromosome 9) was associated with leaf rolling score and drying score.
- Among them, RM 490 and RM259 showed co-location of QTLs for leaf temperature and chlorophyll stability index and RM259 & RM1026 showed co-location of QTLs for leaf drying score. The Q-Q plot also confirmed the association of these markers with phenotypic traits. Thus indicating that these markers are the most consistent markers. Consequently, consistent QTLs can be useful for improving drought resistance in rice through marker assisted breeding programmes (MAB) and cloning strategies.
- Association genetic analysis is a valuable method to identify molecular markers for complex traits such as drought resistance using diverse rice genotypes.

REFERENCE

Abd, A. 2009. Genetic studies on leaf rolling and some root traits under drought conditions in rice (*Oryza sativa* L.). *Afr. J. Biotechnol.* 8 (22): 6241–6248.

Abdurakhmonov, I.Y. and Abdukarimov, A. 2008. Application of Association Mapping to Understanding the Genetic Diversity of Plant Germplasm Resources. *Int. J. Plant Genomics*. 3: 1–18.

Agarie, S. and Kaufman, P. 1995. Measurement of Cell Membrane Stability Evaluated by Electrolyte Leakage as a Drought and Heat Tolerance Test in Rice (*Oryza sativa* L.) *J. Fac. Agricul*. 40:102-114.

Agarwal, P., Parida, S.K., Raghuvanshi, S., Kapoor, S., Khurana, P., Khurana, J.P. and Tyagi, A.K. 2016. Rice Improvement Through Genome-Based Functional Analysis and Molecular Breeding in India. *Rice*. 9 (1):34-43.

Agrama H A and Eizenga, G C. 2007. Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of *Oryza sativa* and its wild relatives. *Euphytica*. 160 (3): 339–355.

Agrama, H.A. and Eizenga, G.C. 2007. Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of *Oryza sativa* and its wild relatives. *Euphytica*. 160 (3): 339–355.

Ahadiyat, Y. and Susanto, P. 2014. Drought tolerance, phosphorus efficiency and yield characters of upland rice
lines. *Emirates. J. Food. Agric*. 26 (1): .25-32.

Ahmadikhah, A. and Marufinia, A. 2016. Effect of reduced plant height on drought tolerance in rice. *Biotech*. 6 (2): 23-33.

Ahmed, M., Haq, M., Hossain, Shefat-Al-Maruf and Hasan, M. 2017. Performance of Four Different Rice Cultivars under Drought Stress in the North-Western Part of

Bangladesh. *Int. J. Agric .For.*7 (6); 134–139.

Akram, H. M., Ali, A., Sattar, A., Rehman, H. S. U., and Bibi, A. 2013. Impact of water deficit stress on various physiological and agronomic traits of three basmati rice (*Oryza sativa* L.) cultivars. *J. Anim. Plant Sci.* 23 (5): 1415-1423.

Alghabari, F. and Ihsan, M. 2018. Effects of drought stress on growth, grain filling duration, yield and quality attributes of barley (*Hordeum vulgare* L.). 47 (3): .421–428.

Ananthi, K., Vijayaraghav, H., Karuppaiya, M. and Anand, T. 2013. Drought-induced Changes in Chlorophyll Stability Index, Relative Water Content and Yield of Cotton Genotypes. *Insight Botany*. 3: 1-5.

Anjum, S., Ashraf, U., Zohaib, A., Tanveer, M., Naeem, M., Ali, I., Tabassum, T. and Nazir, U. 2017. Growth and developmental responses of crop plants under drought stress: A review. *Agriculture*. 104: 267-276

Anupam, A., Imam, J., Quatadah, S.M., Siddaiah, A., Prasad Das, S., Variar, M. and Prasad Mandal, N. 2017. Genetic Diversity Analysis of Rice Germplasm in Tripura State of Northeast India Using Drought and Blast Linked Markers. *Rice Science*. 24 (1): 10–20.

Ashkani, S., Rafii, M. Y., Shabanimofrad, M., Azadi, A., Ghasemzadeh, A., Azizi, P. 2015. Allele mining strategies: principles and utilisation for blast resistance genes in rice (*Oryza sativa* L.). *Curr. Issues Mol Biol.* 17: 57–74.

Ashkani, S., Rafii, M.Y., Shabanimofrad, M., Ghasemzadeh, A., Ravanfar, S.A. and Latif, M.A. 2014. Molecular progress on the mapping and cloning of functional genes for blast disease in rice (*Oryza sativa* L.): current status and future considerations. *Critical. Rev. Biotechnol.* 36 (2):353–367.

Ashraf, M. 2010. Inducing drought tolerance in plants: recent advances. *Biotechnol. Adv*. 28: 169-83.

Aswathi, S and Lal. J. P. 2015. Application of molecular markers to improve drought tolerance and yield enhancement in upland rice. *Annals. plant. sci*. 4 (04): *1051-1056.*

Babu, R. C., Nguyen, B. D., Chamarerk, V., Shanmugasundaram, P., Chezhian, P., Jeyaprakash, P., Ganesh, S. R., Palchamy, A., Sarkarung, S., Wade, L. J., and Nguyen, H. T. 2003. Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. *Crop Sci.* 43: 1457-1469.

Bajaj, S. and Mohanty, A. 2005. Recent advances in rice biotechnology-towards genetically superior transgenic rice. *Plant. Biotechnol. J*. 3 (3): 275–307.

Barik, S.R., Pandit, E., Pradhan, S.K., Mohanty, S.P. and Mohapatra, T. 2019. Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. *PLOS ONE*. 14 (12): 23-33.

Barnabás, B., Jäger, K and Fehér, A. 2007. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ*. 31: 11–38.

Basnayake, J., Ouk, M., Thun, V., Kang, S., Pith, K. H., Fukai, S., Men, S., and Fisher, K. 2004. Measurement and management of genotype-environment interaction (GXE) for the improvement of rain-fed lowland rice yield in Cambodia. In: *Proceeding for the 4th International Crop Science Congress*, Brisbane, Australia, 26 September-1 October, p. 239.

Beena, R. 2005. Studies on physio- morphological traits and genetic markers associated with drought responses in rice (*Oryza sativa* L.). Ph.D thesis submitted to Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Beena, R., Praveenkumar,V. P., Vigneshwaran,V., Sindhumol, P. and Narayanankutty, M.C.2017. Phenotyping for root traits and carbon isotope discrimination in rice genotypes of Kerala. *Oryza*. 54 (3): 282-289.

Beena, R., Thandapani, V., and Babu, R. C. 2012. Physio-morphological and biochemical characterization of selected recombinant inbred lines of rice for drought resistance. *Indian J. Plant Physiol.* 17 (2): 189-193.

Behera, S., Rout, R.K., Sinha, B., Padhiary, A., Nayak, A., Behera, D. and Das, T. 2017. Root Phenology and Biochemical Changes in Rice Genotypes under Drought Stress. *Int. J. Current. Microbiol. Appl. Sci.* 6 (11): 1818–1828.

Bernier, J., Atlin, G. N., Serraj, R., Kumar, A. and D. Spaner. 2007. Breeding upland rice for drought resistance. *J. Sci. Food Agric*. 88: 927–939.

Bharathkumar, S. and Kumar, J. 2014. Identification of rice germplasms associated with microsatelite (SSR) markers for heat tolerance at reproductive stage and expression of heat stress related gene Identification of rice germplasms associated with microsatelite (SSR) markers for heat tolerance at reproductive stage and expression of heat stress related gene. *Indian Res. J. Genet. Biotech*, 6 (2): 424–427.

Bhatnagar-Mathur, P., Rao, J.S., Vadez, V., Dumbala, S.R., Rathore, A., Yamaguchi-Shinozaki, K. and Sharma, K.K. 2013. Transgenic peanut overexpressing the DREB1A transcription factor has higher yields under drought stress. *Mol. Breed*. 33 (2): 327–340.

Bhattarai, U. and Subudhi, P.K. 2019. Genetic Diversity, Population Structure, and Marker-Trait Association for Drought Tolerance in US Rice Germplasm. *Plants*. 8 (12): 530-539.

Bhushan, D., Pandey, A., Choudhary, M.K., Datta, A., Chakraborty, S. and Chakraborty, N. 2007. Comparative Proteomics Analysis of Differentially Expressed Proteins in Chickpea Extracellular Matrix during Dehydration Stress. *Mol. Cellular .Proteomics*, 6 (11): 1868–1884.

Bimpong, I. K., R. Serraj, J. H. Chin, J. Ramos, E. M. T. Mendoza, J. E. Hernandez, M. S. Mendioro and D. S. Brar. 2011. Identification of QTLs for Drought-Related Traits

in Alien Introgression Lines Derived from Crosses of Rice (*Oryza sativa* cv. IR64) × *O. glaberrima* under Lowland Moisture Stress. *J. .Plant Biol*. 54: 237-250.

Black C.A. 1965. "Methods of Soil Analysis: Part I Physical and mineralogical properties". American Society of Agronomy, Madison, Wisconsin, USA.

Blum A. 2011. Drought tolerance: Is it a complex trait? *Funct Plant Biol*. 38(10): 753–757.

Blum, A. and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21 (1): 43-47.

Blum, A., Shpiler, L., Golan, G. and Mayer, J. 1989. Yield stability and canopy temperature of wheat genotypes under drought-stress. *Field Crops Res.* 22 (4): 289–296.

Boopathi, N.M., Swapnashri, G., Kavitha, P., Sathish, S., Nithya, R., Ratnam, W. and Kumar, A. 2013. Evaluation and Bulked Segregant Analysis of Major Yield QTL qtl12.1 Introgressed into Indigenous Elite Line for Low Water Availability under Water Stress. *Rice Sci.* 20 (1): 25–30.

Borba, T.C. de O., Brondani, R.P.V., Breseghello, F., Coelho, A.S.G., Mendonça, J.A., Rangel, P.H.N. and Brondani, C. 2010. Association mapping for yield and grain quality traits in rice (*Oryza sativa* L.). *Genet. Mol. Biol*. 33 (3): 515–524.

Bouman, B. A. M., Humphreys, E., Tuong, T. P., and Barker, R. 2007. Rice and water. *Adv. Agron.* 92: 187-237.

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y. and Buckler, E.S. 2007. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*. 23 (19): 2633–2635.

Bunnag, S. and Pongthai, P. 2013. Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. *Am. J. Plant Sci*. 4 (9): 1701-1708.

Cabuslay, G.S., Ito, O. and Alejar, A.A. 2002. Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. *Plant Sci.*163 (4): 815–827.

Cai, Y., Wang, W., Zhu, Z., Zhang, Z., Lang, Y. and Zhu, Q. 2006. Effects of water stress during grain-filling period on rice grain yield and its quality under different nitrogen levels.*J .appl. ecol*. 17: 1201-1210.

Cardon R. L. and Bell J. I. 2001 Association study designs for complex disease. *Nat. Rev. Genet.* 2: 91–99.

Carpentier, C. 1996. Infl uence of high light intensity on photosynthesis: Photoinhibition and energy dissipation. In M. Pessarakli, ed., Handbook of Photosynthesis. Marcel Dekker, New York. 443-450

Chandola, A. 2015.Temperature induction response of tomato cultivars in miatigation of water and heat stress. Ph.D THESIS. Institute of agricultural sciences .Banaras hindu university.144p

Chaturvedi, R., Joshi, J., Jayaraman, M., Govindasamy, B. and Ravindranath, N.H. 2012. Multi-model climate change projections for India under representative concentration pathways. *Current Sci*. 103. 791-802

Cha-um, S., Yooyongwech, S., and Supaibulwatana, K. 2010. Water deficit stress in the reproductive stage of four indica rice (*Oryza sativa* L.) genotypes. *Pak. J. Bot*. 42 (5): 3387-3398

Chen, J., Wang, P., Mi, H., Chen, G.-Y. and Xu, D.-Q. 2010. Reversible association of ribulose-1, 5-bisphosphate carboxylase/oxygenase activase with the thylakoid membrane depends upon the ATP level and pH in rice without heat stress. *J. Exp. Botany*. 61 (11): 2939–2950.

Choudhary, M.K., Basu, D., Datt, A., Chakraborty, N. and Chakraborty, S. 2009. Dehydration responsive nuclear proteome of rice (*Oryza sativa* L.) illustrates protein network, novel regulation of cellular adaptation and evolutionary perspective. *Mol. Cell. Proteomics*. 8: 1579 – 1589.

Chukwu, S.C., Rafii, M.Y., Ramlee, S.I., Ismail, S.I., Oladosu, Y., Okporie, E., Onyishi, G., Utobo, E., Ekwu, L., Swaray, S. and Jalloh, M. 2019. Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (*Oryza sativa* L.). *Biotechnol. Biotechnol Equipment*. 33 (1): 440–455.

Chungada, A.S., Gokhale, N.B., Patil, D.M.,Sawardwkar,SV. and Patil,P.P.2016. Molecular Screening of Rice Germplasm for Biotic and Abiotic Stresses and Their Diversity Study by Using SSR Markers. *J. Indian Soc. Coastal Agric. Res. 34 (2): 7-14*

Clark, L.J., Price, A.H., Steele, K.A. and Whalley, W.R. 2008. Evidence from near-isogenic lines that root penetration increases with root diameter and bending stiffness in rice. *Funct. Plant Biol.* 35 (11): 1163-1173.

Collard, B.C., and Mackill, D.J. 2007. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B*: Biol Sci.* 363 (1491): 557–572.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B. and Pang, E.C.K. 2008. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, 142 (1–2): 169–196.

Courtois, B., Sinha,B.G., Prasad, P.K.,Yadav,K., Rattan and Lishuang,S. 2000. Mapping QTL associated with drought avoidance in upland rice. *Molecular Breeding*. 6: 55-66.

Dellaporta, S. L., Wood, J., and Hicks, J. B. 1983. A rapid method for DNA extraction from plant tissue. *Plant Mol. Biol. Rep*. 1: 19-21.

DES [Directorate of Economics and Statistics]. 2014. Area, Production and Yield of Principal Crops. Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India. Available [on-line]:

Deshmukh, P. S., Kumar, A. R., Bhagat, K., Dhandapani, R., Tuti, M. D., Prakash, G., Kushwaha, S. R., and Singh, T. P. 2007. Strategies for improving abiotic stress

tolerance in crop plants. In: *National Seminar on Physiological and Molecular Approaches for Increasing Yield and Quality of Agricultural, Horticultural & Medicinal Plants Under Changing Environment,* 29-30th November and 1st December 2007. Raipur pp. 146-151.

Deshmukh, V., Mankar, S., Muthukumar, C., Divahar, P., Ayyenar, B., Thomas, H., Rajurkar, A., Sellamuthu, R., Ramalingam, P., Senthivel, S., Ranganathan and Chandrababu. 2019. Genome-Wide Consistent Molecular Markers Associated with Phenology, Plant Production and Root Traits in Diverse Rice (*Oryza sativa* L.) Accessions under Drought in Rainfed Target Populations of the Environment. *Current Sci*. 114: 329-340.

Dien, D.C., Mochizuki, T. and Yamakawa, T. 2019. Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in Rice (*Oryza sativa* L.) varieties. *Plant Production Sci.* 22 (4): 530–545.

Dingkuhn, M., Cruz, R., O'Toole, J. and Drffling, K. 1989. Net photosynthesis, water use efficiency, leaf water potential and leaf rolling as affected by water deficit in tropical upland rice. *Australian J Agricul Res.* 40 (6): 1171-1179.

Donde, R., Mohapatra, S., Baksh, S.Y., Padhy, B., Mukherjee, M., Roy, S., Chattopadhyay, K., Anandan, A., Swain, P., Sahoo, K.K., Singh, O.N., Behera, L. and Dash, S.K. 2020. Identification of QTLs for high grain yield and component traits in New Plant Types of rice. *Int. J. Microbiol*. 345-355.

Dubey, N., Avinashe, H., Jaiwar, S., Chichkhede, L., Mehta, A. 2018. Character Association and Path Analysis Study in Fodder Oat (*Avena sativa* L.). *Australian J Agricul Res.*233-243.

Ehret G. B. 2010. Genome-wide association studies: contribution of genomics to understanding blood pressure and essential hypertension. *Current hypertension reports, 12* (1): 17–25. https://doi.org/10.1007/s11906-009-0086-6

Ehret, G. B. 2010. Genome-wide association studies: contribution of genomics to

understanding blood pressure and essential hypertension. *Current hypertension reports*. 12 (1): 17–25.

Ersoz, E.S., Yu, J. and Buckler, E.S. 2009. Applications of Linkage Disequilibrium and Association Mapping in Maize. *Mol. Genet. Approaches Maize Impr.* 173–195.

Fahad, S., Ihsan, M.Z., Khaliq, A., Daur, I., Saud, S., Alzamanan, S., Nasim, W., Abdullah, M., Khan, I.A., Wu, C., Wang, D. and Huang, J. 2019. Consequences of high temperature under changing climate optima for rice pollen characteristics-concepts and perspectives. *Archives Agrono Soil Sci.* 64 (11): 1473–1488.

Fahramand, M., Mahmoody, M., Keykha, A., Noori, M. and Rigi, K. 2014. Influence of abiotic stress on proline Photosynthetic enzymes and growth. *Int Res J Appl Basic Sci.* 8. 257-265.

FAO [Food and Agriculture Organization]. 2018. Food and Agricultural Organization of United Nations, Rome. Available: http://faostat.fao.org.

Farooq, M., Kobayashi, N., Ito. O., Wahid, A., and Serraj, R. 2010. Broader leaves result in better performance of indica rice under drought stress. *J. Plant Physiol*. 167 (13): 1066–1075.

Fei, X., Jin, Li., Huang, Y., Tong, C., Chen, Y. and Bao, J. 2016. Association mapping of quantitative trait loci for yield-related agronomic traits in rice (*Oryza sativa* L.). *J Int. Agricul.* 15: 2192-2202.

Fei, X., Tang, F., Shao, Y., Chen, Y., Tong, C. and Bao, J. 2014. Genotype × Environment Interactions for Agronomic Traits of Rice Revealed by Association Mapping. *Rice Sci*. 21. 133–141.

Fen, L.L., Ismail, M.R., Zulkarami, B., Rahman, M.S.A. and Islam, M.R. 2015. Physiological and molecular characterization of drought responses and screening of drought tolerant rice varieties. *Biosci J.* 31 (3): 709–718.

Feng, J.Y., Wen, Y.J., Zhang, J. and Zhang, Y.M. 2016. Advances on Methodologies for

Genome-wide Association Studies in Plants. *Acta Agronomica Sinica*, 42 (7): 945-965.

Fischer, K.S., Fukai, S., Kumar, A., Leung, H. and Jongdee, B. 2012. Field Phenotyping Strategies and Breeding for Adaptation of Rice to Drought. *Frontiers Physiol.* 3:33-43.

Fischer, R.A. and Maurer, R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield response. *Australian J. Agric. Res*. 29: 897- 903.

Flint-Garcia, S. A., Thornsberry, J. M. and Buckler, E. S. T. 2003. Structure of linkage disequilibrium in plants. *Annu. Rev. Plant Biol*. 54: 357-374.

Fujino, K., Obara, M., Shimizu, T., Koyanagi, K.O. and Ikegaya, T. 2015. Genome-wide association mapping focusing on a rice population derived from rice breeding programs in a region. *Breeding Sci.* 65 (5): 403–410.

Garg, H. and Bhattacharya, C. 2017. Drought tolerance indices for screening some of rice genotypes. *Int.J.Agric.Biol.Res.* 7 (4): 671–674.

Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S. and McCouch, S. 2005. Genetic Structure and Diversity in *Oryza sativa* L. *Genetics*. 169 (3): 1631–1638.

Garrity, D. P. and O' Toole, J. C. 1995. Selection for reproductive stage drought avoidance in rice, using infrared thermometry. *Agron. J.* 87 (4): 773-779.

Gilavaei, M.D., Lahigi,H.S. and Rabiei. 2018. Evaluation of drought tolerance in rice cultivars and recombinant inbred lines. *Iranian J Plant Breed Genet*. 7 (1):1-9.

Gouda P.K., Varma C.M.K., Saikumar S., Kiran B., Shenoy V., Sashidhar H.E. 2012. Direct selection for grain yield under moisture stress in *Oryza sativa* cv. IR58025B x O. meridionalis population. *Crop Sci.* 52: 644-653.

Gowda,R.P., Amelia,H., Akira,Y., Halagappa ,S. and Rachid,S. 2011. Root biology and genetic improvement for drought avoidance in rice. *Field Crp.Res*. 124. 46-58.

Gupta, P.K., Rustgi, S.and Kulwal, P.L.2005. Linkage disequilibrium and association studies in higher plants: Present status and future prospects. *Plant Mol Biol.* 57: 461–485.

Ha, P. T. T. 2014. Physiological responses of rice seedlings under drought stress. *J. Sci. Dev*. 12 (5): 635–640.

Halder, K. P. and Burrage, S. W 2003. Drought Stress Effects on Water Relations of Rice Grown in Nutrient Film Technique. *Pakistan J Biol Sci* 6 (5): 441–444.

Hall, D., Tegstrom, C. and Ingvarsson, P.K. 2010. Using association mapping to dissect the genetic basis of complex traits in plants. *Briefings. Funct. Genom.* 9 (2); 157–165.

Haque, K. M., Karim, M.D., Bari, M., Islam, M. 2016. Genotypic variation in the effect of drought stress on phenology, morphology and yield of aus rice. *Int J Biosci*. 8. 73-82.

He, J., Zhao, X., Laroche, A., Lu, Z.-X., Liu, H. and Li, Z. 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. *Frontiers in Plant Sci.* 5.33-43.

Hemamalini, G.S., Shashidhar, H.E.and Hittalmani,S. 2000 Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). *Euphytica* 112:69–78.

Henry, A., Dixit, S., Mandal, N.P., Anantha, M.S., Torres, R. and Kumar, A. 2014. Grain yield and physiological traits of rice lines with the drought yield QTL qDTY12.1 showed different responses to drought and soil characteristics in upland environments. *Funct. Plant Biol.* 41 (11): 1066-1074.

Hirayama, M., Wada, Y. and Nemoto, H. 2006. Estimation of Drought Tolerance Based on Leaf Temperature in Upland Rice Breeding. *Breeding Sci.* 56 (1): 47–54.

Hiscox, J.D. and Israelstam, G.F. 1979. Different methods of chlorophyll extraction. *Can. J. Bot*. 57: 1332-1332.

Hittalmani, S., Huang, N., Courtois, B., Venuprasad, R., Shashidhar, H.E., Zhuang, J.-Y., Zheng, K.-L., Liu, G.-F., Wang, G.-C., Sidhu, J.S., Srivantaneeyakul, S., Singh, V.P., Bagali, P.G., Prasanna, H.C., McLaren, G. and Khush, G.S. 2003. Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. *Theor Appl Genet* 107 (4): 679–690.

Hittalmani, S., Shashidhar, H.E., Bagali, P.G., Huang, N., Sidhu, J.S., Singh, V.P. and Khush, G.S. 2002. Molecular mapping of quantitative trait loci for plant growth, yield and yield related traits across three diverse locations in a doubled haploid rice population. *Euphytica*, 125 (2): 207–214.

Hosseini S. J., Tahmasebi Sarvestani Z., and Pirdashti H. 2012. Analysis of tolerance indices in some rice (*Oryza sativa* L.) genotypes at salt stress condition. *Int.Res.J. Applied. Basic Sci.* 3 (1):1-10.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M., Fan, D., Guo, Y., Wang, A., Wang, L., Deng, L., Li, W., Lu, Y., Weng, Q., Liu, K., Huang, T., Zhou, T., Jing, Y., Li, W., Lin, Z., Buckler, E.S., Qian, Q., Zhang, Q.-F., Li, J. and Han, B. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*. 42 (11): 961–967.

Ibrahim, M., Jaafar, H., Karimi, E. and Ghasemzadeh, A. 2019. Impact of Organic and Inorganic Fertilizers Application on the Phytochemical and Antioxidant Activity of Kacip Fatimah (Labisia pumila Benth). *Molecul.*18 (9): 10973–10988.

Iqbal, M.A. and Rahman, M. 2017. Identification of Marker-Trait Associations for Lint Traits in Cotton. *Frontiers in Plant Science*, 8.

IRRI [International Rice Research Institute]. 1996. *International Network for Genetic Evaluation of Rice: Standard Evaluation System for Rice.* International Rice Research Institute, Los Banos, Philippines, 168p.

IRRI. 2014. Standard Evaluation System for Rice. 5th edn. Los Banos, the Philippines: International Rice Research Institute

Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J., Somasundaram, R.and Panneerselvam R. 2009. Drought stress in plants: A review on morphological characteristics and pigments composition. *Int J Agric Biol*.11: 100–105.

Jayaweera, J., Herath, V., Jayatilake, D., Udumulla, G and Wickramasinghe, H. 2016. Physiological, Biochemical and Proteomic Responses of Rice (Oryza sativa L.)Varieties Godaheenati and Pokkali for Drought Stress at the Seedling Stage. *Tropic Agric Res*. 27. 159-170.

Jennings, H. S.1917. The numerical results of diverse systems of breeding, with respect to two pairs of characters, linked or independent, with special relation to the effects of linkage. *Genetics.* 2: 97–154.

Ji, K., Wang, Y., Sun, W., Lou, Q., Mei, H., Shen, S. and Chen, H. 2012. Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *J. Plant Physiol.* 169 (4): 336–344.

Jolliffe, I.T. and Cadima, J. 2016. Principal component analysis: a review and recent developments. *Mathematical, Physical Eng Sci.* 374 (2065): 343-354.

Jones, H.G. and Corlett, J.E. 1992. Current topics in drought physiology. *J Agricul Sci*. 119 (3): 291–296.

Kadan, N. N., Tamilselvan, A., Lawas, L., Quinones, C., Bahuguna, R. N., Thomson, M. J., Dingkuhn, M., Muthurajan, R., Struik, P. C., Yin, X., and Jagadish, S. 2015. Genetic Control of Plasticity in Root Morphology and Anatomy of Rice in Response to Water Deficit. *Plant physiol.174* (4): 2302–2315.

Kadioglu, A. and Terzi, R. 2007. A Dehydration Avoidance Mechanism: Leaf Rolling. *The Botanical Review* .73 (4): 290–302.

Kamoshita, A., Babu, R.C., Boopathi, N.M. and Fukai, S. 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Res.* 109 (1–3): 1–23.

Kamoshita, A., Rodriguez, R., Yamauchi, A., and Wade, L. J. 2004. Genotypic variation in response of rainfed lowland rice to prolonged drought and dewatering. *Plant Produc. Sci.* 7: 406-420

Kanagaraj, P., Prince, S., Annie, S., Joseph. Biji, K., Paul, S., Alagarswamy, Senthil., Ranganathan and Chandrababu. 2010. Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current sci*. 98. 836-839.

Kanbar, A. and Shashidhar, H.E. 2010. Participatory selection assisted by DNA markers for enhanced drought resistance and productivity in rice (*Oryza sativa* L.). *Euphytica*, 178 (1):137–150.

Kang, D.J. and Futakuchi, K. 2019. Effect of Moderate Drought-Stress on Flowering Time of Interspecific Hybrid Progenies (*Oryza sativa* L. × *Oryza glaberrima* Steud.). *J. Crop Sci Biotechnol.* 22 (1): 75–81.

Kang, H.M., Zaitlen, N.A., Wade, C.M., Kirby, A., Heckerman, D., Daly, M.J. and Eskin, E. 2008. Efficient Control of Population Structure in Model Organism Association Mapping. *Genetics*. 178 (3): 1709–1723.

Kardile, P.B., Dahatonde, K.N., Rakshe, M.V. and Burondkar, M.M. 2018. Effect of Moisture Stress on Leaf Relative Water Content (RWC) of Four Cowpea *(Vigna unguiculata* L. walp.) Genotypes at Different Stages of Growth. *Int J Current Microbiol Appl Sci.* 7 (04): 2645–2649.

Kato, Y. and Katsura, K. 2010. Panicle architecture and grain number in irrigated rice, grown

under different water management regimes. *Field Crop Res.* 117 (2–3): 237–244.

Khan,I.M. and Dhurve,O.P. 2016. Drought response indices for identification of drought tolerant genotypes in rainfed upland rice (*oryza sativa* l.). *Int J Sci Environ*. 5 (1): 73 – 83.

Khush, G. S. 2001. Green revolution: the way forward. *Nature Rev*. 2: 815-822.

Khush, G.S. 1997. Origin, dispersal, cultivation and variation of rice. *Plant Mol Biol.* 35: 25–34

Khush, G.S. 2005. What it will take to Feed 5.0 Billion Rice consumers in 2030. *Plant Molecul Biol.* 59 (1): 1–6.

Kondhia, A., Escleto Tabien, R. and Ibrahim, A. 2015. Evaluation and Selection of High Biomass Rice (*Oryza sativa L.*) for Drought Tolerance. *American J Plant Sci*. 06 (12): 1962–1972.

Kumar, A., Bernier, J., Verulkar, S., Lafitte, H.R. and Atlin, G.N. 2008. Breeding for drought tolerance: Direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crop Res.* 107 (3): 221–231.

Kumar, A., Dixit, S., Ram, T., Yadaw, R.B., Mishra, K.K. and Mandal, N.P. 2014. Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. *J Exp Bot*. 65: 6265–6278.

Kumar, A., Sandhu, N., Dixit, S., Yadav, S., Swamy, B. and Shamsudin, N. 2018. Marker-assisted selection strategy to pyramid two or more QTLs for quantitative trait-grainyield under drought. *Rice.*11 (1): 35-45.

Kumar, R., Sreenu, K., Singh, N., Jain, N., Singh, N. K. and Rai, V. 2015. Effect of drought stress on contrasting cultivars of rice. *Int J Trop Agric*. 33 (2): 1559–1564.

Kumar, S., Dwivedi, S. K., Singh, S. S., Bhatt, B. P., Mehta, P., Elanchezhian, R., Singh, V.

P., and Singh, O. N. 2014. Morphophysiological traits associated with reproductive stage drought tolerance of rice (*Oryza sativa* L.) genotypes under rain-fed condition of eastern Indo-Gangetic Plain. *Indian J. Plant Physiol*. 19 (2): 87-93.

Lafitte, H. R. 2002. Relationship between leaf relative water content during reproductive stage water deficit and grain formation in rice. *Field Crop Res*.76: 165–174.

Lafitte, H.R., Price, A.H. and Courtois, B. 2003. Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers. *Theoret Appl Genetics*. 109 (6): 1237–1246.

Lakshmi, P. M., Chen, X., Clarke, J., Salmeron, J. and Nguyen, H. T. 2012. RNAi-mediated disruption of squalene synthase improves drought tolerance and yield in rice. *J Exp. Bot*. 63: 163-75.

Lanceras, J.C., Pantuwan, G., Jongdee, B. and Toojinda, T. 2004. Quantitative Trait Loci Associated with Drought Tolerance at Reproductive Stage in Rice. *Plant Physiol* 135 (1): 384–399.

Lang, N. and Buu, B. 2008. Fine mapping for drought tolerance in rice (*Oryza sativa* L.). *Omonrice*, 16: 9–15.

Lang, N., Nha, C., Ha, P. and Buu, B. 2013. Quantitative trait loci (qtls) associated with drought tolerance in rice (*Oryza sativa* L.). *SABRAO J. Breeding Genet* 45 (3): 409–421.

Larkunthod, P., Nounjan, N., Siangliw, J.L., Toojinda, T., Sanitchon, J., Jongdee, B. and Theerakulpisut, P. 2018. Physiological Responses under Drought Stress of Improved Drought-Tolerant Rice Lines and their Parents. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 46 (2): 679–687.

Lauteri, M., Haworth, M., Serraj, R., Monteverdi, M. C., and Centritto, M. 2014. Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. *PLoS One* 9 (10): 109-154.

Lewontin, R.C.1964. The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics.* 49: 49–67

Li, Z.K. and Xu, J.L. 2007. Breeding for Drought and Salt Tolerant Rice (Oryza Sativa L.): Progress and Perspectives. *Advance Molecu Breedi Drought and Salt Tolerant Crop.* 531–564.

Liang, J., Y. Lu, P. Xiao, M. Sun, H. Corke and J. Bao. 2010. Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. *Theor. Appl. Genet*. 121: 475-87.

Liebler, D. C., Kling, D. D., and Reed, D. J. 1986. Antioxidant protection of phosopholipid bilayers by tocopherol. Control of tocopherol status and lipid peroxidation by ascorbic acid and glutathione. *J. Biol. Chem.* 261: 12114-12119.

Lin, M.H., Lin, C.W., Chen, J.C., Lin, Y.C., Cheng, S.Y., Liu, T.H., Jan,F .J.,Wu,S.,Thenseng,F.S. and Ku,H.M.2007. Tagging Rice Drought-related QTL with SSR DNAMarkers. *Crop. Environ.Bioinform* .4: 65-76.

Lipka, A.E., Kandianis, C.B., Hudson, M.E., Yu, J., Drnevich, J., Bradbury, P.J. and Gore, M.A. 2015. From association to prediction: statistical methods for the dissection and selection of complex traits in plants. *Current Opinion Plant Biol.* 24: 110–118.

Liu, J.X., Liao, D.Q., Oane, R., Estenor, L., Yang, X.E., Li, Z.C. and Bennett, J. 2008. Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. *Field Crops Res* 97 (1): 87–100.

Lu, Q., Zhang, M., Niu, X., Wang, S., Xu, Q., Feng, Y., Wang, C., Deng, H., Yuan, X., Yu, H., Wang, Y. and Wei, X. 2015. Genetic variation and association mapping for 12

agronomic traits in indica rice. *BMC Genomics*, 16 (1). 45-56.

Madhan Mohan, M. 2000. Chlorophyll Stability Index (CSI): its impact on salt tolerance in rice. *Int Rice Res Notes*. 25: 38-39.

Mallikarjuna, B., Nanjappa., Shivakumar., Hittalmani., Shailaja, G.D., Prahalada ., Chandappa., Lohithaswa. and Krishnappa, M.R. 2013. Validation of SSR markers linked to fertility restoration in rice (*Oryza sativa* L.). *Res Crops*. 14: 324-329.

Manickavelu, A., Nadarajan, N., Ganesh, S.K., Gnanamalar, R.P. and Chandra Babu, R. 2006. Drought tolerance in rice: morphological and molecular genetic consideration. *Plant Growth Regul.* 50 (2–3): 121–138.

Manivannan, P., Jaleel, C., Sankar, B., Kishorekumar, A., Somasundaram, R., Lakshmanan, Ganapathy M. and Panneerselvam, R. 2007. Growth, biochemical modifications and proline metabolism in Helianthus annuus L. as induced by drought stress. Colloids and surfaces. *B, Biointerfaces*. 59: 141-149.

Martin, M., Morgan, J., Zerbi G. and Lecain, D. 1997. Water stress imposition rate affects osmotic adjustment and cell wall properties in winter wheat. *Ital. J. Agron.*, 1: 11-20.

Mather, K.A., Caicedo, A.L., Polato, N.R., Olsen, K.M., McCouch, S. and Purugganan, M.D. 2007. The Extent of Linkage Disequilibrium in Rice (*Oryza sativ*a L.). *Genetics*, 177 (4): 2223–2232.

Mathew, I., Shimelis, H., Shayanowako, A.I.T., Laing, M. and Chaplot, V. 2019. Genome-wide association study of drought tolerance and biomass allocation in wheat. *PLOS ONE*, 14(12), p.e0225383.

McCouch, S.R., McNally, K.L., Wang, W. and Sackville Hamilton, R. 2012. Genomics of gene banks: A case study in rice. *American J Bot.* 99 (2): .407–423.

Mehraban, A., Tobe, A., Gholipouri, A., Amiri, E., Ghafari, A. and Rostaii, M. 2018. The Effects of Drought Stress on Yield,Yield Components, and Yield Stabilityat

Different Growth Stages in Bread WheatCultivar (*Triticum aestivum* L.). *Polish J.Environ. Studies.* 28 (2): 739–746.

Mishra, S.S., Behera, P.K., Kumar, V., Lenka, S.K. and Panda, D. 2018. Physiological characterization and allelic diversity of selected drought tolerant traditional rice (*Oryza sativa* L.) landraces of Koraput, India. *Physiol Molecular Biol Plants* 24 (6): 1035–1046.

Mitra, Jiban. 2001. Genetics and genetic improvement of drought resistance in crop plants. *Current Sci*; 48: 683-695.

Moonmoon, S. and Islam, M. 2017. Effect of Drought Stress at Different Growth Stages on Yield and Yield Components of Six Rice (*Oryza sativa* L.) Genotypes. *Fundamental and Appl Agricul.* 2 (3): 13-43.

Mostajeran, A. and Rahimi-Eichi, V. 2009. Effects of Drought Stress on Growth and Yield of Rice (*Oryza sativa* L.) Cultivars and Accumulation of Proline and Soluble Sugars in Sheath and Blades of Their Different Ages Leaves. *J. Agric. Environ. Sci*.5 (2): 264–272.

Musila, R.N. 2015. Genetic analysis for drought tolerance and yield stability in interspecific and *oryza sativa* L. rice germplasm. University of KwaZulu-Natal Republic of South Africa.218p.

Muthukumar, C., Subathra, T., Aiswarya, J., Gayathri, V., and Chandra Babu, R. 2015. Comparative genome-wide association studies for plant production traits under drought in diverse rice (*Oryza sativa* L.) lines using SNP and SSR markers. *Curr. Sci*. 109: 139-147.

Nahakpam, S. 2017. Effectual tolerant traits for grain yield in rice genotypes grown under drought .*J. Pharmacognosy Phytochemistry*. 1:890–897.

Nahakpam, S. 2018. Chlorophyll Stability: A Better Trait for Grain Yield in Rice under

Drought. *Indian J Ecol*. 44.122-134.

Nahar, K., Hasanuzzaman, M., Alam, M. M., Rahman, A., Suzuki, T., and Fujita, M. 2016. Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through up regulating the metal detoxification, antioxidant defense, and methylglyoxal detoxification systems. *Eco .toxicol. Environ. Saf*. 126: 245–255.

Nam, K.H.,Shin,H.J., Pack,I.S.,Park,J.H.,Kim,H.B. and Kim,C.2014. Growth stage-based metabolite profiling of drought-tolerant transgenic rice under well watered and deficit conditions. *Plant omics J*. 587-594.

Nathan, K. K. 2012. Characteristics of Drought in Kerala, India. Drought. *Net. News* 12 (1): 1-5.

Ndjiondjop, M.N., Seck, P.A., Lorieux, M., Futakuchi, K., Yao, N., Djedatin, G., Sow, M., Bocco, R., Cisse, F. and Fatondji, B. 2012. Effect of Drought on *Oryza glaberrima* Rice Accessions and Oryza glaberrima Derived-lines. *Asian J Agric Res.* 6: 144-157.

Ndjionjop, M.N., Manneh, B., Cissoko, M., Drame , N. K., Kakai, R. G., Bocco, R., Baimey, H and Wopereis, M. 2010. Drought resistance in an interspecific backcross population of rice (*Oryza* spp.) derived from the cross WAB56-104 (*O. sativa*) × CG14 (*O. glaberrima*). *Plant Sci*. 179: 364-373.

Neumann, K., Kobiljski, B., Denčić, S., Varshney, R.K. and Börner, A. 2010. Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). *Molecular Breeding*. 27 (1): 37–58.

Nguyen, H.T., Babu, R.C. and Blum, A. 1997. Breeding for Drought Resistance in Rice: Physiology and Molecular Genetics Considerations. *Crop Sci.* 37 (5): 1021-1033.

Nordborg, M. and Tavare, S. 2002. Linkage disequilibrium: what history has to tell us? *Trends Genet*.18: 83–90.

Nordborg, M. and Weigel, D. 2008. Next-generation genetics in plants. *Nature*. 456 (7223): 720–723.

Norton, G.J., Travis, A.J., Douglas, A., Fairley, S., Alves, E.D.P., Ruang-areerate, P., Naredo, M.E.B., McNally, K.L., Hossain, M., Islam, M.R. and Price, A.H. 2018. Genome Wide Association Mapping of Grain and Straw Biomass Traits in the Rice Bengal and Assam Aus Panel (BAAP) Grown Under Alternate Wetting and Drying and Permanently Flooded Irrigation. *Frontiers in Plant Sci.* 9.45-56.

Nounjan, N., Chansongkrow, P., Charoensawan, V., Siangliw, J.L., Toojinda, T., Chadchawan, S. and Theerakulpisut, P. 2018. High Performance of Photosynthesis and Osmotic Adjustment Are Associated With Salt Tolerance Ability in Rice Carrying Drought Tolerance QTL: Physiological and Co-expression Network Analysis. *Frontiers in Plant Sci.* 9. 33-45.

O'Toole, J. C. 2004. Rice and water: the final frontier. In: The first international conference on rice for the future. Bangkok, Thailand: The Rockefeller Foundation

Okami, M., Kato, Y., Kobayashi, N., and Yamagishi, J. 2015. Morphological traits associated with vegetative growth of rice (*Oryza sativa* L.) during the recovery phase after early-season drought. *European J Agronomy*. 64:58-66

Oladosu, Y., Rafii, M.Y., Magaji, U., Abdullah, N., Miah, G., Chukwu, S.C., Hussin, G., Ramli, A. and Kareem, I. 2018. Genotypic and Phenotypic Relationship among Yield Components in Rice under Tropical Conditions. *BioMed Res Int.* 2018. 1–10.

Olsen, K.M., Caicedo, A.L., Polato, N., McClung, A., McCouch, S. and Purugganan, M.D. 2006. Selection under Domestication: Evidence for a Sweep in the Rice Waxy Genomic Region. *Genetics*. 173 (2): 975–983.

Oraibi,A.S., Ibrahim,K.M.and Yousif,S.A. 2014. Screening of some rice *Oryza sativa* L. genotypes for drought tolerance using SSR-PCR technique. J Biotechnol Res Center.8 (2):14-18.

Ouk, M., Basnayake, J., Tsubo, M., Fukai, S., Fischer, K. S., Cooper, M., and Nesbitt, H. 2006. Use of drought response index for identification of drought tolerant genotypes in rainfed lowland rice. *Field Crops Res.* 99 (1): 48-58.

Öztürk-Çolak, A., Moussian, B., Araújo, S.J. and Casanova, J. 2016. A feedback mechanism converts individual cell features into a supracellular ECM structure in Drosophila trachea. *eLife*. 5: 33-45.

Pandey, S. and Bhandari, H. 2007. Drought: economics costs and research implications. In: Serraj, R., Bennet, J., Hardy, B. (Eds.), Drought Frontiers in Rice: Crop Improvement for Increased Rainfed Production. World Scientific Publishing and Los Banos (Philippines): International Rice Research Institute, Singapore, 3–17.

Pandey, S., Bhandari, H. and Hardy, B. 2005. *Economic costs of drought and rainfed rice farmers' coping mechanisms in eastern India. Final project report*.

Pandey, S., Bhandari, H., Ding, S., Prapertchob, P., Sharan, R., Naik, D., Taunk, S.K., and Sastri A .2007. Coping with drought in rice farming in Asia: insights from a cross-country comparative study. *Agric Econ*. 37: 213–224.

Pandey, V. and Shukla, A. 2015. Acclimation and Tolerance Strategies of Rice under Drought Stress. *Rice Sci.* 22 (4).147–161.

Pantuwan, G., Fukai, S., Cooper, M., Rajatasereekul, S. and O'Toole, J. 2002. Yield response of rice (*Oryza sativa* L.) genotypes to different types of drought under rainfed lowlands. *Field Crops Res.* 73 (2–3): 153–168.

Parisa Sharifi 2012. Relationship between drought stress and some antioxidant enzymes with cell membrane and chlorophyll stability in wheat lines. *African J Microbiol Res*. 6 (3).67-77.

Pradhan, B., Mishra, T.K. and Das, S.R.2015 Genetic basis of yield variations in Lowland rice. *Int. J Basic and App. Sci*. 19: 306-318.

Pradhan, S.K., Barik, S.R., Sahoo, A., Mohapatra, S., Nayak, D.K., Mahender, A., Meher,

J., Anandan, A. and Pandit, E. 2016. Population Structure, Genetic Diversity and Molecular Marker-Trait Association Analysis for High Temperature Stress Tolerance in Rice. *PLOS ONE*, 11 (8), p.e0160027.

Price, A.H., Townend, J., Jones, M.P., Audebert, A. and Courtois, B. 2002. Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. *Plant MolecuL Biol* 48 (5/6): 683–695.

Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. and Reich, D. 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics*, 38 (8): 904–909.

Prince, S.J., Beena, R., Gomez, S.M.2015 Mapping Consistent Rice (*Oryza sativa* L.) Yield QTLs under Drought Stress in Target Rainfed Environments. *Rice.* 8: 25-35.

Pritchard J. K., Stephens, M. and Donnelly, P. 2000a. Inference of population structure using multilocus genotype data. *Genetics*. 155: 945–959.

Pritchard, J.K., Stephens, M., Rosenberg, N.A. and Donnelly, P. 2000. Association Mapping in Structured Populations. *American J Human Genetics*. 67 (1). 170–181.

Purbajanti, E.D., Kusmiyati, F. and Fuskhah, E. 2017. Growth, Yield and Physiological Characters of Three Types of Indonesian Rice under Limited Water Supply. *Asian J Plant Sci.* 16 (2): 101–108.

Pushpam, R., Manonmani, S., Varthini, N.V. and Robin, S. 2018. Studies on yield, root characters related to drought tolerance and their association in upland rice genotypes. *Electronic J Plant Breeding*. 9 (3). 816-823.

Quampah A,Wang., R.M. ,Shamsi,I.H, Jilani, G., Zhang, Q., Hua,S. and Xu ,H. 2011.Improving water productivity by potassium application in various rice genotypes. *Int J Agric Biol*. 13: 9- 17.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation

for Statistical Computing, Vienna, Austria

Rafalski, A. 2002. Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol*, 5; 94–100.

Rahimi, M., Dehghani, H., Rabiei, B., & Tarang, A. R. 2013. Evaluation of rice segregating population based on drought tolerance criteria and biplot analysis. *Int J Agricul Crop Sci.* 5(3); 194-204.

Rahman, M. T., Islam, M. T. and Islam, M .O. 2002. Effect of water stress at different growth stages on yield and yield contributing characters of transplanted Aman rice. *Pak J Biol Sci*.5 (2): 169–172.

Rang, Z.W., Jagadish, S.V.K., Zhou, Q.M., Craufurd, P.Q. and Heuer, S. 2011. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. *Environment Experiment Botany*. 70 (1): 58–65.

Ray, D. K., Gerber, J. S., MacDonald, G. K., and West, P. C. 2015. Climate variation explains a third of global crop yield variability. *Nat. Commun*. 6: 59-89.

Rejeth, R.2017. Identification of microsatellite markers associated with root traits for drought tolerance in rice (*Oryza sativa* L.). Kerala agricultural university.132p.

Remington, D.L., Thornsberry, J.M., Matsuoka, Y., Wilson, L.M., Whitt, S.R., Doebley, J., Kresovich, S., Goodman, M.M. and Buckler, E.S. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proceedings National Academy Sci.* 98 (20): 11479–11484.

Sah, S. and Zamora, O. 2005. Effect of Water Deficit at Vegetative and Reproductive Stages of Hybrid, Open Pollinated Variety and Local Maize (*Zea mays* L.). *J Institute Agricul Animal Sci* 26: 37–42.

Sahoo J P, Sharma, V., Verma, R., Chetia, S., Baruah, A., Modi, M. and Yadav, V. 2019. Linkage analysis for drought tolerance in kharif rice of Assam using microsatellite markers. *Indian j traditional knowledge*. 18. 371-375.

Sahoo, J.P., Sharma, V., Verma, R., Chetia, S.,Baruah, A ., Modi, M and Yadav, V. 2019. Linkage analysis for drought tolerance in kharif rice of Assam using microsatellite markers. *Indian j traditional knowledge*. 18. 371-375.

Saikumar, S., Varma, C.M.K., Saiharini, A., Kalmeshwer, G.., Nagendra, K., Lavanya, K. and Ayyappa, D. 2016. Grain yield responses to varied level of moisture stress at reproductive stage in an interspecific population derived from Swarna /O. glaberrima introgression line. *NJAS - Wageningen J Life Sci.* 78: 111–122.

Salunkhe, A.S., Poornima, R., Prince, K.S.J., Kanagaraj, P., Sheeba, J.A., Amudha, K., Suji, K.K., Senthil, A. and Babu, R.C. 2011. Fine Mapping QTL for Drought Resistance Traits in Rice (*Oryza sativa* L.) Using Bulk Segregant Analysis. *Molecular Biotechnol*. 49 (1): 90–95.

Samarah, N.H. 2005. Effects of drought stress on growth and yield of barley. *Agronomy Sustainable Develop.* 25 (1): 145–149.

Sambrook, J. and Russell, D. W. 2000. *Molecular Cloning: A Laboratory Manual*. (3rd Ed.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Pp.345-356.

Sarvestani, Z. T., Pirdashti, H., Sanavy, S. A. M. M. and Balouchi , H. 2008. Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars. *Pak J Biol Sci*. 11(10): 1303–1309.

SAS Institute. Inc. 1990. SAS/STAT user's guide, Version 6 4th edition, vols 1 and 2. SAS institute Inc., Cary, North California, USA.

Savchenko, G.E., Klyuchareva, E.A., Abramchik, L.M. and Serdyuchenko, E.V. 2002.

Effect of periodic heat shock on the membrane system of etioplasts. *Russian J Plant Physiol* 49 (3): 349–359.

Selvaraj, R., Muthurajan, R. and Robin, S. 2016. Mapping QTLs for Physiological Traits Associated with Drought Tolerance in Rice (*Oryza Sativa L.). Rice. 3.*

Serraj R., Kumar A., McNally K.L., Slamet-Loedin I., Bruskiewich R., Mauleon R., Cairns J. and Hijmans R.J. 2009. Improvement of drought resistance in rice. *Advances Agronomy.* 103: 41– 99.

Serraj, R., McNally, K.L., Slamet-Loedin, I., Kohli, A., Haefele, S.M., Atlin, G. and Kumar, A. 2011. Drought Resistance Improvement in Rice: An Integrated Genetic and Resource Management Strategy. *Plant Production Sci.* 14 (1): 1–14.

Shamsudin, N.A.A., Swamy, B.P.M., Ratnam, W., Sta. Cruz, M.T., Raman, A. and Kumar, A. 2016. Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. *BMC Genetics*. 17 (1): 23-33.

Shao, H., Chu, L., Shao, M., Jaleel, C.A. and Hong-mei, M. (2008). Higher plant antioxidants and redox signaling under environmental stresses. *Comptes Rendus Biologies*, 331 (6): 433–441.

Sharifi, P., Amirnia, R., Majidi, E., Hadi, H., Roostaei, M.,Nakhoda, B ., Alipoor, H. and Moradi, F. 2012. Relationship between drought stress and some antioxidant enzymes with cell membrane and chlorophyll stability in wheat lines. *Afric. j. of microbiol res*. 6. 10.5897/AJMR11.1167.

Sheeba, N.K., Viraktamath, B.C., Sivaramakrishnan, S., Gangashetti, M.G., Khera, P. and Sundaram, R.M. 2009. Validation of molecular markers linked to fertility restorer gene(s) for WA-CMS lines of rice. *Euphytica*, 167 (2): 217–227.

Shi, W., Ishimaru, T., Gannaban, R.B., Oane, W. and Jagadish, S.V.K. 2015. Popular Rice (*Oryza sativa* L.) Cultivars Show Contrasting Responses to Heat Stress at Gametogenesis and Anthesis. *Crop Sci.* 55 (2): 589–596.

Shinde, S.R., Kumar, K. and Pawar, R.M. 2015. Genetic studies in upland rice (*Oryza sativa* L.). *Int. J. Plant. Sci.* 10 (1): 33–37.

Shrestha, J. 2019. Drought Stress in Rice (*Oryza Sativa* L.). Retrieved from https://www.linkedin.com/pulse/drought-stress-rice-oryza-sativa-l-jibanshrestha/

Sikuku, P. A., Onyango, J. C. and Netondo, G. W. 2012. Yield components and gas exchange responses of Nerica rice varieties (*Oryza sativa* L.) to vegetative and reproductive stage water deficit. *Global J. Sci. Frontier Res.* 12: 51-62.

Sikuku, P. and Onyango, J. 2012. Physiological and biochemical responses of five nerica rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage. *Agricul Biology J North America*. 3 (3): 93–104.

Singh, A.K., Singh, H., Rai, O.P., Singh, G., Singh, V.P., Singh, N.P. and Singh, R. 2017. Effect of sowing dates and varieties for higher productivity of Indian mustard (Brassica juncea L.). *J. Applied Natural Sci.* 9 (2): 883–887.

Singh, B., Reddy, K.R., Redoña, E.D. and Walker, T. 2017. Screening of Rice Cultivars for Morpho-Physiological Responses to Early-Season Soil Moisture Stress. *Rice Sci.* 24 (6): 322–335.

Singh, N., Dang, T.T.M., Vergara, G.V., Pandey, D.M., Sanchez, D., Neeraja, C.N., Septiningsih, E.M., Mendioro, M., Tecson-Mendoza, E.M., Ismail, A.M., Mackill, D.J. and Heuer, S. 2010. Molecular marker survey and expression analyses of the rice submergence-tolerance gene SUB1A. *Theoret Applied Genet*. 121 (8): 1441–1453.

Singh, S. and Singh, T. N. 2000. Morphological, chemical and environmental factor affecting leaf rolling in rice during water stress. *Indian J. Plant Physiol.* 5: 136-141.

Singh, S., Prasad, S., Yadav, V., Kumar, A., Jaiswal, B., Kumar, A., Khan, N. and Dwivedi, D. 2018. Effect of Drought Stress on Yield and Yield Components of Rice (Oryza

sativa L.) Genotypes. *Int.J.Curr.Microbiol.App.Sci*, 2752–2759.

Singh, S., S. Pradhan, A. Singh and O. Singh. 2012. Marker validation in recombinant inbred lines and random varieties of rice for drought tolerance. *Australian J. of Crop Sci*. 6: 606-612.

Singh, U.M., Babu, B.K. and Kumar,A. 2012. Quantitative trait loci (QTL) mapping: concepts and applications. *Biotech Today.* 2: 37-38.

Singh,B., Reddy, K.R., Redoña, E.D. and Walker, T.2017. Screening of Rice Cultivars for Morpho-Physiological Responses to Early-Season Soil Moisture Stress.*Rice.Sci*.2017.24 (6).322-335

Singh,S., Prasad,S.,Yadav,V., Kumar,A., Jaiswal,B.,Kumar, A., Khan,N.A . and Dwivedi, D.K.2018. Effect of Drought Stress on Yield and Yield Components of Rice (Oryza sativa L.) Genotypes. *Int.J.Curr.Microbiol.App.Sci*. 7: 2752-2759.

Singhal, P., Jan, A.T., Azam, M. and Haq, Q.M.R. 2016. Plant abiotic stress: a prospective strategy of exploiting promoters as alternative to overcome the escalating burden. *Frontiers in Life Sci.*9 (1): 52–63.

Slatkin M. 2008. Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. *Nat Rev Genet* 9: 477–485.

Sokoto, M. B. and Muhammad, A. 2014. Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. *J. Biosci. Med*. 2 (1): 68-74.

Soto-Cerda, B. J. and Cloutier, S. 2012. Association mapping in plant genomes. In M. Çalişkan (ed), Genetic Diversity in Plants (pp. 29–54). Rijeka: InTech.

Srinivasan, S., Gomez, S.M., Kumar, S.S., Ganesh, S.K., Biji, K.R., Senthil, A. and Babu, R.C. 2008. QTLs linked to leaf epicuticular wax, physio-morphological and plant production traits under drought stress in rice (*Oryza sativa* L.). *Plant Growth Regulation*. 56 (3): 245–256.

Srividhya, A., Vemireddy, L. R., Sridhar, S., Jayaprada, M., Ramanarao, P. V., Hariprasad,

A. S., Reddy, H. K., Anuradha, G. and Siddiq, E.. 2011. Molecular Mapping of QTLs for Yield and its Components under Two Water Supply Conditions in Rice (*Oryza sativa* L.). *J. Crop Sci. Biotech*. 14 (1): 45-56

Steele, K.A., Price, A.H., Shashidhar, H.E. and Witcombe, J.R. 2005. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theoret Appl Genet* 112 (2): 208–221.

Subashri, M., Robin, S., Vinod, K.K., Rajeswari, S., Mohanasundaram, K. and Raveendran, T.S. 2008. Trait identification and QTL validation for reproductive stage drought resistance in rice using selective genotyping of near flowering RILs. *Euphytica*, 166 (2): 291–305.

Suh, J.P., Won, Y.J., Ahn, E.K., Lee, J.H., Ha, W.G., Kim, M.K., Cho, Y.C., Jeong, E.G. and Kim, B.K. 2014. Field Performance and SSR Analysis of Drought QTL Introgression Lines of Rice. *Plant Breeding and Biotechnol.* 2 (2): 158–166.

Sullivan, C. Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and method of measurements. In: Rao, N. G. P. and House, L. R. (eds), *Sorghum in the Seventies*. Oxford and IBH Publishers, New Delhi, pp. 247-264.

Sullivan, C. Y. and Ross, W. M. 1979. Stress Physiology in Crop Plants. Wiley, New York, 263–281.

Swamy, B.P.M., Shamsudin, N.A.A., Rahman, S.N.A., Mauleon, R., Ratnam, W., Sta. Cruz, M.T. and Kumar, A. 2017. Association Mapping of Yield and Yield-related Traits under Reproductive Stage Drought Stress in Rice (*Oryza sativa* L.). *Rice*. 10 (1): 33-43.

Swapna, S. and Shylaraj, K.S. 2017. Screening for Osmotic Stress Responses in Rice Varieties under Drought Condition. *Rice Sci.* 24 (5): 253–263.

Tang, Y., Liu, X., Wang, J., Li, M., Wang, Q., Tian, F., and Zhang, Z. 2016. GAPIT version 2: An enhanced integrated tool for genomic association and prediction. *Plant Genome*. 9 (2): 103-123.

Thomson, M.J., de Ocampo, M., Egdane, J., Rahman, M.A., Sajise, A.G., Adorada, D.L., Tumimbang-Raiz, E., Blumwald, E., Seraj, Z.I., Singh, R.K., Gregorio, G.B. and Ismail, A.M. 2010. Characterizing the Saltol Quantitative Trait Locus for Salinity Tolerance in Rice. *Rice*. 3 (2–3). 148–160.

Tiwari, A., Prasad, S.,Mishra, A., Pradesh, I., Singh. and Singh, K. 2017. Physio-Molecular approach towards developing rice variety for dual resistance (Drought and Submergence) under rainfed lowland ecosystem. *J.Pharmacogonosy Phytochem.*3: 06-12.

Todaka, D., Shinozaki, K. and Yamaguchi-Shinozaki, K. 2015. Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Frontiers in Plant Sci.* 6: 56-67.

Tripathi,J., Thagele, S.N., Sharma,A.S., Tripathi,N., Tripathi,P. and Singh,A.K.2018. Tolerance ability in rice carrying drought tolerance QTL; physiological and co-expression network analysis towards genetically superior transgenic rice. *Plant Biotechnol. J*. 3: 33-45.

Tripathy, J. N., Zhang, J., Robin, S., Nguyen, T. T., and Nguyen, H. T. 2000. QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theor. App. Genet.* 100: 1197-1202.

Turner, N. C. 1979. Drought resistance and adaptation to water deficits in crop plants. In: Mussell H, Staples CR, eds. Stress physiol. in crop plants. New York: John Wiley and Sons. 343–372.

Turner, N. C. 1981. Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* 58 (1): 339-366.

Tyagi, A., Kumar, N., and Sairam, R. K. 1999. Efficacy of RWC, membrane stability, osmotic potential, endogenous ABA and root biomass as indices for selection against water stress in rice. *Indian J. Plant Physiol.* 4: 302-306.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y., Wu, J., Matsumoto, T., Takai, T., Okuno, K. and Yano, M. 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*. 45 (9): 1097–1102.

Vanniarajan, C., Vinod, K.K. and Pereira, A. 2012. Molecular evaluation of genetic diversity and association studies in rice (*Oryza sativa* L.). *J. Genetics*. 91 (1): 9–19.

Vasant, D.V. 2012. Genome wide association mapping of drought resistance traits in rice (*Oryza sativa* L.). Master Thesis submitted to Tamil Nadu Agricultural University, Coimbatore, India.

Venuprasad, R., Bool, M.E., Dalid, C.O., Bernier, J., Kumar, A. and Atlin, G.N. 2009a. Genetic loci responding to two cycles of divergent selection for grain yield under drought stress in a rice breeding population. *Euphytica*, 167 (2): 261–269.

Venuprasad, R., Dalid, C.O., Valle, M.D., Zhao, D., Espiritu, M., Cruz, M.T.S., Amante, M., Kumar, A. and Atlin, G.N. 2009b. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoret ApplGenet.* 120 (1): 177–190.

Venuprasad, R., Shashidhar, H.E., Hittalmani, S. and Hemamalini, G.S. 2002. Tagging quantitative trait loci associated with grain yield and root morphological traits in rice (*Oryza sativa* L.) under contrasting moisture regimes. *Euphytica*, 128 (3): 293–300.

Verma, H., Borah, J.L. and Sarma, R.N. 2019. Variability Assessment for Root and Drought Tolerance Traits and Genetic Diversity Analysis of Rice Germplasm using SSR Markers. *Scientific Reports*. 9 (1): 103-123.

Verma, S., Saxena, R., Xalxo, M.S. and Verulkar, S. 2014. QTL for grain yield under water stress and non-stress conditions over years in rice (*Oryza sativa* L.). *Australian J Crop Sci.* 8. 916-926.

Verulkar, S. B. and Shrivastava, M. N. 2009. Breeding rice for drought-prone areas of eastern India: accomplishments in the recent past and current scenario. *Limited Proc.* 14: 3-12.

Verulkar, S. B., Mandal, N. P., Dwivedi, J. L., Singh, B. N., Sinha, P. K., Mahato, R. N., Dongre, P., Singh, O. N., Bose, L. K., Swain, P.. Robin, S., Chandrababu, R., Senthil, S., Jain, A., Shashidhar, H. E., Hittalmani, S., Vera Cruz, C., Paris, T., Raman, A., Haefele, S., Serraj, R., Atlin, G. and Kumar, A. 2010. Breeding resilient and productive genotypes adapted to drought-prone rainfed ecosystem of India. *Field Crops Res*. 117: 197-208

Wang, Y., and Li, J. 2011. Branching in rice. *Curr. Opin. Plant Biol*. 14: 94–99.

Wang, Z., Xu, Y., Wang, J., Yang, J. and Zhang, J. 2012. Polyamine and ethylene interactions in grain filling of superior and inferior spikelets of rice. *Plant Growth Regul.* 66: 215–228.

Wassmann, R., Jagadish, S. V. K. S., Heuer, S., Ismail, A., Redona, E., Serraj, R., Singh, R. K., Howell, G., Pathak, H., and Sumfleth, K. 2009. Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. *Adv. Agron*. 101: 59-121.

Wei, S., Hu, W., Deng, X. Zhang, Y., Liu, X., Zhao,X., Luo, Q., Jin, Z., Li, Y., Zhou,S.,Sun, T., Wang, L., Yang, G and He, G. 2014. A rice calcium-dependent protein kinase OsCPK9 positively regulates drought stress tolerance and spikelet fertility. *BMC Plant Biol.* 14: 133-145.

Wopereis, M.C.S., Kropff, M.J., Maligaya, A.R. and Tuong, T.P. (1996). Drought-stress responses of two lowland rice cultivars to soil water status. *Field Crops Res*. 46 (1–3): 21–39.

Xangsayasane, P., Jongdee, B., Pantuwan, G., Fukai, S., Mitchell, J.H., Inthapanya, P. and Jothiyangkoon, D. 2014. Genotypic performance under intermittent and terminal drought screening in rainfed lowland rice. *Field Crops Res.* 156: 281–292.

Xiang, J.J., Zhang, G.H., Qian, Q. and Xue, H.W. 2012. SEMI-ROLLED LEAF1 Encodes a Putative Glycosylphosphatidylinositol-Anchored Protein and Modulates Rice Leaf Rolling by Regulating the Formation of Bulliform Cells. *Plant Physiol.* 159 (4): 1488–1500.

Xing, Y., Tan, Y., Hua, J., Sun, X., Xu, C. and Zhang, Q. 2010. Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theoret Appl Genetic*. 105 (2): 248–257.

Xu, J. L., Lafitte, H. R., Gao, Y. M., Fu, B. Y., Torre, R., and Li, Z. K. 2005. QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. *Theor. Appl. Genet.* 111(8): 1642-1650.

Xu, Y. 2002. Global view of QTL: rice as a model. In: Kang MS (ed) Quantitative genetics, genomics and plant breeding. *CAB Int*. l; 109–134.

Yang Q., He H., Li H., Tian H., Zhang J., Zhai L., et al. (2011). NOA1 functions in a temperature-dependent manner to regulate chlorophyll biosynthesis and rubisco formation in rice. *PLOS ONE*6:e20015.

Yang, Y., Xu, W., Hou, P., Liu, G., Liu, W., Wang, Y., Zhao, R., Ming, B., Xie, R., Wang, K. and Li, S. 2019. Improving maize grain yield by matching maize growth and solar radiation. *Scientific Reports*. 9 (1): 89-100.

Yu, J. and Buckler, E.S. 2006. Genetic association mapping and genome organization of maize. *Curr Opin Biotechn.* 17:155-160.

Yu, J., Pressoir, G., Briggs, W.H., Vroh Bi, I., Yamasaki, M., Doebley, J.F., McMullen, M.D., Gaut, B.S., Nielsen, D.M., Holland, J.B., Kresovich, S. and Buckler, E.S.

2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, 38 (2): 203–208.

Yue, B., Xue, W.Y., Luo, L.J. and Xing, Y.Z. 2006. QTL Analysis for Flag Leaf Characteristics and Their Relationships with Yield and Yield Traits in Rice. *Acta Genetica Sinica*, 33 (9): 824–832.

Zain, N., Ismail, M., Mahmood, M., Puteh, A. and Ibrahim, M. 2014. Alleviation of Water Stress Effects on MR220 Rice by Application of Periodical Water Stress and Potassium Fertilization. *Molecules*, 19 (2): 1795–1819.

Zaman, N.K., Abdullah, M.Y., Othman, S. and Zaman, N.K. 2018. Growth and Physiological Performance of Aerobic and Lowland Rice as Affected by Water Stress at Selected Growth Stages. *Rice Sci*. 25 (2): 82–93.

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M. and Jin, J. 2018. Effect of Drought on Agronomic Traits of Rice and Wheat: A Meta-Analysis. *Int J Environl Res Public Health*. 15 (5): 745-756.

Zhang, L., Yu, S., Zuo, K., Luo, L. and Tang, K. 2012. Identification of Gene Modules Associated with Drought Response in Rice by Network-Based Analysis. *PLoS ONE*, 7 (5), p.e33748.

Zhang, P., Zhong, K., Tong, H., Shahid, M.Q. and Li, J. 2016. Association Mapping for Aluminum Tolerance in a Core Collection of Rice Landraces. *Frontiers in Plant Sci*. 7: 112-123.

Zhao, K., Aranzana, M., Kim, S., Lister, C. and Shindo, C. 2008. An Arabidopsis example of association mapping in structured samples. *PLoS Genet* 3 (1): 43-56.

Zhou, L., Xu, H., Mischke, S., Meinhardt, L.W., Zhang, D., Zhu, X., Li, X. and Fang, W. 2014. Exogenous abscisic acid significantly affects proteome in tea plant (*Camellia sinensis*) exposed to drought stress. *Horticulture Res*. 1 (1): 112-122.

Zhu, C., Gore, M., Buckler, E.S. and Yu, J. 2008. Status and Prospects of Association Mapping in Plants. *The Plant Genome*, 1 (1): 5–20.

Zhu, H., Li, Y., Liang, J., Luan, X., Xu, P., Wang, S., Zhang, G. and Liu, G. 2018. Analysis of QTLs on heading date based on single segment substitution lines in rice (*Oryza Sativa* L.). *Scientific Reports*. 8 (1): 112-123.

Zhu, J., Zhou, Y., Liu, Y., Wang, Z., Tang, Z., Yi, C., Tang, S., Gu, M. and Liang, G. 2010. Fine mapping of a major QTL controlling panicle number in rice. *Molecular Breeding*, 27 (2): 171–180.

Zhu, Q.H., Ramm, K., Shivakkumar, R., Dennis, E.S. and Upadhyaya, N.M. 2004. The ANTHER INDEHISCENCE1 Gene Encoding a Single MYB Domain Protein Is Involved in Anther Development in Rice. *Plant Physiol.* 135 (3); 1514–1525.

Zubaer, M., Chowdhury, A., Islam, M., Ahmed, T., Hasan, M. and Hasan, A. 2007. Effects of Water Stress on Growth and Yield Attributes of Aman Rice Genotypes. *Int. J. Sustain. Crop Prod*, 2 (6): 25–30.

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE, VELLAYANI
DEPARTMENT OF PLANT PHYSIOLOGY
DOCTORAL DEFENSE SEMINAR

Nithya N.
2016-21-008

Date: 10-03-2020
Time: 10.00-10.40

Abstract

The present investigation entitled "Identification of molecular markers and Quantitative Trait Loci (QTLs) associated with drought tolerant and plant production traits in rice (*Oryza sativa* L.*)* using association genetic analysis" was conducted at Department of Plant Physiology, College of Agriculture, Vellayani and RARS, Pattambi during 2016-18 . The objective of the study was to identify molecular markers, Quantitative trait loci (QTLs) associated with drought tolerance and plant production traits in rice under drought condition.

The extend of variation for water stress indicators for morpho-physiological and plant production traits were assessed by evaluating 81 rice genotypes collected from RARS, Pattambi and NRRI, Cuttack under water stress and irrigated conditions in the open field. Each genotype was raised in four rows of 2m length and was exposed to water stress at panicle initiation stage for a period of 25 consecutive days by withdrawing irrigation along with irrigated control and replicated twice. The morpho- physiological, and plant production traits were recorded after imposing water stress. Significant variation was observed for these traits under water stress condition. Genotyping of 81 rice genotypes were done using 100 SSR primers. Quality and quantity of DNA was checked. Amplification pattern of 100 primers were scored as per standard procedure. Population structure was calculated using the software "STRUCTURE" with genotypic score value. The molecular markers/ QTLs linked to drought tolerance and plant production traits were identified using the software, TASSEL 5.

The result of the study revealed that morphological parameters such as the plant height at flowering was observed to be highest in PTB1 and lowest in ASD-16 under water stress condition in both experiments. The number of tillers were maximum for PTB7 (10.5

plant^{-1}) and minimum for Kuttithekkan under water stress condition in both experiments. Delayed days to 50% flowering (7 days) was observed for the genotypes under water stress in two experiments compared to irrigated control.

Physiological parameters such as Relative Water Content (RWC) decreased where as leaf temperature increased significantly in most of the genotypes under water stress condition. Highest leaf rolling (score – 7.78) was observed in Prathyasha in both experiment I (7.78) and experiment II (7.84). But the genotypes PTB55 (1.78) & PTB 29 (1.11) showed least leaf rolling symptoms in both experiment I and II respectively. Among the genotypes, the RWC was recorded to be highest in PTB15 (78.72%) while the lowest was recorded in Prathyasha (57.34%) under water stress condition in experiment I and in experiment II maximum relative water content was observed in PTB27 (72.98%) and minimum in ADT37 (50.25%). Membrane stability index was highest in PTB29 in experiment I (94.36 %) and in PTB27 in the experiment II (92.24%). Maximum leaf temperature was observed in PTB1 and minimum in Prathyasha under water stress condition in both experiments. Among the genotypes, chlorophyll stability index was recorded to be highest in PTB27 (95.59%) while the lowest in Pandichempan (80.36%) in experiment I, and in experiment II maximum was recorded in variety PTB7 (92.85%) and minimum in Pandichempan (75.52 %).

The grain yield per plant under water stress condition was positively correlated with parameters such as tiller numbers, days to 50% flowering, relative water content, membrane stability index, chlorophyll stability index , spikelet fertility percentage and 1000 grain weight where as negatively correlated with leaf temperature, leaf rolling score, leaf drying score and percentage relative yield reduction.

Under water stress the first principal component showed 41.77% variation, while second component exhibited 16.57% variation. Among the 14 morpho-physiological and plant production traits, relative water content, cell membrane stability index and chlorophyll stability index contributed towards maximum diversity. Under irrigated control condition the first principal component revealed 48.9 % variation, while the second component showed 16.57% variation. Among the 8 morpho-physiological and plant production traits studied, the

relative water content and spikelet fertility percentage contributed towards maximum diversity.

Clustering by Ward method was done to establish a relationship among the 81 rice genotypes. Similar types of genotypes were clustered together based on the phenotypic data. All the genotypes were clustered mainly into 5 clusters under water stress as well as irrigated control condition.

Genotyping of eighty one rice genotypes were done using 100 SSR markers. Among them, 40 primers which demonstrated polymorphism among the genotypes were used to classify the genomic regions (QTLs) associated with drought tolerance and plant production traits through association genetic analysis. The marker trait association for all the traits under water stress and irrigated control condition were calculated using GLM and MLM model of TASSEL5 software. A total of 136 marker trait associations were observed while performing GLM analysis and MLM analysis resulted in 48 marker trait association based on the *P* and r^2values in water stress as well as irrigated control condition.

LD was distributed unequally on each chromosome and more concentrated on chromosomes 1 and 7. LD analysis in the whole population showed that there were significant LD pairs ($P<0.05$). A total of 52 LD pairs were observed under water stress and irrigated control conditions and out of these, there were 46 inter chromosomal LD pairs and 6 intra chromosomal LD pairs. The markers RM3825, RM455, RM490, RM259 and RM1026 showed significant associations with many phenotypic traits in water stress as well as irrigated control conditions. The Q-Q (Quantile-Quantile) plot also confirmed the association of these markers with phenotypic traits.

In summary, there was significant variation for morpho-physiological and plant production traits among rice genotypes under water stress condition. Genotypes having higher relative water content, cell membrane stability index and chlorophyll stability index were found to be tolerant to drought. In the present study, the genotypes *viz* PTB28, PTB29, PTB30, PTB15, PTB7, PTB55, N-22 and Chomala identified as drought tolerant can be used in breeding programmes to improve drought tolerance in rice. From this study, 29 significant

(P<0.05) marker trait associations were detected using mixed linear model (MLM). The identified most significant markers and their corresponding QTLs are, RM455 (Chromosome7) was linked to tiller number, leaf temperature, relative water content, yield per plant and spikelet fertility percentage. RM490 (Chromosome1) was associated with leaf temperature and chlorophyll stability index. Marker RM259 (Chromosome1) was associated with leaf temperature, chlorophyll stability index, leaf drying score, yield per plant and spikelet fertility percentage. RM1026 (Chromosome 9) was associated with leaf rolling score and drying score. Among them, RM 490 and RM259 showed co-location of QTLs for leaf temperature and chlorophyll stability index and RM259 & RM1026 showed co-location of QTLs for leaf drying score.