IDENTIFICATION OF MICROSATELLITE MARKERS ASSOCIATED WITH ROOT TRAITS FOR DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Identification of microsatellite markers associated with root traits for drought tolerance in rice (*Oryza sativa* L.)"** 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.

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LIST OF ABBREVIATIONS

DES	Directorate of Economics and Statistics
IMD	Indian Meteorological Department
DNA	Deoxyribo Nucleic Acid
BLA	Bulked Line Analysis
Mb	Mega base
FAO	Food and Agricultural Organization
Mha	Million hectares
IRRI	International Rice Reseach Institute
ψ_L	Leaf Water Potential
VPD	Vapour Pressure Deficit
PEG	Polyethylene Glycol
SCMR	SPAD Chlorophyll Meter Reading
OPV	Open Pollinated Variety
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
U. S. A	United States of America
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

SE(m)	Standard Error (Mean)
G	Genotype
T	Treatment
G*T	Genotype x Treatment
GxE	Genotype x Environment
%	per cent
^0C	Degree celsius
m H_2O moles m^{-2} s^{-1}	milli H_2O moles $meter^{-2}$ $second^{-1}$
μ CO_2 moles m^{-2} s^{-1}	micro CO_2 moles $meter^{-2}$ $second^{-1}$
μ moles/g tissue	micro moles/gram tissue
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

Introduction

1. INTRODUCTION

Rice, (*Oryza sativa* L.) is the world's most important wetland food crop belonging to the family Poaceae and subfamily Oryzoidae. Asia accounts for more than 90% of world's rice production and consumption. Worldwide, rice is cultivated in an area of 164 million hectares with an annual production of 772.8 million tons (FAO, 2013). Globally, India ranks first in cultivable area (43.92 million hectares) under rice and second in production (91.61 million tons) after China (Pandey *et al.*, 2010).

Rice is grown under more diverse environmental conditions than any other major food crops in the world. Distribution of world's rice area can be categorized into four major ecosystems *viz.* irrigated (55%), rainfed lowland (25%), upland (12%), and flood prone (8%) ecosystems. Rice is a semi aquatic plant and its production is water intensive (Wassmann *et al.*, 2009, Bouman *et al.*, 2007). It is estimated that 5000 litres of water is required to produce one kg of rice (Serraj *et al.*, 2011). Total worldwide withdrawals of fresh water for agriculture is estimated at 3,600 km^3 annually, of which 2,500 km^3 is used to irrigate rice crop alone. (Falkenmark and Rockstrom, 2004). Since the crop depends purely on rainfall for its water supply under rainfed condition, drought is the most severe abiotic stress that limits rice productivity in rainfed lowland and upland ecosystems (Bimpong *et al.*, 2011).

Drought stress is the most important constraint to rice production and yield stability in rainfed regions, affecting 10 million hectares of upland rice and over 13 million hectares of rainfed lowland rice in Asia alone (Pandey *et al.*, 2007). In India, from the beginning of the green revolution era in rice in 1965 till 2009, on 14 occasions, rice production failed to achieve the expected production level. Drought was the factor for lower production on 11 of these 14 occasions (DES, 2009). Severe drought occured in 2002 and 2009 caused a significant reduction in rice as well as total food production in India. In 2002, 29% of the geographical area suffered from

drought due to 19% annual rainfall deficiency. Compared to the previous year, rice production fell by 21.5 million tons. Similarly in 2009, total rice production declined by approximately 10.02 million tons in India.

In Kerala, the decreasing rainfall over the region, late onset of the monsoon, failure of the monsoon and break in the monsoon in the state lead to many drought situations. Kerala had severe dry spells and droughts in 1983. 1985. 1986 and 1987 even though the state has a wet climate (Nathan, 2000). Indian Meteorological Deportment (IMD) has reported that Kerala as a whole had a rainfall deficit of 39% during the year 2012.

It has been estimated that the rice demand in 2010 will be 100 million tons and in 2025, the demand will be 140 million tons (Misra, 2004). This increased demand for rice can no longer be met only from irrigated areas. Greater efforts are needed to enhance the contribution of rainfed areas to overall agricultural production.

The major breeding objective in rainfed ecosystems is to improve drought resistance in rice plants, but little progress has been achieved in improving yield under stress due to poor knowledge of the genetic control of drought resistance. However, phenotypic selection for secondary traits is labour intensive. Molecular marker technology serves as a tool for selecting such complex traits and allows breeders to track genetic loci controlling drought resistance traits without having to measure the phenotype, thus reducing the need for extensive field testing over space and time. Molecular markers are also not affected by environmental and can be detected at all stages of plant growth.

Identification of DNA markers linked to drought resistance traits is usually carried out with a large population, each of which has to be genotyped with several markers. This is time and labour intensive and cost ineffective. Various techniques have been reported to reduce the number of plants to be genotyped. Bulked Line Analysis (BLA) is one such technique in which the process of genotyping aids in reducing the sample size to two DNA samples by grouping plants according to their

high or low expression of a particular trait (Tan *et al.*, 1998). BLA measures the variation in pools of different genotypes that have sorted according to phenotype and uses the correlation to assign a likely map location. By linking genetic polymorphism to root growth at depth, molecular markers could be identified to improve drought tolerance in rice. Hence the present study was carried out with the following objectives

1. To validate the role of root traits for drought tolerance in rice.
2. To identify the microsatellite markers associated with root traits for drought tolerance in rice.

Review of Literature

2. REVIEW OF LITERATURE

Rice, (*Oryza sativa* L.) is the most important food crop for nearly half of the world's population (Sellamuthu *et al.*, 2011). More than 90% of the world's rice is grown and consumed in Asia, where 60% of the earth's people live. Rice belongs to the tribe Oryzeae of the family Poaceae and subfamily Oryzoideae, The genus *Oryza*, to which cultivated rice belongs probably originated at 1east 130 million years ago and spread as a wild grass in Gondwanaland the super continent that eventually broke up and drifted apart to become Asia, Africa, America, Australia and Antarctica (Chang, 1976). Today's species of genus *Oryza* are distributed in all of these continents except Antarctica. There are twenty-one wild species in the genus *Oryza*. Nine of the wild species are tetraploid. Remaining wild species as well as the cultivated species are diploid. There are two cultivated species of rice. *O. sativa*, the Asian rice, is grown worldwide. *O. glaberrima*, the African rice, is grown on a limited scale in West Africa. Indica and japonica are the two subspecies coming under *Oryza sativa* (Oka, 1988). Indica is predominantly a tropical subspecies, while Japonica consists of temperate and tropical types.

Rice has a genome size of approximately 430 Mb (Chen *et al.*, 2002). Rice contains 12 1inkage groups and 24 chromosomes in diploid condition (2n = 24). Rice though has a relatively small genome, it has high polymorphism in DNA. The size of the rice genome is estimated approximately 260 Mb, larger than the fully sequenced dicot plant model *Arabidopsis thaliana*. Because of small genome size rice is considered as the most ideal monocot for molecular mapping and map based cloning of agriculturally important genes. With its synteny with most other cereals, the findings in rice can be applied to other crops as well (Ahn *et al.*, 1993).

2.1 IMPACT OF DROUGHT ON RICE PRODUCTION

Worldwide, rice is cultivated in an area of 154 million hectares with an annual production of 700 million tons (FAO, 2011). Globally, India ranks first in cultivable

area under rice and second in production, producing 131 million tons. Rainfed area occupies about 45% of global rice area (IRRI, 2004). India has 46 Mha of the total rice area out of which 35% is in rainfed lowlands and 16% in upland (IRRI, 2005). These areas frequently experience severe water deficit due to uncertain and uneven rainfall distribution patterns and yields is seriously affected.

The variations in climate are adversely affecting water resources and the frequency of occurrence of drought and floods are expected to increase in future. The yield of crop depends on specific climatic conditions and is highly influenced by variations in climate. The variability in overall rice productivity due to climate change over last three decades was estimated by Ray *et al.* (2015) and they observed that approximately 54% of rice producing regions of the world experience the influence of climate variability on yield at the rate of about 0.1 t/ha/year.

In earlier times, India witnessed a lot of famines and rural poverty due to drought. Kumar *et al.* (2013) studied variability of monsoon droughts across India using a drought monitoring index, namely the Standardized Precipitation Evapo-transpiration Index (SPEI) for the period 1918 to 2010. Higher frequency of multi-year droughts (24 months) was during the period 1951–2010. During the period 1951–2010, there were 12 multi-year droughts (24 months), while during the period 1901–1950, there were only three such long-lived droughts. In years like 1918 and 2002, more than 60% of the country was affected by moderate drought on a shorter time scale. In some years like 1902, 1905, 1966, 1987, 2002, 2003 and 2010, multi-year droughts have affected more than 40% of the country. The country experienced 3 years of consecutive droughts during 2000–2002. The cumulative adverse effect was however, seen in the agricultural crop production. In 2002, total crop yield during the *Kharif* season was less than 50 million tones and the lowest in the period of data used (1966–2004).

2.2 ADAPTATION MECHANISM TO DROUGHT TOLERANCE IN RICE

Drought tolerance is a complex phenomenon and it is a combination of morphological, physiological, biochemical and molecular characters. The adaptation mechanism of drought tolerance and the pathways regulating water stress tolerance in rice have been extensively studied. According to Levitt (1980) drought resistance can be divided into drought avoidance (maintenance of tissue water potential), drought escape (flowering to complete life cycle before drought), and drought tolerance and further defines drought tolerance in to dehydration avoidance and dehydration tolerance.

2.3 IMPACT OF DROUGHT ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

2.3.1 Leaf rolling

Leaf rolling is one of the drought avoidance mechanisms to prevent water loss during drought stress (O'Toole and Cruz, 1980).

Turner *et al.* (1986) reported that leaf rolling can be used as a criteria for scoring drought tolerance in tall and semi dwarf rice cultivars. Also, they observed that rice varieties differ in their ability to roll leaves under similar water deficit. Dingkuhn *et al.* (1991) also find out that there is a variation in the extent to which the leaves of rice roll in response to low water potential.

Leaf rolling is induced by the loss of turgor maintenance and is a component of dehydration avoidance (Blum, 1989). Rolled leaves of rice transpire 41% less water than did the unrolled ones (Singh and Singh, 2000).

2.3.2 Relative water content

Relative water content is considered as a measure of water status of plant, indicating the metabolic activity in tissues. It can be used as the most meaningful index for dehydration tolerance. The capacity to maintain higher relative water content (RWC) under moisture stress condition is obviously a drought resistance mechanism in rice (O'Toole and Moya, 1978). Relatively high RWC have been reported in drought tolerant cultivars of rice. Fischer (1989) found that RWC was directly related to soil water content. A substantial decrease in the relative water content, leaf water potential and transpiration rate, and a simultaneous increase in leaf temperature were observed when rice plants were exposed to drought stress (Akram *et al.*, 2013).

Biswas and Choudhuri (1984) studied the effect of water stress at various developmental stages of rice. They reported that the relative water content (RWC) and leaf water potential (ψ_L) decreased with the increase in plant age and development, both under stress and non-stress conditions, but under stress condition the decrease was more pronounced.

Baruah *et al.* (1998) reported depletion in the relative leaf water content of all the traditional 'ahu' rice genotypes subjected to water stress. High yielding variety, Govind maintained relatively higher leaf water potential and relative leaf water content under water stress. Apart from Govind, Annada also exhibited high leaf water potential and relative leaf water content.

Cha-um *et al.* (2010) reported that relative water content is positively correlated with soil water content. Relative water content (RWC) in the flag leaf of PT1 (lowland irrigated cultivar) and IR20 (negative check) rice cultivars (drought susceptible) dropped significantly in plants exposed to mild water-deficit with 25% soil water content (SWC) and RWC recovery was delayed during re-watering. The RWC in both KDML105 (moderately drought tolerant) and NSG19 (positive check)

was maintained in mild water stress, but reduced significantly when plants were exposed to severe water shortage (7% SWC) and increased quickly after re-watering.

Beena *et al.* (2012) reported a remarkable reduction in the mean leaf relative water content to 53.1% in selected recombinant inbred lines (RIL's) of IR20 x Nootripathu and their parents when they were exposed to water stress at panicle initiation stage. The reduction was higher in IR20 (48.9%) than Nootripathu (65.2%).

2.3.3 Membrane stability index

Cell membrane stability (CMS) is a physiological index widely used for the evaluation of drought and temperature tolerance (Blum and Ebercon, 1981). This method was developed for a drought and heat tolerance assay in sorghum and measures the amount of electrolyte leakage from leaf segments (Sullivan, 1972). Lower membrane stability or higher injury reflects the extent of membrane lipid peroxidation, which in turn is a consequence of higher susceptibility to oxidative stress due to various environmental stresses including drought (Leibler *et al.*, 1986). The movement of molecules across membranes is accelerated by heat stress and thereby loosening chemical bonds within molecules of biological membranes. This makes the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated fatty acids (Savchenko *et al.*, 2002). The increased solute leakage as an indication of decreased cell membrane thermostability (CMT), has long been used as an indirect measure of heat-stress tolerance in diverse plant species wheat (Blum *et al.*, 2001), sorghum (Marcum, 1998) and barley (Wahid and Shabbir, 2005).

Tyagi *et al.* (1999) reported that the MSI was higher in tolerant genotypes under water stress. Tolerant genotypes CR 143-2-2 and N 22 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.

2.3.4 Leaf temperature

Sensing the infrared radiation emitted by the leaf is one way of measuring water stress. Blum *et al.* (1978) observed a rise in leaf temperature associated with the decrease of transpiration rate, reflecting the degree of water stress in sorghum and indicated the possibility of selecting for drought tolerance based on the leaf temperature.

Jones and Corlett (1992) reported that leaf temperature is associated with the plant stress level. They also observed that leaf temperature is influenced by stomatal and boundary layer resistances, as well as by meteorological conditions. The rate of leaf transpiration is only one of many components of the canopy energy balance that affect canopy temperature factors such as radiation, wind speed, air temperature, humidity, and VPD all have major effects.

Garrity and O'Toole (1995) were able to screen rice varieties for reproductive stage drought-avoidance traits, using canopy temperature as a surrogate trait for plant water status under stress. They showed that grain yield and spikelet fertility were highly correlated with midday canopy temperature on the day of flowering, and lines with high drought-avoidance scores consistently remained the coolest under stress.

As water becomes limiting, leaf temperature increases above air temperature because transpiration is reduced. Differences in canopy temperature among rice

cultivars are known to be related to drought avoidance based mainly on the potential to maintain transpiration under stress and canopy temperature was shown to be negatively co-related with biomass and grain yield under stress in rice (Blum, 1988). Plants with a deeper root system would maintain cooler canopy temperature and ultimately higher yield under drought. Canopy temperature was found to have a positive correlation with leaf rolling and leaf drying and negative correlation with root thickness in rice (Babu *et al.*, 2003).

2.3.5 Stomatal conductance

Dingkuhn *et al.* (1999) conducted a study in upland rice under the irrigated and drought condition. They concluded that stomatal conductance was controlled by a soil moisture dependent root signal under drought conditions.

Stomatal (*gs*) and mesophyll conductance (*gm*) to CO_2 often decrease in response to drought (Centritto *et al.*, 2009). Thus, the ability to maintain the *gm* values under water-deficits determines the drought tolerance of rice varieties (Lauteri *et al.*, 2014).

Stomatal conductance was drastically reduced under drought in all genotypes, possibly as a strategy to curtail water loss and maintain plant water status (Lo Gullo *et al.*, 2003).

Farooq *et al.* (2010) reported that stomatal conductance and the amount of water transpired decreased substantially under drought than in well-watered conditions in a study conducted in indica rice under drought stress.

Stomatal closure is generally accepted to be the main determinant for decreased photosynthesis under mild to moderate drought stress (Medrano *et al.*, 2002). Ji *et al.* (2012) reported that stomatal conductance (gs) decreased in Zhenshan97B (drought susceptible) and IRAT109 (drought tolerant) with respect to control after drought

stress treatment. However, the drought-stressed plants of IRAT109 showed lower rates of gs than those of Zhenshan97B. Therefore, more intensive stomatal closure was observed in IRAT109, which exhibited 58% decrease in gs. However, the gs of Zhenshan97B decreased 19%, the decrease in gs from 0.20 to 0.13 mol H_2O m^{-2} s^{-1} was paralleled by a decline in Pn. Therefore, stomatal closure seems to be the main cause of decreased photosynthesis in drought tolerant cultivar IRAT109.

2.3.6 Photosynthetic rate

Photosynthesis is the main metabolic process determining crop production and is affected by drought stress. The major components, limiting photosynthesis are the CO_2 diffusional limitation due to early stomatal closure, reduced activity of photosynthetic enzymes, the biochemical components related to triose-phosphate formation and decreased photochemical efficiency of Photosystem II. Change in any of these components alters the final pbotosynthetic rate.

Uprety and Sirohi (1985) studied the effect of water stress on the photosynthesis of wheat varieties C-306 and Kalyaansona. They showed that drought affected both stomatal and non stomatal components of photosynthesis. The comparatively higher photosynthesis in variety C-306 under drought condition might probably be by the maintenance of higher turgor due to the higher water potential of its leaves. Sairam *et al.* (1990) also reported that the tolerant genotypes generally had higher photosynthesis than the susceptible genotypes. Tiwari *et al.* (1998) observed in rice (*Oryza sativa* L.) that photosynthetic rate declines and stomatal resistance goes up under water stress condition.

Ji *et al.* (2012) reported that the photosynthetic rate (Pn) of flag leaves decreased 47% from 12.37 to 5.62 μmol CO_2 m^{-2} s^{-1} in Zhenshan97B and 60% from 12.31 to 3.72 μmol CO_2 m^{-2} s^{-1} in IRAT109 under the drought stress compared to control.

Yang *et al.* (2014) reported that drought stress reduced photosynthetic rate (Pn) of all rice lines, but the Pn in diploid rice decreased sharper than that of corresponding autotetraploid rice as drought stress increased. The Pn of autotetraploid rice was significantly higher than those of corresponding diploid rice under drought stress and under high light intensity (>800 μ mol mol-2 s·').

2.3.7 Transpiration rate

Pal and Varade (1980) reported that the transpiration rate remained nearly constant at high soil moisture contents and decrease as the soil moisture content start decreasing.

Sairam (1994) studied the effect of moisture stress imposed at tillering and anthesis stages on four drought susceptible (HD-2339, Hd-2001, WL- 711, WH-147) and four drought tolerant (C-306, NI-5439, WH-147) and Pissi local and DL-153-2 genotypes of wheat. He reported that transpiration rate decreased under moisture stress. Tolerant genotypes generally had lower rates of transpiration than the susceptible genotypes.

Cabuslay *et al.* (2002) conducted a study in 27 rice cultivars in order to determine physiological traits that contribute to tolerance for water deficit. They discovered that cultivars tolerant of mild water stress had a high relative transpiration (transpiration under stress compared with that under non-stressed conditions). Relative transpiration data showed that water stress caused more than 50% reduction in cumulative transpiration in all cultivars. Transpiration rate was highest in IR20 and KDML 105 while lowest values were obtained in M55 and PI 163575.

2.3.8 Proline content

It has been suggested that accumulation of proline contributes to maintain proper balance between extra and intra- cellular osmolarity under conditions of water

stress (Madhusudhan *et al.*, 2002). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants.

Sheela and Alexander (1995) reported that drought tolerant varieties like Tulasi and M-102 had a high accumulation of free proline than susceptible variety, Jaya. Also, they found out that seed hardening using water for 24 hours showed an increase in proline accumulation than control.

Hsu *et al.* (2003) investigated the regulation of proline accumulation in polyethylene glycol (PEG–1.5 MPa) treated rice leaves. Proline accumulation caused by PEG was related to protein hydrolysis, an increase in ornithine–δ–amino–transferase activity, an increase in the content of ammonia, and an increase in the content of precursors of proline biosynthesis. Results also showed that abscisic acid accumulation is not required for proline accumulation in PEG–treated rice leaves.

Beena *et al.* (2012) conducted a study on the effect of water deficit on various physio-morhological and biochemical traits during panicle initiation stage using selected recombinant inbred lines (RIL's) of IR20 x Nootripathu and their parents. Water stress caused a reduction in SCMR value (11.9%), plant height (10.4%), biomass (29.7%) and an increase in proline (89.6%) across the RIL's as compared to control.

Bunnag and Pongthai (2013) reported that the rice varieties KDML 105, IR62266 and IR52561 were found to accumulate more amount of proline compared with the normal level of proline found in CT9993 and BT under mild stress (after 20 days of the treatment) and severe stress conditions (after 60 days of the treatment).

2.4 IMPACT OF DROUGHT ON ROOT TRAITS

2.4.1 Root length

The possession of a deep and thick root system which allows access to water deep in the soil profile is crucially considered important in determining drought tolerance in upland rice and substantial genetic variation exists for this trait (Ekanayake *et al.*, 1985; Fukai and Cooper, 1995; OToole, 1982; Yoshida and Hasegawa, 1982).

Puckridge and O'Toole. (1981) found that a deep-rooting cultivar, Kinandang Patong, extracted more water at 40-70 cm depth than the two cultivars IR20 and IR36 which were shallow rooted. Chang *et al.* (1986) also found that rice with a deep root system avoided drought better than rice with a shallow root system.

Lilley and Fukai (l994a) showed also under upland conditions, the variation in water extraction among four cultivars was directly related to the variation in root length density.

Lines with large root length tend to have high leaf water potential and delayed leaf death during drought (Mambani and Lal, 1983; Cruz and O'Toole, 1985 and Ekanayake *et al.*, 1985). This favorable plant water status may result in larger grain yield under water limiting conditions (Mambani and Lal, 1983), although this is not always observed (Puckridge and O'Toole, 1981).

In the case of Lilley and Fukai (l994b), there was an indication that the cultivar with the greatest root length performed better than others under mild stress conditions, but there was no direct relationship between total root length and grain yield when there was only one period of prolonged drought.

Kamoshita *et al.* (2004) observed a positive relationship between root length density and soil water extraction rate by the end of a shorter drought period in the 40-

50 cm layer, showing the advantage of deep root development for extracting water from deep soils when drought period is not extended. Thus, showed that there is a large genotypic variability for root traits and a clearer definition of drought development (eg. rate of soil drying) may be needed to understand genotype by environment interaction for deep root development.

Steele *et al.* (2006) mapped a QTL on chromosome 9 involved in root length and thickness. Uga *et al.* (2011) identified a major QTL *Dro 1* on chromosome 9 playing a crucial role in deep rooting.

Ji *et al.* (2012) reported that IRAT109 (drought tolerant) showed more drought-induced root growth in depth than Zhenshan97B (drought susceptible).

2.4.2 Root dry weight

Cruz *et al.* (1986) observed in rice that mild water stress at vegetative stage significantly reduced the total root dry matter as well as root length density.

Sah and Zamora (2005) investigated the effects of water deficit on vegetative and reproductive stages of hybrid, open pollinated (OPV) and local varieties of maize. They found out that there was significant difference in the root dry matter per plant between the varieties in the 25-75 cm depth, but not in 0- 25 cm depth at 47 DAP. Local variety (Tiniguib) had significantly higher root dry matter per plant in 25-75 cm depth as compared to Hybrid (IPB 911) but at par with OPV. At 67 DAP, the root dry matter of Hybrid was higher than OPV but at par with Local variety in the 0-25 cm depth.

Anbumalarmathi *et al.* (2008) evaluated 13 parents and their 40 hybrids for root traits contributing to drought tolerance in rice under PVC pipe condition. Among the parents, Vellaichitraikar, Norungan, Nootripathu, Kallurundaikar, Chandaikar and PMK 3 showed significantly superior mean values than the grand mean for most of

the root traits. Kallurundaikar (L1) and Chandaikar (L4) registered significantly superior mean performance for five traits each *viz*., root thickness, root volume, total number of roots, root:shoot ratio and root dry weight. Hybrids Kallurundaikar/ASD 18 (L1 x T3) and Chandaikar/Co 47 (L4 x T1) showed high *per se* performance for five traits.

Ji *et al.* (2012) reported drought stress caused a reduction of 61% and 43% in root dry weight (0–30 cm) for Zhenshan97B and IRAT109, respectively. The root dry weight (30–90 cm) decreased 14% in Zhenshan97B, but increased 72% in IRAT109.

2.4.3 Root volume

Zuno *et al.* (1990) assessed root volume, determined by the displacement of water in a 1 litre cylinder, in 44 day old seedlings of 13 varieties. The Japanese upland variety Rikuto Norin 12 had the greatest root volume (31 ml) while IR20, a lowland variety, had the least (10 ml). Root volume was negatively associated with damage caused by drought in the reproductive phase (r = -0.85). Root volume was significantly and positively correlated with both root and shoot length (r = 0.87 and 0.68, respectively).

Raisagar (2003) conducted a study on root traits associated with drought tolerance in 112 recombinant inbred lines derived from a cross between Safri-17 x Kranti. She observed relatively high significant difference for root traits, especially root volume between the parents as well as RILs. The average root volume for all 112 lines was 43.7 ml standard deviation was 23.33, with the range of 92.5 ml. The parents also exhibited differences for this trait. Safari 17 had 90 ml and Kranti had 60 ml of the root volume. The maximum value of root volume was 100 cm^3 for line no. 29 while the minimum value was 7.5 ml for line no.71.

Nag (2008) studied the variation in root traits of rice germplasm in rainfed and irrigated environments. The result showed that root volume was highly significant 32

due to different water regimes. Mean root volume of rice germplasm was measured 109.19 ml under irigated condition, whereas mean root volume of rice germplasm was 127.90 ml in under rainfed condition. Among the genotypes the overall reduction in root volume was 17.1 percent due to less moisture availability.

Kar (2014) conducted a study on identification of drought tolerant rice (*Oryza sativa* L.) genotypes for rainfed lowland ecology using microsatellite markers linked to drought. The study was carried out using Recombinant Inbred Lines (RILs) developed by a cross between BPT-5204 (drought susceptible) and Sahbhagi Dhan (drought tolerant) genotypes. Further, root studies were carried out using selected 20 phenotypically highly drought tolerant RILs and found out significant differences in all the root traits *viz.*, root length, root volume, root thickness and root to shoot ratio. Root volume ranged from 1.53-14.00 cc. Root volume was found to be negatively, but significantly correlated with biomass (-0.627).

2.4.4 Root shoot ratio

Boyer (1985) reported that increased root to shoot ratio was observed in plants during soil moisture deficit.

Cruz *et al.* (1986) presented that mild stress condition during vegetative stage in rice can cause more reduction in root dry weight than shoot dry weight and thereby decreasing root to shoot ratio.

Abscisic acid influences the relative growth rates of many plant parts such as an increase in the root to shoot dry weight ratio, inhibition of leaf area development and production of prolific and deeper roots (Sharp *et al.*, 1994).

Insalud *et al.* (2006) assessed the responses of rice roots to low phosphorus supply in aerated and stagnant nutrient solution and observed that the plants in stagnant solution had up to 19% more adventitious roots, 24% greater root porosity and 26% higher root/shoot ratio.

2.5 IMPACT OF DROUGHT ON MORPHOLOGICAL AND YIELD PARAMETERS

2.5.1 Plant height

Babu *et al.* (2003) conducted a study on identification of quantitative trait loci (QTL) linked to drought tolerance in rice using double haploid lines (DH) of CT9993-5-10-1-M/ IR62266-42-6-2. They observed that the mean plant height was reduced by 3.8cm under stress in DHs. Among the parents, CT9993 showed no significant reduction in plant height, while IR62266 had a 4.2 cm reduction in plant height under stress.

Ji *et al.* (2012) 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% in Zhenshan97B and 3% in IRAT109. The decrease was less in IRAT109 which further indicates its tolerance to water stress.

Beena *et al.* (2012) reported that plant height reduced 10.4% across the RIL's as compared to irrigated control during drought stress.

Bunnag and Pongthai (2013) reported that the seven rice cultivars under study showed a slight reduction in the growth rate of the stems when they were subjected to mild drought stress (after 20 days of the treatment) at vegetative stage (42 DAS). Also, the growth rate reduction became more dramatic under severe stress (after 60 days of the treatment).

Water stress during vegetative stage reduces plant height, tiller number and leaf area (Sokoto and Muhammad, 2014).

2.5.2 Days to 50% flowering

Woperesis *et al.* (1996) reported that water stress during flowering induction and inflorescence development lead to delay in flowering (anthesis) or even to complete inhibition as apical morphogenesis is sensitive to water deficit.

Pantuwan *et al.* (2002) reported that the drought stress developed prior to flowering generally delayed the flowering of genotypes and such a delay was associated with drought susceptibility in rice.

Deshmukh *et al.* (2007) observed that in rice, water stress during flowering can reduce the harvest index by as much as 60%, largely as a result of a reduction in grain set. Five panicles in stress plants fail to full exert (emerge) from the flag leaf sheath, flowering is delayed and the percentage of spikelet that open at anthesis is reduced. The failure of panicle exertion alone accounts for approximately 25% to 30% of spikelet sterility because the unexerted spikelet cannot complete anthesis and shed pollen, even when development is otherwise normal.

A delay in flowering under stress as compared with that under non-stress has been reported to be one of the parameters associated with drought tolerance (Bernier *et al.*, 2007 and Venuprasad *et al.*, 2007).

Verulkar and Shrivastava (2009) reported that delay in flowering under drought conditions was related to low water status and was an indicator of drought susceptibility. Delay in flowering was also associated with higher spikelet sterility.

2.5.3 Tiller number

Kumar (1992) reported that in upland rice cultivars, tiller number and panicle length had positive significant association while negative association between plant

height and panicle length. Further, they emphasized that the plant height and tiller number might be used as a selection criterion for yield improvement.

Park *et al.* (1999) investigated the growth stages in the influences of soil moisture stress in the cultivar Japonica and Dongjinbyeo. The cultivar were subjected to soil moisture stress at five growth stages until the initial wilting point (about 10% soil moisture content) and was reirrigated. At maturity the plant height, leaf area, tiller number, spikelet numbers per panicle and panicle numbers per hill was reduced significantly due to moisture stress. All these characters were found responsible for the decrease in grain yield under drought.

2.5.4 Productive tiller number

Reddy *et al.* (1995) showed a significant positive relationship of grain yield with productive tiller number, root length and root dry weight.

2.5.5 Panicle length

Sen *et al.* (2000) studied defoliation of flag leaf at 0, 2, 4, 6, 8, 10, 12, 14, 18 and 20 days after panicle emergence (DAE) and no defoliation (control) in cv. Pusharjali. They observed that defoliation up to 4-6 DAE reduced the relative growth rate of panicle, but after 4 days it had no adverse effect on panicle growth. All the yield-attributing characters, except panicle length, were significantly reduced when flag leaf was detached within 4-6 DAE. It seems that critical period of flag leaf duration was only up to the 4-6 days of panicle emergence.

Ji *et al.* (2012) reported that panicle length remained unchanged in drought susceptible variety Zhenshan97B, but it increased 8% in IRAT109, drought tolerant variety under water stress condition compared to well watered control.

2.5.6 Yield per plant

Classen and Shaw (1970) observed that water deficit at vegetative stage caused a significant grain yield reduction (12-15%) and 53% grain yield reduction due to water deficit at 75% milking stage in maize.

Row *et al.* (1983) conducted an experiment in yield component of rice varieties *viz*, Rasi and IR-20 under moisture stress condition. The reproductive and ripening phase were vulnerable and crucial for moisture stress, which resulted in permanent damage to growth and yield.

Woperesis *et al.* (1996) reported that drought at mid-tillering, panicle initiation and flowering strongly reduced yields to below 200 g m^{-2}. They also argued that the lower yield obtained in drought stressed plants was due to the larger percentage of unfilled grains.

Yanbao and Ingram (1988) reported that the water deficit in the vegetative phase had no significant effect on grain yield. Yield reduction up to 88% was noticed when plants were exposed to a 15-day stress period in the reproductive phase resulting from a reduction in the number of spikelets per plant and an increase in the percentage of unfilled spikelets.

Lafitte *et al.* (2004a) reported that the number of grains per panicle had no influence on grain yield. Basnayake *et al.* (2004) observed 9 to 51% and Ouk *et al.* (2006) observed 12 to 46% yield reduction due to drought in rice.

Sah and Zamora (2005) observed that water deficit at vegetative as well as reproductive stages significantly reduced the grain yield per plant in maize as compared to well-watered plant. The reduction was 19.5% and 48.5% due to water

deficit in vegetative and reproductive stages, respectively, as compared to well watered plants.

2.5.7 Spikelet fertility percentage

The drought-induced inhibition of panicle exertion has been identified as a consequence of a decrease in peduncle elongation, which can usually account for 70-75% spikelet sterility under water deficit (O'Toole and Namuco, 1983).

Ekanayake *et al.* (1989) showed that water potential is the important parameter that determines spikelet sterility in rice.

Jongdee *et al.* (1998) studied genotypic differences for grain yield in response to drought at flowering stage in rice and found out that genotypes that had lower leaf water potential at flowering under drought conditions had a higher spikelet sterility.

Lafitte and Courtois (2002) showed the advantages of early flowering over later flowering in terms of higher spikelet fertility, higher harvest index and higher yield.

Liu *et al.* (2006) reported a significant difference in number of pollen grains between IR64 and Moroberekan in the top four rachis under drought conditions. The variation in spikelet fertility between the genotypes was mainly due to the difference in locule-wall structure, and to variation in the number of pollen grains on stigma.

According to Serraj *et al.* (2009), the strong effects of drought on rice grain yield are largely due to reduction in spikelet fertility and panicle exertion.

2.5.8 1000 grain weight

Woperesis *et al.* (1996) reported that 1000 grain weight is considerably reduced in rice genotypes exposed to water stress at panicle initiation stage.

Ji *et al.* (2012) reported that the rate of filled grain and 1000-grain weight were reduced under the drought stress in Zhenshan97B and IRAT109. The changes of the rate of filled grain were consistent with 1000-grain weights in both the cultivars.

2.6 CORRELATION OF PHYSIO-MORPHOLOGICAL TRAITS AND YIELD COMPONENTS WITH GRAIN YIELD UNDER DROUGHT CONDITION

Shahid *et al.* (1994) observed that positive correlation existed between root length, root dry weight, shoot dry weight, stomata frequency and drought tolerance, whereas negative correlation existed between shoot length and stomata size.

Babu *et al.* (2003) detected positive correlation of biomass under stress with yield, percent spikelet fertility, number of grains per panicles, harvest index, and relative yield. On the other hand, leaf drying scores had negative correlation with yield and harvest index.

Kumar *et al.* (2004) discovered that in rice there is a negative association between delay in flowering and grain yield, relative water content and post flowering dry matter production under rainfed condition. A direct relationship was observed between delay in flowering and sterility. Leaf water status governed flowering delay under drought stress condition.

Lafitte *et al.* (2004b) found out that plant height is negatively correlated with yield under stress. Tall lines yield poorly in the rain-fed experiment, but there is no significant association between yield and height under control condition.

Singh *et al.* (2004) reported that significant and positive correlation existed between grain yield per plant and yield contributing traits, effective flag leaf breadth and total grains per panicle. A negative correlation was found between grain yield and plant height.

Gomez *et al.* (2005) observed that root breadth and root weight were positively correlated with root length; root breadth was significantly and positively correlated with root weight and biological yield; and plant height was positively correlated with grain number/panicle. Leaf area per plant showed the highest positive direct effect on root weight, followed by biological yield and root breadth. Selection based on biological yield may help identify drought-resistant types.

2.7 MOLECULAR MARKER TECHNOLOGY

Molecular marker technology offers a wide range of novel approaches to improve the selection strategies in cereal breeding. The development of molecular marker technology and the consequent identification of many marker loci has generated renewed interest in genetic mapping. Based on polymorphism in nucleotide sequence, molecular marker analysis allows identifying genome segment contributing to the genetic variance of trait and then selecting superior genotype at these traits in the early stage of plant development without uncertainties regarding genotypes due to environmental interaction and error. Applications of these markers for genetic studies of cereals have been so far many more diverse.

Main uses include

(i) Assessment of genetic variability and characterization of germplasm

(ii) Estimation of genetic distance between population and inbreeds

(iii) Identification and fingerprinting of genotypes

(iv) Detection of monogenic and quantitative trait loci

(v) Marker assisted selection

2.8 MICROSATELLITES OR SIMPLE SEQUENCE REPEATS (SSRS)

Litt & Lutty coined the term microsatellites in 1989. It is also known as Simple Sequence Repeats (SSRs). These are sections of DNA and consist of tandemly repeating nucleotide units that are arranged throughout the genomes of eukaryotic species and some prokaryotes. Simple Sequence Repeats (SSRs) developed from genomic libraries and these sequences are especially used to distinguish closely related genotypes. Polymorphism by microsatellite markers can be detected by PCR. If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers can be designed to amplify the microsatellite by PCR.

2.9 MICROSATELLITE MARKERS IN RICE

Simple sequence repeat (SSR) or microsatellite markers are co-dominant and PCR based markers used for genetic studies in rice. Microsatellite markers are reproducible and site specific.

In rice more than 2500 microsatellite markers have been developed and used to construct a genetic map (McCouch *et al.*, 2002).

Steele *et al.* (2006) identified the target segment on chromosome 9 significantly increased root length under both irrigated and drought stress regions using microsatellite markers.

Kanagaraj *et al.* (2010) identified markers linked to drought resistance using 23 recombinant inbred (RI) lines of IR20/Nootripathu, two indicia ecotypes with the extreme drought response. Parents were screened using 1206 rice microsatellite primer and 134 SSR primers produced polymorphism between parents. Also, three polymorphic primers between the bulks showed cosegregation among the individual RI lines forming the bulks.

Salunkhe *et al.* (2011) reported that the region, RM212-RM302-RM8085-RM3825 on chromosome 1, harbors large effect QTLs for drought resistance traits across several genetic backgrounds in rice.

Lang *et al.* (2013) identified quantitative trait loci (QTLs) associated with drought tolerance in rice. A total of 229 lines (BC2F2) derived from the cross of OM1490/WAB880-1-38-18-20-P1- HB was evaluated for drought at flowering, root dry weight (RDW), and root length (RL).

Mukherjee *et al.* (2013) reported that the microsatellite markers were distributed among 10 chromosomes of rice. The primers RM12921, RM18384, RM23877, RM23744, RM257, RM25181, RM25735 and RM5479 showed polymorphism in rice varieties.

Sangodele *et al.* (2014) developed inter-varietal backcross inbred lines (BILs) of Swarna x WAB 450 using 58 polymorphic SSR markers.

Muthukumar *et al.* (2015) studied marker trait associations using 1168 simple sequence repeat (SSR) markers and 911,153 single nucleotide polymorphisms (SNPs) with 17 diverse rice lines from different geographical regions.

Ramadan *et al.* (2015) studied genotypes revealed high level of genetic diversity indicating the availability of materials for rice breeding program for drought tolerance. The results indicated that among SSR markers used, 43 SSR loci were polymorphic and produced 127 alleles.

All these reports suggest that we can use proper marker for identifying drought tolerance gene.

2.10 BULKED LINE ANALYSIS

Bulked line analysis (BLA), was developed for identification of the molecular markers associated with a target gene. Instead of segregating progenies, conventional lines sharing the same trait were bulked by the BLA method. This method is an alternative approach to the identification of DNA markers linked with a target gene. A major advantage of this method is time saving for genetic stock development. The advantage is very significant for organisms having a long generation period.

Using Bulked Line Analysis (BLA), Kumar *et al.* (2005) identified two primers RM223 and RM263, associated with drought tolerance in rice.

Anitha *et al.* (2008) identified co-segregated marker RM 314, out of 25 polymorphic primers from 20 rice varieties (10 drought resistant and 10 drought susceptible rice varieties) using Bulked Line Analysis. And RM314 has been mapped and found to be linked to many root traits.

Prasad *et al.* (2016) identified three primers *viz.* RM 1092, RM 129 and RM 157B associated with drought tolerant traits using Bulked Line Analysis in 36 rice genotypes from diverse genetic background. The genomic regions flanked by these markers were found to be associated with various drought tolerant traits in rice.

Bulked segregant analysis (BSA) is another strategy which serves as an alternative approach for rapid identification of markers associated with drought resistance traits. Kanagaraj *et al.* (2010) used this technique to identify markers linked to drought resistance using 23 recombinant inbred (RIL) lines of IR20/Nootripathu, two indica ecotypes with extreme drought response. Out of 134 SSR polymorphic primers between parents, three primers showed polymorphism between bulks. Those three primers co segregated among the individual RI lines constituting the respective bulks.

Vikram *et al.* (2012) identified major effect QTLs for grain yield under drought in rice using phenotypic and genotypic data of two recombinant inbred line populations, Basmati334/Swarna and N22/MTU1010. The BSA approach successfully detected consistent effect drought grain yield QTLs $qDTY_{1.1}$ and $qDTY_{8.1}$ by whole population genotyping (WPG) and selective genotyping (SG).

Boopathi *et al.* (2013) performed Bulked Segregant Analysis (BSA) with SSR markers, and the marker RM27933 was found to be segregated perfectly well in individual components of drought resistant and drought susceptible bulks which were bulked based on yield under water stress among F2:3 lines.

2.11 IDENTIFICATION OF QTL FOR DROUGHT TOLERANCE IN RICE

QTL (Quantitative Trait Loci) are the regions within genomes that contain genes associated with a particular quantitative trait. Molecular approaches to drought tolerance have been widely applied to rice, beginning with QTL analysis. QTLs have been identified for many traits that are associated with drought response such as root characters, membrane stability, osmotic adjustment and morphological and physiological traits where tolerance is measured as yield under drought.

Venuprasad *et al.* (2001) worked on IR64 x Azucena DH mapping population of rice in three diverse environments and detected QTLs for ten traits at a threshold. The QTLs were spread across six chromosomes 1, 3, 4, 5, 6 and 7. Three QTLs for grain yield were detected one each on chromosome 3, 4 and 5.

Hittalmani *et al.* (2002) detected a total of 34 quantitative trait loci (QTLs) for 11 traits across three locations; one QTL was identified for grain yield per plant.

Price *et al.* (2002) reported 24 QTLs for various root growth traits in population of a cross between Azucena and Bala in rice for drought tolerance.

Hittalmani *et al.* (2003) reported a genomic region of 7.9 cM (from 135.8 to 143.7 cM) on chromosome 1 which was associated with drought resistance traits such as leaf rolling, number of spikelets, heading date and harvest index in IR64/Azucena rice DH lines. Similar result was found by Kanagaraj *et al.* (2010) by using 23 recombinant inbred (RI) lines of IR20/ Nootripathu.

Lafitte *et al.* (2004) identified 31 QTLs for yield and its components under rice drought on working with used population of a cross between Bala x Azucena.

Lanceras *et al.* (2004) used population of a cross between CT9993 and IR62266 and identified 77 QTLs for yield; yield components, panicle sterility etc. in rice.

Xu *et al.* (2005) identified 36 QTLs in introgression indica lines of rice for yield and its components under drought.

Gomez *et al.* (2006) identified 24 QTLs for various physio-morphological and plant production traits under drought stress. The number of QTLs per trait under stress was: 5 for leaf rolling, 4 for leaf drying, 3 for days to 50% flowering, 5 for plant height, 2 for number of productive tillers, 1 for panicle length, 3 for grain yield and 1 for straw yield.

Bernier *et al.* (2007) using population of 436 random F3 derived line from a cross between the upland rice cultivar Vandana and Way Rarem reported identification of QTL (*qtl12.1*) on chromosome 12 with large effect on grain yield under stress. They also reported that under stress also increases harvest index, biomass yield and plant height while reducing the number of days to flowering.

Kumar *et al.* (2007) reported detection of a QTL on chromosome 1 near *sd1* that explained 32% of the genetic variation for yield under stress, but only 4% under nonstress. They also found their effect consistence across years.

Kanjivavila *et al.* (2008) conducted an experiment in India for identifying genomic regions contributing to drought resistance. Total of 11 QTLs was identified for various plant phenology and production traits under rainfed and irrigated conditions.

Khowaja *et al.* (2009) reported QTL for rolling, stomatal conductance, dimentional traits, drought avoidance, plant height, plant biomass, leaf morphology and root traits.

Xing *et al.* (2010) reported QTLs for grain yield on chromosome 1, 5, 6 and 7, and for grain weight on chromosome 1, 3, 5 and 7 by using Zhenshan97/ Minghui63. They also identified gene *gw2* on short arm of chromosome 2 which controlling grain width and weight and gene *gs3* which responsible for grain length.

Chakraborty *et al.* (2011) conducted an experiment on QTL Mapping for days to flowering under drought condition in rice (*Oryza sativa* L.) and found that rice is very sensitive to moisture stress during flowering resulting in high floret sterility.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Identification of microsatellite markers associated with root traits for drought tolerance in rice (*Oryza sativa* L.)" was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani during 2016-17 with the objective to validate the role of root traits for drought tolerance in rice and to identify the microsatellite markers associated with drought tolerance in rice using Bulked Line Analysis. The details of the materials used and methods adopted for the rainout shelter experiment as well as Bulked Line Analysis and procedures followed for laboratory analysis during the course of experimentation are described in this chapter.

3.1 EVALUATION OF SELECTED 35 RICE GENOTYPES FOR ROOT TRAITS AND DROUGHT TOLERANCE

3.1.1 Plant materials

The rice accessions used in the present study consist of land races and improved local strains collected from RARS, Pattambi (Table 1).

3.1.2 Location

The study was conducted in the rainout shelter of Department of Plant Physiology, College of Agriculture, Vellayani during 2016-17 (Plate 1).

3.1.3 Experimental details

The details of the rainout shelter experiment are given in the table 2.

Plate 1. General view of experimental unit

Plate 2. View of experimental unit with rice plants inside rain out shelter

Table 1. List of rice accessions used in the study

Sl. No.	Genotypes	Sl. No.	Genotypes
1.	Aryan (Ptb1)	19.	Athikkiraya (Ptb19)
2.	Ponnaryan (Ptb2)	20.	Vadakkan Chitteni (Ptb20)
3.	Eravapandy (Ptb3)	21.	Thekkan (Ptb21)
4.	Vellari (Ptb4)	22.	VeluthaVattan (Ptb22)
5.	Velutharikayama (Ptb5)	23.	Cheriya Aryan (Ptb23)
6.	Athikkiraya (Ptb6)	24.	Chuvanna Vattan (Ptb24)
7.	Parambuvattan (Ptb7)	25.	Thonnooran (Ptb25)
8.	Thavalakkannan (Ptb8)	26.	Chenkayama (Ptb26)
9.	Thavalakkannan (Ptb9)	27.	Kodiyan (Ptb27)
10.	Thekkancheera (Ptb10)	28.	Kattamodan (Ptb28)
11.	Vyshak (Ptb 60)	29.	Karutha Modan (Ptb29)
12.	Thekkan Chitteni (Ptb12)	30.	Chuvanna Modan (Ptb30)
13.	Kayama (Ptb13)	31.	Elappapoochampan (Ptb31)
14.	Maskathi (Ptb14)	32.	Aruvakkari (Ptb32)
15.	Kavunginpoothala (Ptb15)	33.	Arikkirai (Ptb33)
16.	Harsha (Ptb 55)	34.	Valiya Champan (Ptb34)
17.	Jeddu Halliga (Ptb17)	35.	Chomala
18.	Eravapandy (Ptb18)		

Table 2. Particulars of rainout shelter experiment

1. Crop	Rice : 35 genotypes
2. Design	Completely Randomized Design (CRD)
3. Number of treatments	Two 1. Water stress from panicle initiation to 15 consecutive days (by withholding irrigation) 2. Control
4. Replication	Three

3.1.4 Methodology

In this study, plants were raised in polythene tubes of 25cm diameter and 1 meter height in rainout shelter (Plate 2). Separate set of plants with three replications were maintained for the two treatments. Irrigation was given regularly for both the control and water stress treatments up to panicle initiation stage according to their duration and then irrigation was withholded to a period of 15 days to create drought condition. The control plants were well irrigated up to maturity. Observations on root traits, physiological and biochemical parameters were taken at this stage. Rewatering was done when the leaves were completely rolled and started drying at tips and margins (after 15 days of drought imposition). Plants were kept upto maturity following rewatering. At the time of harvest, morphological and yield parameters were taken.

3.1.5 Preparation of potting mixture and transplanting

Polythene tubes of 1m height were filled with 50kg of potting mixture prepared by mixing soil, sand, and FYM in the ratio of 3:2:1. For the experiment, 10 grams of seeds of each genotype obtained from RARS, Pattambi were sown in plastic trays (30cm x 15cm dimension) filled with soil and coir pith in the ratio 2:1. Twenty one days old seedlings were transplanted to the polythene tubes at the rate of three seedlings per tube. Gap filling was done on 8th day after transplanting and one healthy seedling was maintained in each tube. Foliar spray of 19:19:19 mixture was given on 15th day after transplanting. Crop was applied with recommended dose of fertilizer as per package of practices of Kerala Agricultural University, Thrissur. The cultural operations including weeding and plant protection measures were carried out as per *ad hoc* recommendations of Kerala Agricultural University, Thrissur.

3.1.6 Observations

3.1.6.1 Physiological and biochemical parameters

3.1.6.1.1 Leaf rolling score

Leaf rolling was observed under rainout shelter after imposing water stress condition 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 5th 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

3.1.6.1.2 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 sample leaves 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.

3.1.6.1.3 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.

3.1.6.1.4 Leaf temperature

Leaf temperature was measured at morning time between 9 am and 11 am using portable photosynthetic system (CIRAS-3, PP systems U.S.A) and were expressed in ^{0}C.

3.1.6.1.5 Stomatal conductance

Stomatal conductance was measured at morning time between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and were expressed in m H_2O moles m^{-2} s^{-1}.

3.1.6.1.6 Photosynthetic rate

Photosynthetic rate was measured at morning time between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and were expressed in μ CO_2 moles m^{-2} s^{-1}.

3.1.6.1.7 Transpiration rate

Transpiration rate was measured at morning time between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and were expressed in m H_2O moles m^{-2} s^{-1}.

3.1.6.1.8 Proline content

Proline content was estimated as per the procedure described by Bates *et al.,* (1973). A known amount (0.5g) of mid-leaf portion was homogenized with 10ml of 3% aqueous sulphosalicylic acid and centrifuged at 3000 rpm for 15 minutes. 2ml of the supernatant was taken and mixed with an equal amount of glacial acetic acid and acid ninhydrin. The contents were allowed to react at 100^{0}C for one hour in water

bath. The reaction was terminated by keeping it in ice bath for 10 min. The reaction mixture was mixed with 4ml toluene using vortex mixture for 15 – 20 seconds. The chromophore containing toluene was aspirated from aqueous phase, warmed to room temperature and the optical density was read at 520nm with toluene as blank. A standard curve was drawn using concentration verses absorbance.

The concentration of proline was determined from graph and expressed as

$$\mu \text{ moles/g tissue} = \{[(\mu g \text{ proline / ml}) \times \text{ml toluene}] / 115.5\} \times (5 / \text{g sample}),$$

where 115.5 is the molecular weight of proline.

3.1.6.2 Root traits

Roots were collected from water stressed and control plants after 15 days of stress imposition by carefully tearing the polythene bags. The soil particles adhered to the root surface was removed by washing with high jet of water.

3.1.6.2.1 Root length

Root length was measured from the cut end to the tip of the longest rootlet by using a centimetre scale and expressed in cm.

3.1.6.2.2 Root dry weight

Roots collected were dried moisture free in a hot air oven at 80°C for 48 hours (till attaining constant weight). Then the dry weights were recorded in grams by using an electronic balance.

3.1.6.2.3 Root volume

Root volume was determined in cubic centimetre by water displacement method. Roots were immersed in water in a 1000 ml measuring cylinder after

removing from the soil and cleaning thoroughly. The displaced volume of the water was taken as the volume of the roots.

3.1.6.2.4 Root shoot ratio

The shoot weight was recorded separately after drying the shoot portion in hot air oven at 80°C for 48 hours till reaching constant weight. Root shoot ratio was calculated as follows

Root shoot ratio = Root dry weight / Shoot dry weight

3.1.6.3 Morphological and yield parameters

3.1.6.3.1 Plant height

Plant height was measured from the base of the plant to the tip of the primary panicle at the time of maturity and expressed in centimeters.

3.1.6.3.2 Days to 50% flowering

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

3.1.6.3.3 Tiller number

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

3.1.6.3.4 Productive tiller number

In each replication, the number of panicle bearing tillers at the time of harvest was counted and recorded.

3.1.6.3.5 Panicle length

The length of the primary panicle from each plant was measured from the neck node to the tip of the apical grain using a centimeter scale and expressed in cm.

3.1.6.3.6 Yield per plant

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

3.1.6.3.7 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

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of fertile spikelets}}{\text{Total number of spikelets}} \times 100$$

3.1.6.3.8 1000 grain weight

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

3.1.7 Statistical analysis

Statistical analysis was carried out using the SAS program (SAS institute Inc., 1990).

3.1.8 Correlation analysis

Correlation coefficient (r) was calculated for grain yield and yield contributing characters by using the standard procedure given by Searle (1961).

$$r(x,y) = \frac{Cov.(x,y)}{\sqrt{Var(x).Var(y)}}$$

where,

$r(x,y)$ = Correlation coefficient between characters x and y

$Cov.(x,y)$ = Phenotypic covariance between x and y

$Var(x)$ = Variance of x character

$Var(y)$ = Variance of y character

3.2 IDENTIFICATION OF MICROSATELLITE MARKERS ASSOCIATED WITH DROUGHT TOLERANT TRAITS IN RICE USING BULKED LINE ANALYSIS

3.2.1 Plant sample

A set of 10 genotypes, which showed better performance and another 10 genotypes with poor performance from the 35 genotypes under water stress condition were selected for Bulked Line Analysis (Table 3).

Table 3. List of rice genotypes selected for Bulked Line Analysis

Sl. No.	Tolerant genotypes	Sl. No.	Susceptible genotypes
1.	Aryan (Ptb-1)	1.	Eravapandy (Ptb-3)
2.	Parambuvattan (Ptb-7)	2.	Thavalakkannan (Ptb-8)
3.	Thekkancheera (Ptb-10)	3.	Thekkan Chitteni (Ptb-12)
4.	Kavunginpoothala (Ptb-15)	4.	Jeddu Halliga (Ptb-17)
5.	Kattamodan (Ptb-28)	5.	Velutha Vattan (Ptb-22)
6.	Karuthamodan (Ptb-29)	6.	Cheriya Aryan (Ptb-23)
7.	Chuvannamodan (Ptb-30)	7.	Chuvanna Vattan (Ptb-24)
8.	Harsha (Ptb-55)	8.	Kodiyan (Ptb-27)

Cont...

9.	Vaishak (Ptb-60)	9.	Aruvakkari (Ptb-32)
10.	Chomala	10.	Valiya Champan (Ptb-34)

3.2.2 Genomic DNA isolation

Genomic DNA from the selected 20 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 : isoamylalcohol mixture was added and mixed well for 15 minutes. Centrifuged at 12,000 rpm for 15 minutes and aqueous phase was transferred to another microcentrifuge tube without disturbing the inter phase. Two volumes 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 12,000 rpm 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.

3.2.3 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). The ratio between the readings at 260nm and 280nm (OD 260/OD 280) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have 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.

3.2.4 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 to be diluted, M_2 is the concentration of working solution and V_2 is the volume of working solution 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°C till further use.

3.2.5 Preparation of DNA bulks

10µl of diluted DNA each from ten drought tolerant and ten drought susceptible rice genotypes were taken and pooled into drought tolerant and drought susceptible bulks, respectively.

3.2.6 PCR amplification using SSR primers

3.2.6.1 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 (1U) - 0.3µl
e) Forward primer (10pM) - 0.75µl
f) Reverse primer (10pM) - 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^{0}C for 3 minutes

Denaturation - 94^{0}C for 1 minute
Primer annealing - 53^{0}C to 55^{0}C for 1 minute
Primer extension - 72^{0}C for 1 minute
} 35 cycles

Final extention - 72^{0}C for 5 minutes

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

3.3.7 Detection of polymorphism between the bulks using SSR primers

One hundred and fifty primers combinations were screened by PCR and their sequence are listed in Table 4. 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 bulks. Putative polymorphic markers between the bulks were checked for similar product size among the individual genotypes constituting the tolerant and susceptible bulks.

Table 4. List of SSR primers used for Bulked Line Analysis

S. No.	Marker	Primer F	Primer R
1.	RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
2.	RM1003	GATTCTTCCTCCCCTTCGTG	TTCCTGTCAGAACAGGGAGC
3.	RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC
4.	RM313	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC
5.	RM1054	TGCATATGTACCGCAACCTC	TTTCTGCATGATCCCCTCTG
6.	RM122	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC
7.	RM161	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG
8.	RM169	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
9.	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
10.	RM130	TGTTGCTTGCCCTCACGCGAAG	GGTCGCGTGCTTGGTTTGGTTC
11.	RM112	GGGAGGAGAGGCAAGCGGAGAG	AGCCGGTGCAGTGGACGGTGAC
12.	RM7119	AGGCTGAGGCTTATAGGCAG	GGATGATACAACTTGACCCC
13.	RM170	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC
14.	RM164	TCTTGCCCGTCACTGCAGATATC	GCAGCCCTAATGCTACAATTCTTC
15.	RM111	CACAACCTTTGAGCACCGGGTC	ACGCCTGCAGCTTGATCACCGG
16.	RM108	TCTCTTGCGCGCACACTGGCAC	CGTGCACCACCACCACCACCAC
17.	RM311	TGGTAGTATAGGTACTAAACAT	TCCTATACACATACAAACATAC
18.	RM102	AACTTTCCCACCACCACCGCGG	AGCAGCAGCAAGCCAGCAAGCG
19.	RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG

20.	RM7039	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC
21.	RM467	GGTCTCTCTCTCTCTCTCTCTC	CTCCTGACAATTCAACTGCG
22.	RM474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
23.	RM80	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG
24.	RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
25.	RM525	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG
26.	RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
27.	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
28.	RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
29.	RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
30.	RM9	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC
31.	RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG
32.	RM283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
33.	RM287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
34.	RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
35.	RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
36.	RM433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
37.	RM507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
38.	RM84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
39.	RM85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
40.	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
41.	RM514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
42.	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
43.	RM520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG
44.	RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
45.	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
46.	RM455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
47.	RM454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
48.	RM302	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
49.	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC
50.	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
51.	RM7555	AAAGGATAAATGTGGGGATC	ATAACCGTCTGGTTTCACTG
52.	RM1130	AGATCGGATTGGGATGGC	ACCCAACCAATTAGTGCCAC
53.	RM1201	TTACCGCGCCACATATACAC	CGTACGAGCCCTAGTTACCG
54.	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
55.	RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC

56.	RM153	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG
57.	RM11	TCGTCTACTGTTGGCTGCAC	ATAGCGGGCGAGGCTTAG
58.	RM303	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT
59.	RM13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG
60.	RM265	CGAGTTCGTCCAAGTGAGC	CATCCACCATTCCACCAATC
61.	RM7121	GGAGATGGCACACGTCAAAC	AGGATCCCGTTTTGTAGCAG
62.	RM14	CCGAGGAGAGGAGTTCGAC	GTGCCAATTTCCTCGAAAAA
63.	RM212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
64.	RM6836	TGTTGCATATGGTGCTATTTGA	GATACGGCTTCTAGGCCAAA
65.	RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
66.	RM3894	TATGCTCTCTCCTTCAGGCC	CTTACCAACTCCGCACTTGC
67.	RM120	CACACAAGCCCTGTCTCACGACC	CGCTGCGTCATGAGTATGTA
68.	RM7117	AGTTGGCTGGTTGCTACCAC	AGGGTTCCCTGGCTACTCAC
69.	RM552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC
70.	RM348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
71.	RM417	CGGATCCAAGAAACAGCAG	TTCGGTATCCTCCACACCTC
72.	RM203	CCTATCCCATTAGCCAAACATTGC	GATTTACCTCGACGCCAACCTG
73.	RM243	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
74.	RM202	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
75.	RM133	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC
76.	RM3515	GGAAAGAAGATATGCCATGC	AGAGAGAATCAGAAACACCAAC
77.	RM317	CATACTTACCAGTTCACCGCC	CTGGAGAGTGTCAGCTAGTTGA
78.	RM6144	TGGAACTCAACGGGAGTCTC	GAAGTAGTGGAATCGGCGAG
79.	RM166	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG
80.	RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
81.	RM255	TGTTGCGTGTGGAGATGTG	CGAAACCGCTCAGTTCAAC
82.	RM1018	ATCTTGTCCCACTGCACCAC	TGTGACTGCTTTTCTGTCGC
83.	RM254	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
84.	RM118	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG
85.	RM178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC
86.	RM169	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
87.	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
88.	RM7119	AGGCTGAGGCTTATAGGCAG	GGATGATACAACTTGACCCC
89.	RM311	TGGTAGTATAGGTACTAAACAT	TCCTATACACATACAAACATAC
90.	RM108	TCTCTTGCGCGCACACTGGCAC	CGTGCACCACCACCACCACCAC
91.	RM7039	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC

92.	RM1090	GTTATAGCGCACCCTGGATG	GAACCGAAGGGACATGTGTG
93.	RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
94.	RM315	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
95.	RM349	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG
96.	RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC
97.	RM3042	CAAAAAGGAATCAATGTGAA	GGCTGTTGAGAGGTAGAGAA
98.	RM48	TGTCCCACTGCTTTCAAGC	CGAGAATGAGGGACAAATAACC
99.	RM485	CACACTTTCCAGTCCTCTCC	CATCTTCCTCTCTTCGGCAC
100.	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
101.	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
102.	RM151	GGCTGCTCATCAGCTGCATGCG	TCGGCAGTGGTAGAGTTTGATCTGC
103.	RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
104.	RM136	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC
105.	RM338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC
106.	RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
107.	RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
108.	RM47	ACTCCACTCCACTCCCCAC	GTCAGCAGGTCGGACGTC
109.	RM537	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC
110.	RM104	GGAAGAGGAGAGAAAGATGTGTGTC	TCAACAGACACACCGCCACCGC
111.	RM17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
112.	RM328	CATAGTGGAGTATGCAGCTGC	CCTTCTCCCAGTCGTATCTG
113.	RM222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG
114.	RM236	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC
115.	RM278	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
116.	RM246	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT
117.	RM144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGC
118.	RM225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
119.	RM536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
120.	RM462	ACGGCCCATATAAAAGCCTC	AAGATGGCGGAGTAGCTCAG
121.	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
122.	RM149	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG
123.	RM461	GAGACCGGAGAGACAACTGC	TGATGCGGTTTGACTGCTAC
124.	RM1090	GTTATAGCGCACCCTGGATG	GAACCGAAGGGACATGTGTG
125.	RM163	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
126.	RM408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
127.	RM6314	GACTTTGATCTTTGGTGGACG	GGTTCAGGGACGAATTTCAG

128.	RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC
129.	RM334	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG
130.	RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
131.	RM312	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
132.	RM284	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC
133.	RM489	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
134.	OSR13	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC
135.	OSR14	AAATCCACGCACACTTTGCG	AGGTAAACGAGCTTGAGGTG
136.	OSR16	AAAACTAGCTTGCAAAGGGGA	TGCCGGCTGATCTTGTTCTC
137.	OSR17	GCTGGTTGATTCAGCTAGTC	GCCTCGTTGTCGTTCCACAC
138.	RM1920	CAAACACAGTGTTGACAGAA	GCTATTGACTTATCCGTTCA
139.	RM1925	AATTCATTCAAGCCTTGATA	ATTAGTTTCACCAAAGCAAC
140.	RM1940	ACTATCGATCAAAATGCTAG	AAACGAATGGTTAAATGTTA
141.	RM1942	CTGCTCAATGATACAGGA	GGCATCCACTAAATTTAGATA
142.	RM1869	CGTTTCACAATGTAAGACTT	CTCCGTTTTACAATGTAAGA
143.	RM1896	GGACAGGGTAAAGTGTTAGA	CCTAAGACCTATCAACTCCA
144.	RM1937	AATAAATAAAAATCCAGCAC	AGATCAGATATGGCATTAAG
145.	RM2770	TAGGCCCTGATTAGTTTCC	ATATATGTGTCCCTTCTCCATAC
146.	RM2814	AATACCTGTTTGTATGTGTC	CACTTATAGGTTAATTATGTGA
147.	RM2819	AATGTTGCTAGATTTAAAAC	CAGTAGGATATCTTACAACC
148.	RM2887	GATCAATATGATTTTTTTCA	TAGTCGATTACTATTGGGTA
149.	RM2972	GAGCCAATATGTTGTCTTGA	GTTCAGATCATGATGCCTAC
150.	RM3103	CAGACAACTTGTAATGTACG	ATGTCATGGGAGATAATTAA

Results

4. RESULTS

The experiment was conducted to evaluate the role of root traits for drought tolerance in selected 35 rice genotypes and to identify the microsatellite markers associated with root traits for drought tolerance using Bulked Line Analysis in the Department of Plant Physiology, College of Agriculture, Vellayani during 2016-17. The rice plants were exposed to water stress condition at panicle initiation stage along with an irrigated control and replicated thrice. The physio-morphological, biochemical and yield characters were recorded after stress imposition in both stress and control plants. Bulked Line Analysis was carried out using selected drought tolerant and susceptible lines to identify the microsatellite markers linked to root traits for drought tolerance in rice. The data were statistically analyzed and the results are presented in this chapter with suitable tables.

4.1 EFFECT OF WATER STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

4.1.1 Leaf rolling score

Leaf rolling is considered as an adaptive mechanism in cereal crops to avoid water loss through transpiration during water stress. The scores on leaf rolling revealed the capacity of rice genotypes to maintain leaf water potential throughout the drought condition. The results of leaf rolling scores are presented in the table 5.

The results showed that the rate of rolling significantly increased over days of drought. Mean leaf rolling scores of rice genotypes was recorded 5.2 at water stress condition and 1.0 at irrigated condition. Complete leaf rolling (score – 9) was observed in Ptb-7 (Plate 3) and Ptb-13 while the genotypes Ptb-29 and Ptb-30 showed no leaf rolling symptoms (score – 1) under water stress condition. All the genotypes exhibited a score of 1 (fully unrolled, turgid leaf) under irrigated condition.

Table No 5. Leaf rolling score of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	1.0	4.0	2.5
2.	Ponnaryan(Ptb2)	1.0	8.7	4.8
3.	Eravapandy (Ptb3)	1.0	3.7	2.3
4.	Vellari (Ptb4)	1.0	2.7	1.8
5.	Velutharikayama (Ptb5)	1.0	7.7	4.3
6.	Athikkiraya(Ptb6)	1.0	8.0	4.5
7.	Parambuvattan(Ptb7)	1.0	9.0	5.0
8.	Thavalakkannan(Ptb8)	1.0	7.7	4.3
9.	Thavalakkannan(Ptb9)	1.0	8.0	4.5
10.	Thekkancheera (Ptb10)	1.0	2.7	1.8
11.	Vyshak (Ptb 60)	1.0	4.7	2.8
12.	ThekkanChitteni (Ptb12)	1.0	4.7	2.8
13.	Kayama (Ptb13)	1.0	9.0	5.0
14.	Maskathi (Ptb14)	1.0	8.0	4.5
15.	Kavunginpoothala (Ptb15)	1.0	2.7	1.8
16.	Harsha (Ptb 55)	1.0	1.7	1.3
17.	JedduHalliga (Ptb17)	1.0	8.7	4.8
18.	Eravapandy (Ptb18)	1.0	8.0	4.5
19.	Athikkiraya(Ptb19)	1.0	3.7	2.3
20.	VadakkanChitteni (Ptb20)	1.0	8.7	4.8
21.	Thekkan (Ptb21)	1.0	2.7	1.8
22.	VeluthaVattan (Ptb22)	1.0	7.7	4.3
23.	Cheriya Aryan (Ptb23)	1.0	3.7	2.3
24.	Chuvanna Vattan (Ptb24)	1.0	7.7	4.3
25.	Thonnooran (Ptb25)	1.0	4.0	2.5
26.	Chenkayama (Ptb26)	1.0	2.7	1.8
27.	Kodiyan (Ptb27)	1.0	4.7	2.8
28.	Kattamodan (Ptb28)	1.0	1.7	1.3
29.	Karutha Modan (Ptb29)	1.0	1.0	1.0
30.	Chuvanna Modan (Ptb30)	1.0	1.0	1.0
31.	Elappapoochampan (Ptb31)	1.0	5.7	3.3
32.	Aruvakkari (Ptb32)	1.0	6.7	3.8
33.	Arikkirai (Ptb33)	1.0	4.7	2.8
34.	ValiyaChampan (Ptb34)	1.0	3.7	2.3
35.	Chomala	1.0	2.7	1.8
	Mean	**1.0**	**5.2**	
		C.D. (5%)	**SE(m)**	
	G	0.5	0.2	
	T	0.1	0.0	
	G*T	0.7	0.3	

Plate 3. Leaf rolling symptoms of Ptb-7 at flowering stage under irrigated and water stress conditions

The leaf rolling score indicates the reduction in leaf water content due to water stress condition. The genotypes Ptb-29, Ptb-30 and Ptb-55 which showed minimum leaf rolling (score 1-2), maintained higher leaf water content, whereas Ptb-7 and Ptb-13 which showed maximum (score 8-9) leaf rolling score maintained lower leaf water content.

4.1.2 Relative Water Content

The results related to relative water content at flowering stage are presented in table 6. The results showed that relative water content decreased significantly in all the genotypes under water stress condition compared to irrigated control. Among the genotypes, the RWC was recorded to be highest in Ptb-4 (78.8%) followed by Ptb-10 (78.4%) while the lowest was recorded in Ptb-13 (37.4%) under water stress condition. Chomala showed the highest value of relative water content, i.e. 91.5% under irrigated condition followed by Ptb-23 (89.3%) while the lowest value was found in Ptb-14 (70.8%). The mean relative water content was 66.0 and 84.2 percent at flowering stage under water stress and irrigated conditions respectively. Maximum percent reduction in relative water content was observed in Ptb-22 (32.7%) whereas minimum percent reduction was in Ptb-15 (1.6 %). The overall reduction in relative water content at flowering stage was noticed by 21.6 percent under water stress condition over irrigated condition.

4.1.3 Cell membrane stability index

The results related to cell membrane stability index at flowering stage are being presented in table 7. It was found that among the genotypes Ptb-29 (98.5%) showed higher membrane stability followed by Ptb-10 (98.1%) while the lowest value was recorded in Ptb-2 (79.1%).

Table No 6. Relative water content (%) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	89.8	72.0	80.9
2.	Ponnaryan(Ptb2)	80.3	60.2	70.2
3.	Eravapandy (Ptb3)	84.9	63.5	74.2
4.	Vellari (Ptb4)	82.2	78.8	80.5
5.	Velutharikayama (Ptb5)	83.3	63.6	73.5
6.	Athikkiraya(Ptb6)	74.7	55.5	65.1
7.	Parambuvattan(Ptb7)	81.1	40.9	61.0
8.	Thavalakkannan(Ptb8)	84.8	64.3	74.6
9.	Thavalakkannan(Ptb9)	87.1	69.9	78.5
10.	Thekkancheera (Ptb10)	86.9	78.4	82.6
11.	Vyshak (Ptb 60)	82.5	73.9	78.2
12.	ThekkanChitteni (Ptb12)	82.6	68.0	75.3
13.	Kayama (Ptb13)	84.0	37.4	60.7
14.	Maskathi (Ptb14)	70.8	51.3	61.0
15.	Kavunginpoothala (Ptb15)	77.3	76.1	76.7
16.	Harsha (Ptb 55)	87.8	77.6	82.7
17.	JedduHalliga (Ptb17)	83.7	71.1	77.4
18.	Eravapandy (Ptb18)	87.4	59.5	73.4
19.	Athikkiraya(Ptb19)	86.9	73.7	80.3
20.	VadakkanChitteni (Ptb20)	85.3	69.9	77.6
21.	Thekkan (Ptb21)	84.9	66.1	75.5
22.	VelthaVattan (Ptb22)	87.8	59.1	73.4
23.	Cheriya Aryan (Ptb23)	89.3	76.1	82.7
24.	Chuvanna Vattan (Ptb24)	76.0	62.1	69.0
25.	Thonnooran (Ptb25)	84.9	74.3	79.6
26.	Chenkayama (Ptb26)	83.9	72.5	78.2
27.	Kodiyan (Ptb27)	88.8	67.9	78.3
28.	Kattamodan (Ptb28)	83.3	58.9	71.1
29.	Karutha Modan (Ptb29)	84.8	76.7	80.7
30.	Chuvanna Modan (Ptb30)	86.5	71.4	79.0
31.	Elappapoochampan (Ptb31)	84.6	52.7	68.7
32.	Aruvakkari (Ptb32)	86.9	66.1	76.5
33.	Arikkirai (Ptb33)	85.3	59.1	72.2
34.	ValiyaChampan (Ptb34)	84.9	68.5	76.7
35.	Chomala	91.5	74.6	83.1
	Mean	**84.2**	**66.0**	
		C.D. (5%)	**SE(m)**	
	G	3.6	1.3	
	T	0.9	0.3	
	G*T	5.1	1.8	

Table No. 7. Membrane stability index (%) of rice genotypes at flowering stage

Sl. No.	Genotypes	MSI (Mean)
1.	Aryan (Ptb1)	85.2±0.3
2.	Ponnaryan(Ptb2)	79.1±4.2
3.	Eravapandy (Ptb3)	81.6±3.0
4.	Vellari (Ptb4)	83.3±4.5
5.	Veluthari kayama (Ptb5)	81.9±1.6
6.	Athikkiraya(Ptb6)	82.4±2.3
7.	Parambuvattan(Ptb7)	92.8±0.8
8.	Thavalakkannan(Ptb8)	82.5±0.2
9.	Thavalakkannan(Ptb9)	85.7±3.9
10.	Thekkancheera (Ptb10)	98.1±3.4
11.	Vyshak (Ptb 60)	95.9±4.4
12.	Thekkan Chitteni (Ptb12)	83.2±1.7
13.	Kayama (Ptb13)	83.0±1.4
14.	Maskathi (Ptb14)	87.9±6.1
15.	Kavunginpoothala (Ptb15)	96.8±0.3
16.	Harsha (Ptb 55)	81.3±5.2
17.	Jeddu Halliga (Ptb17)	85.0±0.1
18.	Eravapandy (Ptb18)	87.2±1.3
19.	Athikkiraya(Ptb19)	80.4±1.0
20.	Vadakkan Chitteni (Ptb20)	88.9±5.4
21.	Thekkan (Ptb21)	82.1±1.8
22.	Velutha Vattan (Ptb22)	82.4±3.0
23.	Cheriya Aryan (Ptb23)	84.3±1.1
24.	Chuvanna Vattan (Ptb24)	82.8±1.4
25.	Thonnooran (Ptb25)	85.1±2.2
26.	Chenkayama (Ptb26)	85.1±0.6
27.	Kodiyan (Ptb27)	82.2±1.0
28.	Kattamodan (Ptb28)	96.0±9.4
29.	Karutha Modan (Ptb29)	98.5±1.6
30.	Chuvanna Modan (Ptb30)	92.2±0.2
31.	Elappapoochampan (Ptb31)	89.2±6.2
32.	Aruvakkari (Ptb32)	85.9±6.1
33.	Arikkirai (Ptb33)	82.1±3.1
34.	Valiya Champan (Ptb34)	86.3±2.7
35.	Chomala	94.3±3.1
	C.D. (5%)	9.8
	SE(m)	3.5

4.1.4 Leaf temperature

A significant difference was observed among the genotypes and treatments for leaf temperature. The results obtained for the leaf temperature at flowering stage under irrigated and water stress conditions are presented in table 8. Among the genotypes, the maximum leaf temperature was observed in Ptb-1 (31.7^0C) followed by Ptb-2 (31.2^0C) and the minimum in Ptb-7 (27.8^0C) under water stress condition, whereas in irrigated condition also, Ptb-1 showed highest leaf temperature (29.1^0C). The lowest leaf temperature was recorded in Ptb-4 (27.0^0C) under irrigated condition which can be attributed to its high relative water content. The mean decrease in leaf temperature between irrigated and water stress conditions was 3.8%. The highest percentage increase in leaf temperature under water stress condition compared to irrigated condition was observed in Ptb-1 (8.9%), while the lowest was in Ptb-55 (1.4%) and chomala (1.4%).

4.1.5 Stomatal conductance

The results related to stomatal conductance at flowering stage are presented in table 9. The results showed that stomatal conductance decreased significantly at flowering stage under water stress condition compared to irrigated condition. The mean stomatal conductance of rice genotypes was 451.9 m H_2O moles m^{-2} s^{-1} under irrigated condition, whereas under water stress condition it was 257.7 m H_2O moles m^{-2} s^{-1}. Among the genotypes, stomatal conductance was recorded to be highest in Ptb-30 (674 m H_2O moles m^{-2} s^{-1}) while the lowest in Ptb-20 (92 m H_2O moles m^{-2} s^{-1}) under water stress condition. In irrigated condition, maximum stomatal conductance was attained by Ptb-30 (885.0 m H_2O moles m^{-2} s^{-2}) followed by Ptb-15 (783.0 m H_2O moles m^{-2} s^{-2}), while the minimum stomatal conductance was attained by Ptb-22 (224.0 m H_2O moles m^{-2} s^{-2}).

Table No 8. Leaf temperature (^{0}C) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	29.1	31.7	30.4
2.	Ponnaryan(Ptb2)	28.7	31.2	30.0
3.	Eravapandy (Ptb3)	27.8	28.7	28.3
4.	Vellari (Ptb4)	27.0	28.6	27.8
5.	Veluthari kayama (Ptb5)	27.6	28.9	28.3
6.	Athikkiraya(Ptb6)	27.7	28.8	28.3
7.	Parambuvattan(Ptb7)	27.1	27.8	27.5
8.	Thavalakkannan(Ptb8)	27.9	28.7	28.3
9.	Thavalakkannan(Ptb9)	27.3	28.1	27.7
10.	Thekkancheera (Ptb10)	27.9	28.4	28.2
11.	Vyshak (Ptb 60)	28.3	28.8	28.6
12.	Thekkan Chitteni (Ptb12)	27.8	28.8	28.3
13.	Kayama (Ptb13)	28.2	29.1	28.7
14.	Maskathi (Ptb14)	28.4	29.2	28.8
15.	Kavunginpoothala (Ptb15)	27.7	28.7	28.2
16.	Harsha (Ptb 55)	28.4	28.8	28.6
17.	Jeddu Halliga (Ptb17)	27.4	28.6	28.0
18.	Eravapandy (Ptb18)	27.9	28.8	28.4
19.	Athikkiraya(Ptb19)	28.1	28.9	28.5
20.	Vadakkan Chitteni (Ptb20)	27.6	28.5	28.1
21.	Thekkan (Ptb21)	27.4	28.6	28.0
22.	Velutha Vattan (Ptb22)	27.8	28.7	28.3
23.	Cheriya Aryan (Ptb23)	27.4	28.6	28.0
24.	Chuvanna Vattan (Ptb24)	27.6	28.8	28.2
25.	Thonnooran (Ptb25)	27.7	28.9	28.3
26.	Chenkayama (Ptb26)	27.3	28.7	28.0
27.	Kodiyan (Ptb27)	27.1	28.6	27.9
28.	Kattamodan (Ptb28)	28.0	28.5	28.3
29.	Karutha Modan (Ptb29)	28.2	28.7	28.5
30.	Chuvanna Modan (Ptb30)	27.8	28.3	28.1
31.	Elappapoochampan (Ptb31)	27.5	28.6	28.1
32.	Aruvakkari (Ptb32)	28.3	29.0	28.7
33.	Arikkirai (Ptb33)	27.8	29.1	28.5
34.	Valiya Champan (Ptb34)	28.4	29.2	28.8
35.	Chomala	28.3	28.7	28.5
	Mean	27.8	28.9	
		C.D. (5%)	**SE(m)**	
	G	0.8	0.3	
	T	0.2	0.1	
	G*T	N/A	0.4	

Table No 9. Stomatal conductance (m H_2O moles m^{-2} s^{-1}) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	432.0	192.0	312.0
2.	Ponnaryan(Ptb2)	483.0	220.0	351.5
3.	Eravapandy (Ptb3)	457.0	198.0	327.5
4.	Vellari (Ptb4)	524.0	349.0	436.5
5.	Veluthari kayama (Ptb5)	345.0	152.0	248.5
6.	Athikkiraya(Ptb6)	239.0	148.0	193.5
7.	Parambuvattan(Ptb7)	648.0	488.0	568.0
8.	Thavalakkannan(Ptb8)	356.0	278.0	317.0
9.	Thavalakkannan(Ptb9)	284.0	168.0	226.0
10.	Thekkancheera (Ptb10)	565.0	367.0	466.0
11.	Vyshak (Ptb 60)	598.0	341.0	469.5
12.	Thekkan Chitteni (Ptb12)	712.0	399.0	555.5
13.	Kayama (Ptb13)	476.0	174.0	325.0
14.	Maskathi (Ptb14)	253.0	153.0	203.0
15.	Kavunginpoothala (Ptb15)	783.0	512.0	647.5
16.	Harsha (Ptb 55)	612.0	423.0	517.5
17.	Jeddu Halliga (Ptb17)	441.0	259.0	350.0
18.	Eravapandy (Ptb18)	494.0	285.0	389.5
19.	Athikkiraya(Ptb19)	267.0	164.0	215.5
20.	Vadakkan Chitteni (Ptb20)	229.0	92.0	160.5
21.	Thekkan (Ptb21)	254.0	136.0	195.0
22.	Velutha Vattan (Ptb22)	224.0	148.0	186.0
23.	Cheriya Aryan (Ptb23)	263.0	124.0	193.5
24.	Chuvanna Vattan (Ptb24)	467.0	211.0	339.0
25.	Thonnooran (Ptb25)	412.0	149.0	280.5
26.	Chenkayama (Ptb26)	452.0	157.0	304.5
27.	Kodiyan (Ptb27)	248.0	135.0	191.5
28.	Kattamodan (Ptb28)	724.0	413.0	568.5
29.	Karutha Modan (Ptb29)	682.0	420.0	551.0
30.	Chuvanna Modan (Ptb30)	885.0	674.0	779.5
31.	Elappapoochampan (Ptb31)	279.0	184.0	231.5
32.	Aruvakkari (Ptb32)	321.0	102.0	211.5
33.	Arikkirai (Ptb33)	242.0	138.0	190.0
34.	Valiya Champan (Ptb34)	419.0	245.0	332.0
35.	Chomala	747.0	420.0	583.5
	Mean	**451.9**	**257.7**	
		C.D. (5%)	**SE(m)**	
	G	11.5	4.1	
	T	2.7	1.0	
	G*T	16.3	5.8	

4.1.6 Photosynthetic rate

Photosynthetic rate was reduced significantly in all the genotypes under water stress condition compared to irrigated control condition. The results related to the photosynthetic rate at flowering stage of rice genotypes are presented in the table 10.

Under water stress condition, the highest photosynthetic rate was observed in Ptb-30 (15.2 μ CO_2 moles m^{-2} s^{-1}), while the lowest was observed in Ptb–6 (3.4 μ CO_2 moles m^{-2} s^{-1}). Ptb-15 (25.2 μ CO_2 moles m^{-2} s^{-1}) showed a highest photosynthetic rate in irrigated condition where as the lowest was in Ptb-6 (12.6 μ CO_2 moles m^{-2} s^{-1}). The mean photosynthetic rate of rice genotypes was 18.2 μ CO_2 moles m^{-2} s^{-1} and 8.4 μ CO_2 moles m^{-2} s^{-1} under irrigated and water stress conditions respectively. The percentage reduction in photosynthetic rate was highest in Ptb-6 (73%) and lowest in Ptb-10 (40%).

4.1.7 Transpiration rate

Transpiration rate showed significant reduction in all the genotypes under water stress condition compared to irrigated condition. The results of transpiration rate at flowering stage of rice genotypes are presented in the table 11.

Among the genotypes, the transpiration rate was found to be highest in Ptb-4 (1.4 m H_2O moles m^{-2} s^{-1}) followed by Ptb-2 (0.93 m H_2O moles m^{-2} s^{-1}) while the lowest was recorded in Ptb-31 (0.05 m H_2O moles m^{-2} s^{-1}) under water stress condition. Ptb-4 showed the highest value of transpiration rate, *i.e.* 2.52 m H_2O moles m^{-2} s^{-1} under irrigated condition, while the lowest value was found in Ptb-9 (0.69 m H_2O moles m^{-2} s^{-1}). The mean transpiration rate was 0.52 m moles m^{-2} s^{-1} and 1.28 m H_2O moles m^{-2} s^{-1} at flowering stage under water stress and irrigated conditions respectively. The maximum percent reduction in transpiration rate was observed in Ptb-31 (95.7%) whereas minimum percent reduction was in Ptb-2 (21.8%).

Table No 10. Photosynthetic rate (μ CO_2 moles m^{-2} s^{-1}) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	17.7	7.5	12.6
2.	Ponnaryan(Ptb2)	21.7	10.4	16.1
3.	Eravapandy (Ptb3)	18.2	8.4	13.3
4.	Vellari (Ptb4)	22.1	12.9	17.5
5.	Veluthari kayama (Ptb5)	15.4	5.3	10.4
6.	Athikkiraya(Ptb6)	12.6	3.4	8.0
7.	Parambuvattan(Ptb7)	24.3	14.5	19.4
8.	Thavalakkannan(Ptb8)	15.9	5.8	10.9
9.	Thavalakkannan(Ptb9)	14.4	5.1	9.8
10.	Thekkancheera (Ptb10)	22.8	13.6	18.2
11.	Vyshak (Ptb 60)	23.0	13.5	18.3
12.	Thekkan Chitteni (Ptb12)	24.5	14.1	19.3
13.	Kayama (Ptb13)	20.6	9.0	14.8
14.	Maskathi (Ptb14)	14.1	4.8	9.5
15.	Kavunginpoothala (Ptb15)	25.2	15.0	20.1
16.	Harsha (Ptb 55)	23.4	13.2	18.3
17.	Jeddu Halliga (Ptb17)	16.7	6.2	11.5
18.	Eravapandy (Ptb18)	21.9	11.8	16.9
19.	Athikkiraya(Ptb19)	13.8	5.3	9.6
20.	Vadakkan Chitteni (Ptb20)	12.8	3.6	8.2
21.	Thekkan (Ptb21)	13.6	4.3	9.0
22.	Velutha Vattan (Ptb22)	13.9	4.4	9.2
23.	Cheriya Aryan (Ptb23)	13.2	4.0	8.6
24.	Chuvanna Vattan (Ptb24)	18.7	7.5	13.1
25.	Thonnooran (Ptb25)	14.5	4.8	9.7
26.	Chenkayama (Ptb26)	15.0	4.9	10.0
27.	Kodiyan (Ptb27)	13.5	4.3	8.9
28.	Kattamodan (Ptb28)	24.5	14.3	19.4
29.	Karutha Modan (Ptb29)	23.2	13.8	18.5
30.	Chuvanna Modan (Ptb30)	25.7	15.2	20.5
31.	Elappapoochampan (Ptb31)	13.3	5.4	9.4
32.	Aruvakkari (Ptb32)	12.9	3.6	8.3
33.	Arikkirai (Ptb33)	14.1	4.5	9.3
34.	Valiya Champan (Ptb34)	16.4	6.1	11.3
35.	Chomala	24.6	14.2	19.4
	Mean	**18.2**	**8.4**	
		C.D. (5%)	**SE (m)**	
	G	0.5	0.2	
	T	0.2	0.1	
	G*T	0.7	0.2	

Table No 11. Transpiration rate (m H_2O moles m^{-2} s^{-1}) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	1.15	0.78	0.97
2.	Ponnaryan(Ptb2)	1.19	0.93	1.06
3.	Eravapandy (Ptb3)	1.96	0.44	1.20
4.	Vellari (Ptb4)	2.52	1.40	1.96
5.	Veluthari kayama (Ptb5)	1.21	0.31	0.76
6.	Athikkiraya(Ptb6)	1.78	0.17	0.98
7.	Parambuvattan(Ptb7)	1.59	0.38	0.99
8.	Thavalakkannan(Ptb8)	1.36	0.61	0.98
9.	Thavalakkannan(Ptb9)	0.69	0.37	0.53
10.	Thekkancheera (Ptb10)	0.71	0.45	0.58
11.	Vyshak (Ptb 60)	1.81	0.69	1.25
12.	Thekkan Chitteni (Ptb12)	1.79	0.65	1.22
13.	Kayama (Ptb13)	1.33	0.62	0.98
14.	Maskathi (Ptb14)	1.32	0.59	0.96
15.	Kavunginpoothala (Ptb15)	0.95	0.50	0.73
16.	Harsha (Ptb 55)	0.83	0.46	0.64
17.	Jeddu Halliga (Ptb17)	0.94	0.67	0.81
18.	Eravapandy (Ptb18)	1.05	0.61	0.83
19.	Athikkiraya(Ptb19)	1.41	0.72	1.07
20.	Vadakkan Chitteni (Ptb20)	1.68	0.30	0.99
21.	Thekkan (Ptb21)	1.39	0.16	0.78
22.	Velutha Vattan (Ptb22)	0.95	0.60	0.77
23.	Cheriya Aryan (Ptb23)	1.27	0.49	0.88
24.	Chuvanna Vattan (Ptb24)	1.22	0.39	0.81
25.	Thonnooran (Ptb25)	1.30	0.34	0.82
26.	Chenkayama (Ptb26)	1.28	0.42	0.85
27.	Kodiyan (Ptb27)	1.34	0.71	1.03
28.	Kattamodan (Ptb28)	1.33	0.45	0.89
29.	Karutha Modan (Ptb29)	1.27	0.67	0.97
30.	Chuvanna Modan (Ptb30)	1.16	0.35	0.76
31.	Elappapoochampan (Ptb31)	1.15	0.05	0.60
32.	Aruvakkari (Ptb32)	1.14	0.31	0.73
33.	Arikkirai (Ptb33)	0.98	0.76	0.87
34.	Valiya Champan (Ptb34)	0.82	0.44	0.63
35.	Chomala	0.76	0.28	0.52
	Mean	1.28	0.52	
		C.D. (5%)	**SE(m)**	
	G	0.03	0.01	
	T	0.02	0.01	
	G*T	0.04	0.02	

4.1.8 Proline content

Proline accumulation is reported to be an adaptive mechanism of plants to mitigate the harmful effects caused by water stress. The results of proline content of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 12 .

The results revealed that proline content increased significantly in all the genotypes under water stress condition compared to irrigated condition. Among the genotypes, Ptb-27 (167.9 μ moles / g tissue) recorded the maximum proline accumulation followed by Ptb-15 (150.5 μ moles / g tissue) under water stress condition, while the minimum proline accumulation was recorded in Ptb-22 (5.0 μ moles / g tissue). In irrigated condition, the highest proline content was observed in chomala (96.6 μ moles / g tissue), while the lowest was observed in Ptb-24 (4.1 μ moles / g tissue). The percent increase in proline content was more in Ptb-21 and less in Ptb-5. The average proline content of the rice genotypes at flowering stage was 47.7 μ moles / g tissue and 31.1 μ moles / g tissue under water stress and irrigated conditions respectively.

4.2 EFFECT OF WATER STRESS ON ROOT TRAITS

4.2.1 Root length

The results of root length of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 13 .

Root length showed significant difference among the genotypes under water stress and control conditions. The mean root length of rice genotypes was measured 38.7cm under irrigated condition, whereas the mean root length of rice genotypes was measured 41.5 cm under water stress condition. The maximum root length was

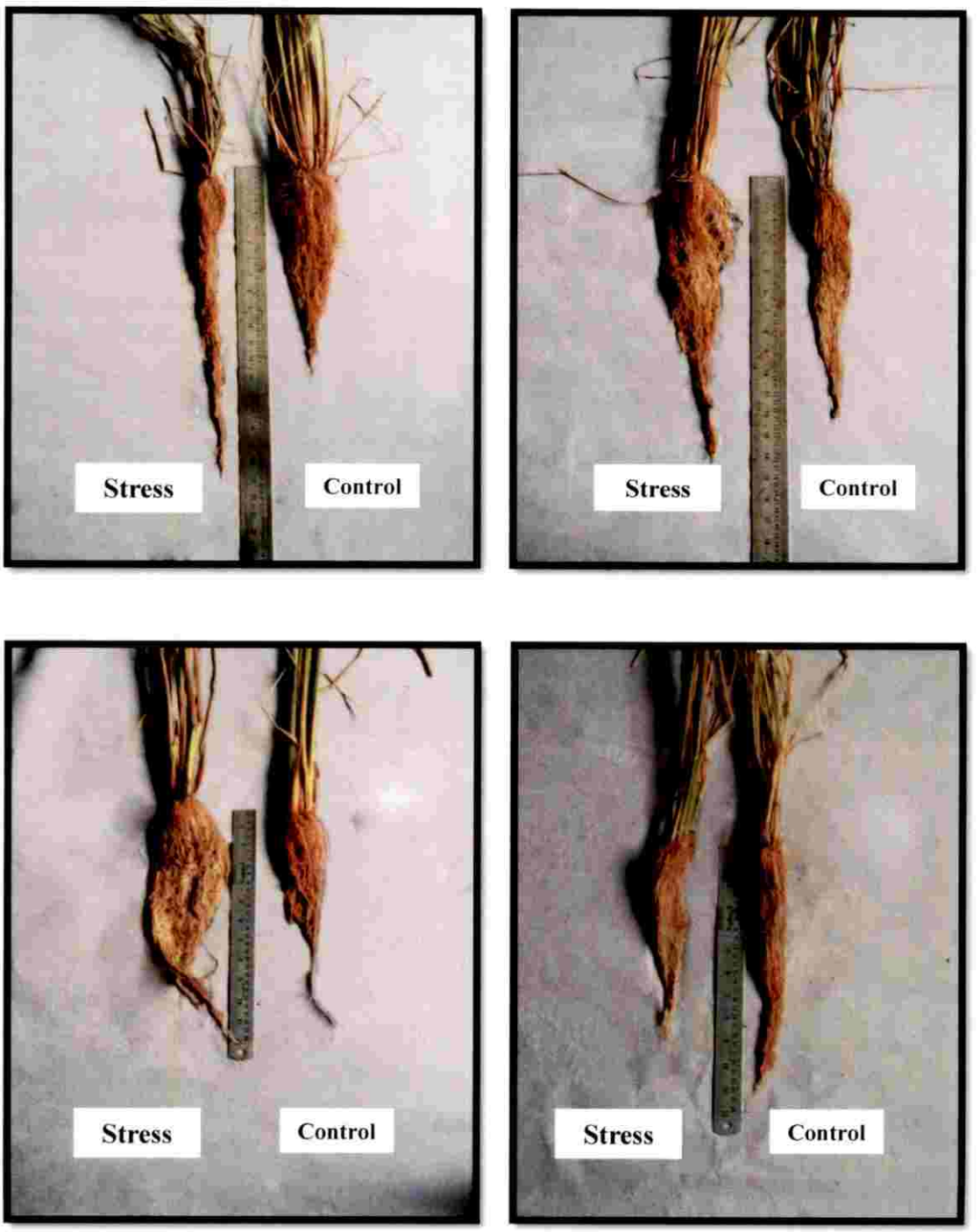
Table No 12. Proline content (μ moles / g tissue) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	53.1	76.1	64.6
2.	Ponnaryan(Ptb2)	12.5	28.5	20.5
3.	Eravapandy (Ptb3)	12.1	19.9	16.0
4.	Vellari (Ptb4)	16.5	23.3	19.9
5.	Veluthari kayama (Ptb5)	43.2	46.5	44.8
6.	Athikkiraya(Ptb6)	6.6	8.1	7.3
7.	Parambuvattan(Ptb7)	38.8	46.4	42.6
8.	Thavalakkannan(Ptb8)	8.0	13.6	10.8
9.	Thavalakkannan(Ptb9)	8.6	11.4	10.0
10.	Thekkancheera (Ptb10)	51.6	67.2	59.4
11.	Vyshak (Ptb 60)	39.9	58.2	49.1
12.	Thekkan Chitteni (Ptb12)	19.8	23.7	21.8
13.	Kayama (Ptb13)	10.3	20.5	15.4
14.	Maskathi (Ptb14)	34.4	55.4	44.9
15.	Kavunginpoothala (Ptb15)	71.1	150.5	110.8
16.	Harsha (Ptb 55)	41.4	50.5	45.9
17.	Jeddu Halliga (Ptb17)	35.6	45.4	40.5
18.	Eravapandy (Ptb18)	5.9	7.3	6.6
19.	Athikkiraya(Ptb19)	12.9	19.4	16.1
20.	Vadakkan Chitteni (Ptb20)	25.1	30.8	27.9
21.	Thekkan (Ptb21)	60.2	146.9	103.6
22.	Velutha Vattan (Ptb22)	4.3	5.0	4.6
23.	CheriyaAryan (Ptb23)	10.7	12.9	11.8
24.	Chuvanna Vattan (Ptb24)	4.1	6.3	5.2
25.	Thonnooran (Ptb25)	5.5	6.6	6.1
26.	Chenkayama (Ptb26)	12.1	16.5	14.3
27.	Kodiyan (Ptb27)	82.4	167.9	125.2
28.	Kattamodan (Ptb28)	76.2	110.5	93.4
29.	Karutha Modan (Ptb29)	66.2	101.7	83.9
30.	Chuvanna Modan (Ptb30)	44.6	60.6	52.6
31.	Elappapoochampan (Ptb31)	33.7	39.7	36.7
32.	Aruvakkari (Ptb32)	27.5	30.2	28.9
33.	Arikkirai (Ptb33)	7.5	9.7	8.6
34.	Valiya Champan (Ptb34)	9.8	14.1	11.9
35.	Chomala	96.6	136.6	116.6
	Mean	**31.1**	**47.7**	
		C.D. (5%)	**SE(m)**	
	G	1.7	0.6	
	T	0.4	0.1	
	G*T	2.4	0.9	

Table No 13. Root length (cm) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	34.3	49.0	41.7
2.	Ponnaryan(Ptb2)	38.0	43.6	40.8
3.	Eravapandy (Ptb3)	30.5	27.7	29.1
4.	Vellari (Ptb4)	34.0	29.2	31.6
5.	Veluthari kayama (Ptb5)	44.3	38.8	41.6
6.	Athikkiraya(Ptb6)	40.0	44.9	42.5
7.	Parambuvattan(Ptb7)	39.9	49.0	44.5
8.	Thavalakkannan(Ptb8)	32.4	27.7	30.1
9.	Thavalakkannan(Ptb9)	45.2	43.2	44.2
10.	Thekkancheera (Ptb10)	37.8	47.0	42.4
11.	Vyshak (Ptb 60)	36.0	45.0	40.5
12.	Thekkan Chitteni (Ptb12)	42.0	30.1	36.1
13.	Kayama (Ptb13)	43.0	50.8	46.9
14.	Maskathi (Ptb14)	45.0	41.6	43.3
15.	Kavunginpoothala (Ptb15)	47.0	58.8	52.9
16.	Harsha (Ptb 55)	38.0	41.0	39.5
17.	Jeddu Halliga (Ptb17)	42.0	37.0	39.5
18.	Eravapandy (Ptb18)	36.3	40.6	38.5
19.	Athikkiraya(Ptb19)	42.6	48.0	45.3
20.	Vadakkan Chitteni (Ptb20)	42.2	45.2	43.7
21.	Thekkan (Ptb21)	40.0	38.6	39.3
22.	Velutha Vattan (Ptb22)	34.2	36.3	35.3
23.	Cheriya Aryan (Ptb23)	48.3	45.4	46.9
24.	Chuvanna Vattan (Ptb24)	44.6	49.2	46.9
25.	Thonnooran (Ptb25)	32.0	37.2	34.6
26.	Chenkayama (Ptb26)	32.0	39.0	35.5
27.	Kodiyan (Ptb27)	31.4	34.4	32.9
28.	Kattamodan (Ptb28)	38.8	46.6	42.7
29.	Karutha Modan (Ptb29)	44.3	52.8	48.6
30.	Chuvanna Modan (Ptb30)	41.5	50.3	45.9
31.	Elappapoochampan (Ptb31)	35.6	39.8	37.7
32.	Aruvakkari (Ptb32)	36.2	28.2	32.2
33.	Arikkirai (Ptb33)	38.0	41.0	39.5
34.	Valiya Champan (Ptb34)	30.7	33.6	32.2
35.	Chomala	36.0	43.0	39.5
	Mean	**38.7**	**41.5**	
		C.D. (5%)	**SE(m)**	
	G	1.3	0.4	
	T	0.3	0.1	
	G*T	1.7	0.6	

Plate 4. Variation in root length (cm) of rice genotypes at flowering stage under irrigated and water stress conditions



recorded in Ptb-15 (58.8cm) (Plate 4) and Ptb-29 (52.8cm) under water stress condition, whereas the minimum root length was exhibited by Ptb-3 (27.7cm) (Plate 5) and Ptb-8 (27.7cm) (Plate 6). In irrigated condition, the maximum root length was attained by Ptb-23 (48.3cm) and Ptb-15 (47.0cm), while the minimum root length was attained by Ptb-3 (30.5cm). The percent increase in root length was observed to be highest in Ptb-1(42.9%) (Plate 7) and lowest in Ptb-22(6.1%).

4.2.2 Root dry weight

A significant difference was observed among the genotypes and treatments for root dry weight. The results of root dry weight of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 14 .

The highest root dry weight was recorded in Ptb-13 (12.3g) followed by Ptb-10 (10.2g) under water stress condition while the lowest root dry weight was recorded in Ptb-31 (1.7g). In irrigated condition, the maximum root dry weight was obtained in Ptb-2 (17.4g), followed by Ptb-3 (16.4g) and Ptb-1 (16.2g), while the minimum root dry weight was obtained in Ptb-55 (2.6g). The mean root dry weight of rice genotypes was measured 9.5g under irrigated condition, whereas the mean root dry weight of rice genotypes was measured 6.4g under water stress condition. The percent increase in root dry weight was observed to be highest in Ptb-55 (73.1%) and lowest in Ptb-2 (1.3%).

4.2.3 Root volume

The results of root volume of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 15.

Root volume differed significantly in several genotypes under water stress compared to irrigated condition. The mean root volume of rice genotypes was measured 38.9cm^3 under irrigated condition, whereas the mean root volume of rice genotypes was measured 29.6cm^3 under water stress condition. The maximum root

Table No 14. Root dry weight (g) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	16.2	8.9	12.5
2.	Ponnaryan(Ptb2)	17.4	9.0	13.2
3.	Eravapandy (Ptb3)	16.4	5.8	11.1
4.	Vellari (Ptb4)	12.5	6.4	9.5
5.	Velutharikayama (Ptb5)	10.8	10.0	10.4
6.	Athikkiraya(Ptb6)	8.2	8.3	8.3
7.	Parambuvattan(Ptb7)	5.0	6.9	5.9
8.	Thavalakkannan(Ptb8)	10.9	8.8	9.9
9.	Thavalakkannan(Ptb9)	7.9	6.3	7.1
10.	Thekkancheera (Ptb10)	8.1	10.2	9.2
11.	Vyshak (Ptb 60)	8.8	4.9	6.8
12.	ThekkanChitteni (Ptb12)	14.5	5.1	9.8
13.	Kayama (Ptb13)	14.5	12.3	13.4
14.	Maskathi (Ptb14)	10.7	5.3	8.0
15.	Kavunginpoothala (Ptb15)	12.4	8.3	10.3
16.	Harsha (Ptb 55)	2.6	4.5	3.6
17.	JedduHalliga (Ptb17)	12.1	6.2	9.2
18.	Eravapandy (Ptb18)	8.6	7.5	8.1
19.	Athikkiraya(Ptb19)	13.2	9.4	11.3
20.	VadakkanChitteni (Ptb20)	12.3	8.0	10.1
21.	Thekkan (Ptb21)	9.4	7.8	8.6
22.	VelthaVattan (Ptb22)	6.9	2.4	4.6
23.	Cheriya Aryan (Ptb23)	10.3	6.3	8.3
24.	Chuvanna Vattan (Ptb24)	9.2	4.9	7.0
25.	Thonnooran (Ptb25)	4.8	3.6	4.2
26.	Chenkayama (Ptb26)	8.5	4.4	6.4
27.	Kodiyan (Ptb27)	6.2	7.5	6.9
28.	Kattamodan (Ptb28)	8.5	2.5	5.5
29.	Karutha Modan (Ptb29)	12.4	6.3	9.3
30.	Chuvanna Modan (Ptb30)	6.2	4.2	5.2
31.	Elappapoochampan (Ptb31)	8.5	1.7	5.1
32.	Aruvakkari (Ptb32)	3.5	2.8	3.2
33.	Arikkirai (Ptb33)	4.9	6.5	5.7
34.	ValiyaChampan (Ptb34)	4.7	7.0	5.8
35.	Chomala	6.4	4.7	5.6
	Mean	**9.5**	**6.4**	
		C.D. (5%)	**SE(m)**	
	G	0.2	0.1	
	T	0.1	0.0	
	G*T	0.3	0.1	

Table No 15. Root volume (cm^3) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	60.0	30.6	45.3
2.	Ponnaryan(Ptb2)	62.0	34.0	48.0
3.	Eravapandy (Ptb3)	43.3	28.4	35.9
4.	Vellari (Ptb4)	55.0	23.0	39.0
5.	Veluthari kayama (Ptb5)	40.0	35.0	37.5
6.	Athikkiraya(Ptb6)	35.0	28.0	31.5
7.	Parambuvattan(Ptb7)	20.0	33.3	26.7
8.	Thavalakkannan(Ptb8)	43.0	28.9	36.0
9.	Thavalakkannan(Ptb9)	40.0	25.0	32.5
10.	Thekkancheera (Ptb10)	20.0	39.0	29.5
11.	Vyshak (Ptb 60)	22.7	18.0	20.4
12.	Thekkan Chitteni (Ptb12)	54.0	18.4	36.2
13.	Kayama (Ptb13)	43.3	38.0	40.7
14.	Maskathi (Ptb14)	63.0	35.0	49.0
15.	Kavunginpoothala (Ptb15)	57.0	44.0	50.5
16.	Harsha (Ptb 55)	15.0	20.0	17.5
17.	Jeddu Halliga (Ptb17)	32.0	18.0	25.0
18.	Eravapandy (Ptb18)	40.0	36.6	38.3
19.	Athikkiraya(Ptb19)	48.2	39.0	43.6
20.	Vadakkan Chitteni (Ptb20)	48.0	36.6	42.3
21.	Thekkan (Ptb21)	54.0	46.0	50.0
22.	Velutha Vattan (Ptb22)	32.0	13.3	22.7
23.	Cheriya Aryan (Ptb23)	46.0	43.3	44.7
24.	Chuvanna Vattan (Ptb24)	42.0	36.6	39.3
25.	Thonnooran (Ptb25)	29.5	23.3	26.4
26.	Chenkayama (Ptb26)	28.0	23.3	25.7
27.	Kodiyan (Ptb27)	23.0	36.6	29.8
28.	Kattamodan (Ptb28)	43.0	20.0	31.5
29.	Karutha Modan (Ptb29)	32.0	16.6	24.3
30.	Chuvanna Modan (Ptb30)	36.0	33.3	34.7
31.	Elappapoochampan (Ptb31)	40.0	16.6	28.3
32.	Aruvakkari (Ptb32)	24.2	20.0	22.1
33.	Arikkirai (Ptb33)	35.0	36.6	35.8
34.	Valiya Champan (Ptb34)	29.0	40.0	34.5
35.	Chomala	25.6	20.0	22.8
	Mean	**38.9**	**29.6**	
		C.D. (5%)	**SE(m)**	
	G	1.1	0.4	
	T	0.3	0.1	
	G*T	1.6	0.6	

volume was recorded in Ptb-21 (46.0cm^3) followed by Ptb-15 (44.0cm^3) under water stress condition, whereas the minimum root volume was exhibited by Ptb-22 (13.3cm^3) and Ptb-31 (16.6cm^3). In irrigated condition, the maximum root volume was attained by Ptb-14 (63.0cm^3) and Ptb-2 (62.0cm^3), while the minimum root volume was attained by Ptb-55 (15cm^3). The percent increase in root volume was observed to be highest in Ptb-10 (95.0%) and lowest in Ptb-33 (4.6%).

4.2.4 Root shoot ratio

The results of the root shoot ratio of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 16. There was a significant increment in the root shoot ratio under water stress condition over irrigated condition.

The mean root shoot ratio of rice genotypes was measured 0.28 under irrigated condition, whereas the mean root shoot ratio of rice genotypes was measured 0.36 under water stress condition. The highest root shoot ratio was recorded in Ptb-29 (0.48) and Ptb-30 (0.48) under water stress condition, whereas the lowest root shoot ratio was exhibited by Ptb-31 (0.17) and Ptb-4 (0.22). In irrigated condition, the highest root shoot ratio was attained by Ptb-29 (0.39) followed by Ptb-28 (0.38) and Ptb-7 (0.38), while the minimum root volume was attained by Ptb-31 (0.11). The percent increase in the root shoot ratio was observed to be highest in Ptb-14 (88.2%) and lowest in Ptb-13 (10.5%).

4.3 EFFECT OF WATER STRESS ON MORPHOLOGICAL AND YIELD PARAMETERS

4.3.1 Plant height

Plant height was measured at maturity stage from the base of the shoot to panicle tip. The results related to plant height of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 17.

Table No 16. Root shoot ratio of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	0.36	0.47	0.41
2.	Ponnaryan(Ptb2)	0.24	0.32	0.28
3.	Eravapandy (Ptb3)	0.20	0.27	0.23
4.	Vellari (Ptb4)	0.17	0.22	0.20
5.	Velutharikayama (Ptb5)	0.27	0.36	0.31
6.	Athikkiraya(Ptb6)	0.32	0.39	0.36
7.	Parambuvattan(Ptb7)	0.38	0.46	0.42
8.	Thavalakkannan(Ptb8)	0.33	0.40	0.37
9.	Thavalakkannan(Ptb9)	0.25	0.33	0.29
10.	Thekkancheera (Ptb10)	0.33	0.38	0.36
11.	Vyshak (Ptb 60)	0.36	0.45	0.40
12.	ThekkanChitteni (Ptb12)	0.16	0.23	0.20
13.	Kayama (Ptb13)	0.38	0.42	0.40
14.	Maskathi (Ptb14)	0.17	0.32	0.25
15.	Kavunginpoothala (Ptb15)	0.35	0.44	0.40
16.	Harsha (Ptb 55)	0.24	0.30	0.27
17.	JedduHalliga (Ptb17)	0.25	0.30	0.27
18.	Eravapandy (Ptb18)	0.23	0.33	0.28
19.	Athikkiraya(Ptb19)	0.28	0.37	0.33
20.	VadakkanChitteni (Ptb20)	0.30	0.36	0.33
21.	Thekkan (Ptb21)	0.32	0.39	0.36
22.	VelutheVattan (Ptb22)	0.23	0.27	0.25
23.	Cheriya Aryan (Ptb23)	0.27	0.33	0.30
24.	Chuvanna Vattan (Ptb24)	0.31	0.38	0.35
25.	Thonnooran (Ptb25)	0.26	0.36	0.31
26.	Chenkayama (Ptb26)	0.23	0.27	0.25
27.	Kodiyan (Ptb27)	0.24	0.33	0.28
28.	Kattamodan (Ptb28)	0.38	0.47	0.43
29.	Karutha Modan (Ptb29)	0.39	0.48	0.44
30.	Chuvanna Modan (Ptb30)	0.37	0.48	0.42
31.	Elappapoochampan (Ptb31)	0.11	0.17	0.14
32.	Aruvakkari (Ptb32)	0.23	0.29	0.26
33.	Arikkirai (Ptb33)	0.36	0.41	0.39
34.	ValiyaChampan (Ptb34)	0.30	0.38	0.34
35.	Chomala	0.27	0.33	0.30
	Mean	**0.28**	**0.36**	
		C.D. (5%)	**SE(m)**	
	G	0.01	0.00	
	T	0.00	0.00	
	G*T	0.01	0.01	

Table No 17. Plant height (cm) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	141.5	136.3	138.9
2.	Ponnaryan(Ptb2)	133.1	122.5	127.8
3.	Eravapandy (Ptb3)	128.8	103.0	115.9
4.	Vellari (Ptb4)	132.6	108.2	120.4
5.	Veluthari kayama (Ptb5)	131.4	111.5	121.4
6.	Athikkiraya(Ptb6)	138.3	124.0	131.1
7.	Parambuvattan(Ptb7)	100.7	99.9	100.3
8.	Thavalakkannan(Ptb8)	94.3	92.8	93.5
9.	Thavalakkannan(Ptb9)	130.7	109.7	120.2
10.	Thekkancheera (Ptb10)	124.0	120.4	122.2
11.	Vyshak (Ptb 60)	121.8	113.0	117.4
12.	Thekkan Chitteni (Ptb12)	102.7	98.3	100.5
13.	Kayama (Ptb13)	129.5	128.9	129.2
14.	Maskathi (Ptb14)	122.5	119.9	121.2
15.	Kavunginpoothala (Ptb15)	144.9	128.5	136.7
16.	Harsha (Ptb 55)	95.8	92.0	93.9
17.	Jeddu Halliga (Ptb17)	134.3	129.6	132.0
18.	Eravapandy (Ptb18)	135.3	130.7	133.0
19.	Athikkiraya(Ptb19)	134.5	127.9	131.2
20.	Vadakkan Chitteni (Ptb20)	108.8	104.0	106.4
21.	Thekkan (Ptb21)	120.0	107.0	113.5
22.	Velutha Vattan (Ptb22)	137.8	125.8	131.8
23.	Cheriya Aryan (Ptb23)	122.4	118.0	120.2
24.	Chuvanna Vattan (Ptb24)	125.4	117.8	121.6
25.	Thonnooran (Ptb25)	118.3	112.7	115.5
26.	Chenkayama (Ptb26)	128.0	123.0	125.5
27.	Kodiyan (Ptb27)	138.0	129.9	134.0
28.	Kattamodan (Ptb28)	121.0	118.8	119.9
29.	Karutha Modan (Ptb29)	126.0	124.3	125.2
30.	Chuvanna Modan (Ptb30)	114.3	111.4	112.9
31.	Elappapoochampan (Ptb31)	130.0	124.0	127.0
32.	Aruvakkari (Ptb32)	120.2	117.2	118.7
33.	Arikkirai (Ptb33)	132.1	128.7	130.4
34.	Valiya Champan (Ptb34)	76.3	71.8	74.1
35.	Chomala	99.0	88.7	93.8
	Mean	**122.7**	**114.9**	
		C.D. (5%)	**SE(m)**	
	G	3.5	1.2	
	T	0.8	0.3	
	G*T	4.9	1.8	

In general, a significant reduction in plant height was observed among the rice genotypes when the plants were exposed to water stress at panicle initiation stage. The varieties Ptb-15 (144.9cm) and Ptb-1 (141.5cm) were observed to maintain maximum plant height under well irrigated conditions where as Ptb-34 (74.3cm) and Ptb-8 (94.3cm) maintained minimum plant height. When stress was imposed at panicle initiation stage, the maximum height was recorded in Ptb-1 (136.3cm) followed by Ptb-18 (130.7cm). The minimum plant height at water stress condition was recorded in Ptb-34 (71.8cm). The mean plant height of rice genotypes was measured 122.7cm and 114.9cm under irrigated and water stress conditions respectively. The percent reduction in plant height was observed to be highest in Ptb-3 (20.0%) and lowest in Ptb-13 (0.5%).

4.3.2 Days to 50% flowering

The results related to days to 50% flowering of rice genotypes under irrigated and water stress conditions are presented in the table18.

In most of the genotypes early flowering was observed under water stress condition. Early flowering can be attributed to drought escape mechanism in rice.

4.3.3 Tiller number

A significant difference was observed among the genotypes and treatments for the number of tillers per plant. Among the genotypes, tiller number per plant ranged from 6 to 14 under well watered conditions (Table 19). There was a significant reduction in the number of tillers among the rice genotypes due to water stress when compared to irrigated conditions.

The highest number of tillers was recorded in Ptb-7 (11 plant^{-1}) followed by Ptb-15, Ptb-21 and Ptb-27 (8 plant^{-1}) under water stress condition. The minimum tiller number at water stress condition was recorded in Ptb-3 (4 plant^{-1}) and Ptb-28 (4 plant^{-1}). Under irrigated condition, Ptb-7 was observed to have highest number of

Table No 18. Days to 50% flowering of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	108.0	104.7	106.3
2.	Ponnaryan(Ptb2)	106.0	98.0	102.0
3.	Eravapandy (Ptb3)	100.0	87.7	93.8
4.	Vellari (Ptb4)	109.7	104.0	106.8
5.	Velutharikayama (Ptb5)	105.0	105.7	105.3
6.	Athikkiraya(Ptb6)	110.0	83.3	96.7
7.	Parambuvattan(Ptb7)	88.0	81.7	84.8
8.	Thavalakkannan(Ptb8)	102.0	102.0	102.0
9.	Thavalakkannan(Ptb9)	109.7	90.0	99.8
10.	Thekkancheera (Ptb10)	75.0	81.7	78.3
11.	Vyshak (Ptb 60)	76.3	80.3	78.3
12.	ThekkanChitteni (Ptb12)	115.3	91.7	103.5
13.	Kayama (Ptb13)	105.0	98.0	101.5
14.	Maskathi (Ptb14)	96.0	91.0	93.5
15.	Kavunginpoothala (Ptb15)	118.3	81.3	99.8
16.	Harsha (Ptb 55)	80.0	75.7	77.8
17.	Jeddu Halliga (Ptb17)	110.0	91.0	100.5
18.	Eravapandy (Ptb18)	98.0	80.7	89.3
19.	Athikkiraya(Ptb19)	103.0	84.7	93.8
20.	VadakkanChitteni (Ptb20)	97.0	77.0	87.0
21.	Thekkan (Ptb21)	100.3	84.7	92.5
22.	VelthaVattan (Ptb22)	86.0	75.7	80.8
23.	Cheriya Aryan (Ptb23)	85.7	76.7	81.2
24.	Chuvanna Vattan (Ptb24)	87.7	82.0	84.8
25.	Thonnooran (Ptb25)	92.0	80.7	86.3
26.	Chenkayama (Ptb26)	95.0	83.7	89.3
27.	Kodiyan (Ptb27)	96.0	79.3	87.7
28.	Kattamodan (Ptb28)	90.0	83.7	86.8
29.	Karutha Modan (Ptb29)	78.0	73.3	75.7
30.	Chuvanna Modan (Ptb30)	80.0	75.0	77.5
31.	Elappapoochampan (Ptb31)	82.0	74.3	78.2
32.	Aruvakkari (Ptb32)	93.0	84.3	88.7
33.	Arikkirai (Ptb33)	105.0	93.0	99.0
34.	ValiyaChampan (Ptb34)	70.0	73.3	71.7
35.	Chomala	102.0	90.7	96.3
	Mean	**95.9**	**85.7**	
		C.D. (5%)	**SE(m)**	
	G	2.4	0.8	
	T	0.6	0.2	
	G*T	3.4	1.2	

Table No 19. Tiller number of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	7	5	6
2.	Ponnaryan(Ptb2)	8	5	6
3.	Eravapandy (Ptb3)	5	4	4
4.	Vellari (Ptb4)	9	7	8
5.	Velutharikayama (Ptb5)	9	7	8
6.	Athikkiraya(Ptb6)	9	6	7
7.	Parambuvattan(Ptb7)	14	11	12
8.	Thavalakkannan(Ptb8)	9	5	7
9.	Thavalakkannan(Ptb9)	6	5	5
10.	Thekkancheera (Ptb10)	9	7	8
11.	Vyshak (Ptb 60)	7	5	6
12.	ThekkanChitteni (Ptb12)	6	5	5
13.	Kayama (Ptb13)	10	7	9
14.	Maskathi (Ptb14)	9	5	7
15.	Kavunginpoothala (Ptb15)	10	8	9
16.	Harsha (Ptb 55)	9	6	8
17.	JedduHalliga (Ptb17)	8	5	6
18.	Eravapandy (Ptb18)	9	7	8
19.	Athikkiraya(Ptb19)	11	5	8
20.	VadakkanChitteni (Ptb20)	7	5	6
21.	Thekkan (Ptb21)	9	8	8
22.	VelthaVattan (Ptb22)	8	6	7
23.	Cheriya Aryan (Ptb23)	10	6	8
24.	Chuvanna Vattan (Ptb24)	8	5	6
25.	Thonnooran (Ptb25)	11	7	9
26.	Chenkayama (Ptb26)	11	7	9
27.	Kodiyan (Ptb27)	15	8	11
28.	Kattamodan (Ptb28)	8	4	6
29.	Karutha Modan (Ptb29)	10	7	9
30.	Chuvanna Modan (Ptb30)	6	5	5
31.	Elappapoochampan (Ptb31)	6	5	6
32.	Aruvakkari (Ptb32)	7	5	6
33.	Arikkirai (Ptb33)	7	5	6
34.	ValiyaChampan (Ptb34)	9	7	8
35.	Chomala	7	5	6
	Mean	**8**	**6**	
		C.D. (5%)	**SE(m)**	
	G	1.1	0.4	
	T	0.3	0.1	
	G*T	1.5	0.6	

tillers (14 plant^{-1}) followed by Ptb-19, Ptb-25 and Ptb-26 (11 plant^{-1}), whereas the lowest number of tillers was recorded in Ptb-3 (5 plant^{-1}). The mean number of tillers per plant of rice genotypes was measured 8 and 6 under irrigated and water stress conditions respectively. The percent reduction in the number of tillers per plant was observed to be highest in Ptb-28 (50.0%) and lowest in Ptb-22 (11.1%).

4.3.4 Productive tiller number

The results related to the productive tiller number of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 20.

The results revealed that productive tiller number decreased significantly in all the genotypes due to water stress at panicle initiation stage compared to irrigated control. The mean productive tiller number of rice genotypes was measured 5 plant^{-1} under irrigated condition, whereas the mean productive tiller number of rice genotypes was measured 3 plant^{-1} under water stress condition. The highest number of productive tillers was recorded in Ptb-15 (6 plant^{-1}) followed by Ptb-4 (5 plant^{-1}) and Ptb-7 (5 plant^{-1}) under water stress condition, whereas the lowest number of productive tillers was exhibited by Ptb-31, Ptb-20, Ptb-24, Ptb-25 (2 plant^{-1}each). In irrigated condition, the highest number of productive tillers was attained by Ptb-7 (8 plant^{-1}) and Ptb-15 (8 plant^{-1}) while the lowest was attained by Ptb-1, Ptb-9, Ptb-12, Ptb-14 (4 plant^{-1}). The percent decrease in the productive tiller number was observed to be highest in Ptb-8 (66.7%) and lowest in Ptb-34 (10.5%).

4.3.5 Panicle length

Panicle length showed comparatively less reduction in rice genotypes due to water stress at panicle initiation stage. The results obtained for panicle length of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 21.

Table No 20. Productive tiller number of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	4	3	3
2.	Ponnaryan(Ptb2)	5	2	3
3.	Eravapandy (Ptb3)	3	3	3
4.	Vellari (Ptb4)	7	5	6
5.	Velutharikayama (Ptb5)	5	4	4
6.	Athikkiraya(Ptb6)	5	3	4
7.	Parambuvattan(Ptb7)	8	5	6
8.	Thavalakkannan(Ptb8)	6	2	4
9.	Thavalakkannan(Ptb9)	4	3	4
10.	Thekkancheera (Ptb10)	6	3	4
11.	Vyshak (Ptb 60)	5	3	4
12.	ThekkanChitteni (Ptb12)	4	2	3
13.	Kayama (Ptb13)	5	4	4
14.	Maskathi (Ptb14)	4	2	3
15.	Kavunginpoothala (Ptb15)	8	6	7
16.	Harsha (Ptb 55)	5	3	4
17.	JedduHalliga (Ptb17)	4	2	3
18.	Eravapandy (Ptb18)	6	3	5
19.	Athikkiraya(Ptb19)	5	3	4
20.	VadakkanChitteni (Ptb20)	4	2	3
21.	Thekkan (Ptb21)	6	4	5
22.	VelthaVattan (Ptb22)	4	3	3
23.	Cheriya Aryan (Ptb23)	4	3	3
24.	Chuvanna Vattan (Ptb24)	5	2	3
25.	Thonnooran (Ptb25)	4	2	3
26.	Chenkayama (Ptb26)	5	3	4
27.	Kodiyan (Ptb27)	5	3	4
28.	Kattamodan (Ptb28)	4	2	3
29.	Karutha Modan (Ptb29)	5	4	4
30.	Chuvanna Modan (Ptb30)	4	2	3
31.	Elappapoochampan (Ptb31)	3	3	3
32.	Aruvakkari (Ptb32)	5	4	4
33.	Arikkirai (Ptb33)	5	4	4
34.	ValiyaChampan (Ptb34)	4	4	4
35.	Chomala	4	3	3
	Mean	**5**	**3**	
		C.D. (5%)	**SE(m)**	
	G	0.73	0.26	
	T	0.18	0.06	
	G*T	1.03	0.37	

Table No 21. Panicle length (cm) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	33.9	32.6	33.2
2.	Ponnaryan(Ptb2)	27.7	24.7	26.2
3.	Eravapandy (Ptb3)	23.6	20.5	22.1
4.	Vellari (Ptb4)	23.0	18.8	20.9
5.	Veluthari kayama (Ptb5)	26.0	23.7	24.9
6.	Athikkiraya(Ptb6)	26.1	24.3	25.2
7.	Parambuvattan(Ptb7)	22.9	20.9	21.9
8.	Thavalakkannan(Ptb8)	21.7	18.1	19.9
9.	Thavalakkannan(Ptb9)	25.2	20.6	22.9
10.	Thekkancheera (Ptb10)	21.9	20.2	21.1
11.	Vyshak (Ptb 60)	21.8	21.0	21.4
12.	Thekkan Chitteni (Ptb12)	21.9	18.4	20.2
13.	Kayama (Ptb13)	25.7	24.7	25.2
14.	Maskathi (Ptb14)	23.8	21.9	22.9
15.	Kavunginpoothala (Ptb15)	33.7	31.8	32.8
16.	Harsha (Ptb 55)	23.1	21.0	22.0
17.	Jeddu Halliga (Ptb17)	27.0	25.8	26.4
18.	Eravapandy (Ptb18)	22.0	20.4	21.2
19.	Athikkiraya(Ptb19)	25.0	24.0	24.5
20.	Vadakkan Chitteni (Ptb20)	22.6	21.0	21.8
21.	Thekkan (Ptb21)	20.6	18.8	19.7
22.	Velutha Vattan (Ptb22)	26.0	24.8	25.4
23.	Cheriya Aryan (Ptb23)	25.0	21.0	23.0
24.	Chuvanna Vattan (Ptb24)	25.8	21.7	23.8
25.	Thonnooran (Ptb25)	26.0	24.7	25.4
26.	Chenkayama (Ptb26)	26.0	25.1	25.6
27.	Kodiyan (Ptb27)	22.8	19.8	21.3
28.	Kattamodan (Ptb28)	26.0	24.6	25.3
29.	Karutha Modan (Ptb29)	22.0	21.2	21.6
30.	Chuvanna Modan (Ptb30)	22.0	21.6	21.8
31.	Elappapoochampan (Ptb31)	24.0	21.0	22.5
32.	Aruvakkari (Ptb32)	24.2	21.5	22.9
33.	Arikkirai (Ptb33)	26.0	23.2	24.6
34.	Valiya Champan (Ptb34)	23.0	21.3	22.2
35.	Chomala	16.6	16.0	16.3
	Mean	**24.4**	**22.3**	
		C.D. (5%)	**SE(m)**	
	G	0.7	0.3	
	T	0.2	0.1	
	G*T	0.1	0.4	

The results revealed that stress imposed at panicle initiation stage was found to have greater impact on panicle length. The highest panicle length was recorded in Ptb-1 (32.6cm) followed by Ptb-15 (31.8cm) under water stress condition, while the lowest panicle length was recorded in chomala (16.0cm). Under irrigated condition, also Ptb-1 was observed to have highest panicle length (33.9cm) followed by Ptb-15 (33.7cm), whereas lowest panicle length was recorded in chomala (16.6cm). The mean panicle length of rice genotypes was measured 24.4cm and 22.3cm under irrigated and water stress conditions respectively. The percent reduction in panicle length was observed to be highest in Ptb-4 (18.3%) and Ptb-9 (18.3%) lowest in Ptb-30 (1.8%).

4.3.6 Yield per plant

The results related to yield per plant of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 22.

The results showed that there was a significant reduction in yield per plant of rice genotypes under water stress condition compared to irrigated condition. The average mean yield of rice genotypes was 16.5g in irrigated condition, whereas the mean yield of rice genotypes were 12.0g in water stress condition. The grain yield was highest in Ptb-55 (17.5g) followed by Ptb-2 (16.8g) and Ptb-30 (16.4g) under water stress condition. The variety Ptb-21 was found to have lowest yield (5.4g) under water stress condition. Under irrigated condition, Ptb-24 was observed to have highest grain yield (24.3g) followed by Ptb-28 (22.3g) where as the lowest grain yield was recorded in Ptb-21 (7.4g). Maximum percent reduction in grain yield was observed in Ptb-32 (52.7%), whereas minimum percent reduction was in Ptb-28 (7.6%).

Table No 22. Yield (g) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	20.4	15.3	17.9
2.	Ponnaryan(Ptb2)	19.5	16.8	18.1
3.	Eravapandy (Ptb3)	19.2	12.9	16.1
4.	Vellari (Ptb4)	12.8	10.3	11.6
5.	Veluthari kayama (Ptb5)	13.4	9.7	11.6
6.	Athikkiraya(Ptb6)	12.5	9.3	10.9
7.	Parambuvattan(Ptb7)	18.0	12.3	15.2
8.	Thavalakkannan(Ptb8)	13.9	10.0	12.0
9.	Thavalakkannan(Ptb9)	12.0	8.7	10.4
10.	Thekkancheera (Ptb10)	14.4	10.8	12.6
11.	Vyshak (Ptb 60)	21.0	15.7	18.4
12.	Thekkan Chitteni (Ptb12)	17.6	15.3	16.5
13.	Kayama (Ptb13)	15.7	9.2	12.5
14.	Maskathi (Ptb14)	12.7	7.6	10.1
15.	Kavunginpoothala (Ptb15)	8.5	7.2	7.8
16.	Harsha (Ptb 55)	21.7	17.5	19.6
17.	Jeddu Halliga (Ptb17)	16.4	12.9	14.6
18.	Eravapandy (Ptb18)	20.0	13.2	16.6
19.	Athikkiraya(Ptb19)	15.0	9.8	12.4
20.	Vadakkan Chitteni (Ptb20)	20.4	13.9	17.2
21.	Thekkan (Ptb21)	7.4	5.4	6.4
22.	Velutha Vattan (Ptb22)	17.3	14.3	15.8
23.	Cheriya Aryan (Ptb23)	20.8	12.9	16.9
24.	Chuvanna Vattan (Ptb24)	24.3	20.5	22.4
25.	Thonnooran (Ptb25)	10.8	5.8	8.3
26.	Chenkayama (Ptb26)	14.2	9.7	11.9
27.	Kodiyan (Ptb27)	14.3	10.4	12.4
28.	Kattamodan (Ptb28)	22.3	20.6	21.5
29.	Karutha Modan (Ptb29)	20.2	14.5	17.4
30.	Chuvanna Modan (Ptb30)	18.2	16.4	17.3
31.	Elappapoochampan (Ptb31)	12.6	7.2	9.9
32.	Aruvakkari (Ptb32)	18.4	8.7	13.5
33.	Arikkirai (Ptb33)	20.8	10.9	15.9
34.	Valiya Champan (Ptb34)	15.3	11.8	13.5
35.	Chomala	15.0	12.9	13.9
	Mean	**16.5**	**12.0**	
		CD (%)	**SE(m)**	
	G	0.5	0.2	
	T	0.1	0.0	
	G*T	0.1	0.2	

4.3.7 Spikelet fertility percentage

The results related to spikelet fertility of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 23.

Number of filled grains and unfilled grains were observed as major attributes affected drastically under water stress condition. The spikelet fertility percentage was found to be significantly reduced due to water stress at panicle initiation stage. The mean spikelet fertility percentage of rice genotypes was measured 80.9% and 68.7% under irrigated and water stress conditions respectively. The highest spikelet fertility percentage was recorded in Ptb-25 (84.6%) (Plate 8) followed by Ptb-55 (80.2%) under water stress condition, while the lowest spikelet fertility percentage was recorded in Ptb-1 (53.7%) (Plate 9). Under irrigated condition, Ptb-19 was observed to have highest spikelet fertility (89.1%) followed by Ptb-13 (87.2%), whereas the lowest spikelet fertility was recorded in Ptb-10 (67.7%). The percent reduction in spikelet fertility percentage was observed to be highest in Ptb-14 (32.9%), while percent reduction in spikelet fertility percentage was lowest in Ptb-25 (1.16%).

4.3.8 1000 grain weight

The results of 1000 grain weight of rice genotypes at flowering stage under irrigated and water stress conditions are presented in the table 24 .

The results showed that there was a significant reduction in 1000 grain weight of rice genotypes under water stress condition compared to irrigated condition. The mean 1000 grain weight of rice genotypes was 24.9g in irrigated condition, whereas the mean 1000 grain weight of rice genotypes was 24.1g in water stress condition. Thousand grain weight was highest in Ptb-28 (27.5g) followed by Ptb-30 (27.2g) under water stress condition. The variety Ptb-17 was found to have lowest 1000 grain weight (17.5g) under water stress condition. Under irrigated condition, Ptb-28 was observed to have highest grain yield (28.0g) followed by Ptb-22 (27.8g) where as

Table No 23. Spikelet fertility percentage (%) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	75.7	53.7	64.7
2.	Ponnaryan(Ptb2)	77.2	61.0	69.1
3.	Eravapandy (Ptb3)	79.0	61.1	70.0
4.	Vellari (Ptb4)	82.0	67.2	74.6
5.	Veluthari kayama (Ptb5)	80.0	64.4	72.2
6.	Athikkiraya(Ptb6)	79.1	62.8	71.0
7.	Parambuvattan(Ptb7)	66.7	69.8	68.2
8.	Thavalakkannan(Ptb8)	80.0	66.2	73.1
9.	Thavalakkannan(Ptb9)	83.2	71.1	77.2
10.	Thekkancheera (Ptb10)	67.7	62.7	65.2
11.	Vyshak (Ptb 60)	82.0	79.5	80.7
12.	Thekkan Chitteni (Ptb12)	79.7	65.1	72.4
13.	Kayama (Ptb13)	87.2	66.7	77.0
14.	Maskathi (Ptb14)	84.9	57.0	71.0
15.	Kavunginpoothala (Ptb15)	83.5	74.1	78.8
16.	Harsha (Ptb 55)	84.2	80.2	82.2
17.	Jeddu Halliga (Ptb17)	84.1	75.0	79.5
18.	Eravapandy (Ptb18)	80.0	78.7	79.3
19.	Athikkiraya(Ptb19)	89.1	67.6	78.4
20.	Vadakkan Chitteni (Ptb20)	83.5	62.4	73.0
21.	Thekkan (Ptb21)	77.4	62.7	70.0
22.	Velutha Vattan (Ptb22)	78.8	61.6	70.2
23.	Cheriya Aryan (Ptb23)	82.0	70.3	76.1
24.	Chuvanna Vattan (Ptb24)	84.3	63.2	73.7
25.	Thonnooran (Ptb25)	85.6	84.6	85.1
26.	Chenkayama (Ptb26)	86.6	73.2	79.9
27.	Kodiyan (Ptb27)	72.6	56.3	64.4
28.	Kattamodan (Ptb28)	85.2	77.0	81.1
29.	Karutha Modan (Ptb29)	83.5	71.8	77.7
30.	Chuvanna Modan (Ptb30)	79.3	74.3	76.8
31.	Elappapoochampan (Ptb31)	81.0	66.7	73.8
32.	Aruvakkari (Ptb32)	83.7	71.4	77.6
33.	Arikkirai (Ptb33)	82.6	78.6	80.6
34.	Valiya Champan (Ptb34)	79.1	69.2	74.2
35.	Chomala	82.0	67.2	74.6
	Mean	**80.9**	**68.7**	
		C.D. (5%)	**SE(m)**	
	G	2.1	0.7	
	T	0.5	0.2	
	G*T	2.9	1.1	

Plate 5. Variation in spikelet fertility percentage (%) of rice genotypes at flowering stage under irrigated and water stress conditions

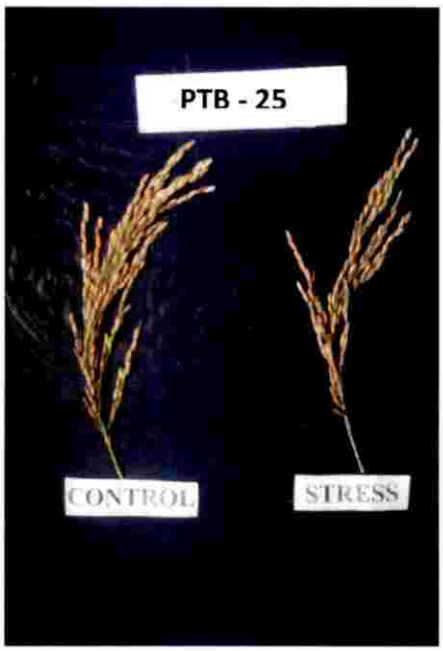


Table No 24. 1000 grain weight (g) of rice genotypes at flowering stage under irrigated and water stress conditions

Sl. No.	Genotypes	Irrigated	Water stress	Mean
1.	Aryan (Ptb1)	25.6	25.0	25.3
2.	Ponnaryan(Ptb2)	26.3	25.7	26.0
3.	Eravapandy (Ptb3)	25.5	23.8	24.6
4.	Vellari (Ptb4)	26.0	25.2	25.6
5.	Velutharikayama (Ptb5)	22.3	21.5	21.9
6.	Athikkiraya(Ptb6)	24.5	23.3	23.9
7.	Parambuvattan(Ptb7)	25.4	25.0	25.2
8.	Thavalakkannan(Ptb8)	23.8	23.0	23.4
9.	Thavalakkannan(Ptb9)	24.5	23.7	24.1
10.	Thekkancheera (Ptb10)	25.0	24.6	24.8
11.	Vyshak (Ptb 60)	23.6	23.2	23.4
12.	ThekkanChitteni (Ptb12)	26.6	25.0	25.8
13.	Kayama (Ptb13)	23.6	23.0	23.3
14.	Maskathi (Ptb14)	22.4	21.4	21.9
15.	Kavunginpoothala (Ptb15)	19.0	18.5	18.8
16.	Harsha (Ptb 55)	23.7	23.3	23.5
17.	JedduHalliga (Ptb17)	18.2	17.5	17.9
18.	Eravapandy (Ptb18)	27.3	26.4	26.9
19.	Athikkiraya(Ptb19)	24.6	23.9	24.2
20.	VadakkanChitteni (Ptb20)	27.2	26.5	26.9
21.	Thekkan (Ptb21)	26.8	26.1	26.4
22.	VelthaVattan (Ptb22)	27.8	26.4	27.1
23.	Cheriya Aryan (Ptb23)	22.2	21.7	22.0
24.	Chuvanna Vattan (Ptb24)	24.4	24.0	24.2
25.	Thonnooran (Ptb25)	26.7	26.0	26.4
26.	Chenkayama (Ptb26)	24.0	23.2	23.6
27.	Kodiyan (Ptb27)	26.4	25.7	26.1
28.	Kattamodan (Ptb28)	28.0	27.5	27.7
29.	Karutha Modan (Ptb29)	27.0	26.5	26.8
30.	Chuvanna Modan (Ptb30)	27.5	27.2	27.4
31.	Elappapoochampan (Ptb31)	25.5	25.0	25.3
32.	Aruvakkari (Ptb32)	25.0	24.3	24.7
33.	Arikkirai (Ptb33)	26.2	25.7	25.9
34.	ValiyaChampan (Ptb34)	27.6	26.8	27.2
35.	Chomala	20.0	19.4	19.7
	Mean	**24.9**	**24.1**	
		C.D. (5%)	**SE(m)**	
	G	0.7	0.2	
	T	0.2	0.1	
	G*T	N/A	0.3	

lowest 1000 grain weight was recorded in Ptb-17 (18.2g). The maximum percent reduction in 1000 grain weight was observed in Ptb-3 (6.3%), whereas minimum percent reduction was in Ptb-30 (1.1%).

4.4 CORRELATION STUDIES

Correlation coefficient is a statistical measure, which is used to know the degree and direction of relationship between two or more variables. The degree of association also affects an effectiveness of selection process. The data on various traits which were recorded under the irrigated and water stress conditions in rice genotypes were subjected to correlation analysis. The results of correlation between some of the characters under both the conditions are presented in table 25 and table 26.

4.4.1 Correlation between drought resistant traits and yield under water stress condition

Correlation study revealed that grain yield per plant under water stress condition was positively correlated with parameters such as relative water content (r = 0.086), membrane stability index (r = 0.128), proline content (r = 0.042), stomatal conductance (r = 0.393*), photosynthetic rate (r = 0.470**), transpiration rate (r = 0.208), root length (r = 0.199), root shoot ratio (r = 0.219), spikelet fertility % (r = 0.063), and 1000 grain weight (r = 0.238), where as negatively correlated with leaf temperature (r = -0.179), leaf rolling score (r = -0.108), root volume (r = -0.237), root dry weight (r = -0.215), plant height (r = -0.084), days to 50% flowering (r = -0.121), and panicle length (r = -0.004). Significant positive correlation were found out between yield and stomatal conductance (r = 0.393*) and photosynthetic rate (r = 0.470**).

Table 25. Correlation of traits with grain yield under water stress condition

	Y	LR	RWC	MSI	PR	LT	g_s	A	T	RL	RDW	RV	RSR	PH	DF	TN	PTN	PL	SF	1000GW
Y	1	-.108	.086	.128	.042	-.179	.393*	.465**	.208	.199	-.215	-.237	.219	-.084	-.121	.364*	.398*	-.004	.063	.238
LR		1	-.637**	-.358*	-.454**	.086	-.454**	-.412*	-.047	-.105	.253	.066	-.152	.149	.287	.004	-.166	.046	-.279	-.166
RWC			1	.178	.220	-.004	.197	.155	.186	-.049	-.101	-.106	-.050	-.145	-.142	-.145	-.031	-.036	.215	-.119
MSI				1	.468**	-.304	.614**	.608**	-.186	.508**	-.172	-.136	.448**	-.012	-.376*	.145	.177	-.004	.231	-.031
PR					1	-.042	.347*	.338*	-.089	.292	.044	.121	.319	.069	-.101	.251	.251	.031	-.150	-.137
LT						1	-.246	-.106	.363*	.060	.264	.091	.082	.265	.451**	-.270	-.144	.512**	-.329	.067
g_s							1	.912**	.089	.343*	-.126	-.149	.356*	-.256	-.168	.159	.148	-.083	.321	-.016
A								1	.237	.351*	-.034	-.164	.291	-.174	-.052	.143	.180	-.092	.270	.016
T									1	-.128	.242	-.045	-.051	.219	.455**	-.027	.117	.108	-.052	.050
RL										1	.264	.316	.651**	.386*	-.231	.181	.163	.456**	.090	-.102
RDW											1	.657**	.350*	.136	.456**	.304	.254	.174	-.348*	-.168
RV												1	.348*	.063	.049	.336*	.239	.109	-.247	-.006
RSR													1	.093	-.047	.193	.140	.312	.144	.128
PH														1	.077	-.034	.008	.585**	-.089	-.059
DF															1	-.159	.040	.140	-.303	-.288
TN																1	.640**	.013	.038	.049
PTN																	1	.153	.090	-.131
PL																		1	.009	-.181
SF																			1	-.009
1000GW																				1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

GY – Grain Yield ; LR – Leaf rolling; MSI – Membrane stability index; RWC – Relative water content ; PR – Proline content ; LT – Leaf temperature ; g_s – Stomatal conductance ; A – Photosynthetic rate ; T – Transpiration rate ; RL – Root length ; RDW – Root dry weight ; RV – Root volume ; R:S R – Root : Shoot ratio ; PH – Plant height ; DF – Days to 50% flowering; TN – Tiller number ; PTN – Productive tiller number ; PL – Panicle length ; SF – Spikelet fertility percentage ; 1000GW – 1000 grain weight.

4.4.2 Correlation between drought resistant traits and yield under irrigated condition

Under irrigated condition, grain yield was found to be positively correlated with parameters such as relative water content (r = 0.170), leaf temperature (r = 0.340*), stomatal conductance (r = 0.208), photosynthetic rate (r = 0.271), transpiration rate (r = 0.014), root length (r = 0.034), root shoot ratio (r = 0.218), root dry weight (r = 0.026), spikelet fertility % (r = 0.040) and 1000 grain weight (r = 0.244). Similarly, negative correlation was observed between root volume (r = -0.159), plant height (r = -0.122), days to 50% flowering (r = -0.339), and panicle length (r = -0.019) with yield under irrigated condition.

4.5 SELECTION OF RICE ASSESSIONS FOR BULKED LINE ANALYSIS

On the basis of physio-morphological and biochemical parameters such as proline content, membrane stability index, leaf rolling, relative water content, root length, root shoot ratio, plant height, spikelet fertility percentage and 1000 grain weight drought tolerant and susceptible rice genotypes were selected. Ten drought tolerant and ten drought susceptible genotypes were identified after the detailed analysis of these traits (Table 3). Genotypes were selected for bulked line analysis based on procedure described as follows:

Genotypes which are having extreme values on either side of grand mean is given as either positive (+) or negative (-) sign for each trait studied. The genotypes which have scored nearer value to either side of grand mean is omitted and were not considered for bulking in order to have two very distinct bulks amongt the genotypes. Therefore, the genotypes which scored high value in all the traits and low scores for leaf rolling is considered for tolerant bulk and those scored lower or negative in all the parameters and high scores for leaf rolling were used to form susceptible bulk.

Table 26. Correlation of traits with grain yield under irrigated condition

	GY	RWC	PRL	LT	g_s	A	T	RL	RDW	RV	R:S R	PH	DF	TN	PTN	PL	SF	1000GW
GY	1	.170	-.108	.340*	.208	.271	.014	.034	.026	-.159	.218	-.122	-.339*	.242	.246	-.019	.040	.244
RWC		1	.181	.059	-.002	-.027	-.324	-.335*	-.200	-.364*	.029	-.139	-.197	-.065	-.246	-.246	-.120	.185
PRL			1	.096	.453**	.404*	-.191	.075	-.088	-.138	.265	-.021	-.075	.152	.126	-.133	-.234	-.212
LT				1	.174	.211	-.281	-.094	.189	.138	.197	-.147	-.136	-.316	-.261	.160	.129	.035
g_s					1	.949**	-.021	.082	.026	-.132	.318	-.254	-.126	-.087	.241	-.105	-.078	-.088
A						1	.023	.097	.104	-.073	.306	-.206	-.097	-.067	.341*	-.132	-.177	-.044
T							1	-.125	.389*	.301	-.145	.079	.221	.044	.142	-.126	.011	.221
RL								1	.224	.335*	.223	.189	.202	-.028	.123	.179	.249	-.380*
RDW									1	.747**	-.062	.380*	.510**	-.205	-.063	.337*	.097	-.126
RV										1	-.161	.318	.570**	-.249	-.024	.394*	.207	.000
R:S R											1	-.067	-.157	.193	.335*	.147	-.128	.037
PH												1	.420*	.037	.069	.577**	.094	-.125
DF													1	-.078	.177	.386*	.180	-.346*
TN														1	.537**	.036	-.216	-.046
PTN															1	.077	-.308	-.145
PL																1	.142	-.196
SF																	1	-.240
1000GW																		1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

GY – Grain Yield ; RWC – Relative water content ; PRL – Proline content ; LT – Leaf temperature ; g_s – Stomatal conductance ; A – Photosynthetic rate ; T – Transpiration rate ; RL – Root length ; RDW – Root dry weight ; RV – Root volume ; R:S R – Root : Shoot ratio ; PH – Plant height ; DF – Days to 50% flowering; TN – Tiller number ; PTN – Productive tiller number ; PL – Panicle length ; SF – Spikelet fertility percentage ; 1000GW – 1000 grain weight.

4.6 QUALITY AND QUANTITY ASSESSMENT OF DNA SAMPLES

Quantity and purity of DNA samples obtained for selected 10 drought tolerant and 10 drought susceptible rice genotypes for Bulked Line Analysis are presented in the table 27. Quality of DNA samples were assessed from the gel picture showing DNA bands (Plate 6).

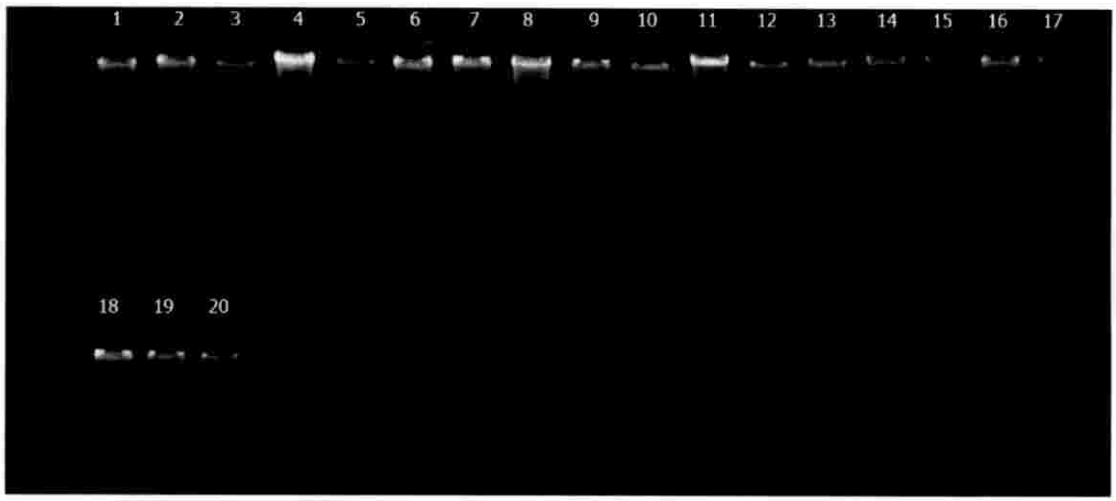
4.7 IDENTIFICATION OF MICROSATELLITE MARKERS ASSOCIATED WITH DROUGHT TOLERANT TRAITS IN RICE USING BULKED LINE ANALYSIS

A total of 150 microsatellite pimers representing different chromosomes were selected randomly and used to amplify the SSR regions between the bulked 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 polymorphic or monomorphic. Out of the 150 microsatellite primers screened only one marker, *i.e.* RM 474 showed polymorphism between the tolerant and susceptible bulks (Plate 7). This primer which produced polymorphism between the bulks were checked in all the 20 individual rice genotypes along with two bulked samples. The primer produced a product size of approximately 252 bp for the tolerant bulk and the individual lines which constituted the tolerant bulk. At the same time, the primer produced a product size of approximately 300 bp for the susceptible bulk and the individual genotypes forming the susceptible bulk (Plate 8). All the ten genotypes, which were considered as drought tolerant, produced similar product size (252 bp) as produced in tolerant bulk, and this product size was different from the susceptible bulk and susceptible genotypes (~ 300 bp).

Table No. 27: Quality and quantity of DNA samples of rice genotypes selected for Bulked Line Analysis

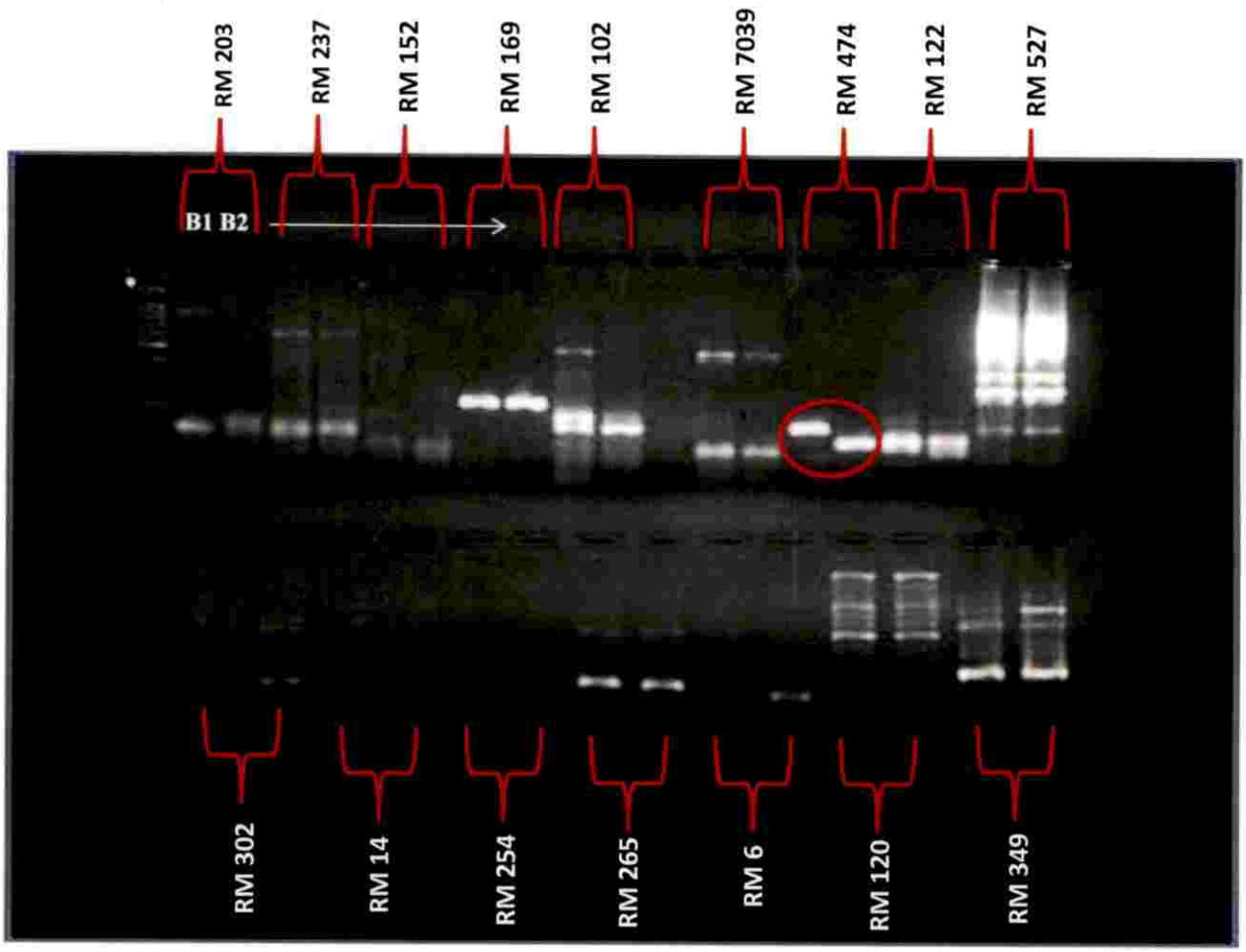
Sl. No	Variety	A_{260}/A_{280} value	DNA concentration (ng/µl)
1	Ptb-1	1.8	2307.0
2	Ptb-3	1.6	1161.0
3	Ptb-7	1.8	1167.0
4	Ptb-8	1.8	1329.0
5	Ptb-10	1.8	4458.0
6	Ptb-60	1.7	552.0
7	Ptb-12	1.9	294.0
8	Ptb-15	1.9	372.0
9	Ptb-55	1.7	471.0
10	Ptb-17	1.9	453.0
11	Ptb-22	1.8	4629.0
12	Ptb-23	1.8	2301.0
13	Ptb-24	1.8	3483.0
14	Ptb-27	1.6	2667.0
15	Ptb-28	1.8	1638.0
16	Ptb-29	1.8	474.0
17	Ptb-30	1.6	2241.0
18	Ptb-32	1.8	2343.0
19	Ptb-34	1.7	3075.0
20	Chomala	1.6	7905.0

Plate 6. Gel profile with DNA bands of rice genotypes selected for Bulked Line Analysis



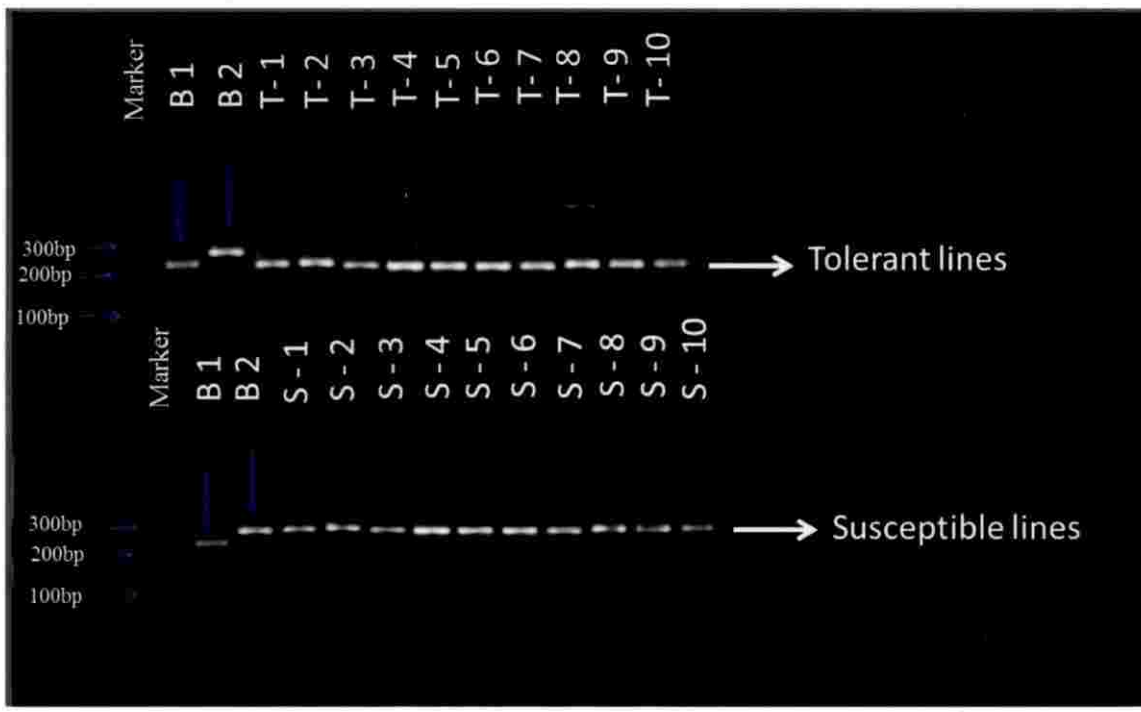
1–Ptb 1, 2–Ptb 7, 3–Ptb 15, 4–Ptb 10, 5–Ptb 8, 6–Ptb 3, 7–Ptb 12, 8–Ptb 17, 9–Ptb 22, 10–Ptb 23, 11–Ptb 24, 12–Ptb 28, 13–Ptb 29, 14–Ptb 30, 15–Chomala, 16–Ptb 55, 17–Ptb 60, 18–Ptb 27, 19–Ptb 32, 20–Ptb 34

Plate 7. Gel profile showing monomorphic and polymorphic bands of tolerant and susceptible bulks for various primers



B1- Susceptible bulk; B2-Tolerant bulk

Plate 8. SSR profile of bulks and individuals using primer RM 474



B1-tolerant bulk; B2-susceptible bulk; T-1-Ptb 1; T-2-Ptb 7; T-3-Ptb 10; T-4-Ptb 15; T-5-Ptb 28; T-6-Ptb 29; T-7-Ptb 30; T-8-Ptb 55; T-9-Ptb 60; T-10-Chomala.
S-1- Ptb3; S-2-Ptb 8; S-3-Ptb 12; S-4-Ptb 17; S-5-Ptb 22; S-6-Ptb23 ; S-7-Ptb 24; S-8-Ptb 27; S-9-Ptb 32; S-10-Ptb 34.

Discussion

5. DISCUSSION

Climate change threatens the sustainability of modern agriculture. Constantly changing climatic conditions around the world demand constant efforts to understand and adapt to environmental challenges for sustainable crop production. The challenge is even greater for crops such as rice (*Oryza sativa* L.), which is the staple food of more than half of the world's population and grown under diverse environmental conditions. In the light of recent climate change, in the near future, water deficit is predicted to be a major challenge for sustainable rice production (Wassmann *et al.*, 2009).

Rainfed rice accounts for around 45% of the world's total rice area (Maclean *et al.*, 2002). The rainfed rice ecosystem is highly vulnerable to drought due to abnormal distribution of rainfall over the years. It is estimated that 4.3% of rice yields are lost every year because of drought in Asia (Dey and Upadhyaya, 1996). Even though rice is vulnerable to drought, it has developed several mechanisms to mitigate harmful effects of drought. The inherent capacity of rice for wider adaptation in varied hydrological ecosystems has developed much scope for improvement of drought tolerance. But, there has been little success in developing drought tolerant rice cultivars (Fukai and Cooper, 1995). Two main reasons were recognized for the slow progress in this field. The first and the foremost reason is that drought tolerance is a complex phenomenon and is controlled by more than one gene. Secondly, due to the incidence of large genotype by environment (GxE) interaction, which result from a combination of differences in the genotype adaptation and the heterogenous environment within the target areas.

Alternatively, improvement in yield could be achieved by identifying plant characteristics that allows a plant to escape, avoid or tolerate stress and selecting for these traits in breeding programs (O'Toole, 1987). The effectiveness of selection using secondary traits for yield improvement under stress has been demonstrated in

rice (Babu *et al.*, 2003; Lanceras *et al.*, 2004). However, phenotypic selection for these traits is labour intensive and time consuming process. Considering these limitations, molecular marker technology serves as a powerful tool for selecting such complex traits. Identification of DNA markers linked to drought tolerant traits is carried out using large mapping population where each progeny has to be genotyped with several markers. This is highly time consuming and often costly. Several strategies have been reported to reduce the number of plants to be genotyped. Tan *et al.*, (1998) developed a technique called Bulked Line Analysis (BLA) by pooling DNAs of genotypes from diverse genetic backgrounds but sharing similar phenotypes (e.g. Drought tolerant or sensitivity). This technique resulted in rapid identification of DNA markers linked with drought tolerance in rice.

In the present study, 35 rice assession collected from RARS, Pattambi were evaluated for physio-morphological and yield parameters and the genotypes having better drought tolerant capacity were grouped into drought tolerant lines and the genotypes having poor drought tolerance capacity were grouped into drought susceptible lines. Then, Bulked Line Analysis was carried out by using the bulked DNA samples of tolerant and susceptible genotypes inorder to find out the polymorphic primers between the bulks. 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 AND BIOCHEMICAL PARAMETERS

In rice, various physiological and biochemical processes are affected by drought stress and it induces several physiological responses which help them to adapt to such water limiting environmental conditions. One of the important physiological responses in rice is its ability to maintain turgor pressure by reducing osmotic potential. In this study, various physiological and biochemical parameters

were studied for identifying drought tolerant genotypes and this section explains the basis of results obtained.

Leaf rolling is one of the visible physiological responses to plant water deficit (Singh and Singh, 2000). In this study, the degree of leaf rolling among the rice genotypes under drought stress showed the extent of drought tolerance (Figure 1). Complete leaf rolling in two rice genotypes, Ptb-7 and Ptb-13 may be attributed to their failure in maintaining leaf water content under stress. Rice genotypes which showed leaf rolling score of 1 may have the capacity to maintain turgor pressure under stress. Similar results were obtained by Dingkuhn *et al.* (1991), who reported that leaf rolling is an adaptive mechanism found in rice plants to escape the drought. Blum, (1989) reported that delayed leaf rolling is associated with better osmotic adjustment and avoidance of dehydration under water stress in rice. Even though, varieties showing early leaf rolling symptoms are considered as drought susceptible, it can also be a mechanism to avoid transpiration loss by reducing the leaf area (Maji *et al.*, 2001). In the present study, the genotypes which showed a leaf rolling score (1-3) were considered as drought tolerant and genotypes which showed a leaf rolling score (7-9) were considered as drought susceptible. Similar selection was done by Kanagaraj *et al.*, 2010, who selected 11 RILs which performed well (low score 1-2) and 12 RILs which performed very poorly (high score 8-9) out of 330 RI lines under water stress condition and grouped in to drought tolerant and susceptible lines.

It has been suggested that the variation in drought tolerance among the rice cultivars mostly reflects the variation in plant water status during stress periods. Relative water content is a measure of plant water status, which can differ significantly among rice cultivars exposed to the same level of water stress (O'Toole and Moya, 1978). In the present study, all the genotypes showed a significant reduction in relative water content which may be a result of decrease in soil water potential due to drought imposition (Figure 2). Similar results were obtained by Fischer (1989), who reported that RWC was positively correlated to soil water

Figure 1 : Leaf rolling score of rice genotypes at flowering stage under irrigated and water stress conditions

Figure 2: Variation in relative water content (%) of rice genotypes at flowering stage under irrigated and water stress conditions

content. Also the genotypes, which maintained better relative water content under water stress showed lesser leaf rolling symptoms.(e.g. The variety Ptb-29 which showed lesser leaf rolling under stress (score 1) maintained comparatively higher RWC (76.7%)). So, it can be suggested that leaf rolling is associated with leaf water potential. Similar findings were given by Beena et al., (2012) who reported that Nootripathu, a drought tolerant variety had higher relative water content (61.7%) than IR20 (55.7%), a drought susceptible variety.

Cell membrane stability has been reported to be associated with water and high temperature tolerance in various crop plants (Blum and Ebercon, 1981). In this study, several genotypes such as Ptb-29 (98.6%) and Ptb-10 (98.1%) showed higher membrane stability, which may be due to the presence of more amount of saturated fatty acid in their membrane or due to the maintenance of relatively high leaf water content (Figure 3). These findings were supported by Savchenko *et al.*, (2002) who reported that drought stress affects the fluidity of cell membrane by either denaturation of protein or increase in unsaturated fatty acids. However, genotypes such as Ptb-2 (79.1%) showed very low membrane stability which can be attributed to more lipid peroxidation in the membrane which is on par with the findings of Leibler *et al.*, (1986) who reported that the lower membrane stability or higher injury reflects the extent of membrane lipid peroxidation, which in turn is a consequence of higher susceptibility to oxidative stress due to various environmental stresses including drought.

Leaf temperature is considered as an index to measure water stress in crop plants. As soil water diminishes, leaf temperature increase because transpiration is reduced (Blum, 1988). In the present study overall increase in leaf temperature among rice genotypes under water stress was 3.95% as compared to control condition (Figure 4). This may be due to reduction in leaf water content and decreased transpiration rate caused by drought stress. The ability to maintain lower leaf temperature is an indication of high transpiration and photosynthetic rates and

Figure 3: Variation in membrane stability index (%) of rice genotypes at flowering stage

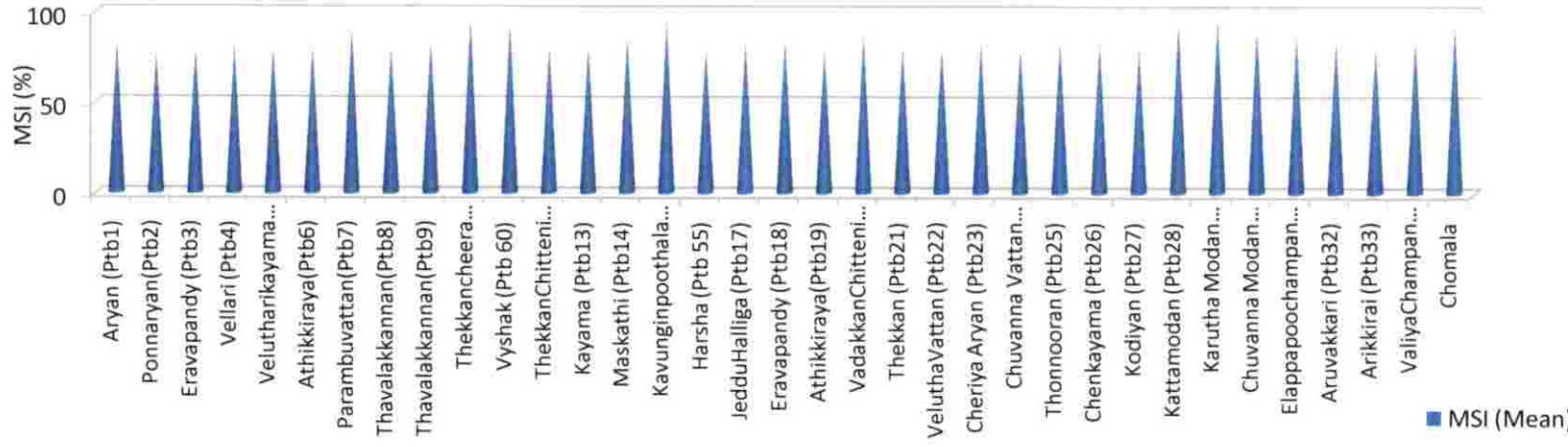
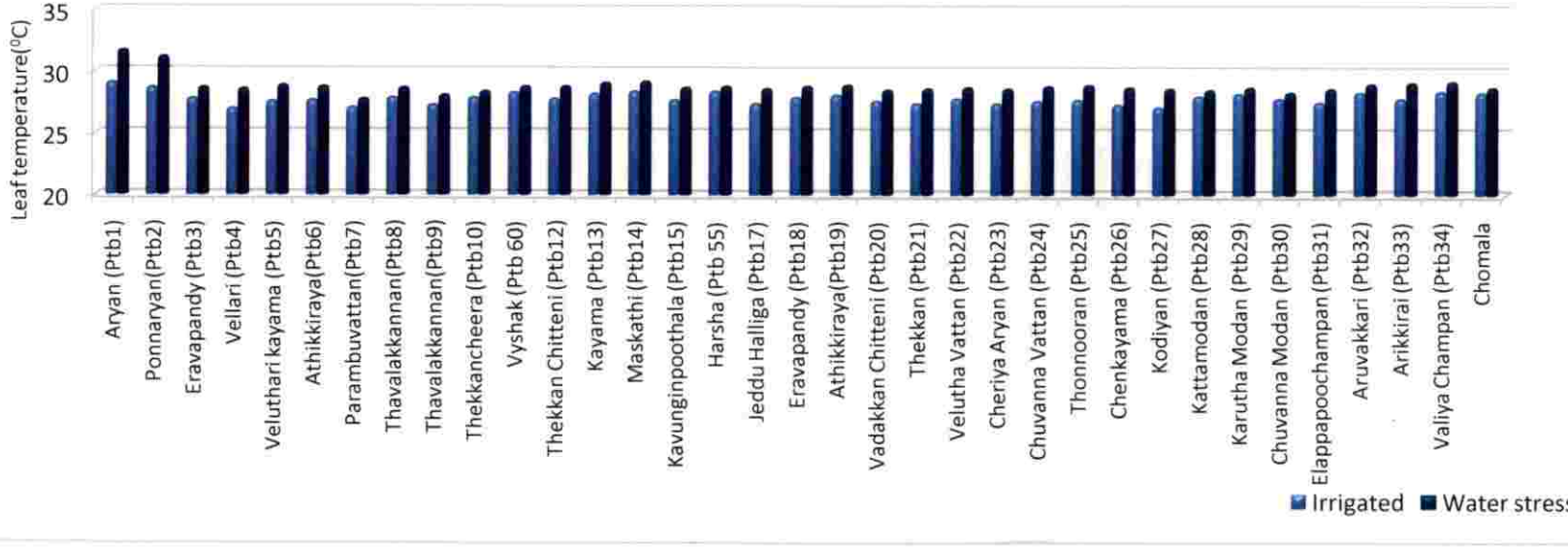


Figure 4: Variation in leaf temperature (^{0}C) of rice genotypes at flowering stage under irrigated and water stress conditions



productivity under drought. Similar findings were reported by Garrity and O'Toole (1995) and Hirayama *et al.* (2006). They showed that grain yield and spikelet fertility were highly correlated with midday canopy temperature on the day of flowering, and lines with high drought-avoidance scores consistently remained the coolest under stress. Deep rooted cultivars which are capable of absorbing soil moisture from deeper soil layers are having lesser leaf temperatures (Nemotto *et al.*, 1988). In this study deep rooted cultivars such as Ptb- 15 maintained lesser leaf temperature (28.7 ^{0}C) under water stress condition.

Stomatal regulation in response to water stress has been found to be triggered by root to shoot chemical or hydraulic signaling (Tardieus and Davies, 1993). It is considered as a key adaptation strategy to avoid tissue dehydration under drought. In the present study, all the genotypes showed significant reduction in stomatal conductance in water stress compared to irrigated control, which may be due to the stomatal closure in response to decreasing soil moisture status (Figure 6). The rate of stomatal closure varies within the genotypes according to their ability to tolerate drought. Similar results were derived by (Lo Gullo et al., 2003) who reported a drastic reduction in stomatal conductance under water stress in rice genotypes.

The decrease in photosynthetic rate under drought can be attributed to many factors such as early stomatal closure, decline in Rubisco activity, and reduced efficiency of PS II. In the present study, overall reduction of 53.8% in photosynthetic rate was observed in rice genotypes exposed to water stress with respect to control. Also, the mean decrease in stomatal conductance from 451.9 m H_2O moles m^{-2} s^{-1} to 257.7 m H_2O moles m^{-2} s^{-1} among the rice genotypes is on par with a decrease in photosynthetic rate from 18.2 μ CO_2 moles m^{-2} s^{-1} to 8.4 μ CO_2 moles m^{-2} s^{-1} (Figure 7). Therefore stomatal closure seems to be the main cause of the decrease in photosynthetic rate among the rice genotypes under water stress. Similar results were obtained by Ji *et al.*, (2012). This study also witnessed some genotypes which showed comparatively higher photosynthetic rate under water stress condition compared to

Figure 5: Variation in stomatal conductance (m moles m^{-2} s^{-1}) of rice genotypes at flowering stage under irrigated and water stress conditions

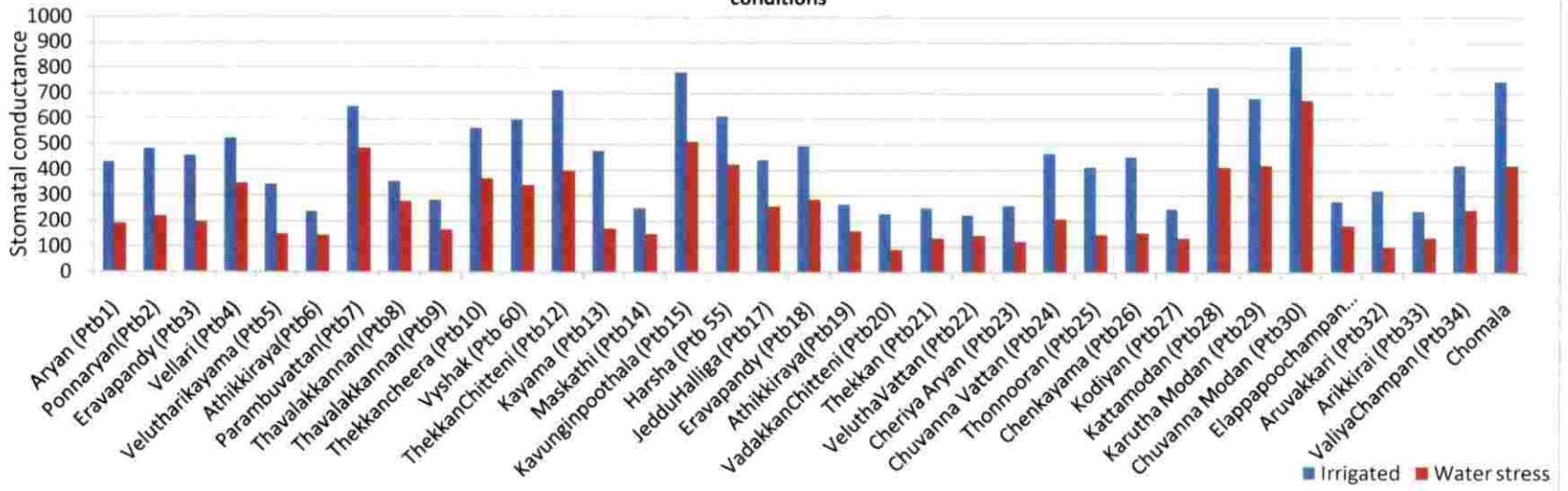
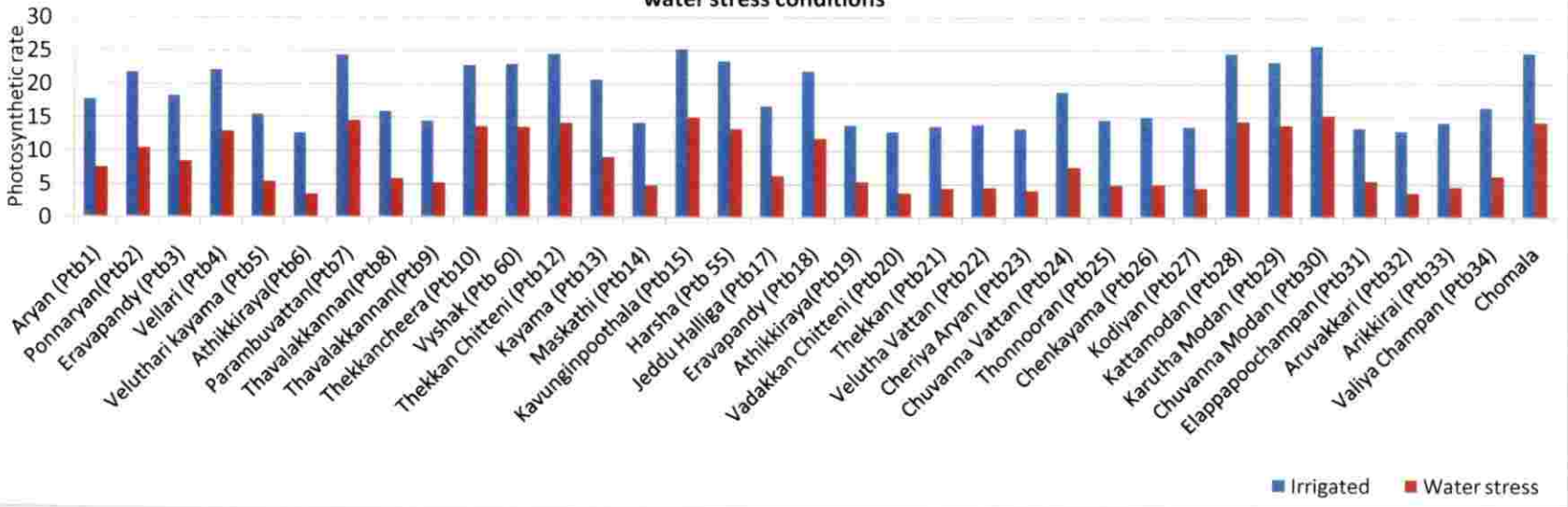


Figure 6: Variation in photosynthetic rate (μ moles m^{-2} s^{-1}) of rice genotypes at flowering stage under irrigated and water stress conditions



control. These varieties can be considered as drought tolerant considering the findings of Uprety and Sirohi (1985) and Sairam *et al.* (1990) who reported that tolerant genotypes generally showed higher photosynthetic rate than susceptible ones.

The transpiration rate seems to be decreasing in rice genotypes under water stress condition. An overall reduction of 59.3% in transpiration rate was observed in the rice genotypes under water stress condition compared to control (Figure 8). Decrease in stomatal conductance can be a probable reason for this. Similar results were reported by Cabuslay et al., (2002). Varietal variation in transpiration rate under water stress was reported in this study. Tolerant genotypes generally had lower rates of transpiration than the susceptible genotypes (Sairam, 1994).

Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. In the present study, water stress caused an overall increase of 53.3% in proline content among the genotypes (Figure 9). This result was in line with Bunnag and Pongthai, (2013) that water stress caused twice increase in free proline content in rice. Similar observations were also reported in rice by Beena *et al.* (2012) and Sheela and Alexander, (1995).

5.2 EFFECT OF WATER STRESS ON ROOT TRAITS

It has been observed that under mild water stress root length of rice genotypes increases (Lilley and Fukai, 1994b), although this is not always observed in severe drought conditions (Puckridge and O'Toole, 1981). In the present study water stress was given for a period of 15 days, which resulted in severe drought condition. Some genotypes showed significant increase in root length. The average increase in root length among these rice genotypes was 7.23% under water stress condition (Figure 10). The genotypes which showed an increment in root length may have a better root penetration ability which is in accordance with the findings of Yu *et al.*, (1995). The reduction in root length among few rice genotypes may be due to their inability to

Figure 7: Variation in transpiration rate (m moles m^{-2} s^{-1}) of rice genotypes at flowering stage under irrigated and water stress conditions

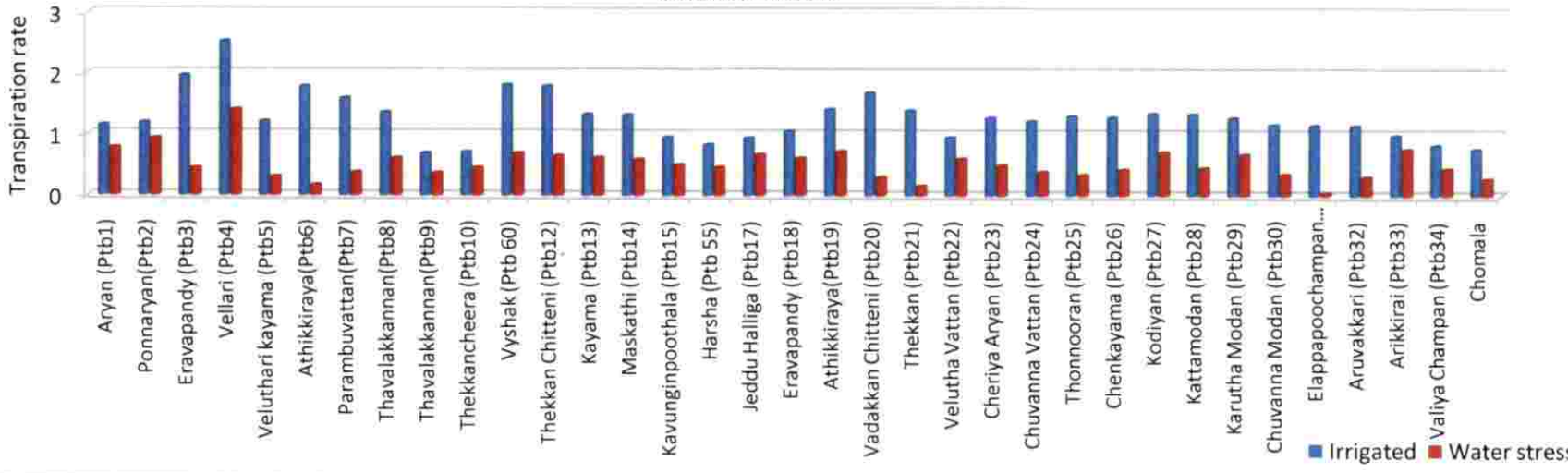
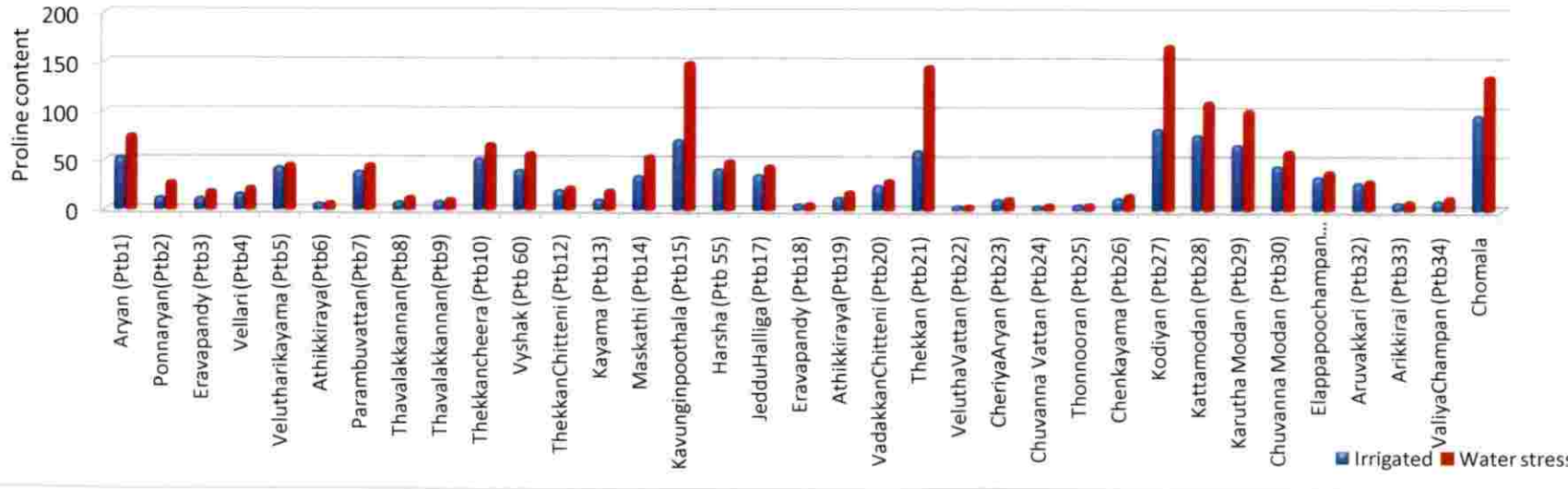


Figure 8: Variation in proline content (µ moles / g tissue) of rice genotypes at flowering stage under irrigated and water stress conditions



penetrate the hard pan of soil which is formed due to drought. Deep rooted rice cultivars tolerate drought better than shallow rooted cultivars (Chang *et al.*, 1986) because of their ability to extract moisture from the deeper layers of soil (Fukai and Cooper, 1995). So, the varieties like Ptb-15 which showed highest root length (58.8cm) can be considered as drought tolerant.

Root volume was significantly and positively associated with root length in rice (Zuno *et al.*, 1990), but in the case of susceptible genotypes this trend might not be always exists. In the present study, an overall decrease in root volume from 38.9cm^3 (irrigated condition) to 29.6 cm^3 (water stress condition) occurred among the genotypes. The reason for this decrease in root volume is due to the decrease in moisture availability under water stress (Figure 11). Similar results were reported by Nag, (2008), who observed 17.1 reduction in root volume due to less moisture availability. Eventhough root volume decreased in most of the genotypes under stress, genotypes such as Ptb-10, Ptb-7 and Ptb-55 showed an increase in root volume under stress condition compared to control condition. This increase in root volume can be attributed to their ability to increase root biomass in order to extract moisture from deeper layers of soil. So, these genotypes have the ability to tolerate drought. This can be confirmed from the findings of Ekanayake *et al.*, 1985; Fukai and Cooper, 1995; O'Toole, 1982; Yoshida and Hasegawa, 1982 who reported that the possession of a deep and thick root system which allows access to water deep in the soil profile is crucially considered important in determining drought tolerance in upland rice.

The increase in root biomass under water stress condition is a function of the ability to tolerate drought in rice (Cruz *et al.*, 1986). The result of root dry weight showed an overall decrease of 32.6% in rice genotypes due to water stress condition compared to control condition (Figure 12). This reduction in root dry weight may be due to the decreased supply of photosynthates to roots which is a result of decrease in leaf water potential under water stress condition (Cruz and O'Toole, 1985). These

Figure 9: Variation in root length (cm) of rice genotypes at flowering stage under irrigated and water stress conditions

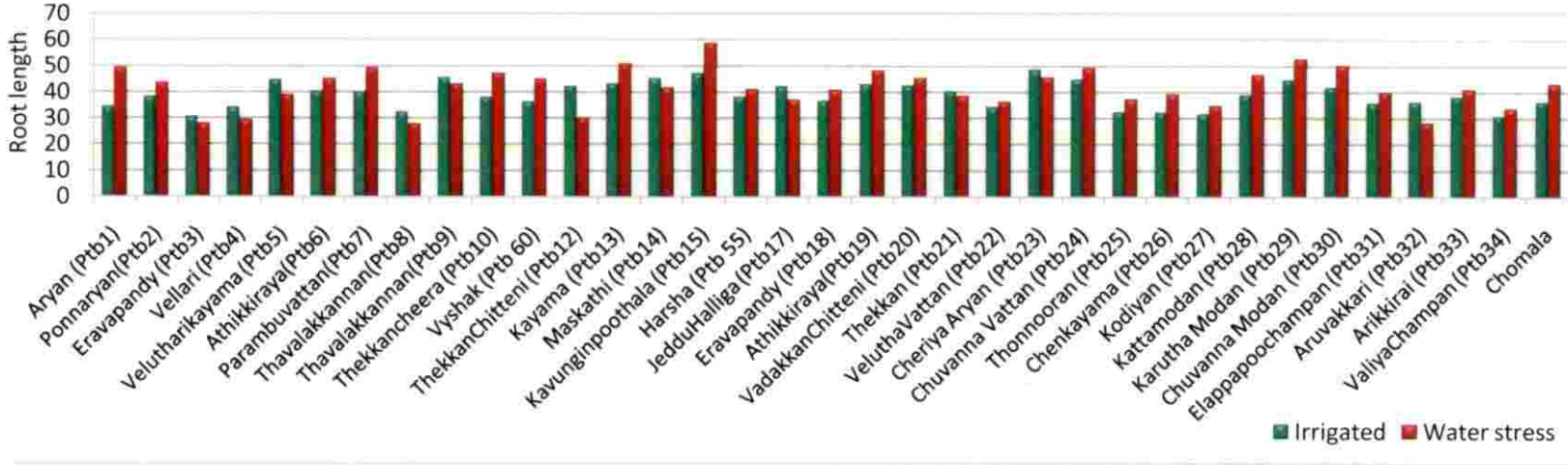
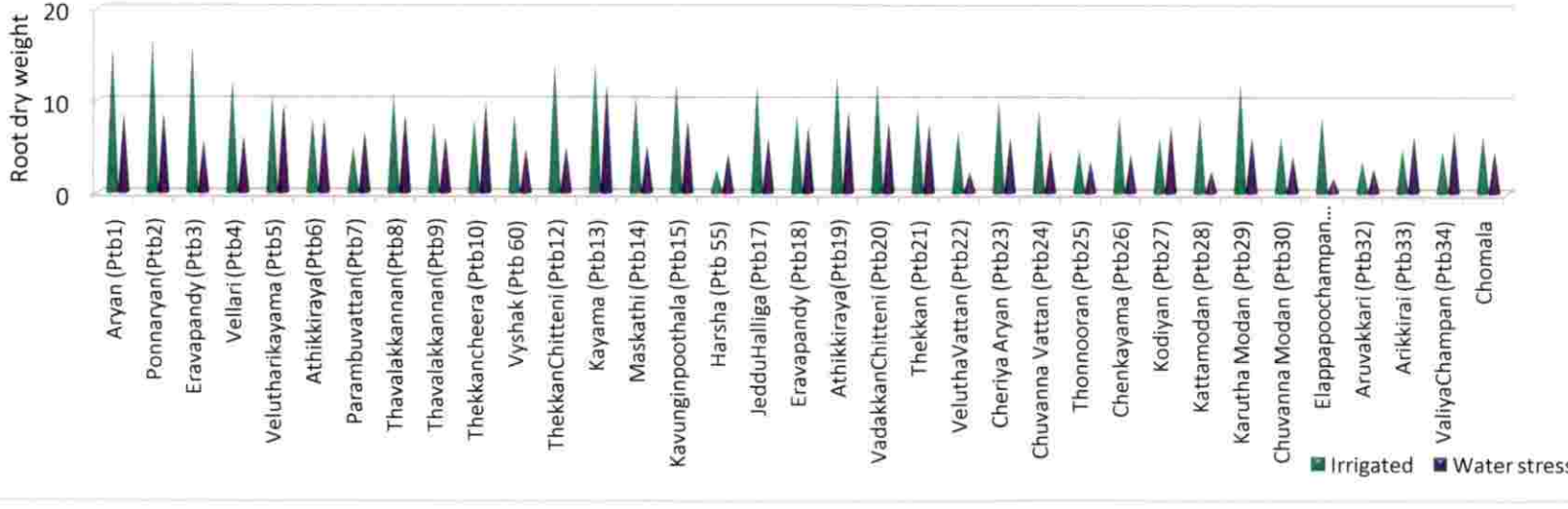


Figure 10: Variation in root dry weight (g) of rice genotypes at flowering stage under irrigated and water stress conditions



findings were in line with the findings of Srividhya *et al.* (2011) and Ji *et al.* (2012). However, genotypes such as Ptb-7, Ptb-10, Ptb-55 etc. showed an increase in root dry weight under stress condition compared to control condition. These increment in root dry weight can be connected with their maintenance of high leaf water potential under drought condition. Thus it is obvious that these genotypes possesses the capacity to tolerate drought considering the findings of Cruz *et al.*, (1986).

Root to shoot ratio can be considered as an important parameter in determining drought tolerance in rice. In the present study, a significant increase in root shoot ratio of all the rice genotypes were observed (Figure 13). An increase of 28.6% in root shoot ratio among the rice genotypes under water stress condition was there compared to control condition. Such an increase in root shoot ratio can be linked with maintenance of leaf water status under drying soil. Similar results were reported by Boyer (1985) who observed an increase in root shoot ratio under soil moisture deficit. But, this may not happens in every condition. Cruz *et al.* (1986) presented that mild stress condition during vegetative stage in rice can cause more reduction in root dry weight than shoot dry weight and thereby decreasing root to shoot ratio.

5.3 EFFECT OF WATER STRESS ON MORPHOLOGICAL AND YIELD PARAMETERS

Drought stress directly affects the growth of rice plants by reducing plant height and the number of tillers per plant because plants are unable to absorb soil water when soil water becomes inadequate, resulting in the essential elements being less available to the plants. The plant cells become less turgid, leading to a reduction in cell division and expansion. Therefore, the growth of the stems is retarded (Hsiao *et al.* 1984). In the present study, an overall reduction of 6.4% in plant height was observed among the rice genotypes exposed to water stress compared to control (Figure 14). These findings was in accordance with Beena *et al.*, (2012) who reported a 10.4% reduction in plant height due to water stress among recombinant inbreed

Figure 11: Variation in root volume (cm^3) of rice genotypes at flowering stage under irrigated and water stress conditions

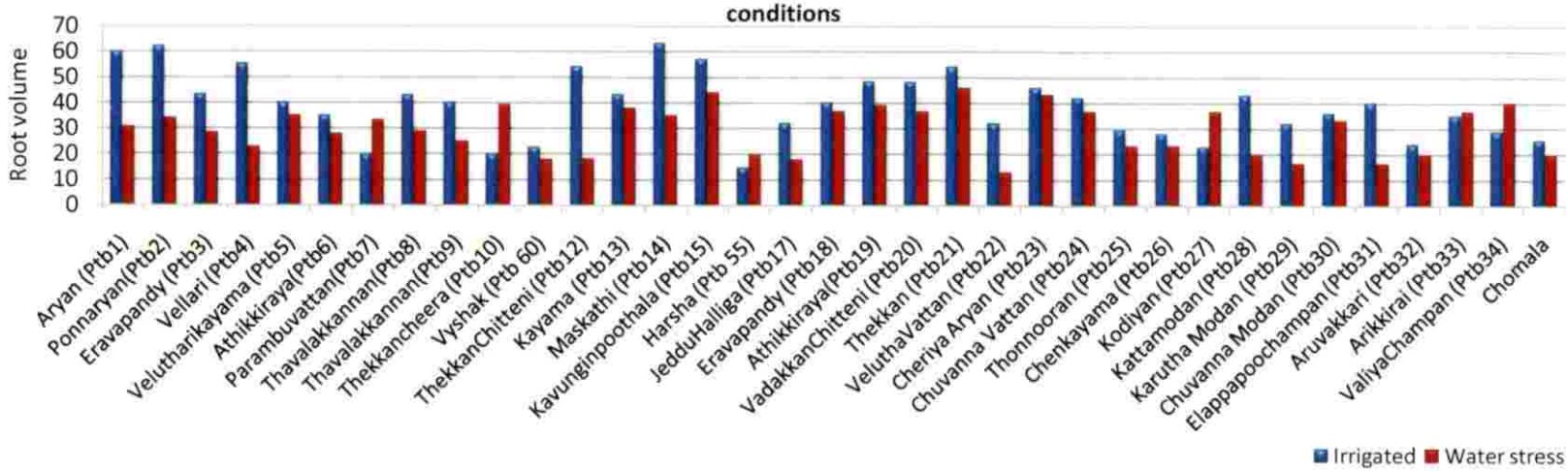
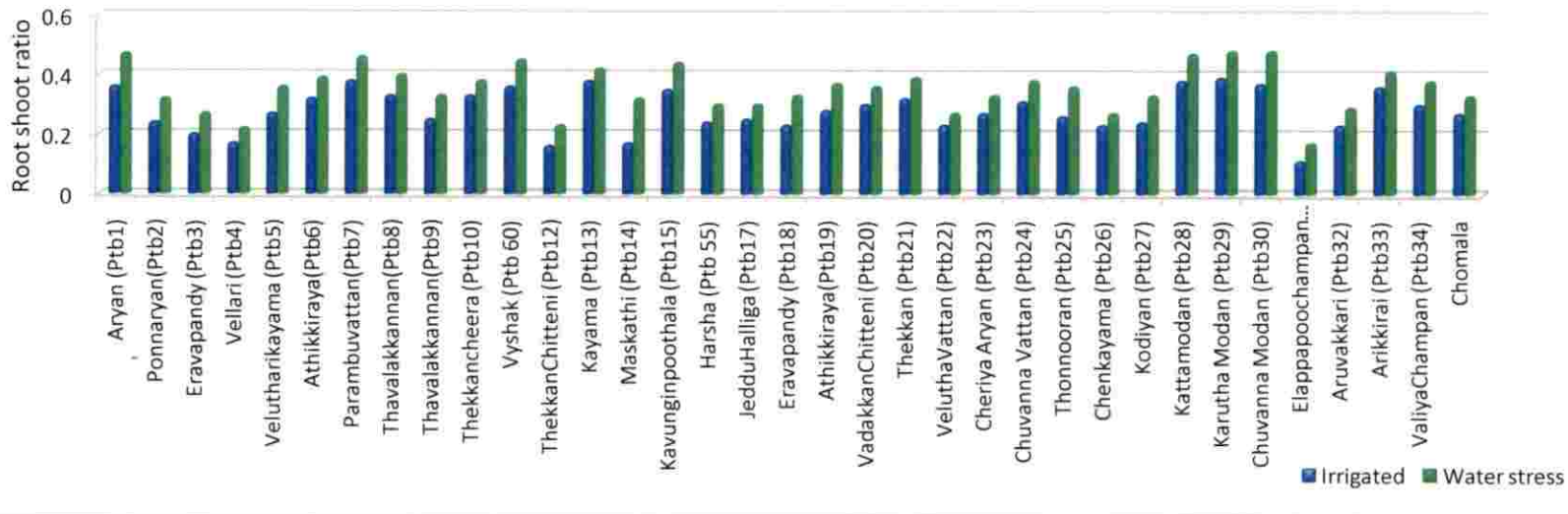


Figure 12: Variation in root shoot ratio of rice genotypes at flowering stage under irrigated and water stress conditions



lines of IR20 x Nootripathu. Similar results were reported by Ji *et al.* (2012), Bunnag and Pongthai (2013) and Kumar *et al.* (2014).

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, early flowering was noticed in most of the varieties which can be attributed to their mechanism of drought escape (Figure 15). Also, varieties such as Ptb-5, Ptb-8 and Ptb-34 showed delay in flowering which is a function of low plant water status and higher delay indicates drought susceptibility.

For morphological traits such as tiller number and productive tiller number significant variation was observed among the genotypes under both conditions. Water stress caused an overall reduction of 40% in number of productive tillers across the genotypes as compared to irrigated condition. The higher reduction in productive tiller number can be connected with lower water status due to water stress. Similar results were also reported earlier in rice by Prince *et al.*, (2015).

Water stress at flowering stage is a serious problem that affects yield and yield related traits because it adversely affects pollination, flower and grain development, and causes increase in percentage of unfilled grains (Hsiao *et al.*, 1976). In the present study, water stress at panicle initiation stage resulted in an overall reduction of 8.6% in panicle length, 27.3% in yield per plant, 15.1% in spikelet fertility, and 3.2% in 1000 grain weight among the rice genotypes as compared to control(Figure 17, figure 18, figure 19 and figure 20). This reduction in yield components might be due to decrease in translocation of assimilates towards reproductive organs (Rahman *et al.*, 2002). Davatgar *et al.*, 2009 observed that drought at reproductive stage increased percentage of unfilled grains in rice. These findings are in line with the findings of Swain *et al.*, (2010) and Singh *et al.*, (2010).

Figure 13: Variation in plant height (cm) of rice genotypes at flowering stage under irrigated and water stress conditions

Figure 14: Variation in days to 50% flowering of rice genotypes at flowering stage under irrigated and water stress conditions

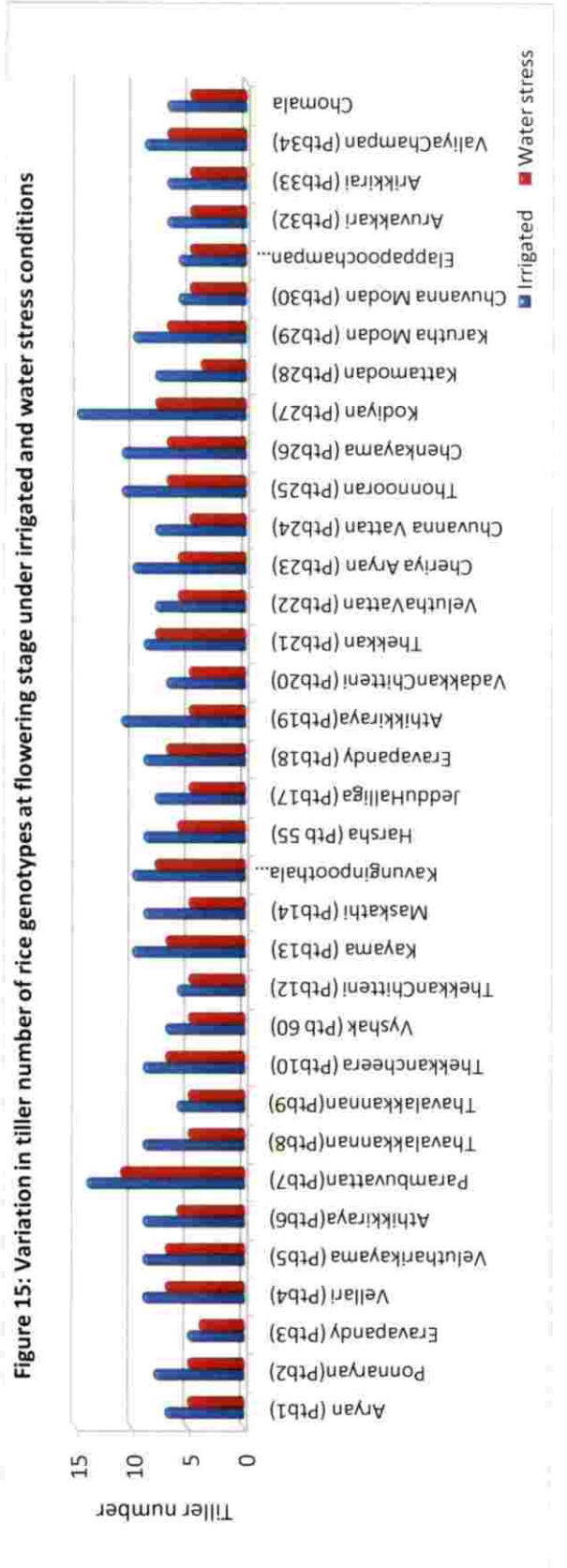


Figure 15: Variation in tiller number of rice genotypes at flowering stage under irrigated and water stress conditions

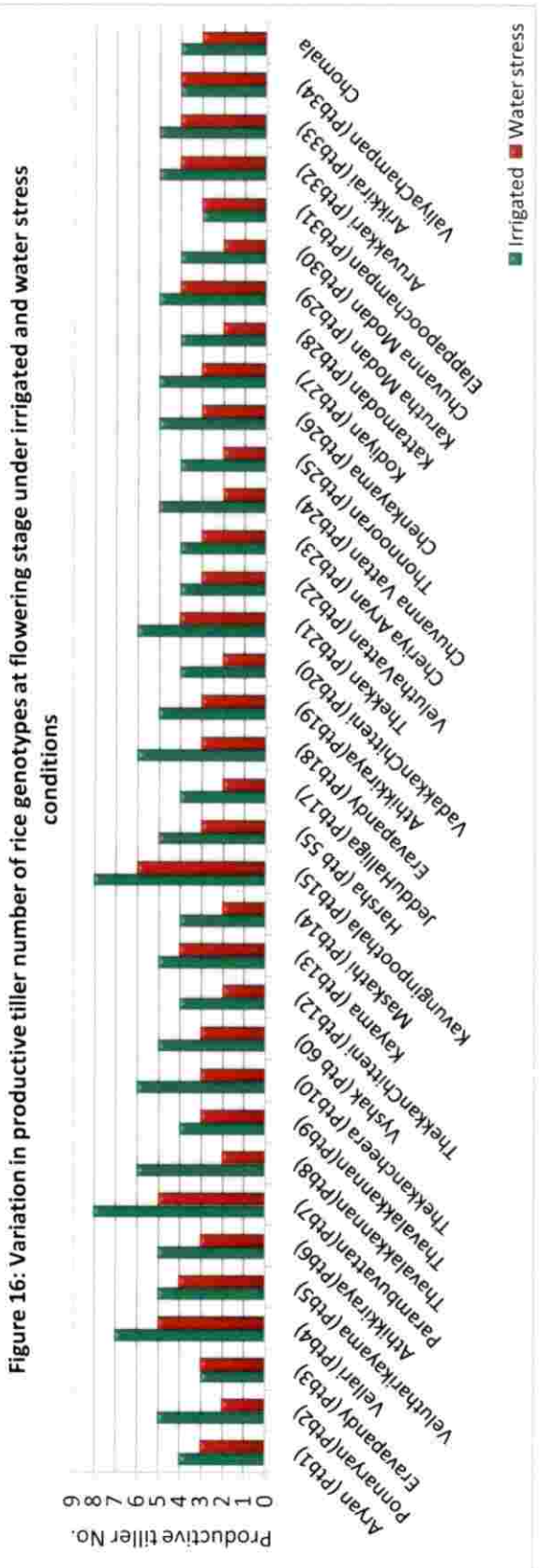


Figure 16: Variation in productive tiller number of rice genotypes at flowering stage under irrigated and water stress conditions

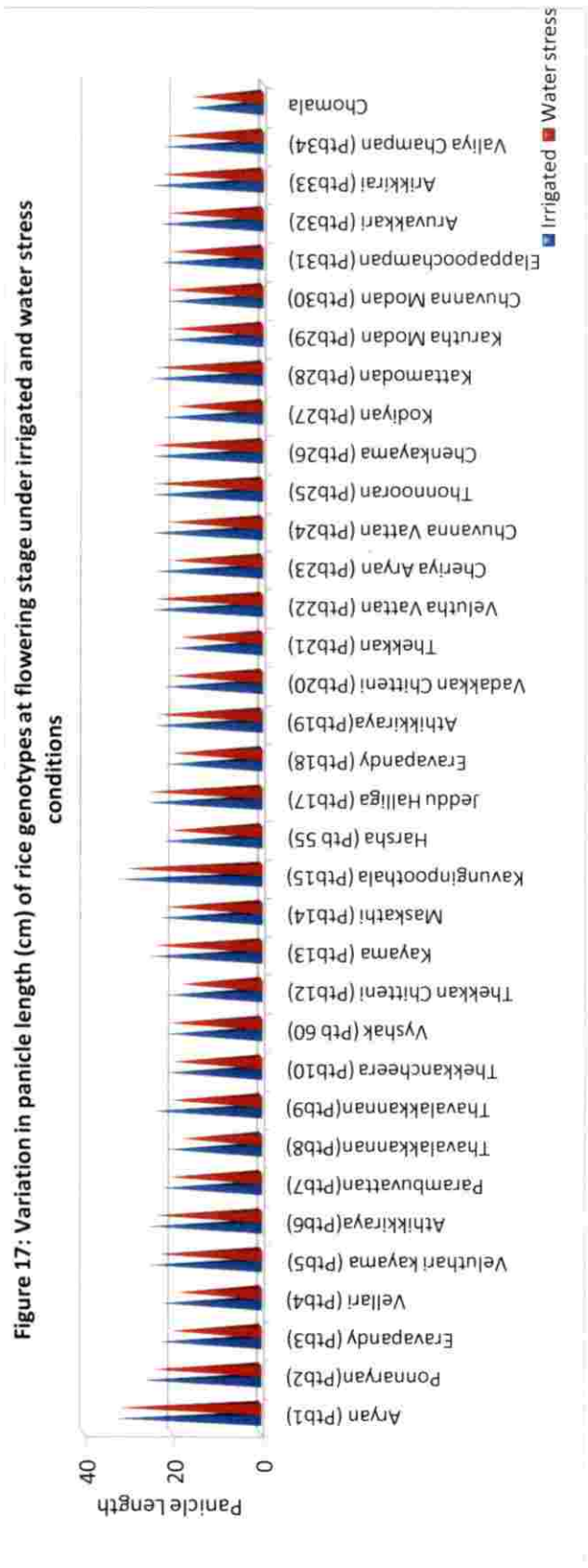


Figure 17: Variation in panicle length (cm) of rice genotypes at flowering stage under irrigated and water stress conditions

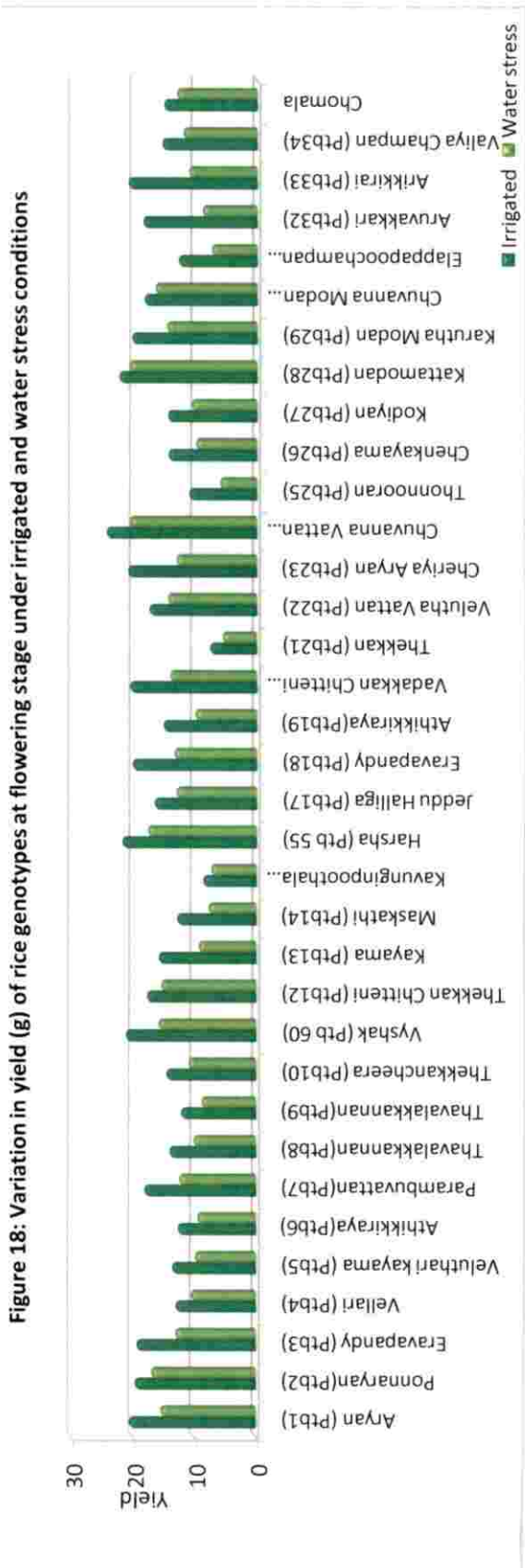


Figure 18: Variation in yield (g) of rice genotypes at flowering stage under irrigated and water stress conditions

Figure 19: Variation in spikelet fertility percentage (%) of rice genotypes at flowering stage under irrigated and water stress conditions

Figure 20: Variation in 1000 grain weight (g) of rice genotypes at flowering stage under irrigated and water stress conditions

5.4 CORRELATION STUDY

Correlation gives an idea on nature and depth of relationship among various physio-morphological traits under water stress and irrigated conditions. The importance of correlation studies in drought related experiments is that improvement of yield can be achieved by improving any of the traits which is associated with it. Correlation study revealed that grain yield per plant under water stress condition was positively correlated with parameters such as relative water content, membrane stability index, proline content, stomatal conductance, photosynthetic rate, transpiration rate, root length, root shoot ratio, spikelet fertility % and 1000 grain weight where as negatively correlated with leaf temperature, leaf rolling score, root volume, root dry weight, plant height, days to 50% flowering and panicle length. In irrigated condition, grain yield was positively associated with parameters such as relative water content, leaf temperature, stomatal conductance, photosynthetic rate, transpiration rate, root length, root dry weight, root shoot ratio, spikelet fertility % and 1000 grain weight where as negatively correlated with proline content root volume, plant height, days to 50% flowering and panicle length.

In this study, positive correlation was observed between plant production traits such as total number of tillers, total number of productive tillers, spikelet fertility percentage and1000 grain weight with yield under stress. Positive and significant correlation between total number of tillers, total number of productive tillers, and 1000 grain weight with grain yield were reported in rice by Kumar *et al.*, (2009), Rao *et al.*, (2014); Shinde *et al.*, (2015) and Islam *et al.*, (2016). Positive correlation between spikelet fertility percentage and grain yield under stress was reported by Saikumar *et al.*, (2014) and Pradhan *et al.*, (2015). Physiological parameters such as as relative water content, membrane stability index, proline content, stomatal conductance, photosynthetic rate and transpiration rate showed positive correlation with yield under stress. Plant production traits such as plant height, panicle length, and days to 50% flowering were negatively correlated with

grain yield under stress. Similar results were reported in rice by Babu *et al.*, (2012); Pradhan *et al.*, (2015) and Savitha and Usharani, (2015). Root traits such as root length and root shoot ratio showed positive correlation with grain yield under drought. This observation was in accordance with the findings of Manickavelu *et al.*, (2006). Leaf temperature and leaf rolling were negatively correlated with grain yield under stress. Similar observations were reported by Babu *et al.*, (2003) and Boopathi *et al.*, (2013). All the root traits such as root length, root volume, root dry weight and root shoot ratio were positively correlated with proline content under water sress condition which implies that rice plants with better root traits can maintain osmotic balance in their cells and thereby drought tolerance.

5. 5 BULKED LINE ANALYSIS

In the present study, Bulked Line Analysis (BLA) was carried out to find out the molecular markers linked to drought tolerant traits in rice. Ten drought tolerant and ten drought susceptible genotypes were selected from the germplasm collection of 35 genoypes based on phenotypic evaluation of traits contributing to drought tolerance. Genotypes which showed higher values for root length, root shoot ratio, membrane stability index, relative water content, proline content, spikelet fertility percentage, 1000 grain weight, grain yield per plant as well as lesser leaf rolling score and leaf temperature under water stress were selected as drought tolerant genotypes.The genotypes which showed lower values for root length, root shoot ratio, membrane stability index, relative water content, proline content, spikelet fertility percentage, 1000 grain weight, grain yield per plant and higher leaf rolling score and leaf temperature were selected as drought susceptible ones. The selection was done on the basis of the fact that individual plants which shows similar phenotypic expression under a particular environment will have a specific gene which controls that particular phenotype. So, the grouping of plants based on higher or lower level of expression of a particular trait and genotyping using several primers may results in identification of new markers linked to a particular trait.

Eventhough identification of DNA markers linked to target gene can be effectively done by using Near isogenic lines and Bulked segregant analysis (BSA), BLA method permits the genetic stock to be prepared more quickly. Eventhough localization of genes cannot be done by using BLA method, it can be effectively used to identify molecular markers linked to desired gene. By means of such markers, the linked traits can be precisely localized if the markers used have been previously mapped. In the present study, the primer RM474 (252 bp) produced polymorphism between drought tolerant and susceptible bulks. The same primer produced similar product size (252 bp) among the individual genotypes forming tolerant bulk which was different from susceptible bulk and genotypes forming susceptible bulk (~ 300 bp). Considering the findings of Temnykh *et al.* (2001), the primer RM 474, which is identified in this study is located on rice chromosome 10 (Figure 21). Various studies were reported in rice where, several locus in chromosome 10 were found to be associated with drought tolerant traits. Some of these studies are discussed here.

Zhang *et al.* (2001) reported that the region between RG257 and ME5_16 which flanked by the marker RM 222 in chromosome 10 of rice, to be associated with total root dry weight in a double haploid (DH) population of CT9993, an upland *japonica* type possessing a deep and thick root system and IR62266, an *indica* type with a shallow root system. Also, Verma (2010) reported that chromosome 10 contains QTLs for leaf rolling, grain yield and spikelet fertility after evaluating two hundred seventy Recombinant inbred lines (RILs) of two *indica* genotypes, Danteshwari x Dagad Deshi under both rainfed and terminal stage drought (TSD) conditions.

Kanagaraj *et al.* (2010) conducted BSA using 23 RIL's of IR20/Nootripathu and found out three primers *viz.* RM212, RM302, and RM3825 which co-segregated among the individual RI line forming the tolerant and susceptible bulks. They also reported that the region where these primers were located to be associated with

various drought tolerant traits such as plant height, leaf drying, RWC, root to shoot ratio, grain yield.

Salunkhe *et al.* (2011) reported that the region, RM212-RM302-RM8085-RM3825 on chromosome 1, harbors large effect QTLs for drought resistance traits across several genetic backgrounds in rice. Using Bulked Line Analysis (BLA), Kumar *et al.* (2005) identified two primers RM223 and RM263, associated with drought tolerance in rice.

Anitha *et al.* (2008) identified co-segregated primer RM 314, out of 25 polymorphic primers from 20 rice varieties (10 drought resistant and 10 drought susceptible rice varieties) using Bulked Line Analysis. And RM314 has been mapped on chromosome 6 of rice and found to be linked to many root traits.

Prasad *et al.* (2016) identified three primers *viz.* RM 1092, RM 129 and RM 157B associated with drought tolerant traits using Bulked Line Analysis in 36 rice genotypes from diverse genetic background. The genomic regions flanked by these markers were found to be associated with various drought tolerant traits in rice.

Thus, BLA method can be effectively used for identifying molecular markers linked to drought tolerant traits in rice. The primer RM 474 which is identified in this study can be used for marker assisted selection for drought tolerance in rice. Various markers (including RM 474) which were located in different chromosomes of rice were found to be linked to drought tolerance in rice. So, fine mapping of loci harbouring these markers can be done to find out the genes conferring drought tolerance in rice.

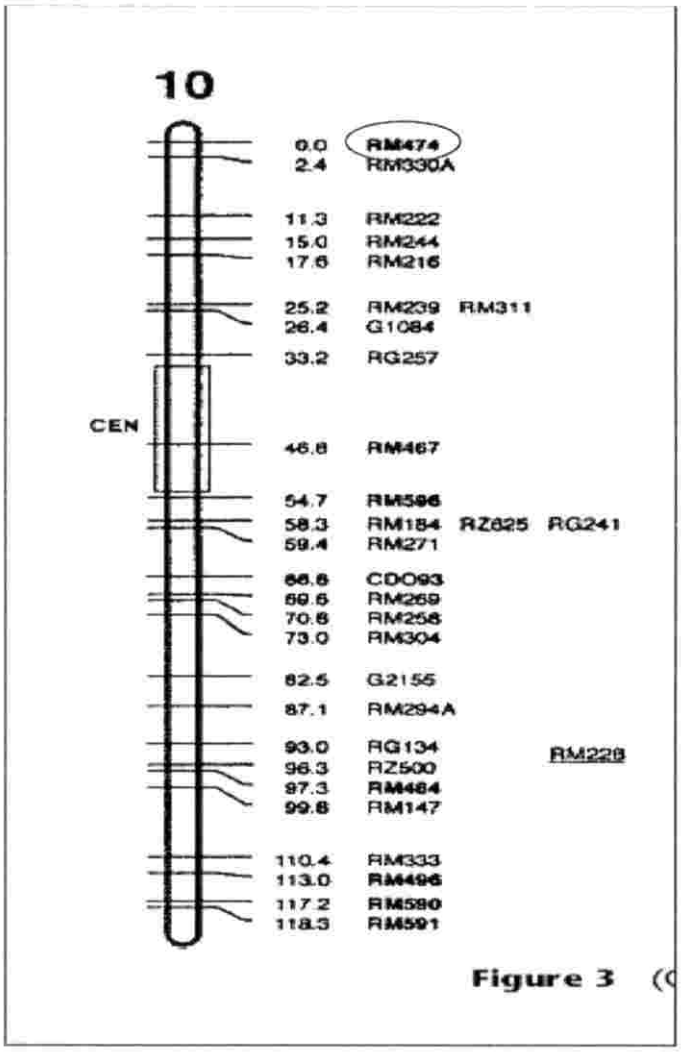


Figure 21. Chromosome 10 of rice showing the position of marker RM 474 (Temnykh *et al*. 2001)

Summary

6. SUMMARY

The salient findings of the present study to validate the role of root traits for drought tolerance in rice and to identify the microsatellite markers associated with drought tolerance in rice using Bulked Line Analysis are summarized here:

- In the first experiment, 35 rice genotypes consisting of improved varieties and landraces were evaluated for their performance for physio-morphological traits under water stress condition.
- Physiological parameters such as relative water content (RWC), photosynthetic rate, transpiration rate and stomatal conductance decreased whereas proline content and leaf temperature increased significantly in most of the genotypes under water stress condition.
- Some genotypes were found to maintain the higher relative water content with the depleting soil moisture under water stress condition.
- The leaf rolling score throughout the drought imposition period indicated that the reduction in leaf water content due to water stress condition is correlated with leaf rolling.
- Leaf temperature was less than ambient temperature in most of the genotypes under water stress.
- The genotypes which maintained higher membrane stability index, showed a higher leaf water status under drought.
- The average reduction in photosynthetic rate was 53.8% in water stress compared to control condition.
- Proline content was increased under stress condition in all the genotypes with maximum accumulation in Ptb-27.
- Among the root traits, root length and root shoot ratio were found to be improved in water stress condition where as root volume and root dry weight were significantly reduced under water stress condition.

- All the yield parameters were significantly reduced under water stress compared to control.
- Correlation study revealed that grain yield under stress exhibited highly positive correlation with root traits such as root length and root shoot ratio. In irrigated condition grain yield showed positive correlation with root length, root shoot ratio and root dry weight.
- Physiological parameters such as photosynthetic rate, transpiration rate and stomatal conductance showed positive and significant correlation with grain yield under both conditions.
- Morphological and yield parameters such as plant height, days to 50% flowering and panicle length were negatively correlated with yield where as spikelet fertility percentage and 1000 grain weight were positively correlated with yield under both conditions.
- In Bulked Line Analysis, SSR primer, RM 474 showed polymorphism between the tolerant and susceptible bulks. The same primer showed similar product size among the individual lines constituting the bulks.
- The genomic region flanked by this marker has been identified to be associated with various drought tolerant traits such as root dry weight, leaf rolling, grain yield and spikelet fertility in rice.
- Thus, the rice genotypes evaluated under water stress condition showed significant variation for physio-morphological and plant production traits. Genotypes having higher root characters were found to tolerate drought. The genotypes identified as drought tolerant *viz* Ptb-29, Ptb-30, Ptb-15, Ptb-1, Ptb-55 etc. can be used in breeding programmes to improve drought tolerance in rice.
- Microsatellite marker RM 474 which could distinguish drought tolerant and susceptible bulks can be used for marker assisted selection for drought tolerance in rice.

Future line of work

The drought tolerant genotypes identified in this study can be used as donors for developing new varieties which are high yielding and drought tolerant. More number of markers which are polymorphic between drought tolerant and susceptible genotypes can be identified using the same population and can be used for marker assisted selection for drought tolerance in rice. The drought tolerant genotypes identified in this study can also be used as parents in developing mapping populations for QTL mapping for drought tolerance in rice. These QTLs can be introgressed in popular rice varieties of Kerala.

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Appendices

APPENDICES

I. CHEMICALS FOR PLANT GENOMIC DNA ISOLATION

Dellaporta Extraction Buffer (100 ml)

Tris HCl (1M; pH-8)	5 ml
EDTA (0.5M; pH-8)	5 ml
NaCl (5M)	5 ml
Distilled water	85 ml

5M Potassium Acetate (100 ml)

Potassium acetate	29.6 g
Acetic acid	11.5 ml
Distilled water	28.5 ml

1X TE Buffer (100 ml)

1M Tris-Hcl (pH-8)	1 ml
0.25 EDTA (pH-8)	0.4 ml

Final volume was adjusted to 100 ml and autoclaved.

II. CHEMICALS FOR AGAROSE GEL ELECTROPHORESIS

Gel loading dye

Formamide	50 ml
Xylene cyanol	50 mg

Bromophenol blue	50 mg
0.5 M EDTA	1 ml

10 X TBE Buffer (Tris-Borate-EDTA): 1000 ml

Tris base	107 g
Boric acid	55 g
Na_2EDTA	9.8 g

IDENTIFICATION OF MICROSATELLITE MARKERS ASSOCIATED WITH ROOT TRAITS FOR DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

by

Rejeth R.

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Abstract of the thesis

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ABSTRACT

The present investigation entitled "Identification of microsatellite markers associated with root traits for drought tolerance in rice (*Oryza sativa* L.)" was conducted at Department of Plant Physiology, College of Agriculture, Vellayani during 2016-17. The objective of the study was to validate the role of root traits in rice for drought tolerance and to identify the microsatellite markers associated with root traits for drought tolerance in rice.

The extend of variation for water stress indicators, physio-morphological and yield components were assessed by evaluating 35 rice genotypes collected from RARS, Pattambi under water stress and irrigated conditions in the rainout shelter. The rice accessions grown in polythene tubes of 1 meter height were exposed to water stress at panicle initiation stage for a period of 15 days along with irrigated control. The physio-morphological, biochemical and yield components were recorded on completion of stress period. Significant variation was observed for these traits and ten drought tolerant and ten drought susceptible genotypes were selected. The genomic DNA was isolated from these rice genotypes and were pooled into drought tolerant and susceptible bulks. Bulked line analysis was carried out to identify microsatellite markers linked to drought tolerance in rice.

The result of the study revealed that physiological parameters such as Relative Water Content (RWC), photosynthetic rate, transpiration rate and stomatal conductance decreased where as proline content and leaf temperature increased significantly in most of the genotypes under water stress condition. Highest leaf rolling (score – 9) was observed in Ptb–7 and Ptb-13 while the genotypes Ptb–29 and Ptb-30 showed no leaf rolling symptoms (score – 1). Among the genotypes, the RWC was recorded to be highest in Ptb–4 while the lowest was recorded in Ptb–13 under

water stress condition. The percentage decrease in RWC compared to irrigated control was less in Ptb–15. Membrane stability index was more in Ptb–29 (98.5 %) and Ptb-10 (98.1 %) as compared to other genotypes under water stress condition. Maximum leaf temperature was observed in Ptb–1(31.7^{0}C) and minimum in Ptb–7 (27.8^{0}C) under water stress condition. Among the genotypes, stomatal conductance was recorded to be highest in Ptb–30 (674 m moles m^{-2} s^{-1}) while the lowest in Ptb–20 (92 m moles m^{-2} s^{-1}). The photosynthetic rate decreased significantly under water stress condition with maximum in Ptb–30 (15.2 μ moles m^{-2} s^{-1}) and minimum in Ptb–6 (3.4 μ moles m^{-2} s^{-1}). Under water stress condition, maximum transpiration rate was observed in Ptb–4 (1.4 m moles m^{-2} s^{-1}) and minimum in Ptb–31 (0.05 m moles m^{-2} s^{-1}). Proline content increased at 50% flowering stage in water stress condition with maximum accumulation in Ptb–27 and minimum in Ptb–22.

At flowering stage highest root length was noticed in Ptb-15 and lowest for Ptb–3 and Ptb-8 under water stress condition. Root volume differed significantly in several genotypes with maximum in Ptb–21 and minimum in Ptb-31. Root dry weight decreased in water stress compared to control in most of the genotypes with highest in Ptb-13 and lowest in Ptb-31. Root shoot ratio was found to be highest in Ptb-29 and Ptb-30 and lowest in Ptb-31.

The plant height at maturity was observed to be highest in Ptb-1 and lowest in Ptb-34 under water stress condition. Days to 50% flowering reduced in most of the genotypes under water stress condition compared to irrigated control. Productive tiller number of most of the genotypes significantly reduced in water stress condition with maximum reduction in Ptb-28. Maximum yield under water stress was recorded in Ptb-55 and minimum in Ptb-21. Number of filled grains and unfilled grains were observed as major attributes affected drastically under water stress condition. The spikelet fertility percentage was highest in Ptb-25 and lowest in Ptb-1. Ptb-28 had maximum 1000 grain weight under water stress and minimum was for Ptb-17.

Correlation study revealed that grain yield per plant under water stress condition was positively correlated with parameters such as relative water content, membrane stability index, proline content, stomatal conductance, photosynthetic rate, transpiration rate, root length, root shoot ratio, spikelet fertility % and 1000 grain weight where as negatively correlated with leaf temperature, leaf rolling score, root volume, root dry weight, plant height, days to 50% flowering and panicle length.

In Bulked Line Analysis, out of the 150 microsatellite primers screened only one marker *i.e.,* RM 474 showed polymorphism between the tolerant and susceptible bulks. The same primer showed similar product size (252bp) among the individual lines which constituted respective bulks.

In summary, there was significant variation for physio-morphological and yield components among rice genotypes under water stress condition. Genotypes having higher root length and root shoot ratio were found to be tolerant to drought. The genotypes identified as drought tolerant *viz* Ptb-29, Ptb-30, Ptb-15, Ptb-1, Ptb-55 etc. can be used in breeding programmes to improve drought tolerance in rice. Microsatellite marker RM 474 which could distinguish drought tolerant and susceptible bulks can be used for marker assisted selection for drought tolerance in rice.