PHYSIOLOGICAL AND ANATOMICAL PLASTICITY OF ROOT TRAITS UNDER WATER STRESS AND MOLECULAR CHARACTERIZATION USING ROOT SPECIFIC GENES IN RICE (*Oryza sativa* L.)

by

CHENNAMSETTI LAKSHMI NAGA MANIKANTA.

(2017-11-100)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2019

DECLARATION

I, hereby declare that this thesis entitled **"Physiological and anatomical plasticity of root traits under water stress and molecular characterization using root specific genes 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.

Ch. L. N. Manikanta

Vellayani, **CHENNAMSETTI LAKSHMI NAGA MANIKANTA.**

Date: 6/8/19 (2017-11-100)

CERTIFICATE

Certified that this thesis entitled **"Physiological and anatomical plasticity of root traits under water stress and molecular characterization using root specific genes in rice (*Oryza sativa* L.)"** is a record of research work done independently by Mr. Chennamsetti Lakshmi Naga Manikanta under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellayani,
Date: 6/8/19

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

CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Chennamsetti Lakshmi Naga Manikanta a candidate for the degree of **Master of Science in Agriculture** with major in Plant Physiology, agree that the thesis entitled **"Physiological and anatomical plasticity of root traits under water stress and molecular characterization using root specific genes in rice (*Oryza sativa* L.)"** may be submitted by Mr. Chennamsetti Lakshmi Naga Manikanta, in partial fulfilment of the requirement for the degree.

Dr. Beena, R.
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. M.M. Viji
Professor and Head
Department of Plant Physiology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Roy Stephen
Professor
Department of Plant Physiology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Swapna Alex
Professor & Head
Department of Plant Biotechnology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

External Examiner

ACKNOWLEDGEMENT

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

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

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

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

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

I am extremely grateful to the member of advisory committee, ***Dr. Swapna Alex*** *Professor & Head, Department of Plant Biotechnology for her valuable suggestions and cooperation during the course of present investigation.*

I am thankful to my classmates ***Sayooj S, Lakshmi G Ajay, Raghunath MP, Arya SR*** *and* ***Ammu Alphonso John*** *for their friendship and kind help in times of need.*

I acknowledge the boundless affection, unsolicited help, companionship and moral support rendered by my friends ***Santosh, Vinod Kumar, Suresh, Pavan, Amal, Murgesh, Chandran*** *whom I admire a lot. I warmly remember their role in making the period of my study here a memorable and cherished one. Also, I am thankful to my friends* ***Sowndarya, Yamini,***

Harisha, Bhavana, Zeba, Arathy, Monisha, Golmei *for their love and support during my PG programme.*

My special thanks goes to my entire friends and seniors from my department whom I must name individually; ***Srikanth sir, Gayatri chechi, Yogesh sir, Nithya Chechi, Manasa Akka, Afna chechi, Stephen sir, Saranya Chechi, Sachin sir, Vijay sir, Vipin Sir, Anila chechi, Beena chechi, and Binu chettan.***

I am thanking my juniors ***Bhavana, Amrutha, Sree vardhan, Sree Rag and Sudha rani, Sarath, Rama Mohan, Alekhya, Pravallika*** *for their brotherly affection and kind help without which I may never have completed my research work.*

Finally my special thanks to my besties who are portraying promising roles in my journey, I thank, ***Mahi, Dinesh, Arulnidhi, Ravi, Faizul, Nive, Raghavi, Sowmya, and sruthi.***

At this time of thesis submission, I remember with pleasure the sacrifice and support from my Family, which I couldn't replace at any cost. Thank you for supporting my decisions and for being a precious source of strength throughout the course of my study here.

Once again I am thanking everyone who helped me during my research programme.....

Chennamsetti Lakshmi Naga Manikanta

CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

DES	Directorate of Economics and Statistics
DNA	Deoxyribo Nucleic Acid
Mb	Mega base
FAO	Food and Agricultural Organization
Mha	Million hectares
IRRI	International Rice Reseach Institute
IRMS	Infra-red mass spectroscopy
ψ_L	Leaf Water Potential
Kb	Kilo base pair
PEG	Polyethylene Glycol
PVC	Poly Vinly Chloride
DAS	Days After Sowing
g/m^{-2}	grams/meter^{-2}
SSR	Simple Sequence Repeats
PCR	Polymerase Chain Reaction
BLAST	Basic local alignment search tool
cM	Centimorgan
RARS	Regional Agricultural Research Station
U. S. A	United States of America
IIRR	Indian Institute of Rice Research
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
^{0}C	Degree Celsius
cm	Centimeter
g	Gram
cm^3	cubic centimetre
ml	Milliliter
µl	Microliter
µm	micrometre
mg	microgram
ng/µl	nanogram/microliter
mM	Millimolar
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

Domestication and cultivation of rice (*Oryza sativa*) were the predominant activities on the earth. There are two important cultigens in cultivated rice belonging to the family Poaceae (Gramineae), *Oryza sativa* and *Oryza glaberrima*. The first species can be seen cultivating worldwide, whereas *Oryza glaberrima* is restricted to only African countries. *Oryza sativa* complex consists of the wild and weedy relatives of both rice cultigens. All these wild and weedy relatives of *O. sativa* are found throughout the tropics. Rice is seen in more than a hundred countries i.e., in almost all the continents except Antarctica with a total area of around 158 million hectares, with 700 million tons of annual production (GRiSP, 2013). Ninety percent of global production i.e., 640 million tons are from Asia, whereas sub-Saharan Africa and Latin America contribute 19 & 25 million tons respectively (Manfuls and Acqaah, 2016).

India is having a rice growing tract of around 42.95 million ha, with a production of 112.90 million tons and the productivity is about 2699 kg/ha (DES-2018). In India rice is grown under diverse soil and agro-climatic conditions, which made rice with low productivity in India than other countries. China stood first both in production and productivity, with a production of 205.21 million ha and productivity of 6720 kg/ha. (GRiSP, 2013). The huge difference in the productivity levels of rice in India compared with China is mainly due to the reason, that in china nearly all the rice growing tracts are irrigated whereas in India not even 50% of rice cultivation comes under irrigated and major portion of rice is cultivated under rainfed condition.

Rice is a semi-aquatic crop which generally requires 2-3 folds higher water compared with other cereals such as maize and wheat (Peng *et al.*, 2006). Production of rice requires 30% of the world's freshwater. With its high water requirement, production of rice is a challenging task under the current situation where there is an increase in shortage of water resources.

As an extreme event drought severely affects the overall crop growth and productivity. In Indian subcontinent, drought has been studied since 1960s (Mallik *et al,* 1962). With regard to drought mechanism, it was identified that long term fluctuations and breaks in the southwest monsoon is the major factor responsible for severe summer droughts due to upper tropospheric blocking ridges over East Asia (Raman *et al.,* 1981).

During the period 1871 to 2002, India experienced 22 droughts of which 5 were severe. The drought of 2000 was regarded as the worst drought in past one hundred years and was termed as acute drought caused due to total failure of monsoon rain. Drought had its significant impact on agriculture, human beings, livestock and natural resources in states like Haryana, Rajasthan, Punjab, Kerala, Karnataka, Gujarat, Tamil Nadu, Uttar pradesh, Odisha and Madhya Pradesh are on records (Samra, 2004; Sagari, 2006).

Decreasing and erratic behaviour of the rainfall over the region, late onset and early withdrawal of monsoon as well as monsoon failure in Kerala lead to many drought situations. Kerala had seen severe spells of drought in 1983, 1985, 1986 and 1987, even though the state has a wet climate. During the year 1987, significant damage was recorded due to drought in Kerala (Nathan, 2000).

Drought avoidance is often more concerned with root phenes that are responsible for the better conductance of water to the shoot (Clark *et al.,* 2002; Lynch, 2014). Deep insights into architectural and anatomical phenes that contribute to rooting depth is important for better performance of crop under water stress (Lynch, 2014). The nature and characteristics of root system are considered as the major factors affecting plant response to water stress. Root length, root density and root diameter serve as measures to characterize root system development of rice cultivars. Root length density also influences the potential of water uptake (Sharp and Davis, 1985).

Several traits contributing for drought tolerance in rice have been identified (Kamoshita *et al.,* 2002). However, phenotypic selection for such desirable traits

is labour intensive and time consuming. Hence in such cases, molecular markers serve as a desirable tool in selecting such traits and track genetic loci controlling such traits, without having to measure the phenotype, thus saving time, space and labour (Nguyen *et al.,* 1997).

In rice (*Oryza sativa* L.), two QTLs influencing the root gravitropic response, which alters root growth angle (RGA), have been detected on chromosomes 6 and 10 (Norton and Price, 2009). Three major rice QTLs for RGA, namely *DRO1* (*DEEPER ROOTING 1)*, *DRO2* and *qSOR1* (quantitative trait locus for SOIL SURFACE ROOTING 1), in three different mapping populations (Uga *et al.*, 2011). *DRO1* have been detected on chromosome 9 in recombinant inbred lines (IK-RILs) derived from a cross between the shallow-rooting cultivar IR64 and the deep-rooting cultivar Kinandang Patong (Uga e*t al.*, 2011).

Higher expression of *DRO1* increases the root growth angle, whereby roots grow in a more downward direction. Introducing *DRO1* in to a high yielding shallow rooting rice cultivar by backcrossing enabled the resulting line with avoidance to drought by increasing rooting depth, which maintained high yield potential under drought condition compared to recipient cultivar (Uga *et al.,* 2013).

With these backgrounds the present study was carried out with the following objectives:

1. To evaluate the adaptive plasticity of root morphological and anatomical under water stress.
2. To identify the microsatellite markers associated with root traits for drought tolerance in rice.
3. To study the differential expression of *DRO 1* in different rice genotypes.

Review of Literature

2. REVIEW OF LITERATURE

Rice (*Oryza sativa* L.), the food for billions directly feeding nearly half of the world's population for a longer period of time than any other crop since it was domesticated between 8,000 to 10,000 years ago (Greenland, 1997). In the year 2012, nearly 3 billion people were relying on rice for their dietary requirement every day. Though rice is the second largest cultivated crop in the world, next to wheat majority of population depends on rice than wheat as their staple food. Ninety percent of total production is grown and consumed in Asia. Asia contribute to 90% of the total rice production with India and China as leading producers and the rest of the production is from sub Saharan Africa and Latin American countries (Evans, 1998).

Rice, being unique to its environment, comes in diverse conditions ranging from waterlogged situations where most of the cereals fail to germinate to rain fed conditions. Both under natural and manmade agricultural conditions, plants are prone to various stresses, be it biotic or abiotic. Water accounts for 80-95% of the fresh biomass of plants and plays an important role in plant growth, development and metabolism (Hirt and Shinozaki, 2003). Drought is a most prevalent stress factor for plants across the globe, especially in arid and semi-arid areas (Rao *et al.,* 2006). There might be several factors responsible for water deficit in plants; these include low rainfall, salinity, high intensity of light and high as well as low temperatures.

Amid of various abiotic stresses, drought is the most devastating stress and impairs various morphological parameters that can be noticed in all phenological stages of crop growth (Zhang *et al.,* 2017 and Gaspar *et al.,* 2002). Overall estimation shows that drought will impact 30% of global loss of crop yield by 2025 (Zhang, 2011). There are several reasons for water deficit in plants and these include low rainfall, salinity, high and low temperatures as well as high intensity of light. Drought, a multidimensional stress leads to changes in the physiological,

morphological, ecological, biochemical, and molecular traits of plants (Bhargava *et al.,* 2013).

Plants evoke myriad morphological and biochemical modifications at cellular-levels and whole-plant levels to ward off effects of drought. Noteworthy, among them are the three mechanisms such as (i) drought escape, (ii) drought resistance and (iii) drought avoidance (Yamaguchi-Shinozaki and Shinozaki, 2006).

Drought tolerance is the ability of plants to continue normal cellular metabolism and growth activity at low water potential despite prevailing stress condition and/or ability to recover fast after stress. A crop is considered tolerant, only if it survives drought with minimal yield penalty (Basu *et al.,* 2016).

2.1 Impact of drought on various physio-morphological and biochemical parameters:

2.1.1 Relative water content:

In the middle of the 80s, relative water content was introduced and regarded as the best criteria for plant water status. In later stages it was used in place of plant water potential as RWC referring to its relation with cell volume, and it's accuracy in indicating the balance between water absorbed by plant and lost through transpiration. Better crop growth and productivity during water limited environments depend on relative water content (RWC) and osmotic potential. Higher values of RWC and osmotic adjustment confers for better growth and development of plant (Blum, 2001). Screening for drought tolerance in rice using physio-morphological traits such as RWC, revealed that drought tolerant genotypes showed higher values of RWC than susceptible genotypes (Kumar *et al.,* 2014).

Relative water content reflects a balance between the water available in the leaf tissue and the transpiration rate. Under water stress condition tolerant species

tend to maintain high RWC compared to susceptible ones as reported by Lugojan and ciulca (2011).

Slatyer (1955), observed that the relative water content had a significant impact on photosynthesis. When relative water content falls to a level less than 80%, a reduction in net photosynthesis by more than 50 per cent was recorded.

RWC variation among different rice genotypes could be correlated with their respective variable capacity to absorb water from the soil or the differential capacity of stomata to minimize the water loss in the form of transpiration. Moreover the maintenance of higher RLWC under drought is a resistant mechanism to osmotic stress and it is as a result of more osmotic regulation or less elasticity of tissue cell wall (Kataria and Singh, 2014)

2.1.2 SPECIFIC LEAF AREA:

Specific leaf area has been shown as one of the leaf traits best reflecting the whole plant growth (Cornelissen *et al.*, 2003). It plays a vital role in linking plant carbon (C) and water cycles. SLA deals with the allocation of leaf biomass in relation with leaf area, and thus regarded as unit gain in carbon to unit loss in water, within a plant canopy (Gunn *et al.*, 1999).

SLA was found negatively related to transpiration efficiency in many plants (Virgona *et al.*, 1990). Though the mechanism behind this is not clear, one of the possible reasons could be the presence of more number of mesophyll cells in plants with low SLA resulting higher photosynthetic rate. (Thumma et al., 2001)

Dingkuhn *et al.* (2001), found that a plant is said to be efficient in terms of growth if it maintains higher SLA during early stages of growth and lower SLA during the later stages, such type of plant would have high and early tillering.

2.1.3 CELL MEMBRANE STABILITY INDEX:

Cell membrane is the first site to get damaged under any kind of stress and the tolerance to drought in any plant species can be given by its ability to maintain the integrity and stability under stress (Vassileva *et al.,* 2012). Hence it is used as one of the common indices for drought tolerance.

Blum *et al.* (1999) reported that both osmotic adjustment (OA) and increased cell membrane stability were found to improve tolerance in drought. Cell membrane stability is measured based on electrolyte leakage from leaf segments.

Tripathy *et al.* (2000) reported that CMS has been widely used as an indicator for assessing tolerance to different abiotic stresses and it had shown a promising relationship between tolerance evaluated by CMS and crop yield under certain field conditions.

2.1.4 CARBON ISOTOPE DISCRIMINATION:

Farquhar *et al.* (1983) reported that the overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less compared to the carbon of atmospheric carbon dioxide, indicating that carbon isotope discrimination occurs in the incorporation of CO_2 into plant biomass.

Carbon isotope discrimination has been identified as a precise method and technique for evaluating as well as improving water use efficiency (WUE) in C_3 plants. Carbon isotope discrimination of plants has resulted in significant progress towards understanding the influence of abiotic stresses on carbon dioxide fixation and transpiration (Ehleringer *et al.*, 1993).

Xue *et al.* (2002) observed that, wheat plants grown under less drought stress possess higher carbon isotope discrimination value. C-306 a drought tolerant genotype showed a decrease in discrimination value under water stress in comparison to control, indicating that C-306 genotype experienced more osmotic

stress compared to other genotypes. Richards *et al.* (2002) suggested a necessity of selecting low carbon isotope discrimination types under water stress.

2.2 ROOT PARAMETERS:

Plant roots play an important role in water and nutrient uptake from the surrounding soil. Shoot growth in plants is governed by the extent of physiological and morphological traits of the roots. In rice (*oryza sativa* L.), a well developed root system increases biomass and yield in different water regimes and cultivars in paddy fields (Kang and Morita, 1998).

2.2.1 ROOT LENGTH (cm):

Deeper rooting help the plants to avoid water stress conditions by enabling them to extract water from deep layers of soil (Yoshida and Hasegawa, 1982). To improve drought avoidance in high yielding shallow cultivars of rice, introducing the deep rooting in shallow cultivars is found to be a promising strategy in current breeding programs (Gowda *et al.,* 2011).

Kirkegaard *et al.* (2007) reported that, through field-based direct root and soil water measurements, an extra of 10mm deep soil water can be taken up by the plants for a 30 cm increase in rooting depth at grain development stage. Which resulted in a 0.5 t of grain yield per hectare. Root system with greater proliferation and depth, has a profound influence not alone on transpiration through increased utilization of soil moisture but also shoot biomass production and harvest index (HI) under drought stress.

Uga *et al.* (2013) reported yield difference in two genotypes having contrasting root system, IR 64 a shallow rooting cultivar and DRO1-NIL a deeper rooting one. Yield was higher in case of DRO1- NIL than IR64 mainly due to deeper rooting by DRO1 facilitating better photosynthesis and grain filling.

2.2.2 ROOT VOLUME (ml):

Sandu *et al.* (2013) reported from a phenotypic correlation coefficient analysis of mapping population obtained from a cross between MASARB25 × Pusa Basmati 1460, showed that the Root volume is positively and significantly correlated with yield attributes i.e., panicle length (0.358, p = 0.01).

According to Yoshida and Hasegawa (1982), traits like growth of roots in rice, particularly in terms of total root dry matter, rooting depth, number and volume was found to increase till flowering and then a sharp declining trend was observed towards maturity.

Senthilkumar *et al.* (2017) reported that among the different varieties of tomato selected to study the effect of water mediated stress, PKM-1 recorded higher root volume both at 20 and 40 days after planting followed by Arka Vikas and Arka Meghali. The varieties PKM 1 and Arka Vikas were found to be tolerant and they could perform better under water limited conditions, whereas the adaptive plasticity of Arka Meghali needs further investigation.

2.2.3 ROOT DRY WEIGHT (g):

Boutraa *et al.* (2010) reported that, when a set of wheat cultivars were grown under mild (50%) and severe (30%) water stress conditions there was a significant change in the dry weight of roots. In susceptible wheat genotypes sindy-1 and sindy-2 there was a decrease noted in root dry weight under both water regimes but in contrast to this there was an increase in root dry weight in Al- gaimi a tolerant genotype under mild water stress and there were no significant changes noted under severe water stress suggesting that this particular Al- gaimi genotype had a higher degree of tolerance towards water stress.

Mumbani and Lal , (1983) reported that root traits such as rooting depth and total root dry weight were the best indicators for drought avoidance in upland rice. Genotypes with greater root system have chance to explore larger volume of soil

27

and improve the water uptake from deeper layers of the soil, this strategy adopted by the genotypes would help in maintaining good water potential which had a positive effect on yield under water limited conditions.

Rejeth (2017) conducted experiments on evaluating the role of root traits for drought tolerance in rice and reported that, under water stress conditions some of the cultivars have shown an increase in total root dry weight in comparison with irrigated control. Genotypes such as Ptb-7, Ptb-10 and Ptb-55 have shown an increase in total dry weight and found to be linked with the ability of these genotypes in maintaining high leaf water potential under stress.

2.2.4 ROOT SHOOT RATIO:

Root to shoot ratio and shoot to root ratio are often used as indices to reveal the relative biomass partitioning between shoot and root (Gowda *et al.*, 2011). As proposed by Wilson (1988), there are several factors that govern the relative biomass allocation between shoot and root, such as water availability in different depths of soil, availability of major nutrients, rate of photosynthesis, light, and carbon dioxide. Distribution of carbohydrates among root and shoot may also be associated with corresponding changes in shoot and root.

Li *et al.* (2009) proposed that prolonged periods of drought limits the process of photosynthesis, growth of the plant, productivity and alters the biomass allocation patterns between shoot and root.

Xu (2015) conducted an experiment to reveal underlying mechanism for root shoot plasticity under water stress of two rice cultivars , Zhenshan 97 (drought susceptible) and IRAT109 (drought resistant). Drought stress was imposed hydroponically with polyethylene glycol 6000. Results suggested that there was a significant increase in root shoot ratio in tolerant cultivar under stress compared to its well irrigated control accompanied by proportion of dry matter and soluble sugar of roots markedly increasing under water stress.

Rich and Watt (2013) proposed that plants, during water limited conditions often re-allocate their assimilates from shoot to root, thereby increasing in root extension enabling the plants to explore more volume of soil.

2.2.5 SPECIFIC ROOT LENGTH (cm g^{-1}):

Specific root length is the most commonly measured morphological parameter of root in conditions of water stress since it is believed to characterize the economic aspects of root system. Plants with smaller root diameter as well as smaller specific root length are better adapted to the drying situations (Henry *et al.*, 2012).

2.3 BIOMASS PARTITIONING AMONG LEAF, STEM AND ROOT:

Azhiri-Sigari *et al.* (2000) reported that, under osmotic stress condition induced by polyethylene glycol in rice, an increase in assimilate partitioning was noticed towards root, whereas an increase in assimilate partitioning towards shoot was noticed when the plants were exposed to low humidity conditions. In case of rain fed lowland rice ecosystem, though there was a decrease in assimilate partitioning towards root due to non-availability of photosynthates. The proportion at which the assimilates attributed towards deeper layers increases.

Leaf weight ratio (g g^{-1}) is expressed as the dry weight of leaves to whole plant dry weight (Kvet *et al.*, 1971). Bhagirath (2013) conducted an experiment on rice and two weed species *A. spinosus* and *L. chinensis* maintaining at different field capacity i.e., 12.5%, 25%, 50%, 75%, and 100%, and found *A. spinosus* plants showed tolerance to water stress by increasing leaf weight ratio, whereas the other two i.e., rice and *L. chinensis* showed decreased leaf weight ratio with increased water stress.

Van der Werf *et al.* (1993) based on various conceptual and mathematical models, proposed that in response to given water stress condition plants adapt by adjusting their root weight ratio in order to maximize relative growth rate. Kadam

et al. (2015) conducted experiments on wheat and rice to evaluate the adaptive plasticity of roots and found that there is no significant difference among the genotypes and treatments for root weight ratio, but tolerant rice genotype 'N22' had shown an increase in root weight ratio under water deficit conditions and similar trend was seen in wheat genotypes also reported a variation in leaf and stem weight ratio with a significant effect of drought stress was noticed. From their findings they reported a decrease in leaf weight ratio by 16% and increase in stem weight ratio by 24% under water stress condition from a susceptible rice cultivar ' IR 64', whereas in tolerant rice 'N-22' the biomass partitioning was not altered much by water deficit condition but they noticed an increased trend in root weight ratio.

2.3.4 STARCH ACCUMULATION IN ROOTS:

Starch being an energy constituent and energy reserve found in various parts of the plant, play a crucial role in the development of tolerance against various biotic and abiotic stresses.

Yambao (1992) reported during stress, grain growth is solely relied on reserved food material mainly carbohydrates accumulated before onset of stress and the actual amount of its remobilization. For avoiding such a situation the tolerant rice cultivars accumulate significant amount of carbohydrates before heading, and wide variability exists among genotypes for these characters.

Perez (1971) reported that drought tolerant and susceptible rice genotypes differs in accumulation of starch. Drought tolerant varieties had a higher starch content in comparison with susceptible varieties around the vascular bundles and periphery region of roots after drought. When rice crop undergoes water stress at the flowering stage, substantial quantities of carbohydrates accumulate in different parts of the rice plants.

Singh (2013) conducted an experiment with four drought tolerant and four susceptible cultivars maintaining at two different conditions, viz., control at full irrigated conditions whereas stress was imposed 60 days after planting. Results

revealed that Sita and BPT5204 showed least level of starch accumulation in their roots whereas 'N22' a tolerant cultivar showed the highest level of starch accumulation under drought conditions among all the tested varieties.

2.3.5 PROTEIN PROFILING FROM ROOTS USING SDS PAGE:

Chandler and Robertson (1994) stated that when a plant undergoes water stress numerous physiological and biochemical changes happen in response to drought. The responses varies with plant species classifying them into drought tolerant and susceptible species. Alteration of protein synthesis is one of the most basic metabolically stimulated processes that influence tolerance towards drought.

Close *et al.* (1989) named proteins that were accumulated as a result of water stress as DHN. Now these DHN's are classified based on their homology rather than their expression characteristics in addition to being produced during the later stages of embryogenesis and in seeds.

Singh *et al.* (2015) reported changes in protein bands of SDS-PAGE differentiating tolerant and susceptible varieties under water stress condition. N22 a tolerant cultivar alone had shown a novel protein band of size 39 ± 2 kDa. Three to four bands of range 66–90 kDa were found in tolerant varieties whereas susceptible varieties had shown no such bands also a common protein band of 55 ± 2 kDa was found in both tolerant and susceptible varieties.

2.4 ANATOMICAL PLASTICITY OF ROOTS UNDER WATER STRESS:

Physiological makeup of the cell and anatomy characters that are involved in transmission of water deficit effect to the cells determine the plant tissue responses to water stress (De Micco *et al.*, 2012).

A strong selective pressure, acted on the species that are growing under flooding and water deficit conditions to develop the ability to cope up with the kind of stress imposed by adjusting their anatomy and physiology (Bramley *et al.*, 2009).

Roots are the only point in the plant system for the entry of water and mineral nutrients. This radial and axial movement of water is being influenced by various root anatomical phenes, which are expected to increase the efficiency of water uptake of plants (Lynch, 2014). Axial conductance of water gets affected by xylem vessel traits whereas cortical and the presence of suberized cell layers affects the radial conductance (Gowda *et al.*, 2011). Pitman *et al.* (1983) reported that tissues exposed to water deficit conditions tend to show a reduction in cell size and an increase in vascular tissue thickness and lignification.

2.4.1 ROOT DIAMETER (mm):

Van der Weele *et al.* (2000) conducted experiments to evaluate the role of root traits under water deficit condition in Arabidopsis *thaliana* and reported the following results. After exposing the plants to water stress there is an increase in root diameter from the first day observations. Subsequent results have shown that there was overall thickening in roots in all the water deficit treatments except the most severe one (-0.23MPa) compared with control.

Root diameter had a profound effect in mitigating drought stress, possession of large root diameter enabling the plant roots to penetrate deeper in search of water (Cook *et al.,* 1997).

Fitter (2002) proposed that in plant roots, root diameter is responsible for regulating root length, surface area and increased water uptake under drought. Bengough *et al.* (2011) stated that the large root diameter is advantageous in drying soils, as the drying soils often become harder and the large root diameter gives the ability to penetrate hard soil.

Kadam *et al,* 2015 conducted an experiment on rice cultivars under different water regimes and reported about variations in root diameter. According to Kadam *et al* experiments there was a decrease in root diameter at shoot root junction in drought susceptible varieties i.e., 'IR 64', but there was no significant changes in root diameter in case of tolerant upland varieties Apo and N22.

2.4.2 STELE DIAMETER:

Chimungu *et al.* (2015) proposed that stele anatomical phenes has the capacity to influence root tensile strength, whereas cortical traits influence root bending strength and penetration of root.

Purushothaman *et al.* (2013) studied root anatomical traits and their possible effect towards mitigating the water stress in legumes and reported that the development of stele tissue diameter and root cortical and their proportion significantly influenced the relative plant growth under moisture deficit conditions.

Kadam *et al.* (2015) from his experiments on drought susceptible rice and drought tolerant wheat cultivars made the following observations on stele diameter at root shoot junction. He found that, under stress there is no significant change in stele diameter of two susceptible rice cultivars i.e., IR 64 and Apo, but the tolerant cultivar N22 had shown an increase in stele diameter not at root shoot junction but at 10-15 cm away but in case of wheat cultivars 'SeriM82' had shown an increase in stele diameter at root shoot junction than 'Weebill4'.

2.4.3 XYLEM VESSELS:

Gowda *et al.* (2011) reported that xylem vessel traits such as number, diameter and area affects the axial conductance of water. Presence of thicker roots and larger xylem vessels are considered as a characteristic feature of upland rice and involved in conferring tolerance towards drought.

2.4.3.1 LATE METAXYLEM NUMBER AND DIAMETER:

Kondo *et al.* (2000) conducted experiments on tropical upland and lowland rice varieties and made the following observations on their root anatomy. Kondo *et al.* (2000) stated that traditional upland japonica varieties exhibited largest xylem vessel. Xylem diameter was found to be highest in traditional japonica varieties followed by IRAT216 and IR65598 but in case of indica varieties 'aus' and upland varieties have larger xylem diameter in comparison with low land indica cultivar.

Kadam *et al.* (2015) from his experiments on drought tolerant wheat cultivars and drought susceptible rice cultivars reported root diameter, stele and xylem diameter, and xylem number were found to be more responsive and varying with different positions in nodal roots under water limited conditions in wheat, whereas such variations are relatively conserved in rice cultivars. Tolerant wheat cultivars had shown an increased late metaxylem diameter and reduced metaxylem number near the root tips and decreased metaxylem diameter and increased metaxylem number at root shoot junction facilitating the efficient acquisition and use of available soil moisture but in tolerant rice cultivar i.e., N22 it was observed that compared to other two rice cultivars, IR64 and Apo had an advantage in root morphological and anatomical attributes but lacked plasticity at different positions throughout the root length like in case of wheat under water stress condition.

2.4.3.2 EARLY METAXYLEM NUMBER:

Weerathaworn *et al.* (1992) stated that there exists a constant relationship between the number of cells assigned for the formation of early and late metaxylem number within a cultivar. But under exceptional cases such as drought the number and diameter of these specific metaxylem vessels are somewhat responsive to the supply of water.

2.4.4 WIDTH OF SCLERENCHYMA:

Henry *et al.* (2012) conducted experiments on six diverse genotypes for anatomical plasticity under water stress and reported that under drought conditions suberization and compaction of sclerenchymatous tissue decreased, whereas endodermis suberization was found to be increasing. These observations suggest the differential roles of sclerenchyma and endodermis for retention of oxygen under flooded condition and retention of water under drought condition respectively. They also reported that there is an increase in sclerenchymatous tissue diameter under drought as there is no need for retention of oxygen under drought and the cells are loosely packed resulting the increase in diameter.

Suberization of sclerenchyma was found to decrease upon incidence of water stress as the role of suberization of sclerenchymatous tissue is to reduce the radial loss of water in irrigated conditions. But under water stress conditions as available water in plant system is less, suberization of sclerenchymatous tissue is not required.

2.4.6 WIDTH OF AERENCHYMA:

An interconnected network of air channels i.e., aerenchyma present in root cells provides a space for diffusion of gases enabling the plant to maintain the aerobic respiration and rhizosphere oxygenation. When a plant is grown in flooded conditions i.e., in oxygen deficit conditions, increased aerenchyma is a common adaptive mechanism shown by plants (Jackson and Armstrong, 1999).

Zhu *et al.* (2010) reported that an increased formation of root cortical aerenchyma in maize inbred lines is associated with reduced root respiration, thereby utilizing the conserved metabolic energy in improving the rooting depth for water acquisition, leaf water status, increasing the plant biomass and finally a sustainable increase in yield.

In the case of rice *Oryza sativa* .L formation of aerenchyma under flooded conditions is mainly due to ethylene mediated cell lysis and cell deflation. However additional production of aerenchyma at root cortex region is found to be associated with consumption of mechanical strength needed to resist natural and anthropogenic soil attributes (Engelaar *et al.*, 1993). This aerenchymatous tissue formation is specific to genotypes.

Kundur *et al.* (2015) conducted experiments on rice varieties under water logged and water stressed conditions and found that aerenchymatous tissue formation is more in case of water logged condition than in stressed condition. This is mainly because of plant phenotypic plasticity in response to environmental conditions to survive. Though the exact reason behind the formation of aerenchyma

is not clear, it is speculated as a strategy to kill the non-photosynthesizing cells of root thereby reducing the metabolic energy needed for their maintenance.

2.4.7 STELE DIAMETER TO ROOT DIAMETER:

Guo *et al.* (2008) reported a close association between root diameter and stele diameter, than with the thickness of cortex in 23 Chinese crop species when imposed with water stress.

Kadam *et al.* (2015) conducted experiments on wheat cultivars and low land rice cultivars and reported that the Stele diameter to root diameter was strongly influenced by water deficit conditions in rice and have not shown any variations at tissue position on nodal roots of the rice cultivars. In contrast to this wheat cultivars exhibited a significant variation along the root tissue position in relation with treatment. Wheat cultivars exhibited higher stele diameter to root diameter in comparison with rice cultivars under water deficit condition.

2.5 GROWTH AND YIELD PARAMETERS OF RICE:

2.5.1 PLANT HEIGHT:

Yeo (1998), stated that the drought stress conditions in plants reduces the metabolite activity in plant cells due to non-availability of water and such a condition affects the cell division and elongation of plant cells thereby reducing the plant height.

Singh *et al.* (2018) conducted experiments on five different rice genotypes imposing water stress at 35 DAT and at reproductive stages for 10 days and reported a reduction in plant height under water stress condition irrespective of the genotype but the percent reduction is different among tolerant and susceptible varieties. Highest reduction in plant height has been recorded from Swarna Sub 1 which is around 49.3% whereas low reduction in plant height was noticed from Nagina 22 (17.56%).

Singh *et al.* (2010) evaluated six generations (P1, P2, B1, B2, F1 and F2) of six crosses of rice under irrigated and water stressed condition and found a reduction in several characters including yield traits under water stress condition. They reported an average decrease in plant height from 107.31 cm under irrigated condition to 92.00 cm under water stress condition.

2.5.2 DAYS TO 50% FLOWERING:

Mahalakshmi and Bidinger (1985) reported that the process of apical morphogenesis is sensitive to water deficit and drought stress during flower induction and development of inflorescence leads to delay in anthesis or even complete inhibition of flowering.

Lafitte *et al.* (2003) states that a delay in flowering occurs when the plants are exposed to water stress prior to flowering.

In rice, the duration of delay can be partly related to the extent of stress and the genotypes which when experienced with water stress and with longer delay in flowering will tend to produce lesser grain weight compared to the genotypes that shows not much delay in flowering under water deficit condition (Ravindrakumar *et al.*, 2003). Hanamaratti and Salimath (2012) conducted experiments on association of delay in flowering and drought stress tolerance in upland rice and concluded that any incidence of drought during vegetative stage and just prior to flowering stage have a profound impact on flowering whereas water stress at reproductive stage does not affect the flowering much. From the genotypes under study, susceptible genotypes like, IR- 64 shows more delay in flowering followed by IB NILs and least delay in flowering by TB NILs indicating that TB NILs are least affected by drought stress.

2.5.3 TILLER NUMBER:

Singh *et al.* (2018) reported the reduction in tiller number in all the genotypes of rice studied when they were exposed to water stress but the extent of

37

reduction of tiller number under water stress showed difference between tolerant and susceptible genotypes. Maximum reduction in tiller number was recorded in Swarna sub 1 (25. 82%) while minimum in Nagina (8.76%).

Parfitt *et al.* (2017) reported that the number of tillers per plant got affected by water stress at only vegetative stage but not at reproductive stage. A reduction of 30 and 48% was noticed when water tensions of 100 and 200 kPa were maintained respectively. Recovery under 100 kPa was noticed when the stress is withdrawn whereas under 200 kPa no recovery in tillering was noticed even after the stress condition was withdrawn.

2.5.4 PRODUCTIVE TILLER NUMBER:

Ahmadikhah and Marufinia, (2016) reported that during drought, reproductive stage of rice is more sensitive and shows a reduction in total dry matter production. Reduction in tiller as well as productive tillers were noticed under water stress condition in susceptible genotypes whereas in tolerant genotypes in spite of drought stress, have more number of panicles per plant were noticed indicating the increased number of productive tillers under drought.

2.5.5 PANICLE LENGTH:

Muthurajan *et al.* (2011) stated that there will be a decrease in yield components such as panicle length, primary and secondary branches per panicle, fertility percentage, seed setting, and test weight of grain under water deficit conditions.

2.5.6 YIELD PER PLANT:

Drought is the major environmental constraint that severely affects the rice growth. Especially in rain fed ecosystem it severely curtails the biomass production and yield. Drought stress prevents the crop from reaching the genetically determined theoretical yield (Begg and Turner, 1976).

Singh *et al.* (2018) reported a variation in yield among the rice genotypes under control and water stressed condition. Swarna *sub 1* being a susceptible genotype showed a reduction in grain yield of about 46.07% whereas tolerant genotypes Nagina and NDR had shown the least reduction in grain yield by 19.71 and 20.32% respectively. Drought stress significantly decrease the grain yield /plant in reproductive stage.

Pantuwan *et al.* (2000) reported a decrease in rice yield up to 81% under water stressed condition and this mainly depends on timing, duration and severity of the plant water stress.

2.5.7 SPIKELET FERTILITY PERCENTAGE:

O'Toole and Moya, (1978) reported in rice, water deficit at the time of anthesis results in the failure of panicle exerting from the flag leaf. This reduced exertion of panicle was shown to be governed by plant water status.

Jongdee *et al.* (1998) conducted experiments on genotypic variation for grain yield in response to water stress at flowering stage and reported that the genotypes whose leaf water potential is low at flowering under water deficit tend to have higher spikelet sterility.

2.5.8 1000 GRAIN WEIGHT:

Akram (2011) conducted an experiment with different rice genotypes to study their tolerance to drought and found that the number of grains per panicle was higher in case of Inqlab-91 than Uqab-2000. When these varieties are exposed to different water stress levels, 1000 grain weight was differing significantly among them. Control plants i.e., plants without any water stress recorded the highest 1000 grain weight, whereas 1000 grain weight in plants imposed with drought stress at stem elongation performed better compared with plants imposed with stress at anthesis as well as both stages. At genotypes level Inqlab-91 had higher 1000 grain weight than Uqab-2000.

Zubaer *et al.* (2007) conducted drought studies on three aman rice genotypes of Bangladesh by maintaining them at 100%, 70% and 40% FC and the results indicate that the size of the grain was found to be decreased with increase in levels of water stress from 100% to 40% FC. Implies that 1000 grain weight decreases with decrease in soil moisture levels. This might be due to decrease in translocation of assimilates to the grain under water stress which lowered 1000 grain weight. Along with the water stress genotypes also have an impact on 1000 grain weight. Percent reduction was lower in Binadhan 4 (4.14 to 6.37%) than in Basmoti (6.75to 12.5%) and RD 2585 (4.57 to 14.64%).

2.6 SCREENING OF GENOTYPES FOR DROUGHT TOLERANCE USING MOLECULAR MARKERS (SSR)

Knowledge about the presence of genetic variation among and within the plant populations, their structure and level of expression all these can play a beneficial role in the efficient exploration of plants. (Cole, 2003).

To study the diversity and occurrence of other important traits, agronomic and morphological parameters have been used successfully. During the past few decades advancements in the field of molecular genetics and rapid increase in knowledge about plant genome sequences and the associated role of various plant genes, has revolutionized the molecular genetics and its efficiency in breeding programmes (Hamrick, 1989).

GENETIC MARKERS:

Genetic markers are important tools for the advancements in the field of plant breeding. These genetic markers are a gene or sequence of DNA which are in close association with a known chromosome location controlling a particular gene or trait. Genetic markers act as a flag or a sign to the target gene.

APPLICATION OF MOLECULAR MARKERS:

Molecular markers are widely used in tracking of loci and genome regions in crop breeding programs. A great number of molecular markers that are associated with drought tolerance were available in major crops (Phillips and Vasil, 2001and Jain *et al,* 2010) majority of these molecular markers have been isolated from genomic DNA libraries.

These molecular markers are essential for mapping a gene of interest, marker assisted selection and mapping based cloning strategies- for cloning of genes and gene introgression (Hayashi *et al*, 2004).

MICROSATELLITE MARKERS (SSR):

In rice more than 2500 microsatellite markers have been developed and used to construct a genetic map (Mc Couch *et al.*, 2002). The technical efficiency and multiplex potential of SSRs makes them preferable for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies (Coburn *et al,* 2002), (Cregan *et al,* 1999) and (Mc Couch *et al,* 1997).

SSR markers being co-dominant and multiple allelic can be used reliably in analyzing both *Indica* and *Japonica* rice germplasm, making SSR markers attractive as genetic markers and facilitates the integration of results from independent studies (Chen, 2002).

2.6.1 SCREENING OF RICE GENOTYPES FOR DROUGHT TOLERANCE USING SSR MARKERS:

Gowda *et al.* (2011) stated that extraction of water from deeper layers of soil can be made possible only through the development of deeper root system. To improve avoidance to drought in rice, introgression of QTL responsible for deeper rooting into high yielding shallow cultivars is the most promising strategy.

Kamoshita *et al.* (2002) reported RM212 involved in deeper rooting in rice, increased root thickness, root dry weight and deep root per tiller in CT9993/IR62266 DH lines.

Beena, (2005) reported three SSR markers (RM212, RM302, RM3825) associated with QTL located on rice chromosome 1 that are found to be linked to drought resistance traits and these findings can be employed in breeding programs using marker assisted selection for drought tolerance.

Kanbar and Sashidhar, (2011) reported that the SSR markers RM 472, RM7 and RM201 were found to be associated consistently with maximum root length across the generations. They also reported the linkage of RM 472 and RM7 consistently to root dry weight in F2 and F3 generations.

Boopathi *et al.* (2012) reported from Bulked Segregant Analysis (BSA) that RM27933 as a marker linked to the yield under drought stress in field conditions and is found to be located *qtl12.1* (Bernier *et al.,* 2007).

Deshmukh *et al.* (2018) conducted genotyping of 49 diverse accessions of rice using 599 polymorphic SSR's and reported the following findings. The markers PSM52 located on chromosome 3, RM6909 located on chromosome 4, RM 242 and RM 444 located on chromosome 9 were found to be associated with root traits and grain yield under water stress conditions. Also it was reported that the marker PSM127 located on chromosome 3 and PSM133 located on chromosome 4 were in close association with plant height, spikelet fertility percentage and yield.

Kanagaraj *et al.* (2010) carried BSA to identify the markers associated with tolerance towards drought using 23 RILs (recombinant inbred lines) of rice cultivars IR20/Nootripathu. A set of 1206 SSR primers were used for the study of which 134 pairs showed polymorphism among parents. Of this 134, only three viz., RM212, RM302 and RM3825 were found to be polymorphic between the bulks. All the three primers were located on rice chromosome 1 and are found to be associated with drought tolerance in rice. The QTL to which these markers were

linked were found to be associated with drought resistance traits such as deep root to shoot ratio, basal root thickness, root biomass, tiller number, biomass, plant height, leaf drying and relative water content.

2.6.2 QTL's ASSOCIATED WITH DROUGHT TOLERANCE IN RICE:

QTL (Quantitative Trait Loci) are the regions within genomes that contain genes associated with a particular quantitative trait. QTLs associated with drought response mechanisms have been identified with different traits as root characters, membrane stability, osmotic adjustment and morphological and physiological traits where tolerance is measured as yield under drought.

Lilley *et al.* (1996) reported a QTL associated with osmotic adjustment under water stress on chromosome 8 of rice. Price *et al.* (2002) identified 18 QTLs associated with drought avoidance in rice from all the chromosomes except 9. Li *et al.* (2003) and Courtois, (2003) reported QTLs associated with root and root related traits in all chromosomes of rice.

Babu *et al.* (2015) from their research on Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance, reported QTLs associated with water stress indicators, phenology and production traits.

Nguyen *et al.* (2004) conducted an experiment using 85 markers (50 RFLPs, 5 SSRs, 12 DD cDNA's, 9 ESTs, 8 HSP-encoding cDNA's and one BSA-derived AFLP) for saturation mapping of QTL regions for drought tolerance in drought, to find the candidate genes involved conferring tolerance under stress. Results have shown that one QTL region governing genes responsible for osmotic adjustment on chromosome 3 and 14 and that affect root traits were found on chromosome 1, 2, 4, 5, 6, 7, 8, 9, 10 and 12.

2.7 Expression of genes related to tolerance towards water stress:

Degenkolbe *et al.* (2009) reported in rice that drought stress significantly induced 413 genes and repressed 245 genes. Most of the genes that are induced by water stress are the genes that are coding for metallothionein like protein, and late embryogenesis abundant protein. Three genes that are encoding for serine/threonine protein kinases were found to be highly induced by drought stress. Five genes encoding for cytochrome P450 family were also found to be highly induced by water stress.

Uga, (2011) reported *DRO1* allele of kinandang patong found to be increasing the RDR (Ratio of deep rooting) in the hydroponic cultures and pot cultures, and the line that is homozygous with *DRO1* allele of kinandang patong showed primarily downward rooting. *DRO1* being negatively regulated by auxin and is involved in cell elongation at root tip causing asymmetric root growth and ultimately bending of roots in response to gravity. Higher expression of *DRO1* increases the root angle and thereby enabling the roots to grow downward making it possible for the plant to tide over water deficit conditions by exploiting water from deeper soil layers.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Physiological and anatomical plasticity of root traits under water stress and molecular characterization using root specific genes in rice (*Oryza sativa*)" was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani during 2017-19 with the objective to quantify the adaptive plasticity in root-shoot morphology and physiology, root anatomical plasticity under water stress in selected rice genotypes and molecular characterization using root specific genes. The details of the materials used and methods adopted for the experiment and molecular work protocols followed during the course of experimentation are described in this chapter.

3.1 Evaluation of selected genotypes for adaptive plasticity in root-shoot morphology, physiology and anatomy under irrigated and stressed condition:

3.1.1 Plant materials

The rice accessions used in the present study consists of released rice varieties collected from RARS, Pattambi and Nagina -22 collected from IIRR (Table 1).

3.1.2 Location

The experiment was conducted in Department of Plant Physiology, College of Agriculture, and Vellayani during 2017-19 (Plate 1)

Plate 1: General view of experimental unit.

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

3.1.3 Experimental details

Table 1. List of rice accessions used in the study

S.No	GENOTYPE	S.No	GENOTYPE
1	Nagina-22	4	Annapoorna (Ptb 35)
2	Karuthamodan (Ptb 29)	5	Jyothi (Ptb 39)
3	Chuvannamodan (Ptb 30)	6	Swetha (Ptb 57)

1. Crop	Rice : 6 genotypes
2. Design	Factorial-Completely Randomized Design (CRD)
3 .Number of treatment	Two 1.50% FC throughout the growth period. 2. Control at 100 % FC.
4. Replication	Five

Table 2. Particulars of pot culture experiment

3.1.4 Methodology

In this study, plants were raised in pots of 30 cm height and 25 cm width in rainout shelter. A set of five replication were maintained for each variety under two water level treatments, 100 % and 50% field capacity were maintained regularly in control and stressed plants respectively by following gravimetric method as described by Fontenelli *et al,* (2016). At booting stage observations on root, physiological, biochemical and anatomical parameters were made. Plants were maintained up to maturity at same moisture levels and morphological and yield parameters were made at harvest.

3.1.5 Preparation of potting mixture and transplanting

Pots were filled with potting mixture of around 8 kg potting mixture was prepared by mixing of soil, sand and FYM in the ratio of 3:2:1. Seeds were sown in portrays of 100 cell size filled with coir pith and FYM in 1:1 ratio. Depending on the duration of varieties transplanting was done. Nagina 22 transplanted at 12 days after sowing whereas the rest of the genotypes were transplanted at 18 days after sowing at the rate of two seedling per pot. Thinning was done on 8 days from transplanting and one healthy seedling was maintained in each pot. Foliar application of 19:19:19 was given on 2 weeks after transplanting. Crop was maintained with recommended dose of fertilizers 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 Relative water content (RWC)

Relative leaf water content was measured based on the method described by Turner (1981). RWC measurement was taken from fully expanded leaves. A

known weight of the sample was taken, and then the leaves were immersed in distilled water for about 2 hours. After 2 hours, the leaves were removed from water and the adhering water was blotted off and the turgid weight was recorded. The samples were dried in oven at 70°C for about 48 hours and 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.

3.1.6.1.2 Specific leaf area:

Specific leaf area calculated from plants under 100% and 50% FC soil moisture by selecting a fully expanded leaf. The area of leaf was recorded with the help of graphical method. Leaf sample was oven dried at 70°C for about 48 hours till constant weight was obtained. Specific leaf area was calculated from the following equation as.

$$SLA\ (cm/g) = (LA / DW)$$

Where, LA is the leaf area; DW is the dry 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). Fresh leaf samples were collected from both the treatments washed twice with deionised water to remove electrolytes contamination adhered on the surface. Samples were kept in a vial and incubated in dark for 24 hours at room temperature. The initial conductance was measured with help of a conductivity meter. After this, the vials were autoclaved for 15 min in order to kill the leaf tissues and release the electrolytes. After cooling, the second conductivity was taken. Cell membrane stability index was calculated by using following formula and expressed as percent.

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

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 Carbon isotope discrimination ratio

For determining carbon isotope discrimination ratio, the index leaves i.e., 3rd fully opened leaf were collected from the experimental plants and oven dried at 70°C, the fully dried samples were made into fine powder. For carbon stable isotope studies, the samples were sent to the National Facility at Department of Crop Physiology, GKVK, Bangalore and carbon isotope ratio was measured by using isotope ratio mass spectrometer (IRMS).

3.1.6.2 Root traits.

Roots were collected from 100% (control) and 50% (stressed) FC maintained plants by carefully removing soil mass from pots. The adhering soil particles to the root surface were removed by washing with high jet of water

3.1.6.2.1 Root length:

Root length was measured from plants under 100% and 50% FC soil moisture, from the root shoot junction to the tip of longest rootlet by using a centimetre scale and expressed in cm.

3.1.6.2.2 Root volume:

Root volume was determined in millilitre by water displacement method. Roots after removing from soil cleaned thoroughly and were immersed in a measuring cylinder of volume 100 ml. The amount of water getting displaced while immersing the root is considered as root volume.

57

3.1.6.2.3 Root dry weight

Roots after removing all the adhering soil particles washed thoroughly and were dried in a hot air oven at a temperature of 80°C for 48 hours (till attaining a constant weight). Then the dry weights were recorded using an electronic balance in gram.

3.1.6.2.4 Root shoot ratio:

The root and shoot weight were recorded separately after drying them in hot air oven at 80°C for 48 hours till reaching a constant weight. Root shoot ratio was calculated using following equation as

Root shoot ratio = Root dry weight / Shoot dry weight

3.1.6.2.5 Specific root length

Specific root length was calculated at panicle initiation by measuring the root length from root shoot junction to longest root tip and measuring the oven dry weight by drying the root samples at 80°C for 48 hours till reaching a constant weight using the following equation as

Specific root length = Root length in cm/ root dry weight in g.

3.1.6.3 Biomass partitioning

3.1.6.3.1 Leaf weight ratio:

Leaf weight ratio was measured by drying the leaves of the plant and the whole plant at 80°C for 48 hours till reaching a constant weight using following equation as

Leaf weight ratio = Leaf dry weight / Total biomass

3.1.6.3.2 Stem weight ratio

Stem weight ratio was measured by drying the stem portion of the plant and the whole plant at 80°C for 48 hours till reaching a constant weight using following equation as

Stem weight ratio = Stem dry weight / Total biomass

3.1.6.3.2 Root weight ratio

Root weight ratio was measured by drying the root portion of the plant and the whole plant at 80°C for 48 hours till reaching a constant weight using following equation as

Root weight ratio = Root dry weight / Total biomass

3.1.6.4 Leaf area

Leaf samples from plants grown under 100% and 50% FC were collected and leaf area per plant was found using gravimetric method by following the procedure given by Chaudhary *et al.*, (2102).

3.1.6.5 Starch accumulation in the roots (mg g^{-1})

Starch accumulation in roots under control and stress condition was estimated using anthrone reagent as per the protocol given by Sadasivam and Manickam (1992).

PRINCIPLE:

The root samples were treated with 80% hot alcohol to get rid of sugars and then extraction of starch was done using per chloric acid. In hot acidic medium starch gets hydrolysed to glucose and further dehydration leads to the formation of hydroxymethyl furfural. Hydroxymethyl furfural has the ability to form green coloured product with anthrone.

Material:

- 80% ethanol
- 52% per chloric acid
- Standard Glucose: Stock 1000 mg in 1000 ml water. Working standard – 10ml of stock diluted to 100ml with water.
- Anthrone: Dissolve 200mg anthrone in 100ml of ice-cold 95% sulphuric acid.

Methodology:

1. 0.5 g of sample was taken for extraction and homogenized in hot 80% ethanol to remove sugars. Residue was retained after centrifuging and the procedure was repeated till the washings do not give colour with anthrone reagent. The residue obtained was dried well over a water bath.

2. To the residue obtained 5mL 5 mL of water and 6.5 mL of 52% per chloric acid was added.

3. Extracted the samples at 0°C for 20 min, centrifuged and supernatant was saved.

4. The same extraction was repeated using fresh per chloric acid. Centrifuged and supernatants were pooled and make up to 100 mL.

5. 0.1 mL of the supernatant was taken and made up the volume to 1ml with water.

6. Standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard and made up the volume to 1ml in each tube with water.

7. 4mL of anthrone reagent is added to each tube.

8. Heated for eight minutes in boiling hot water.

9. Cooled rapidly and read the intensity of green to dark green colour at 630nm.

3.1.6.5 Protein profiling from roots using SDS page.

Root samples were collected at panicle initiation stage from control and water stressed plants and washed thoroughly to remove the adhering soil granules. These root samples were made into fine powder by grinding through liquid nitrogen and then extracted at 4^0C using 1.5 ml of cold denaturing buffer. The homogenized samples were centrifuged at 5000 rpm for 15 minutes. The supernatant was pipetted out into a fresh vial and to this equal amounts of acetone chilled is added at 1:1 ratio to precipitate proteins. Discarded the supernatant and the remaining pellet was kept in 50 µl of denaturing buffer and vortexed. The pellet dissolved in buffer was centrifuged at 5000 rpm for 15 minutes. Again the supernatant was mixed with 10 µl of sample buffer and kept for 3 minutes in boiling water bath. Electrophoresis was done using SDS-PAGE.

Reagents

a) Acrylamide stock (30%)

Acrylamide - 29.2 g

Bis-acrylamide - 0.8 g

Double distilled water - 100 ml

b) Separating (resolving) gel buffer stock (1.5M Tris –HCl, pH 8.8)

Tris base (18.15 g) was dissolved in approximately 50 ml of double distilled water. The pH was adjusted to 8.8 with 6 N HCl and the volume was made up to 100 ml with double distilled water and stored at 4^0C.

c) Stacking gel buffer stock (0.5 M Tris – HCl pH 6.8)

Tris base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH was adjusted to 6.8 with 6 N HCl and the volume was made up to 100 ml with double distilled water and stored at 4^0C.

d) Polymersing agents

AmmoniumperSulphate (APS) 10 percemt prepared freshly before use.

TEMED-Fresh from refrigeration

e) Electrode buffer pH 8.3

Tris base	- 6.0 g
Glycine	- 28.8 g
SDS	- 2.0 g
Double distilled water	- 2 L

f) Sample buffer

Double distilled water	- 2.6 ml
0.5 M Tris HCl pH 6.8	- 1.0 ml
2-mercapto ethanol	- 0.8 ml
Glycerol	- 1.6 ml
SDS 20% (w/v)	- 1.6 ml
0.5% Bromophenol blue	- 0.4 ml

g) Staining solution

Coomassie brilliant blue R 250	- 0.1 g
Methanol	- 40.0 ml
Glacial acetic acid	- 10.0 ml
Double distilled water	- 50.0 ml

Destaining solution is the staining solution without coomassie brilliant blue R 250.

Procedure

Separating gel was first casted followed by stacking gel using various chemicals indicated below

a) Preparation of separating gel (12%)

Double distilled water	- 6.7 ml
Tris HCl, pH 8.8	- 5.0 ml
SDS10%	- 0.2 ml
Acrlamide stock	- 8.0 ml

The solution was mixed well and de gassed for 3 minutes and then the following were added immediately.

Freshly prepared 10% Ammonium per Sulphate (APS)	- 0.10 ml
Tetra methyl ethylene diamine (TEMED)	- 0.01 ml

The separating gel was mixed well and poured immediately between glass plates and a layer of water was added above the polymerizing solution to quicken the polymerizing process

b) Preparation of stacking gel

Double distilled water	- 6.1 ml
Tris HCl, pH 6.8	- 2.5 ml
SDS 10%	- 0.2 ml
Acrlamide stock	- 1.3 ml

The solution was mixed well, degassed and the following were added

APS 10%	- O.5 ml
TEMED	- 0.1 ml

The water layer over the separating gel was removed and washed with a little electrode buffer and then stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

The comb was removed after polymerization; samples were loaded in to the wells. A standard with known molecular weights was also loaded to wells. The

57

electrophoresis was performed at 100 V till the dye reached the separating gel. Then the voltage was increased in 200 V and continued till the dye reached the bottom of the gel. Immediately after electrophoresis the gel was removed from the glass plates and incubated in the staining solution overnight with uniform shaking. The gel was transferred to the destaining solution. The protein appeared as bands and gel was photographed after placing it on GEL DOC (G: BOX, Synegene)

3.1.6.2 Anatomical observations made at time of booting stage.

Plants were raised up to booting stage and then uprooted to study the anatomical variations in roots under control and stressed condition. Root samples were washed thoroughly and cross sections were made at 7cm from the tip of root. Cross sections were made using a fine bade and immersed in 2% glycerol solution. Observations from the cross section were made in a compound microscope. LEICA-ICC50 HD. The objective lens was adjusted to 10X magnification and observations on standard anatomical features of root cross section were made.

The measurements that were obtained from microscope were in pixels and need to be expressed in mm. For this in each image a standard scale of about 1mm was fixed and the pixel value of image is converted to mm with the help of this standard scale.

The area measurements were automated by software ImageJ, as per the protocol described by Abramoff, 2004. ImageJ a java based image analyser as base values of pixels were calibrated and stored. Using this software observations were made on 1. Root diameter, 2. Stele diameter, 3. Late metaxylem diameter, 4. Width of sclerenchyma, 5. Width of aerenchyma, 6. Stele diameter to root diameter.

3.1.6.3 Morphological and yield parameters

3.1.6.3.1 Plant height

Plant height measurement was taken from plant base to the tip of the panicle at maturity stage and expressed in cm.

3.1.6.3.2 Days to 50% flowering

The days required for exertion of 50 % panicles in each replication.

3.1.6.3.3 Tiller number

Total number of tillers present in each replication at the time of harvest.

3.1.6.3.4 Productive tiller number

The number of panicle bearing tillers present at the time of harvest in each replication is termed as productive tiller number.

3.1.6.3.5 Panicle length

The distance between neck nodes to the tip of the apical grain of a primary panicle in each plant and expressed in terms of cm.

3.1.6.3.6 Yield per plant

The grain yield obtained from each replication by total number of filled grains expressed in gram.

3.1.6.3.7 Spikelet fertility percentage

The total number of filled and unfilled grains were calculated from 3 randomly selected panicles in the sample population.

Then, Spikelet fertility (%) was calculated by using the formula

Spikelet fertility (%) = (Number of fertile spikelets/ Total number of spikelets)100

3.1.6.3.8 1000 grain weight

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

3.2 MICROSATELLITE MARKERS ASSOCIATED WITH DROUGHT TOLERANT TRAITS IN RICE

3.2.1 Genomic DNA isolation

Genomic DNA from the selected 6 rice genotypes was isolated by raising seedlings in a petri dish using the method as described by Dellaporta *et al.*, (1983) leaf samples were collected from 2 weeks old seedlings separately in a cover. 1 gram of leaf bits excluding midribs were taken in a pre-chilled mortar, ground into fine powder using liquid nitrogen. The powdered samples were then transferred to a 20 ml centrifuge tube. Then 15 ml of extraction buffer was added containing 20µl of ß-mercaptoethanol and 50mg of Polyvinyl pyrollidine and kept at 4^0C.

1% SDS was added to the mixture, mixed thoroughly and incubated at a temperature of 65^0C for 1 hour in a water bath (Beston). To this 5 ml of 5M potassium acetate was added and kept on ice for 20 minutes.

Then the mixture was centrifuged at 12000 rpm for 20 minutes using centrifuge 5430R Eppendorf. Later the clear aqueous phase was transferred to a fresh sterile tube. To this equal volumes of isopropanol was added and mixed gently by inversion and kept in a -20^0C freezer until DNA was precipitated out.

Then the vials are centrifuged at 12000 rpm for 10 minutes and then the pellet obtained was resuspended in 500µl sterile double distilled water. To this, 3µl of RNase was added and incubated at 37^0C for 1 hour. To this mixture a mixture of chloroform : isoamyl alcohol was added at the rate of 500µl, mixed wella for 15 minutes. Then centrifuged at 12000 rpm for a period of 15 minutes. The upper aqueous phase was separated and transferred to another microfuge tube without disturbing the inter phase. Then to this absolute cold ethanol was added at two volumes and 1/10 volume of sodium acetate were added and kept overnight incubation in -20^0C. Again centrifuged at 12000 rpm for 5 minutes and supernatant was discarded. The obtained 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^{0}C for further use.

3.2.3 Quantification and quality assessment of DNA samples

By reading the absorbance of sample at 260 nm and 280 nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer) quantity of DNA present in each sample was determined. The ratio between these two absorbance ie., OD 260/ OD 280 was used as an estimate to evaluate the purity of the DNA sample. According to Sambrook and Russell, (2000) pure preparations of DNA have 260 nm/ 280 nm OD ratio between 1.7 and 1.8. Quality assessment was made by using a gel electrophoresis with a crude DNA sample of 5 µl on a agarose gel (0.8%) and stained with EtBr.

3.2.4 Dilution of DNA samples

The stock DNA samples after quantification were diluted to working standards of 50 ng/µl and used for PCR reaction. Dilutions were made using the following formula as:

$$M_1V_1 = M_2V_2$$

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

The diluted sample preparations were made by transferring appropriate amount of stock to 0.5 ml microcentrifuge tube, and the volume was made to 100µl using TE buffer. Later these DNA working solutions were stored at -20°C for further use.

3.2.5 PCR amplification using SSR primers

3.2.5.1 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	35 cycles
Primer extension	- 72^{0}C for 1 minute	
Final extension	- 72^{0}C for 5 minutes	
Incubation	- 4^{0}C for infinity to hold the sample	

3.2.5.2 Detection of polymorphism between the tolerant and susceptible genotypes using SSR primers

A set of 20 drought specific primers were selected of which 15 primers are from *DRO1* sequence which are highly specific to root traits and the rest are random SSR markers associated with water use efficiency in rice. The primer combinations that are used in study were listed in Table 3. The PCR amplified products were run along with molecular ladder (100bp ladder) on 2% agarose gel using 1X TBE buffer and stained with ethidium bromide.

The gel profile was visualized under UV (312 nm) transilluminator and documented in gel documentation system (Syngene G box documentation system). The so documented SSR gel profile were carefully examined for the polymorphism in banding pattern between the genotypes.

3.2.6. RT- PCR analysis

Expression level of *DRO1 (Deeper Rooting 1)* and a gene associated with water use efficiency was studied in control and stressed plants of a drought tolerant genotype i.e., Chuvannamodan (Ptb 30) and one drought susceptible genotype i.e., Annapoorna(Ptb 35) using RT (Reverse Transcriptase) PCR

Table 3: List of primer combinations used in the study.

Primer	Forward sequence	Reverse sequence
Dro1seq-00	ATATGGGCGTACGGTAGCTG	AGAGATTGGGGAGGGAGAAA
Dro1seq-01	GCTGTGTCCTGTTATCATTCCA	CCTCAAGGAACAGGGAAACA
Dro1seq-02	CTTGCGGCTTAATCGAGTTC	GGAAGAATTTTGCGGGTGTA
Dro1seq-03	AGGGAGTGGAGTAAGCATGG	AGCAACGAAGCGACTGATCT
Dro1seq-04	TGCCACTTTTGTCAATGGAG	TGCCCGTACTGTACCAACAA
Dro1seq-05	AGGGAGTGGAGTAAGCATGG	ATCGGCACGCTTTTGTAAAC
Dro1seq-06	GTAAGCATGGGCAGACATTG	ATCGGCACGCTTTTGTAAAC
Dro1seq-07	TGAAAACATCAGGGAGTGGA	ATCGGCACGCTTTTGTAAAC
Dro1seq-08	GACGATGATGGTGCAAAATG	CCTTTGTCCCAGAACCTCCT
Dro1seq-09	GACGATGATGGTGCAAAATG	GGCAGACAACTCTGGAATCA
Dro1seq-10	GGTGCAAAATGGGTCAAAAC	GGCAGACAACTCTGGAATCA
Dro1seq-11	GATCAGTCGCTTCGTTGCT	ACCTGGCATGAACGAACTAA
RM485	CACACTTTCCAGTCCTCTCC	CACACTTTCCAGTCCTCTCC
RM518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
RM153	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG

RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
RM349	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG

RNA was isolated using TRIZOL reagent.

3.2.6.1. RNA isolation

0.1 gram of root tissue were frozen in liquid nitrogen and ground to a fine powder in a pre-chilled pestle and mortar. To this grounded root sample 1ml of Trizol reagent was added and gently mixed to homogenize the mixture. Later the mixture was incubated at room temperature for 5 minutes in order to dissociate the nucleoproteins complexes completely. After incubating for 5 minutes the homogenate was transferred to a 2ml pre chilled microfuge tube.

Then added with 0.2 ml of chloroform was and shaken vigorously for 15 seconds. Thus obtained mixture was incubated at room temperature for 5 minute then incubated in ice for 10 min. Then centrifuged at 12000 rpm for 15 minutes at 4° C. The aqueous phase thus obtained was transferred to a fresh microfuge tube and added with 0.5 mL of ice cold isopropanol(100%) and incubated at room temperature for 10 minutes. Then centrifuged at 12000 rpm for 10 minutes at 4° C. then the supernatant was excluded and pellet was washed with 1ml of 75% alcohol treated with DEPC water. Sample was briefly vortexed and spun at 7500 rpm for 5 minutes at 4° C. After centrifugation, alcohol was evaporated and the RNA pellet was air dried for 30 minutes. Then the pellet was dissolved in 30 µl RNase free DEPC treated water and incubated at 55 – 60° C for 10 minutes.

3.2.6.2 Quantification and quality assessment of RNA samples

By reading the absorbance of sample at 260 nm and 280 nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer) quantity of RNA present in each sample was determined. The ratio between these two absorbance i.e., OD 260/ OD 280 was used as an estimate to evaluate the purity of the RNA sample. According to Sambrook and Russell, (2000) Pure preparations of RNA have 260 nm/ 280 nm OD ratio between 1.7 and 1.8. Quality assessment was made by using a gel electrophoresis with a crude RNA sample of 1 µl on a agarose gel (2%) and stained with EtBr.

3.2.6.3.Reverse Transcriptase PCR analysis

The cDNA was synthesized using Thermo scientific verso cDNA Synthesis kit Product code. AB-1453/A.

cDNA conversion components as:

5X cDNA synthesis buffer	= 4µl
dNTP mix	= 2µl
anchored oligo dT	= 1µl
RT Enhancer	= 1µl
Verso Enzyme	= 1µ

To this mixture 5 µl of RNA template (1ng of total RNA) were added to an RNAse free tube. Then the volume of the total reaction was made upto 20 µl with the addition of sterile distilled water. The solution is gently vortexed and placed in thermal cycler (Eppendorf Master Cycler) programmed to undergo cDNA synthesis. The following cycling conditions were employed, 30minutes at 42°C and 2 minutes at 95°C. The amplification was done using Thermoscientific amplification kit.

3.2.6.4 Gene expression studies.

The cDNA thus synthesized from mRNA used as a template and a PCR was performed by adding the following components to a new PCR vial.

For a reaction of volume 50 µL

PCR Master Mix (2X)	: 25 µL
Forward primer (0.1-1.0 µM)	: 02 µL
Reverse primer(0.1-1.0 µM)	: 2 µL
Template DNA (10 pg - 1 µg)	: 5 µL

components were made upto 50 µL with sterile distilled Water (nuclease-free).

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	- 95^{0}C for 3 minutes
Denaturation	- 95^{0}C for 30 second.
Primer annealing	- 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

After the amplification, the PCR product was separated by agarose gel electrophoresis.

Table 4. Details of primer used in RT PCR

Primer	Forward sequence	Reverse sequence
RM 518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC

Agarose gel electrophoresis was done at a gel percentage of 1.5%. The gel was loaded with the samples along with gel loading dye and run at 60 V for 1:30 minutes. The gel was visualized using a gel documentation system (E gel imager, Invitrogen).

3.2.6.5 Identification of polymorphism

The SSR marker profiles were generated through electrophoresis of the PCR products on 1.5 percent agarose gel. The profiles were examined in relation to the water use efficiency and any band which is present in tolerant genotype and missing in the susceptible genotype and vice versa was considered as polymorphic.

3.3 Elution of polymorphic band and cleaning (NucleoSpin® Gel and PCR Clean-up kit protocol)

a) Excision of DNA fragment / solubilise gel slice

The DNA fragment was excised from the agarose gel using a sterile scalpel and transferred to a clean to a clean tube after taking the weight of the gel slice (excluding excess agarose). For every 100 mg of 2% agarose gel 200µl buffer NTI was added. Later the samples was incubated at 50 °C for 5 -10 min. Until the gel slice was completely dissolved the sample was briefly vertexed every 2-3 minutes.

b) Binding of DNA

NucleoSpin® Gel and PCR Clean-up Column was placed into a collection tube (2 mL) loaded with 700 µl of sample. The sample was centrifuged at 11,000 x g for 30 s. Discarding the bottom liquid the column was placed back into the collection tube. Then remaining sample is loaded and centrifugation step is repeated.

c) Washing of silica membrane

To the NucleoSpin® Gel and PCR Clean-up Column 700 µl of Buffer NT3 was added. Centrifuged for 30 s at 11,000 x g. Again bottom flow-through was discarded and placed the column back into the collection tube.

d) Drying of silica membrane

Column was centrifuged for 1 min at 11,000 x g to remove Buffer NT3 completely. The contact of spin column with the flow through was avoided while removing it from the centrifuge and collection tube. As residual ethanol is found to be inhibiting the enzyme reactions care was taken to obtain the total removal of ethanol by incubating the columns for 2-5 min at 70 °C prior to elution.

e) Elution of DNA

NucleoSpin® Gel and PCR Clean-up Column was placed into a new 1.5 mL micro centrifuge tube. 15–30 µl Buffer NE was added and incubated at room temperature (18–25 °C) for 1 min centrifuged for 1 min at 11,000 x g.

3.4 Sequencing the eluted product from the polymorphic bands

The eluted DNA product was subjected to PCR re amplification. Nested PCR has been done. The PCR product was run on 2 per cent agarose gel electrophoresis. From the gel picture obtained the product showing single band was sent for sequencing at SciGenom lab. Cochin.

3.5. BLASTn

The sequence generated from this study was analyzed using the nucleotide BLAST at NCBI.

Results

4. RESULTS

The experiment was conducted to evaluate the adaptive plasticity in root-shoot morphology and physiology, root anatomical plasticity under water stress in selected six rice genotypes and molecular characterization using root specific genes in the Department of Plant Physiology, College of Agriculture, Vellayani during 2017-19. The rice plants were exposed to water stress condition throughout the growth period from seedling to maturity by maintaining the plants at 50 percent field capacity soil moisture along with a 100 percent field capacity soil moisture as control and replicated five times. The physio- morphological, biochemical and yield characters were recorded at panicle initiation stage.

Genotyping of selected tolerant and susceptible rice varieties were done using available *DEEPER ROOTING* QTL specific markers, SSR and EST-SSR markers. Expression level of genes linked with water use efficiency in selected genotypes were studied using RT (Reverse Transcriptase) PCR. The data were statistically analysed 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 Relative water content (%)

The results of relative water content were presented in Table 5 (Figure 1). RWC had shown a significant variation among the treatments. Under 100% FC N 22 showed the highest relative water content with 89.34 % followed by Ptb-30 (88.22%) which are on par with Ptb- 29 whereas the least value was recorded in Ptb 39 which is on Par with PTB 35 (83.78%) and Ptb 57 (84.04 %.)

Whereas under water stress condition RWC found to be decreased in all the genotypes but the extent to which it decreased varied among the tolerant and susceptible genotypes. Under 50 % FC Nagina recorded the least reduction in RWC (85.37%) followed by Ptb 29 and Ptb 30 with 83.26 % and 82.943 % respectively. Whereas highest reduction in RWC was recorded from Ptb 35 (71.96%) showing a reduction of 14.11 %. There is an overall reduction of 7.92 % of RWC among the tolerant and susceptible genotypes.

4.1.2 Specific leaf area (cm^2 g^{-1})

The results of specific leaf area were presented in Table 6 (Figure 2). There is a significant variation for SLA among the treatments. Under 100 % FC specific leaf area is found to be highest in Nagina 22 (369.33) followed by Ptb 29 (331.73). Whereas the least was recorded from Ptb 39 (181.02) followed by Ptb 35 (265.72). Under water stress condition the genotypes were expected to curtail their specific leaf area in response to drought. Among the genotypes at 50% FC Nagina-22 had shown a better performance by reducing SLA to 214.90 followed by Ptb 29 (239.45). Whereas genotypes Ptb 35, Ptb 39 and Ptb 57 haven't shown significant reduction in SLA.

4.1.3. Cell membrane stability index (%)

The results of cell membrane stability index were presented in Table 7 (Figure 3). There is a significant variation among the genotypes for cell membrane stability index. Among the genotypes Ptb 30 recorded the highest value of cell membrane stability index with 97.10 % followed by Ptb 29 (96.77%) and N-22 (94.35%) respectively. Whereas genotypes Ptb 39 (83.11%), Ptb 35 (84.36%) and Ptb 57 (84.34%) are on par with each other recorded the least value. There is a mean reduction in cell membrane stability index among the genotypes by 7.9%.

Table 5. Effect of water stress on Relative water content (%) of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	89.34	85.37	- 4.44	87.35
2.	Karuthamodan (Ptb 29)	87.06	83.26	- 4.36	85.16
3.	Chuvannamodan (Ptb 30)	88.22	82.94	- 5.98	85.58
4.	Annapoorna(Ptb 35)	83.78	71.96	- 14.11	77.87
5.	Jyothi (Ptb 39)	83.55	75.39	- 9.76	79.47
6.	Swetha (Ptb 57)	84.04	76.25	- 9.26	80.14
	Mean	86.00	79.19		
	C.D. (0.05)		SE(m) ±		
	G	1.45	0.49		
	T	0.84	0.28		
	G X T	2.06	0.70		

Table 6. Effect of water stress on Specific leaf area (cm^2 g^{-1}) of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	369.63	214.90	- 41.86	292.26
2.	Karuthamodan (Ptb 29)	331.73	239.45	- 27.81	285.59
3.	Chuvannamodan (Ptb 30)	290.47	206.18	- 29.01	248.33
4.	Annapoorna(Ptb 35)	265.72	261.13	- 1.72	263.42
5.	Jyothi (Ptb 39)	181.01	183.73	+1.5	182.37
6.	Swetha (Ptb 57)	308.83	256.30	-17.00	282.56
	MEAN	291.23	226.95		
	C.D. (0.05)		SE(m) ±		
	G	17.99	6.13		
	T	10.39	3.54		
	G X T	25.45	8.63		

Table 7. Effect of water stress on Cell membrane stability index (%) of rice genotypes at booting stage .

Sl. No	Genotype	(MEAN) MSI
1.	Nagina-22	94.35±1.66
2.	Karuthamodan (Ptb 29)	96.77±1.21
3.	Chuvannamodan (Ptb 30)	97.10±0.83
4.	Annapoorna (Ptb 35)	84.36±1.62
5.	Jyothi (Ptb 39)	83.11±1.51
6.	Swetha (Ptb 57)	84.34±1.52
	C.D. (0.05)	4.43
	SE(m) ±	1.43

4.1.4 Carbon isotope discrimination ($\Delta^{13}C$)(mil^{-1})

The carbon isotope discrimination was recorded at booting stage among the genotypes and the results were presented in Table 8 (Figure 4). There is no significant variation among the genotypes for carbon isotope discrimination. The least discrimination in carbon isotope was shown by N-22 (21.84) which is on par with rest of genotypes.

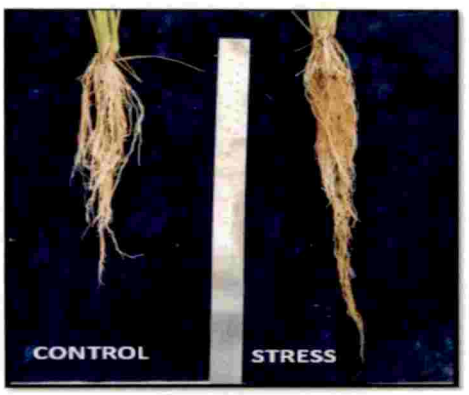
4.1.5. Root length (cm)

The root length was recorded under both control and water stress condition at booting stage were presented in Table 9 (Figure 5). There is a significant variation in root length among the treatments. Under 100% FC highest root length was recorded from N-22 (27.96 cm) followed by Ptb 29 and Ptb 30 with 25.167 and 21.8 cm respectively, whereas least was recorded from Ptb 57 (18.9 cm). Under 50 % FC the highest root length was shown by Ptb 29 (38.46 cm) followed by N-22 (37.00 cm) and Ptb 30 (28.44 cm), whereas rest of genotypes neither shown a decrease in root length nor a significant increase. Ptb 35 recorded the least root length of 14.76 cm. The mean root length recorded in 100 %FC was found to be 23.83 cm whereas under water stress condition mean root length recorded was 27.4 cm. (Plate 3)

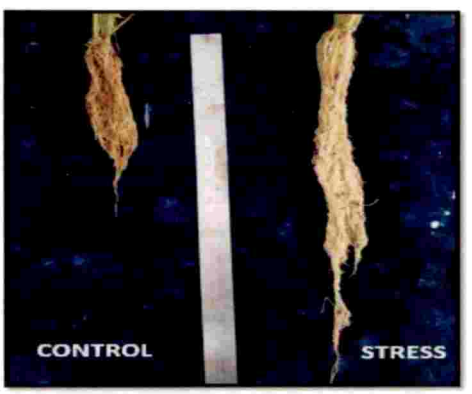
4.1.6. Root volume (ml)

Root volume was recorded at booting stage and presented in Table 10 (Figure 6). There is a significant variation for root volume among the treatments. Under 100 % FC Ptb 29 recorded the highest root volume of 8.91 mL which is on par with N-22 (8.1 mL) and Ptb 30 (7.4 mL) and the least was recorded from Ptb 35 with 3.967 mL, whereas under 50 % FC tolerant genotypes had shown an increase in root volume, the highest root volume shown by Ptb 29 (19.5 mL) and the least was recorded from Ptb 35 (4.46 mL). Genotypes had shown a mean increase of 38.9% increase in root volume under water stress condition.

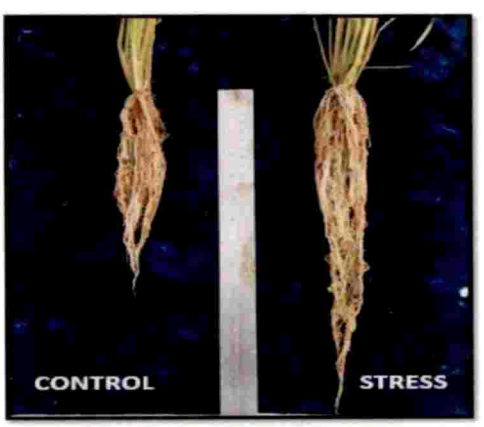
Plate 3. Effect of water stress on root length (cm) of rice genotypes at booting stage.



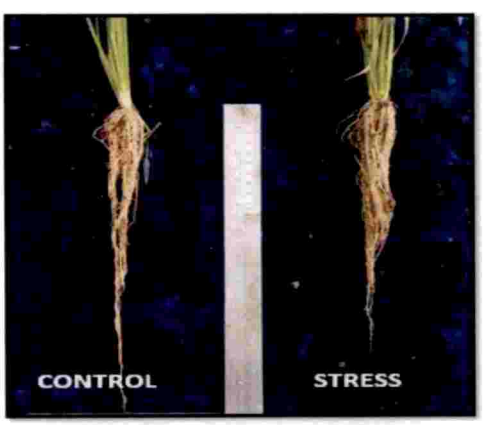
NAGINA 22



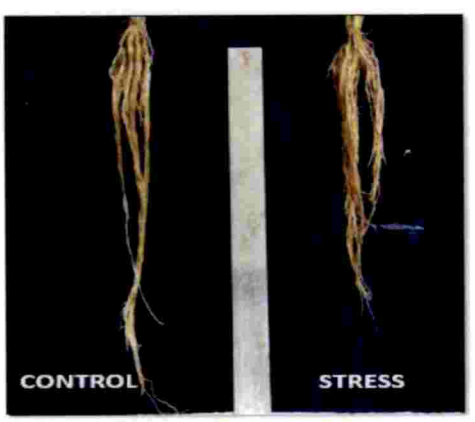
KARUTHAMODAN (PTB 29)



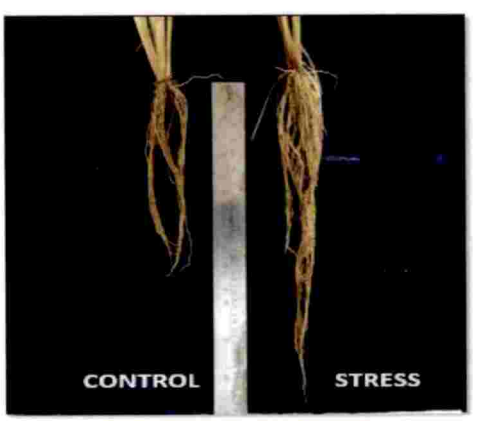
CHUVANNAMODAN(PTB 30)



ANNAPOORNA (PTB35)



JYOTHI (PTB 39)



SWETHA (PTB 57)

Table 8. Carbon isotope discrimination ($\Delta^{13}C$)(mil^{-1}) of rice genotypes at booting stage.

Sl. No	Genotype	(MEAN) MSI
1.	Nagina-22	21.84±0.09
2.	Karuthamodan (Ptb 29)	22.86±0.26
3.	Chuvannamodan (Ptb 30)	22.87±0.06
4.	Annapoorna (Ptb 35)	23.49±0.14
5.	Jyothi (Ptb 39)	23.45±0.13
6.	Swetha (Ptb 57)	22.26±0.03
	C.D. (0.05)	4.42
	SE(m) ±	1.42

Table 9. Effect of water stress on Root length (cm) of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50% FC	%CHANGE	MEAN
1.	Nagina-22	27.96	37.00	+24.41	32.48
2.	Karuthamodan(Ptb 29)	25.16	38.46	+34.57	31.81
3.	Chuvannamodan (Ptb 30)	21.80	30.46	+28.44	26.13
4.	Annapoorna(Ptb 35)	24.53	14.76	-66.21	19.65
5.	Jyothi (Ptb 39)	24.63	20.66	-19.23	22.65
6.	Swetha (Ptb 57)	18.90	23.03	+17.94	20.96
	MEAN	23.83	27.40		
	C.D. (0.05)		SE(m) ±		
	G	2.53	0.86		
	T	1.46	0.49		
	G X T	3.58	1.21		

Table 10. Effect of water stress on Root volume (mL) of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	8.10	13.96	+42.00	11.03
2.	Karuthamodan(Ptb 29)	8.91	19.50	+54.27	14.20
3.	Chuvannamodan (Ptb 30)	7.40	12.97	+42.90	10.18
4.	Annapoorna(Ptb 35)	3.96	4.46	+11.18	4.21
5.	Jyothi (Ptb 39)	4.40	4.86	+9.50	4.63
6.	Swetha (Ptb 57)	4.75	5.66	+16.16	5.20
	MEAN	6.25	10.24		
	C.D. (0.05)		SE(m) ±		
	G	3.44	1.17		
	T	1.98	0.67		
	G X T	4.87	1.65		

4.1.7 Root dry weight (g)

The root dry weight from 100% FC and 50% FC treatments were presented in Table 11 (Figure 7). There is a significant variation for root dry weight among the treatments. Under 100 % FC highest root dry weight was recorded from Ptb 29 (2.88g) whereas least was recorded from Ptb57 (0.690) which is on par with Ptb 35 (0.973 g) and Ptb 39 (1.08 g). Under 50 % FC genotypes had shown an increase in root dry weight, Ptb 29 with 4.273 found highest whereas least was observed from Ptb 39 (0.560). The overall mean root dry weight was found to be highest in Ptb 29 whereas least was recorded from Ptb 57 with dry weights 3.678g and 0.697g respectively.

4.1.8 Root / Shoot ratio.

Observations on root / shoot ratio were made by collecting samples at booting stage and the results were presented in Table 12 (Figure 8). A significant difference in root/ shoot ratio was noticed in control and stressed condition and also among the genotypes. Under 100% FC highest root dry weight was recorded in N 22 (0.243) which is on par with Ptb 30 (0.227) and Ptb 29(0.212). Whereas least was recorded from Ptb 57(0.167). Under stressed condition Ptb 29 (0.431) recorded the highest root/ shoot ratio followed by Ptb 30(0.327) which is on par with N-22 (0.313) and least was recorded from Ptb 35 (0.107).

4.1.9 Specific root length

Specific root length recorded at booting stage was presented in Table 13 (Figure 9). A significant difference in specific root length was noticed in control and stressed condition. Under 100% FC condition the highest specific root length was shown by N-22 (24.06), whereas lowest was recorded from Ptb 39 (15.17) which is on par with Ptb 35 (20.48). Under 50% FC condition specific root length was found to be highest in Ptb 29 (36.36), whereas least was recorded from Ptb 57 (12.54) which is on par with Ptb 39(13.07). Among the genotypes overall specific root length was found to be higher in Ptb 29 (29.569) and least by Ptb 39 (14.126).

Table 11. Effect of water stress on Root dry weight (g) of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	1.75	2.12	+17.25	1.94
2.	Karuthamodan (Ptb 29)	2.88	4.27	+32.52	3.57
3.	Chuvannamodan (Ptb 30)	1.34	1.86	+28.01	1.60
4.	Annapoorna (Ptb 35)	0.97	0.64	- 33.56	0.81
5.	Jyothi (Ptb 39)	1.08	0.56	- 48.30	0.82
6.	Swetha (Ptb 57)	0.69	0.70	+1.8	0.69
	MEAN	1.45	1.69		
	C.D. (0.05)		SE(m) ±		
	G	0.38	0.13		
	T	0.22	0.07		
	G X T	0.54	0.18		

Table 12. Effect of water stress on Root / shoot ratio of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.24	0.31	+13.41	0.27
2.	Karuthamodan (Ptb 29)	0.21	0.43	+50.81	0.32
3.	Chuvannamodan (Ptb 30)	0.22	0.32	+30.61	0.27
4.	Annapoorna (Ptb 35)	0.22	0.10	-51.54	0.16
5.	Jyothi (Ptb 39)	0.18	0.13	-27.71	0.15
6.	Swetha (Ptb 57)	0.13	0.16	+36.32	0.15
	MEAN	0.20	0.24		
	C.D. (0.05)		SE(m) ±		
	G	0.06	0.02		
	T	0.04	0.01		
	G X T	0.09	0.03		

Table 13. Effect of water stress on Specific root length of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	24.06	27.67	+13.03	25.86
2.	Karuthamodan (Ptb 29)	22.77	36.36	+37.36	29.56
3.	Chuvannamodan (Ptb 30)	22.90	31.75	+27.84	27.33
4.	Annapoorna (Ptb 35)	16.82	15.14	-10.02	15.98
5.	Jyothi (Ptb 39)	15.17	13.07	-13.88	14.12
6.	Swetha (Ptb 57)	20.48	28.57	+28.32	16.51
	MEAN	20.37	22.75		
	C.D. (0.05)		SE(m) ±		
	G	4.07	1.38		
	T	2.35	0.80		
	G X T	5.75	1.96		

4.1.10 Leaf weight ratio

Leaf weight ratio was recorded at booting stage and the results were presented in Table 14 (Figure 10). Under 100% FC treatment the highest leaf weight ratio was recorded from genotype Ptb 35 which is on par with N 22, Ptb 30 and Ptb 57 with values 0.47, 0.46, 0.43, and 0.43 respectively. Under 50% FC condition genotypes N 22, Ptb 29 and Ptb 30 retained the leaf weight ratio whereas there is decrease in leaf weight ratio among the susceptible genotypes. Under stress condition highest leaf weight ratio was noticed in N 22 with 0.44, whereas least was recorded from Ptb 57 (0.36).

4.1.11 Stem weight ratio

Stem weight ratio was recorded from genotypes at booting stage and presented in Table 15 (Figure 11). It was found that the overall stem weight ratio was found to be highest in genotypes Ptb 35, Ptb 39 and Ptb 57, whereas genotypes exhibited N 22, Ptb 29 and Ptb 30 least stem weight ratio. Under irrigated conditions Ptb 30 exhibited least stem weight ratio (0.19) whereas highest was from PTB 35 (0.46). Under water stress condition the least stem weight ratio was recorded from N-22 (0.27) and highest from Ptb 57 (0.52) followed by Ptb 35 (0.43) which is on par with Ptb 39 (0.37).

4.1.12 Root weight ratio

Root weight ratio was recorded at booting stage and presented in Table 16 (Figure 12). A significant difference in root weight ratio was noticed among the treatments. At 100% FC. Under 100% FC highest root weight ratio was recorded by N 22 with 0.46 followed by Ptb 30, Ptb 35 and Ptb 57 (0.45) whereas least was recorded from Ptb 29(0.42), whereas under 50% FC the highest root weight ratio was recorded from Ptb 35(0.48) on par with Ptb 39(0.45), whereas least was recorded from Ptb 57 (0.38) on par with Ptb 29(0.39).

Table 14. Effect of water stress on Leaf weight ratio (%) of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.46	0.44	-4.66	0.45
2.	Karuthamodan (Ptb 29)	0.42	0.39	-6.88	0.41
3.	Chuvannamodan (Ptb 30)	0.45	0.40	-9.2	0.43
4.	Annapoorna (Ptb 35)	0.45	0.48	+6.70	0.47
5.	Jyothi (Ptb 39)	0.44	0.45	+3.12	0.45
6.	Swetha (Ptb 57)	0.45	0.38	-17.1	0.42
	MEAN	0.45	0.43		
	C.D. (0.05)		SE(m) ±		
	G	0.03	0.01		
	T	0.02	0.006		
	G X T	0.04	0.01		

Table 15. Effect of water stress on Stem weight ratio (%) of rice genotypes at the booting stage

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.42	0.37	-11.64	0.40
2.	Karuthamodan (Ptb 29)	0.42	0.38	-8.95	0.40
3.	Chuvannamodan (Ptb 30)	0.47	0.42	-11.13	0.45
4.	Annapoorna (Ptb 35)	0.46	0.42	-8.32	0.44
5.	Jyothi (Ptb 39)	0.45	0.44	-2.75	0.45
6.	Swetha (Ptb 57)	0.43	0.50	+14.31	0.47
	MEAN	0.44	0.42		
	C.D. (0.05)		SE(m) ±		
	G	0.04	0.01		
	T	N/S	0.01		
	G X T	N/S	0.02		

Table 16. Effect of water stress on Root weight ratio (%) of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.11	0.18	+38.44	0.14
2.	Karuthamodan (Ptb 29)	0.15	0.21	+27.08	0.18
3.	Chuvannamodan (Ptb 30)	0.07	0.16	+55.85	0.12
4.	Annapoorna (Ptb 35)	0.08	0.09	+7.04	0.08
5.	Jyothi (Ptb 39)	0.10	0.11	+5.88	0.10
6.	Swetha (Ptb 57)	0.11	0.12	+11.22	0.12
	MEAN	10.5	14.6		
	C.D. (0.05)		SE(m) ±		
	G	0.02	0.01		
	T	0.01	0.005		
	G X T	0.03	0.01		

4.1.13 Leaf area:

Leaf area was recorded at booting stage and presented in Table 17 (Figure 13). From the table it was evident that there is a reduction in leaf area among all the genotypes under stress compared with control and the extent of reduction varied among control and stressed plants. Under 100% FC conditions highest leaf area was recorded from genotype Ptb 29 (790. 35 cm^2) which was on par with N-22 (762.99 cm^2) and Ptb 30 (713.43 cm^2). Under 50% FC conditions Ptb 29 recorded the highest leaf area (674.85 cm^2) followed by Ptb 30 (440.23) which is on par with N 22 (406.19), whereas least was recorded from Ptb 57 with 300.22.

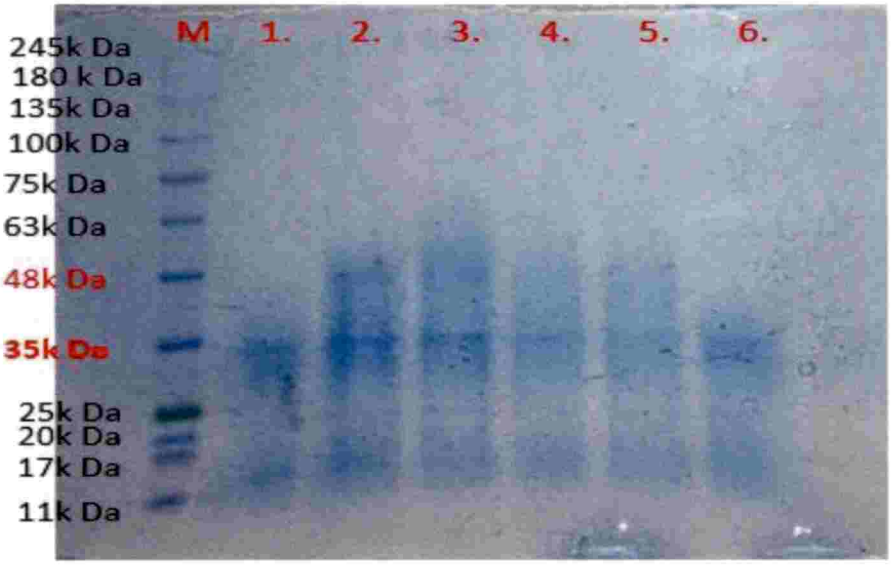
4.1.14 Starch accumulation in the roots:

Accumulation of starch in roots of control and stressed plants were estimated at booting stage and the results were presented in Table 18 (Figure 14). Among the genotypes, a significant increase in starch in roots under stressed conditions was shown by tolerant genotypes i.e., N-22, Ptb 29 and Ptb 30. Under 100% FC the highest accumulation of starch was noticed from N-22 with 1.99 $mg.g^{-1}$, followed by Ptb 30 (1.59 $mg.g^{-1}$) which is on par with rest of genotypes under control condition, whereas under stress condition there is a significant difference in patterns of starch accumulation. Ptb 29 has shown the highest accumulation with 3.52 $mg.g^{-1}$ followed by N-22 (3.05 $mg.g^{-1}$), whereas least was recorded from Ptb 35 (1.35 $mg.g^{-1}$) and is on par with Ptb 39 with 1.87 $mg.g^{-1}$.

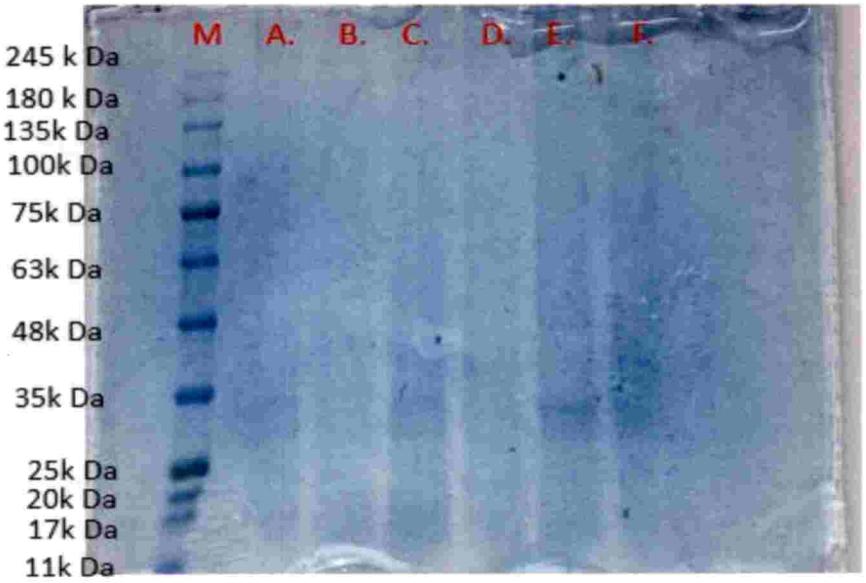
4.1.15 Protein profiling from roots using SDS PAGE:

Among the tolerant and susceptible genotypes, there is a differential accumulation of proteins. All the tolerant genotypes under both the treatments exhibited a protein band of 35kDa, whereas a novel protein band was noticed under N-22 stress with 45kDa which was found to be associated with drought tolerance in rice. Whereas susceptible genotypes haven't shown a clear accumulation patterns of proteins under drought stress condition. (Plate 4)

Plate 4: Protein profiling from roots using SDS Page.



M. 245 k Da Protein Ladder. 1. N-22 CONTROL, 2.N-22 STRESS, 3. PTB 29 CONTROL, 4. PTB 29 STRESS, 5. PTB 30 CONTROL, 6.PTB 30 STRESS



M. 245 k Da Protein Ladder. A. PTB 35 CONTROL, B.PTB 35 STRESS, C. PTB 39 CONTROL, D. PTB 39 STRESS, E. PTB 57 CONTROL, F.PTB 57 STRESS

Table 17. Effect of water stress on Leaf area (cm^2) of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	762.99	406.19	-46.51	584.59
2.	Karuthamodan (Ptb 29)	790.35	674.85	-13.96	732.60
3.	Chuvannamodan (Ptb 30)	713.43	440.23	-38.17	576.83
4.	Annapoorna (Ptb 35)	557.14	484.18	-12.54	520.66
5.	Jyothi (Ptb 39)	452.37	314.24	-29.46	383.31
6.	Swetha (Ptb 57)	481.87	300.22	-35.54	391.05
	MEAN	626.36	436.65		
	C.D. (0.05)		SE(m) ±		
	G	116.41	39.65		
	T	67.21	22.89		
	G X T	N/S	56.07		

Table 18. Effect of water stress on Starch accumulation in the roots (mg g^{-1}) of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	1.99	3.04	+34.65	2.52
2.	Karuthamodan (Ptb 29)	1.50	3.52	+57.46	2.51
3.	Chuvannamodan (Ptb 30)	1.59	2.62	+39.36	2.11
4.	Annapoorna (Ptb 35)	1.33	1.35	+1.40	1.34
5.	Jyothi (Ptb 39)	1.54	1.87	+17.46	1.70
6.	Swetha (Ptb 57)	1.56	2.13	+27.10	1.85
	MEAN	1.58	2.42		
	C.D. (0.05)		SE(m) ±		
	G	0.31	0.11		
	T	0.18	0.06		
	G X T	0.44	0.15		

4.2 EFFECT OF WATER STRESS ON ANATOMICAL PARAMETERS.

4.2.1 Root diameter:

Observations on anatomical parameters were made at booting stage. The details of root diameter were presented in Table 19 (Figure 15). The overall root diameter among the genotypes was found to be varying from 1.402 to 0.971 with Ptb 29 and Ptb 30 forming the extreme positions. At 100% FC condition root diameter varied between 1.008 to 0.891 mm for Ptb 29 and N-22 respectively. Whereas under stress condition the root diameter varied between 1.796 mm to 0.924 mm for Ptb 29 and Ptb 35 respectively.

4.2.2 Stele diameter:

Observations on anatomical parameters were made at booting stage. The details of stele diameter were presented in Table 20 (Figure 16). The overall stele diameter was found to be increasing from 0.367 mm in control to 0.431 mm in water stress condition. Under irrigated control stele diameter varied between 0.427 mm in a susceptible variety Ptb 57 to 0.266mm in tolerant N-22, whereas under stress condition stele diameter varied between 0.531mm to 0.332 mm in Ptb 29 and Ptb 35 respectively.

4.2.3 Late Metaxylem number:

Observations on anatomical parameters were made at booting stage. The details of late metaxylem number were presented in Table 21 (Figure 17). It was found, there is no significant variation among the genotypes but found to be varying among different water regime treatments. Under control conditions the late metaxylem number was varied from 4.667 for Ptb 30 to 3.667 for Ptb 57, whereas under stress condition late metaxylem number increased significantly in tolerant varieties with highest ranging from 5.667 in N-22 and Ptb 29 to least 4 in Ptb 35, Ptb 39 and Ptb 57.

Table 19. Effect of water stress on Root diameter (mm) of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.89	1.55	+97.95	1.22
2.	Karuthamodan (Ptb 29)	1.01	1.80	+43.8	1.40
3.	Chuvannamodan (Ptb 30)	0.96	1.43	+75.6	1.20
4.	Annapoorna (Ptb 35)	0.98	0.92	-5.7	0.95
5.	Jyothi (Ptb 39)	1.01	0.93	-7.0	0.97
6.	Swetha (Ptb 57)	0.97	1.02	+7.1	0.99
	MEAN	0.97	1.28		
	C.D. (0.05)		SE(m) ±		
	G	0.20	0.07		
	T	0.12	0.04		
	G X T	0.28	0.09		

Table 20. Effect of water stress on Stele diameter (mm) of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.27	0.49	+46.15	0.38
2.	Karuthamodan (Ptb 29)	0.36	0.53	+31.38	0.45
3.	Chuvannamodan (Ptb 30)	0.38	0.46	+17.50	0.42
4.	Annapoorna (Ptb 35)	0.37	0.33	-10.01	0.35
5.	Jyothi (Ptb 39)	0.40	0.42	+4.23	0.41
6.	Swetha (Ptb 57)	0.43	0.35	-18.40	0.39
	MEAN	0.37	0.43		
	C.D. (0.05)		SE(m) ±		
	G	N/S	0.03		
	T	0.06	0.02		
	G X T	0.14	0.05		

Table 21. Effect of water stress on Late metaxylem number of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	3.67	5.67	+35.21	4.67
2.	Karuthamodan (Ptb 29)	4.33	5.67	+23.43	5.00
3.	Chuvannamodan (Ptb 30)	4.67	5.33	+12.44	5.00
4.	Annapoorna (Ptb 35)	4.00	4.00	-	4.00
5.	Jyothi (Ptb 39)	4.33	4.00	-7.69	4.17
6.	Swetha (Ptb 57)	3.67	4.00	+8.33	3.83
	MEAN	4.11	4.78		
	C.D. (0.05)		SE(m) ±		
	G	N/S	0.35		
	T	0.59	0.20		
	G X T	N/S	0.49		

4.2.4 Late Metaxylem diameter:

Observations on anatomical parameters were made at booting stage. The details of late metaxylem number were presented in Table 22 (Figure 18). It was found there is a significant difference among the genotypes and also genotype stress interaction for late metaxylem diameter (LMD). The highest diameter under control condition was recorded from Ptb 29 and least from Ptb 57 with 0.060 and 0.049 mm respectively, whereas under water stress condition Ptb 29 recorded the highest LMD with a value of 0.76 mm which is on par with N-22 with 0.069 mm and the least was recorded from Ptb 35 which is on par with Ptb 57 and Ptb 39 with 0.041, 0.046 and 0.043 mm respectively.

4.2.5 Early metaxylem number (EMN):

Observations on anatomical parameters were made at booting stage. The details of early metaxylem number were presented in Table 23 (Figure 19). The overall early metaxylem number varied between 27.33 and 14.167 with Ptb 29 and Ptb 57 forming the extremes. Under irrigated condition Ptb 39 recorded the highest EMN whereas least was shown by Ptb 57 with values 27 and 13.66 respectively. Under stress condition the highest EMN was shown by N-22 with a value of 30.667 which is on par with Ptb 29 and Ptb 30 with 29.33 and 27 respectively whereas least was shown by Ptb 57 with value 14.667.

4.2.6 Width of Sclerenchyma:

Anatomical observations of root were made at booting stage. The variations in width of sclerenchyma were presented in Table 24 (Figure 20). The overall width of sclerenchyma varied from 0.014 to 0.009 among the genotypes. Under irrigated conditions width of sclerenchyma varied from 0.013 mm in Ptb 35 to 0.005 in N-22 whereas under stress condition the width of sclerenchyma varied between 0.024 in N-22 to 0.011 in Ptb 57 which is on par with Ptb 39 and Ptb 35 with 0.011 and 0.012 respectively.

Table 22. Effect of water stress on Late metaxylem diameter of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.05	0.07	+23.03	0.06
2.	Karuthamodan (Ptb 29)	0.06	0.08	+20.92	0.07
3.	Chuvannamodan (Ptb 30)	0.05	0.06	+21.70	0.05
4.	Annapoorna (Ptb 35)	0.05	0.04	-14.55	0.04
5.	Jyothi (Ptb 39)	0.05	0.04	-16.34	0.05
6.	Swetha (Ptb 57)	0.049	0.046	-5.93	0.05
	MEAN	0.05	0.06		
	C.D. (0.05)		SE(m) ±		
	G	0.01	0.003		
	T	N/S	0.002		
	G X T	0.01	0.004		

Table 23. Effect of water stress on Early metaxylem number of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	19.33	30.67	+36.94	25.00
2.	Karuthamodan (Ptb 29)	25.33	29.33	+13.62	27.33
3.	Chuvannamodan (Ptb 30)	17.00	27.00	+37	22.00
4.	Annapoorna (Ptb 35)	21.33	21.00	-1.5	21.17
5.	Jyothi (Ptb 39)	27.00	23.00	-14.81	25.00
6.	Swetha (Ptb 57)	13.67	14.67	+6.77	14.17
	MEAN	0.05	0.06		
	C.D. (0.05)		SE(m) ±		
	G	3.40	1.16		
	T	1.96	0.67		
	G X T	4.80	1.64		

Table 24. Effect of water stress on Width of Sclerenchyma of rice genotypes at the booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.005	0.024	+79.71	0.014
2.	Karuthamodan (Ptb 29)	0.008	0.014	+38.46	0.011
3.	Chuvannamodan (Ptb 30)	0.008	0.012	+30.55	0.010
4.	Annapoorna(Ptb 35)	0.013	0.012	-5.2	0.013
5.	Jyothi (Ptb 39)	0.009	0.011	+21.21	0.010
6.	Swetha (Ptb 57)	0.007	0.011	+36.36	0.009
	MEAN	0.008	0.014		
	C.D. (0.05)		SE(m) ±		
	G	0.002	0.001		
	T	0.001	0.000		
	G X T	0.003	0.001		

4.2.7 Width of aerenchyma :

Anatomical observations of root were made at booting stage. The variations in width of aerenchyma were presented in Table 25 (Figure 21). The overall width of aerenchyma fond to be highest in a susceptible genotype Ptb 57 with 85.687 μm and the least was recorded from a tolerant genotype Ptb 29 with 54.008 μm. Under irrigated condition the width of aerenchyma found least in N-22 with 53.457 μm which is on par with Ptb 29 and Ptb 35 with 58.480 μm and 56.940 μm respectively whereas under stressed condition Ptb 29 recorded the least aerenchyma width with 49.537 μm and the highest from a susceptible genotype Ptb 57 (97.4 μm.)

4.2.8 Stele diameter to root diameter :

Anatomical observations of root were made at booting stage. The variations in stele diameter to root diameter were presented in Table 26 (Figure 22). The stele diameter to root diameter associated with water conduction was found to be varying under control and stressed condition from 0.358 mm in Ptb 30 to 0.311 mm in Ptb 35. Under irrigated condition the highest stele diameter to root diameter was found in Ptb 57 with 0.367 mm which is on par with Ptb 39 with 0.350 mm whereas least was recorded from N-22 with 0.3 mm. Under stress condition Ptb 30 recorded the highest stele diameter to root diameter with 0.401 mm which is on par with Ptb 29 with 0.395 mm and the least was recorded from Ptb 57 with 0.307 mm which conversely recorded the highest stele diameter to root diameter in control. (Plate 5,6,7)

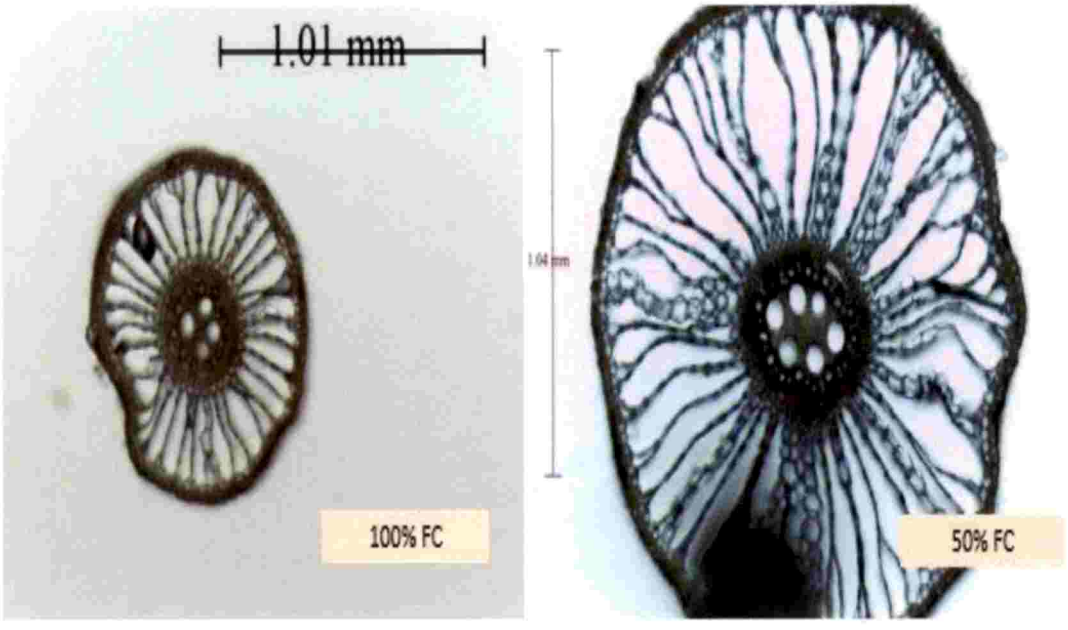
Table 25. Effect of water stress on Width of aerenchyma of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	53.46	78.75	+32.10	66.10
2.	Karuthamodan (Ptb 29)	58.48	49.54	-15.30	54.01
3.	Chuvannamodan (Ptb 30)	75.90	61.88	-18.47	68.89
4.	Annapoorna(Ptb 35)	56.94	75.16	+24.24	66.05
5.	Jyothi (Ptb 39)	79.13	92.25	+14.21	85.69
6.	Swetha (Ptb 57)	71.46	97.40	+26.62	84.43
	MEAN	65.89	75.83		
	C.D. (0.05)		SE(m) ±		
	G	16.43	5.60		
	T	9.49	3.23		
	G X T	N/S	7.91		

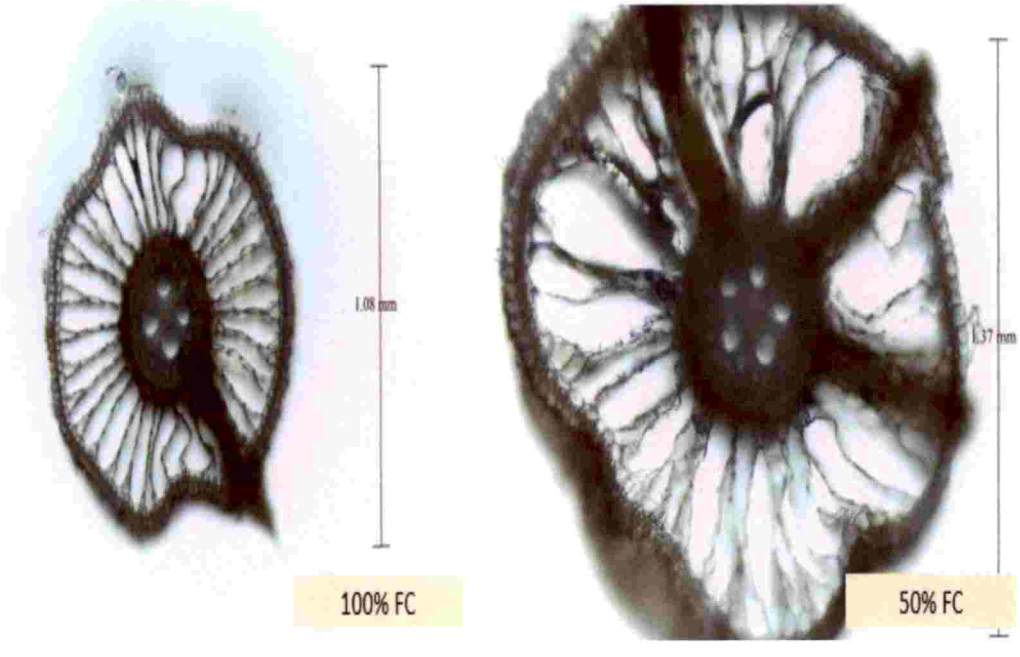
Table 26. Effect of water stress on stele diameter to root diameter of rice genotypes at booting stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	0.30	0.37	+20.19	0.34
2.	Karuthamodan (Ptb 29)	0.33	0.39	+16.65	0.36
3.	Chuvannamodan (Ptb 30)	0.31	0.40	+21.43	0.35
4.	Annapoorna(Ptb 35)	0.32	0.30	-7.09	0.31
5.	Jyothi (Ptb 39)	0.35	0.31	-12.01	0.33
6.	Swetha (Ptb 57)	0.37	0.30	-16.46	0.34
	MEAN	0.33	0.35		
	C.D. (0.05)		SE(m) ±		
	G	0.02	0.01		
	T	0.01	0.005		
	G X T	0.03	0.01		

Plate 5. Effect of water stress on root anatomy of Nagina 22 and Ptb 29 at booting stage .

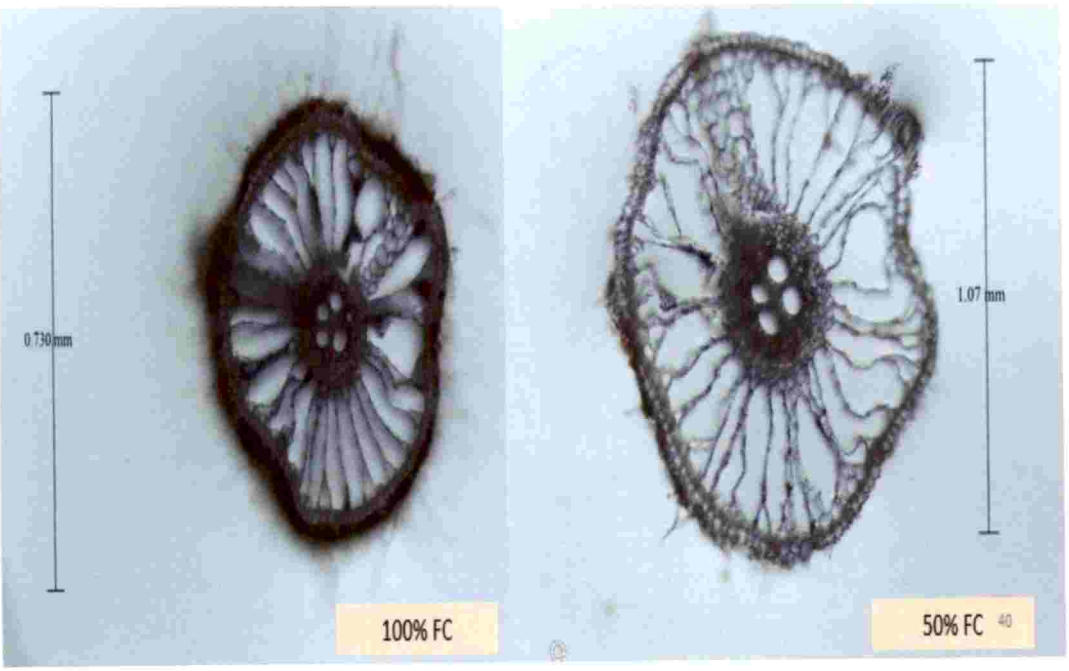


NAGINA 22

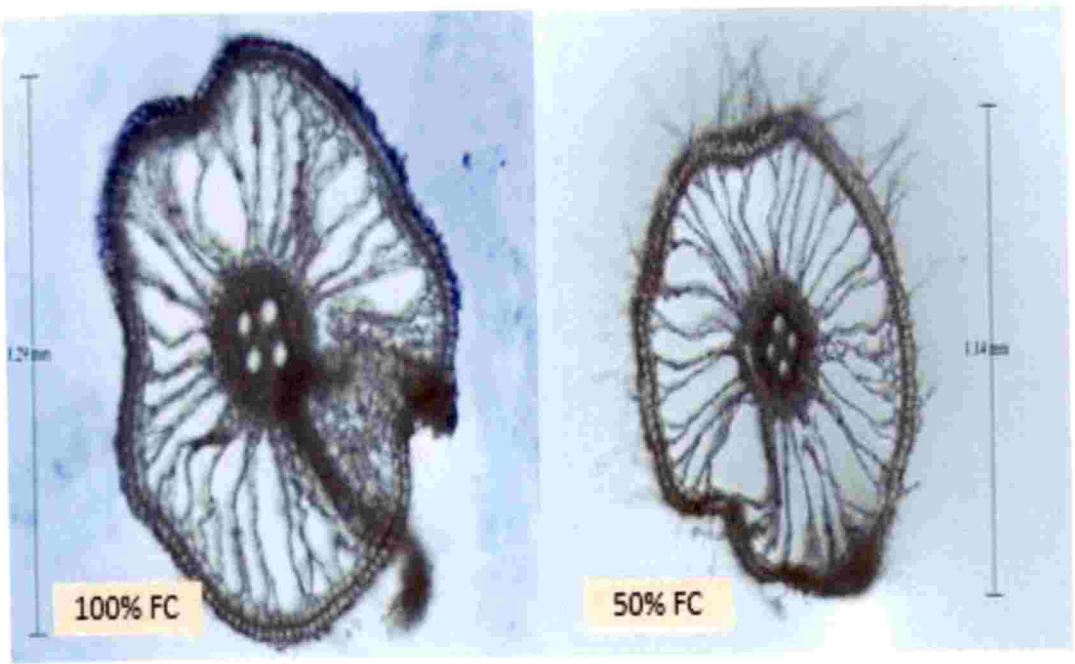


Ptb 29 - KARUTHAMODAN

Plate 6. Effect of water stress on root anatomy of Ptb 30 and Ptb 35 at booting stage.

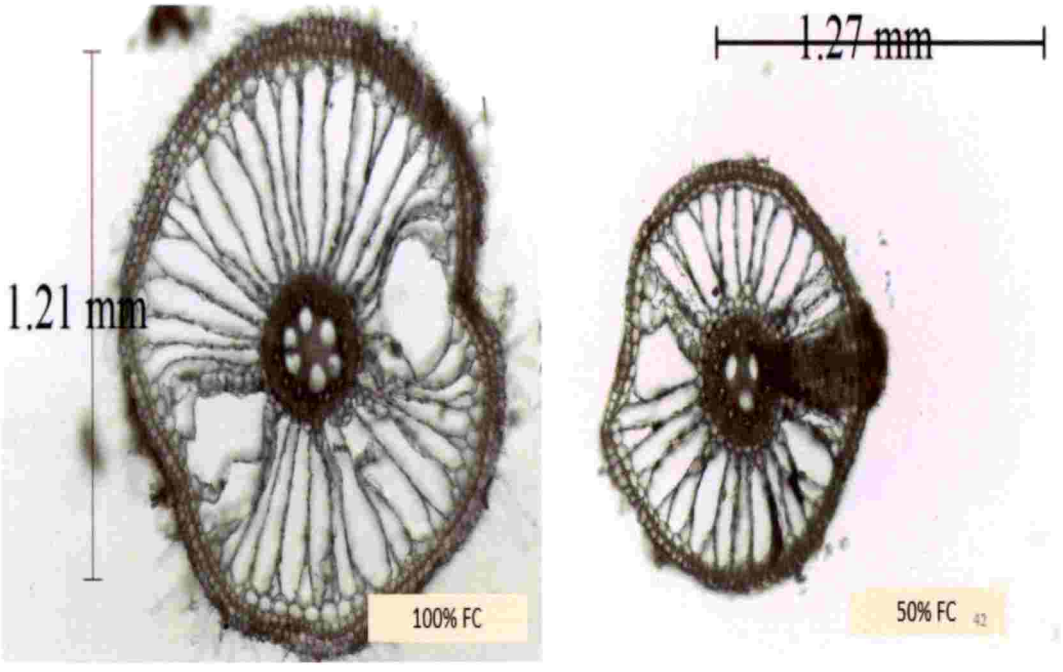


Ptb 30 - CHUVANNAMODAN

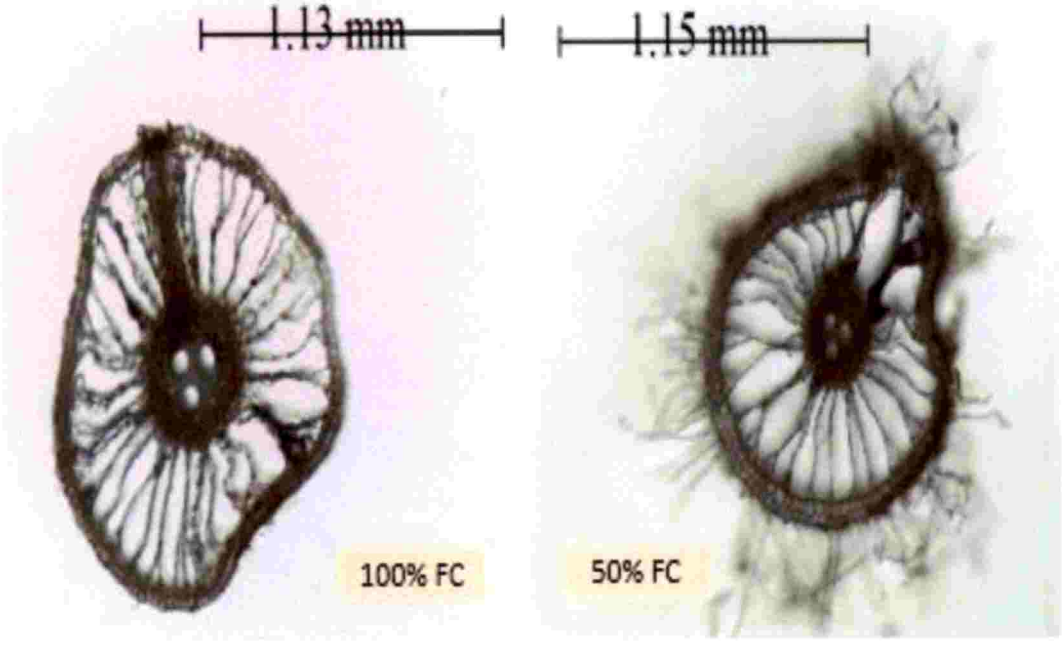


Ptb 35 - ANNAPOORNA

Plate 7. Effect of water stress on root anatomy of Ptb 30 and Ptb 35 at booting stage.

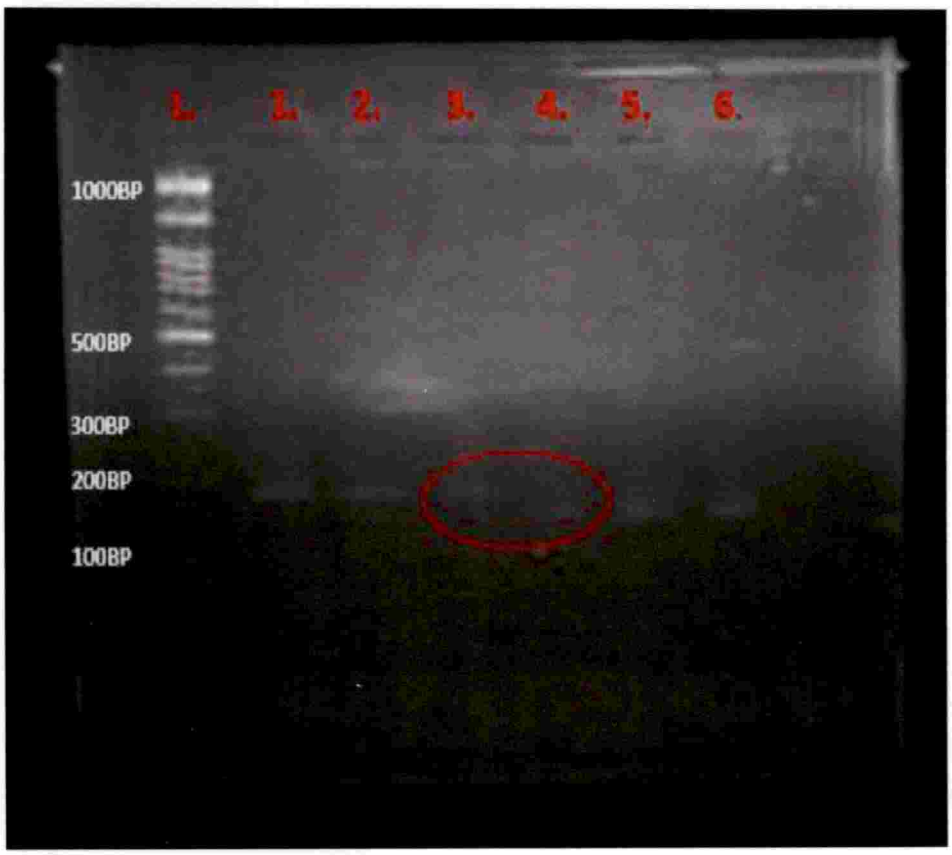


Ptb 39 – JYOTHI



Ptb 57- SWETHA

Plate 9. Gel profile showing polymorphic bands of tolerant and susceptible Genotypes for EST-SSR RM 518.



Loading order: Lane L : Marker 1. N-22., 2. PTB 29., 3.PTB 30., 4.PTB 35., 5. PTB 39., 6. PTB 57

4.3 EFFECT OF WATER STRESS ON GROWTH AND YIELD PARAMETERS.

4.3.1. Plant height (cm):

Growth parameters were made at booting stage. The variations in plant height were presented in Table 27 (Figure 23). Under irrigated condition the highest plant height was found to be highest in N-22 i.e., 138 cm followed by Ptb 30 (111.5 cm) which is on par with Ptb 29 (107.4 cm), whereas least was recorded from Ptb35 (73.33 cm) which is on par with Ptb 39 with 79.00 cm. Under stressed condition the highest reduction in plant height was recorded from Ptb 29 with 80.23 cm a significant reduction of 25.29 % was recorded compared to control, whereas the least reduction in plant height was recorded from Ptb 35 by 1.13% with 72.5 cm.(Plate 8)

4.3.2. Days to 50% flowering:

The results of days to 50% flowering were presented in Table 28 (Figure 24). There is a two way trend in days to 50% flowering. Genotype like N-22, Ptb 35 and Ptb 39 has recorded a decrease in days to 50% flowering, whereas genotypes Ptb 29, Ptb 30 and Ptb 57 had recorded an increase in days to 50% flowering. The highest reduction in days to 50% flowering was noticed in N-22, which had shown a reduction of 15.10 days under 50% Fc, whereas the highest increase in days to 50% flowering under 50% FC was recorded from Ptb 57, which had shown an increase of 6.25 days under 50 % FC compared to their control.

4.3.3. Tiller number:

The results related to variation in tiller number among the genotypes under 100% and 50 % FC were presented in Table 29 (Figure 25). The results show a significant reduction in tiller number among the genotypes under stressed condition. At 100% FC the highest tiller number was recorded from N-22 with 9.66 number which is on par with Ptb29 with 9.33 whereas the least was recorded from Ptb 57 with 6.66 number which is on par with Ptb 35 with 7. At 50 % FC the highest was recorded from Ptb 29 with 8 which is on par with N-22 with 7.33, whereas least

was recorded from Ptb 39 (4) which had shown a severe decline of 39.21 % in tiller number than at 100% FC.

4.3.4. Productive tiller number:

The results related to variation in productive tiller number among the genotypes under 100% and 50 % FC were presented in Table 30 (Figure 26). The results show a significant decrease in productive tiller number among the genotypes. At 100% FC the highest productive tiller number was recorded from N-22 with 7 number which is on par with Ptb 29 with 6.66 whereas least was recorded from Ptb 39 and Ptb 57 with both a value 3.66. At 50 % FC the highest tiller number was recorded from N-22 with 5.33 and the least reduction in productive tiller number was recorded from Ptb 30 with 4.33 showing a reduction of 23.58 percentage which is on par with Ptb 29 with 4.66 whereas highest reduction in productive tiller number was recorded from Ptb 39 with 2.33 productive tiller showing a reduction of 53.84 percentage which is on par with Ptb 35 and Ptb 57.

4.3.5 Panicle length:

The results related to variation in panicle length among the genotypes under 100% and 50 % FC were presented in Table 31 (Figure 27). The results show a significant reduction in panicle length among the susceptible genotypes under water stressed condition. At 100% FC N-22 had recorded highest panicle length of 27.66 cm followed by Ptb 29 with 23.33 which is on par with Ptb 30 with 22.667cm and Ptb 57 with 22.33cm whereas least was recorded from Ptb 35 with 19.67 cm. At 50% FC the highest panicle length was recorded from N-22 with 24.667 cm showing a reduction of 10.86 %. The least reduction in panicle length under 50% FC was recorded from Ptb 29 with 21.66 showing a reduction of 7.17% compared with it's control, whereas the highest reduction in panicle length under stressed condition was noticed from Ptb 39 with 14.67 cm under 50% FC showing a reduction of 27.90% than its control.

Table 27. Effect of water stress on plant height (cm) of rice genotypes at the panicle initiation stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	138.00	115.33	-16.42	126.67
2.	Karuthamodan (Ptb 29)	107.40	80.23	-25.29	93.82
3.	Chuvannamodan (Ptb 30)	111.50	96.73	-13.24	104.12
4.	Annapoorna (Ptb 35)	73.33	72.50	-1.13	72.92
5.	Jyothi (Ptb 39)	79.00	67.67	-14.35	73.33
6.	Swetha (Ptb 57)	93.00	83.00	-10.75	88.00
	MEAN	100.37	85.91		
	C.D. (0.05)		SE(m) ±		
	G	8.26	2.81		
	T	4.77	1.62		
	G X T	11.68	3.98		

Table 28. Effect of water stress on days to 50% flowering of rice genotypes.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	64.00	54.33	-15.10	59.17
2.	Karuthamodan (Ptb 29)	74.67	77.67	+3.8	76.17
3.	Chuvannamodan (Ptb 30)	78.00	81.67	+4.48	79.83
4.	Annapoorna(Ptb 35)	63.33	55.67	-12.11	59.50
5.	Jyothi (Ptb 39)	76.00	82.67	+8.05	79.33
6.	Swetha (Ptb 57)	106.67	100.00	-6.67	103.33
	MEAN	77.11	75.33		
	C.D.(0.05)		SE(m) ±		
	G	2.78	0.95		
	T	1.61	0.55		
	G X T	3.94	1.34		

Table 29. Effect of water stress on tiller number of rice genotypes at the panicle initiation stage.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	9.67	7.33	-24.17	8.50
2.	Karuthamodan (Ptb 29)	9.33	8.00	-14.28	8.67
3.	Chuvannamodan (Ptb 30)	7.67	6.33	-17.43	7.00
4.	Annapoorna (Ptb 35)	7.00	5.33	-33.42	6.17
5.	Jyothi (Ptb 39)	7.67	4.00	-39.21	5.83
6.	Swetha (Ptb 57)	6.67	5.00	-20.5	5.83
	MEAN	8.00	6.00		
	C.D. (0.05)		SE(m) ±		
	G	0.75	0.25		
	T	0.43	0.15		
	G X T	1.06	0.36		

Table 30. Effect of water stress on Productive tiller number of rice genotypes.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	7.00	5.33	-30.47	6.17
2.	Karuthamodan (Ptb 29)	6.67	4.67	-33.32	5.67
3.	Chuvannamodan (Ptb 30)	5.67	4.33	-23.58	5.00
4.	Annapoorna(Ptb 35)	5.33	2.67	-50.12	4.00
5.	Jyothi (Ptb 39)	3.67	2.33	-53.84	3.00
6.	Swetha (Ptb 57)	3.67	2.67	-25	3.17
	MEAN	5.33	3.67		
	C.D. (0.05)		SE(m)±		
	G	0.75	0.25		
	T	0.43	0.15		
	G X T	N/S	0.36		

Table 31. Effect of water stress on Panicle length of rice genotypes.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	27.67	24.67	-10.86	26.17
2.	Karuthamodan (Ptb 29)	23.33	21.67	-7.17	22.50
3.	Chuvannamodan (Ptb 30)	22.67	21.00	-7.35	21.83
4.	Annapoorna (Ptb 35)	19.67	15.33	-22.05	17.50
5.	Jyothi (Ptb 39)	20.33	14.67	-27.90	17.50
6.	Swetha (Ptb 57)	22.33	17.67	-20.92	20.00
	MEAN	22.67	19.17		
	C.D. (0.05)		SE(m) ±		
	G	1.49	0.51		
	T	0.86	0.29		
	G X T	2.11	0.72		

4.3.6 Yield per plant:

The results related to variation in yield per plant among the genotypes under 100% and 50 % FC were presented in Table 32 (Figure 28). There is a significant reduction in yield per plant among the genotypes under stressed condition. At 100% FC the highest yield per plant was recorded from N-22 with 28.66 g followed by Ptb 29 with 23.33g which is on par with Ptb 30 and Ptb 57 with 22.83g and 22.033 g respectively, whereas least was recorded from Ptb 39 with 19.10 g which is on par with Ptb 35 with 20.66 g. At 50% field capacity there is a significant reduction in yield per plant among the genotypes. Under 50% FC the highest yield was recorded from N-22 with 24.667g showing a least reduction of 13.97% followed by Ptb 29 with 18.16 gram per plant, whereas the lowest yield was recorded from Ptb 39 with 10.167g showing a reduction of 47.12% than it's control followed by Ptb 35 with 13.68g with a reduction of 33.80%.

4.3.7 Spikelet fertility percentage:

The results related to variation in yield per plant among the genotypes under 100% and 50 % FC were presented in Table 33 (Figure 29). There is a significant reduction in spikelet fertility percentage among the susceptible genotypes. At 100% FC the highest spikelet fertility percentage was recorded from N-22 with 91% followed by Ptb 29 with 83.33% which is on par with 79.33% whereas the least was noticed from Ptb 39 with a value of 74.93% which is on par with Ptb 57 with 78.16%. At 50% FC the highest spikelet fertility percentage was recorded from N-22 with 85.66% showing a least reduction of 5.86% followed by Ptb 30 with 73.16% showing a reduction of 7.78% which is on par with Ptb 29 with 72.66% whereas least was recorded from Ptb 39 with 60.5 % of spikelet fertility showing the highest reduction of 19.26%.

4.3.8 1000 grain weight:

The results related to variation in 1000 grain weight among the genotypes under 100% and 50 % FC were presented in Table 34 (Figure 30). There is no

significant variation in 1000 grain weight among the genotypes. At 100% FC the highest 1000 grain weight was recorded by Ptb 30 with 27.33g which is on par with Ptb 29 with 27.29g whereas least was recorded from N-22 with 22.167g, whereas at 50% FC the highest 1000 grain weight was recorded from Ptb 30 with 27.23 g showing a minute reduction of 0.36%, followed by Ptb 29 with 26.083g under water stress. The highest reduction in 1000 grain weight was recorded from Ptb 39 with 22.50g with a reduction of 11.87%.

Table 32. Effect of water stress on yield per plant of rice genotypes .

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	28.67	24.67	-13.97	26.67
2.	Karuthamodan (Ptb 29)	23.33	18.17	-22.17	20.75
3.	Chuvannamodan (Ptb 30)	22.83	16.92	-25.94	19.87
4.	Annapoorna (Ptb 35)	20.67	13.68	-33.80	17.17
5.	Jyothi (Ptb 39)	19.10	10.17	-47.12	14.63
6.	Swetha (Ptb 57)	22.03	16.90	-23.29	19.47
	MEAN	22.77	16.75		
	C.D. (0.05)		SE(m)±		
	G	1.36	0.46		
	T	0.78	0.27		
	G X T	1.92	0.65		

Table 33. Effect of water stress on spikelet fertility percentage of rice genotypes.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	91.00	85.67	-5.86	88.33
2.	Karuthamodan (Ptb 29)	83.33	72.67	-12.80	78.00
3.	Chuvannamodan (Ptb 30)	79.33	73.17	-7.78	76.25
4.	Annapoorna (Ptb 35)	76.33	63.33	-17.03	69.83
5.	Jyothi (Ptb 39)	74.93	60.50	-19.26	67.72
6.	Swetha (Ptb 57)	78.17	70.67	-9.60	74.42
	MEAN	80.52	71.00		
	C.D. (0.05)		SE(m) ±		
	G	2.69	0.91		
	T	1.55	0.53		
	G X T	3.80	1.29		

Table 34. Effect of water stress on 1000 grain weight of rice genotypes.

Sl. No	GENOTYPE	100 % FC	50 % FC	% CHANGE	MEAN
1.	Nagina-22	22.17	21.57	-2.70	21.87
2.	Karuthamodan (Ptb 29)	27.30	26.08	-4.44	26.69
3.	Chuvannamodan (Ptb 30)	27.33	27.23	-0.36	27.28
4.	Annapoorna (Ptb 35)	24.03	23.43	-2.49	23.73
5.	Jyothi (Ptb 39)	25.53	22.50	-11.87	24.02
6.	Swetha (Ptb 57)	25.83	24.50	-5.16	25.17
	MEAN	25.37	24.22		
	C.D. (0.05)		SE(m) ±		
	G	0.87	0.30		
	T	0.50	0.17		
	G X T	1.23	0.42		

Plate 8: Effect of water stress on rice genotypes at flowering stage.

NAGINA - 22

Ptb 29-KARUTHAMODAN

Ptb 30- CHUVANNAMODAN

Ptb 35 -ANNAPOORNA

Ptb 39- JYOTHI

Ptb 57 - SWETHA

4.4 Genotyping of the selected tolerant and susceptible rice varieties using available *DEEPER ROOTING* QTL specific microsatellite primers and other drought related primers.

4.4.1 Quality and Quantity of DNA Samples

Quantity and purity of DNA samples obtained for selected genotypes were presented in Table 35. Quality of DNA samples were assessed from the gel picture showing DNA bands.

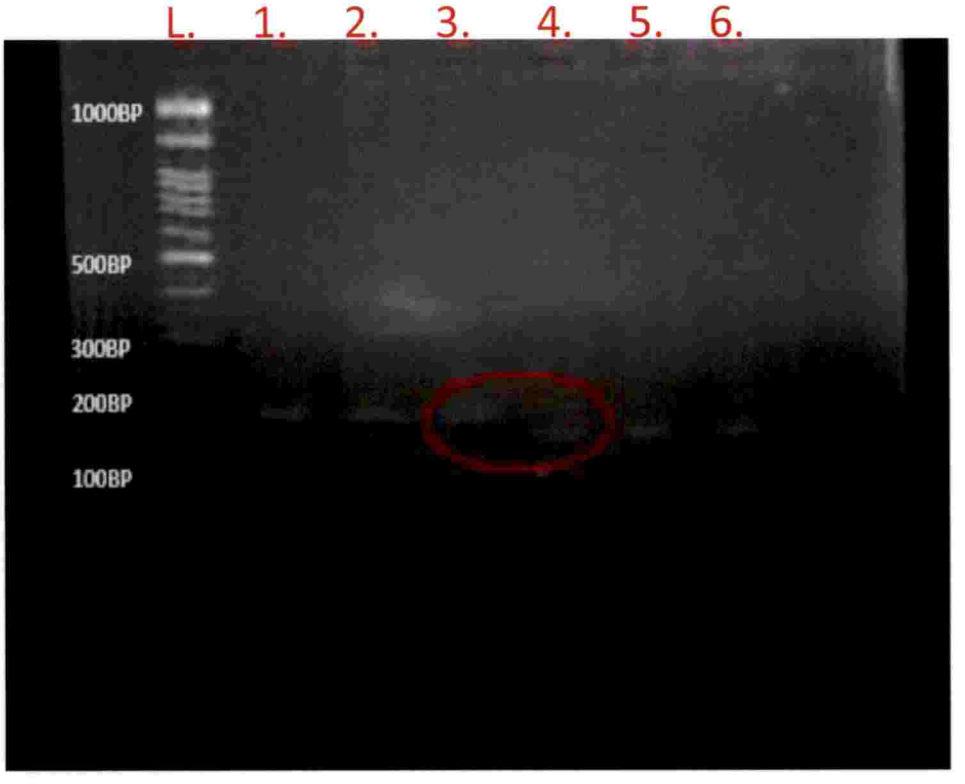
4.4.2 Screening of DEEPER *ROOTING* QTL specific microsatellite primers and other drought related primers.

The genotypes under study were screened with all the available *DEEPER ROOTING QTL* specific markers and other drought related SSR and EST-SSR available. The amplified PCR products were visualized and documented in a gel documentation system. Later the bands were scored either for monomorphic or polymorphic. Of all the 12 *DEEPER ROOTING QTL* specific microsatellite primers, none of them exhibited polymorphism for tolerant and susceptible genotypes. Among the other drought related EST-SSR markers screened one primer RM 518 showed polymorphism between tolerant and susceptible genotypes. The RM 518 produced a product size approximately 180 bp in tolerant genotypes and 170 bp in susceptible genotypes.(Plate 9&10)

Table 35: Quality and Quantity of DNA Samples isolated from rice genotypes.

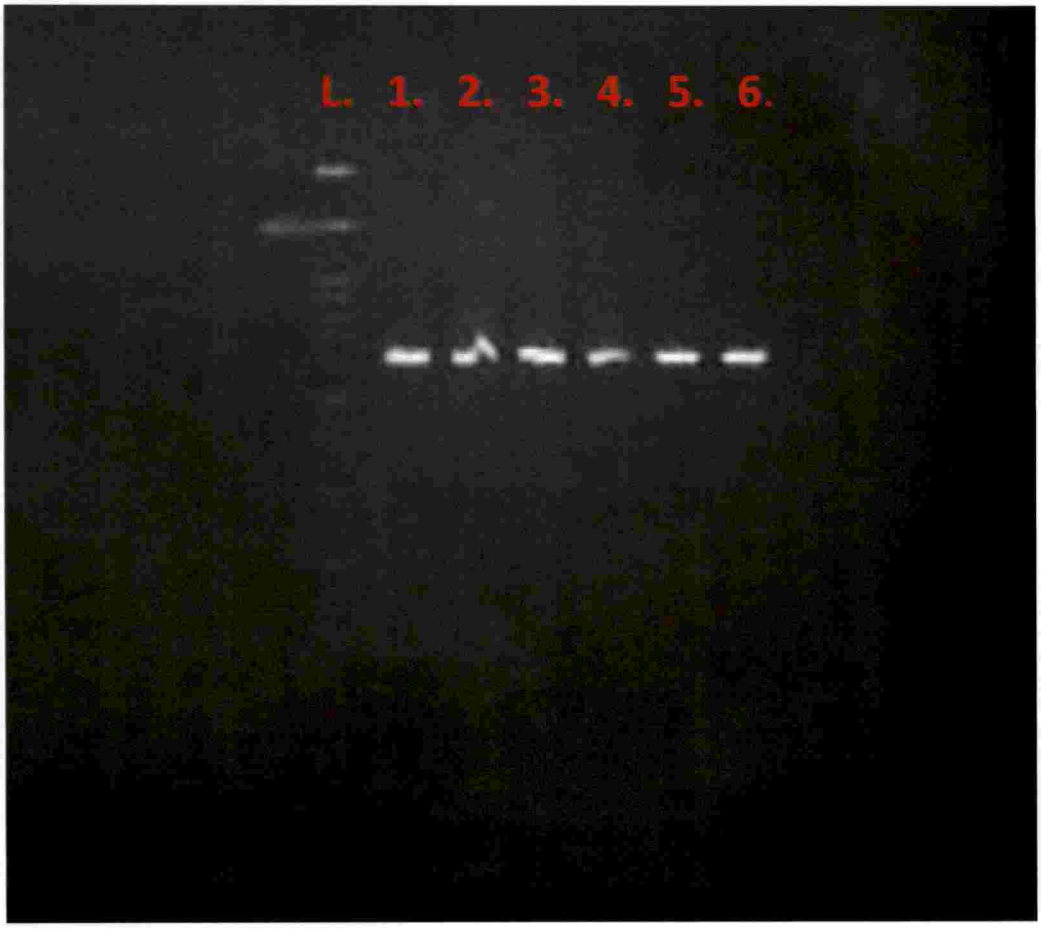
Sl. No.	Genotype	A260/A280 value	DNA Concentration (ng/µL)
1.	Nagina-22	1.70	3048
2.	Karutha modan(Ptb 29)	2.06	1470
3.	Chuvannamodan (Ptb 30)	2.05	2631
4.	Annapoorna(Ptb 35)	2.06	2010
5.	Jyothy (Ptb 39)	1.84	972
6.	Swetha (Ptb 57)	1.68	1512

Plate 9. Gel profile showing polymorphic bands of tolerant and susceptible Genotypes for EST-SSR RM 518.



Loading order: Lane L : Marker 1. N-22., 2. PTB 29., 3.PTB 30., 4.PTB 35., 5. PTB 39., 6. PTB 57

Plate 10. Gel profile showing monomorphic bands of tolerant and susceptible Genotypes for *DRO1-SEQ1*.



Loading order: Lane L : Marker 1. N-22., 2. PTB 29., 3.PTB 30., 4.PTB 35., 5. PTB 39., 6. PTB 57

4.5 Expression studies in the selected genotypes for drought tolerance using RM 518 EST-SSR marker.

4.5.1 Quality and Quantity of RNA Samples

Quantity and purity of RNA samples obtained for selected genotypes i.e., one tolerant genotype Ptb 30 and one susceptible Ptb 35 were presented in Table 36. Quality of DNA samples were assessed from the gel picture showing RNA bands. (Plate 11)

4.5.2 Expression of drought tolerance gene in tolerant and susceptible genotype - RT PCR Analysis.

Expression level of drought tolerance gene was analysed from root samples of Ptb 30 and Ptb 35 at PI stage under both irrigated and stressed conditions and the results were presented in plate 11. An amplicon size of approx. 190 bp was found in tolerant genotype under two conditions. Whereas the susceptible genotype under both conditions produced an amplicon size of 180 bp. Expression of the gene was found in both genotypes under both treatments, but the intensity was less in susceptible genotype and tolerant genotype control condition than tolerant genotype under stress.(Plate 12)

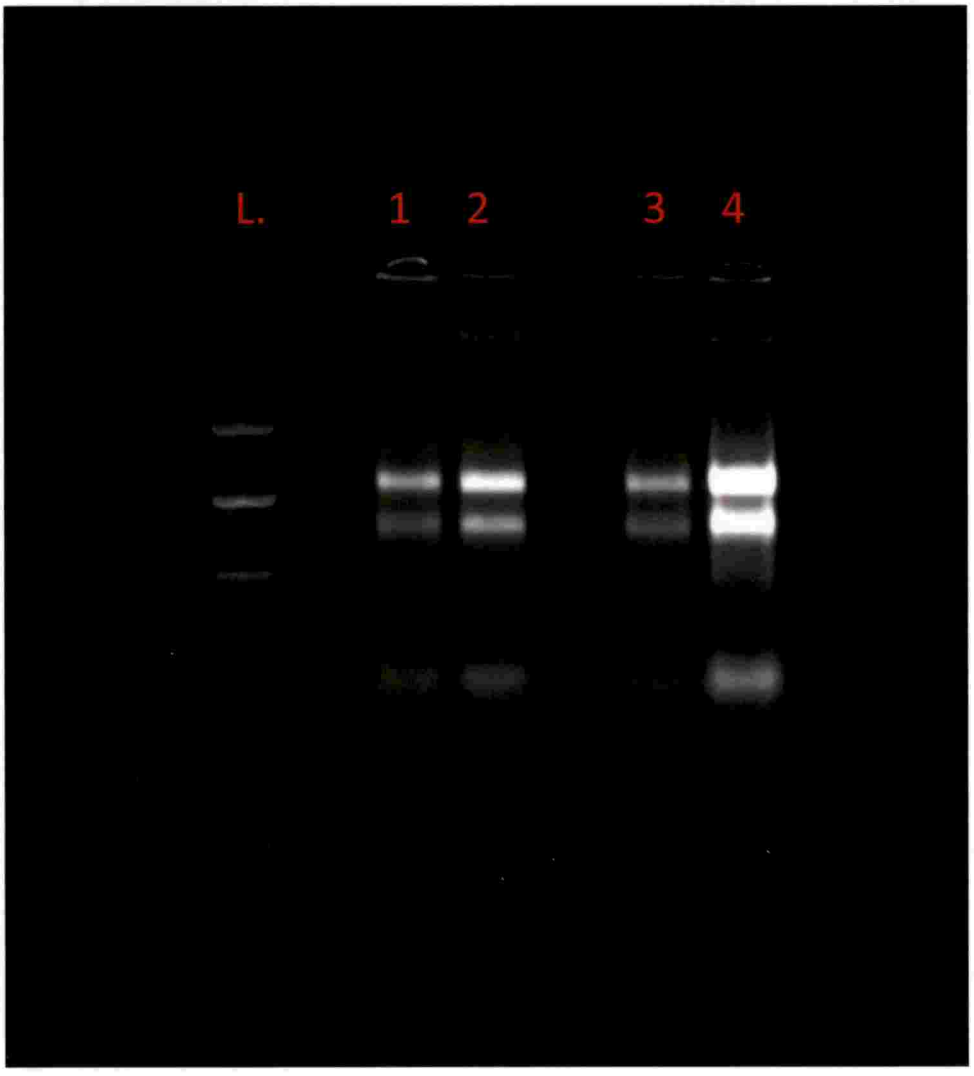
4.5.3 Sequencing and analysis of polymorphism exhibited in Ptb 30 under stress.

The 190 bp sized polymorphic band generated from RM 518 was eluted using Nucleospin® Gel and PCR clean up kit (Macherey-Nagel, Germany). Using agarose gel electrophoresis the quality and quantity of the eluted products were confirmed and was subjected further to reamplification to confirm whether the band contains the amplified product of single region of chromosome.

Table 36: Quality and Quantity of RNA Samples isolated from rice genotypes.

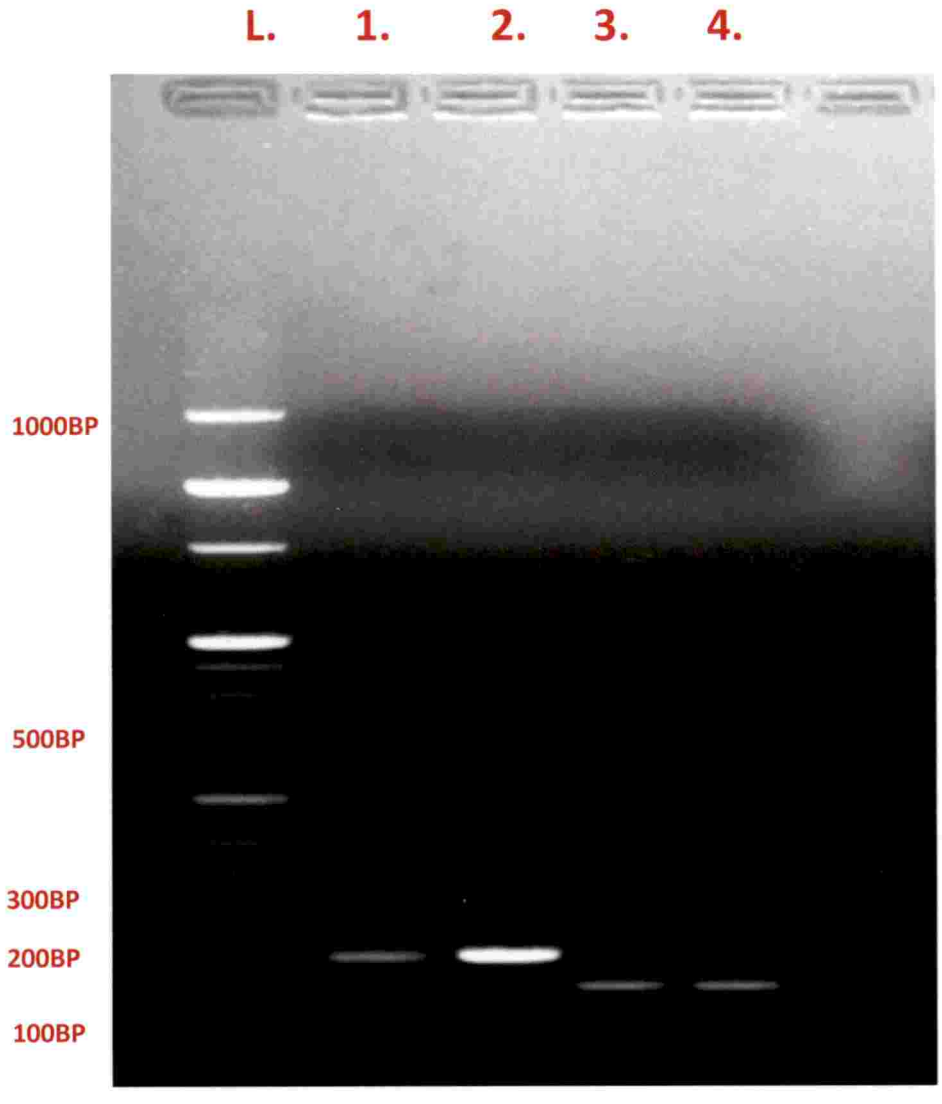
Sl. No.	Genotype	A260/A280 value	RNA Concentration(ng/μL)
1.	Ptb 30 at 100% FC	1.70	2400
2.	Ptb 30 at 50% FC	1.62	1704
3.	Ptb 35 at 100% FC	1.82	1743
4.	Ptb 35 at 50% FC	1.82	1095

Plate 11: Quality of RNA isolated from rice genotypes



L: Ladder 1000bp 1. Ptb 30 at 100% FC 2. Ptb 30 at 50% FC 3. Ptb 35 at 100% FC 4.Ptb 35 at 50%

Plate 12: Expression analysis using RM 518



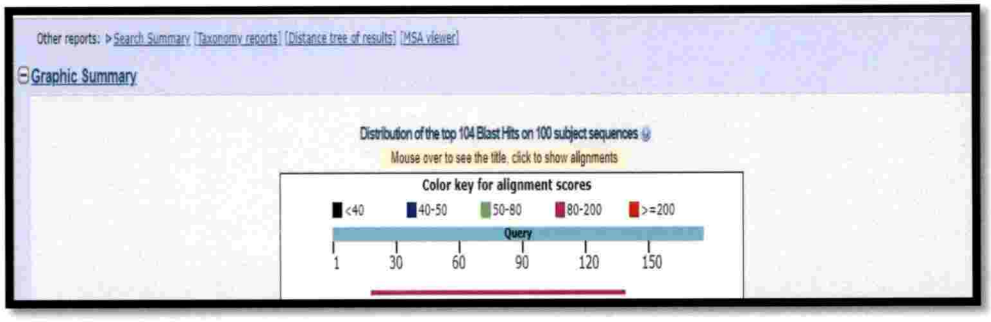
L: Ladder 1000bp 1. Ptb 30 at 100% FC 2. Ptb 30 at 50% FC 3. Ptb 35 at 100% FC 4.Ptb 35 at 50%

Band generated using the nested PCR had shown its suitability for direct sequencing, the product was sent for sequencing with the primer RM 518.

The sequence has 182 base pairs. The sequence generated was 3'-GTGATTGGTGACGACTCGCCTGTTGAGTGTTTTGAGGAGGAACAAAAG ATAGCTGGACAGAGAGAGAGAGAGAGAGAGAGAGAGAGACACCCAC GGTGTGTTCTTCTCCCTCCCGCCCCGGGGTGAGTGAGGGGAGAGACCC CCCCCCCCCCCCCCCCCCTGAAGAGAGGCCCGGG-5'.

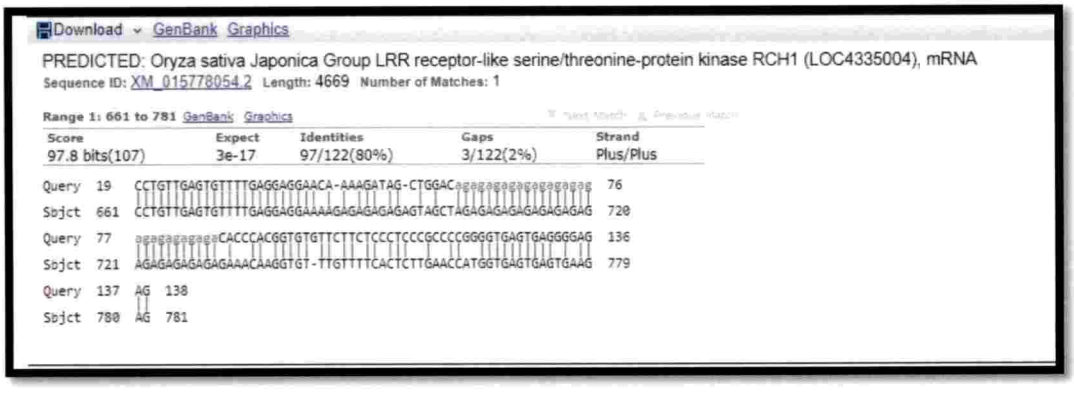
The sequence was subjected to *in silico* analysis, using Nucleotide BLAST (Basic Local Alignment Search Tool). In BLASTN the sequence had shown 79.69 % similarity with *Oryza sativa japonica* group LRR receptor-like serine/threonine-protein kinase RCH1 (LOC4335004), mRNA with accession number XM_015778054.2.

Plate13. Result of nucleotide BLAST of the 182 bp sequence generated using the polymorphic band obtained with EST-SSR RM 518



a. Graphical summary of nucleotide

b. Sequence similarity



c. Alignment

Discussion

5. DISCUSSION

Being a sessile organism, plants encounter a wide range of adverse environmental conditions. Among this wide range of stresses curtailing the crop growth and productivity, water deficit severely affects the sustainable production of a crop (Foley *et al.,* 2011). Rice is a staple food crop for one-third population worldwide, meeting the daily calorie requirements of nearly 80% of these individuals (Khush, 2005). Comparing to other cereals rice is considered as one of the most drought-sensitive crops. Presence of small or shallow root system, thin cuticular wax, and swift stomata closure made it more susceptible to drought stress (Serraj *et al.,* 2011).

Ray *et al.* (2013) reported that variations in climate change severely influence the available sources of water and also influence the frequency of drought and flood occurrence was likely to increase in the near future. As the crop yields depend on the specific climatic factors, any deviations or aberrations would result in a severe decline in production. They also reported from the climatic data of the past 30 years that 53% of rice growing regions are experiencing the influence of climatic variability with a yield penalty of 0.1t/hm.

Waseem *et al.* (2011) reported the responses of plants to drought stress to vary with particular plant genotypes, plant species, age of the plant, it's phase of development and the severity with which the stress is imposed. A plant manifests itself with many mechanisms to adapt to the given drought stress condition, namely drought escape, drought avoidance, and drought tolerance; they adapt on the basis of their molecular responses and morpho-physiological changes (Fukai and Cooper, 1995).

In the present study, six rice genotypes collected from RARS, Pattambi and IIRR were evaluated for physio-morphological, anatomical and yield parameters.

Then genotyping of plant DNA samples were done using *DEEPER ROOTING 1* specific markers, a set of SSR and EST-SSR markers to find out the polymorphic band between the tolerant and susceptible genotypes. Expression

study was carried out in the selected genotypes for drought tolerance using RM 518 EST-SSR marker. Significant variations were observed for all the parameters studied and the results obtained are discussed in this chapter with appropriate support from previous studies.

5.1 EFFECT OF WATER STRESS ON PHYSIOLOGICAL PARAMETERS

In this study, various physiological and biochemical parameters were studied among rice tolerant genotypes and this section explains the basis of results obtained.

Relative water content, one of the methods to study the tissue water status closely related with the leaf water potential (Sinclair and Ludlow, 1985). Kumar *et al.* (2014) stated that screening of genotypes for drought tolerance using morpho-physiological traits such as RWC, revealed that genotypes that are tolerant to drought showed higher RWC than genotypes that are susceptible to drought.

The study indicates a significant variation in RWC among the genotypes under water stress conditions mainly due to inadequate availability of soil moisture under water stress conditions. The genotype N-22 exhibited the least decrease in RWC by 4.44% whereas the Ptb 35, Ptb 39 and Ptb 57 genotypes exhibited a decrease in RWC to a maximum of 14.11% under water stress. Prasad *et al.* (2019) reported a similar kind of results with rice genotypes N 22, NDR 97, Susk Samrat and Swarna Sub1. Among all the genotypes the least reduction in RWC was noticed from N 22 showing a reduction of 4% under stress whereas the highest reduction is noticed from Swarna sub1 with reduction of 25% RWC.

Jahan *et al,* (2013) reported a similar kind of decrease in relative water content among the tolerant and susceptible genotypes under water stress. They also reported that this decrease in RWC will be accompanied by an increase in the osmotic potential of the cells.

Wright *et al,* (1994) reported a significant reduction in SLA was noticed in tolerant genotype under water stress. Higher SLA indicates the more leaf area per unit biomass, resulting in higher transpiration surface and poor photosynthetic machinery as the thickness of the leaf will be less. This kind of result was noticed from susceptible genotypes of the experiment. Under 100% FC genotypes as N 22 and Ptb 29 recorded the highest specific leaf area, whereas under stress these genotypes i.e., N-22 had shown a reduction in SLA to a maximum of 41.86% under stress whereas susceptible genotypes either maintained the same SLA or increased by 1.5% making them prone to transpiration loss of water.

Devi *et al.* (2013) reported similar results by screening of paddy genotypes for high water use efficiency and yield components. From their experiment, the genotypes that recorded lower SLA under water stress viz., NLR 3183, NLR 34242, NLR 3010, NLR 40059 and NLR 33671 had shown higher photosynthetic efficiency as well as WUE.

Cell membrane stability measures the increased permeability and leakage of ions out of the cell as an indicator of drought stress tolerance. Premachandra *et al.* (1979) from their experiment on the relation between electrolyte leakage and the ability of plants to tolerate drought stress, and they concluded that cell membrane stability measured by electrolyte leakage correlates well with the tolerance of other plant processes to stress. A similar trend was noticed from the study where tolerant genotype N-22 recorded the high cell membrane stability index with 94.35 % whereas least was shown by Ptb 39 with 83.11%.

Carbon isotopic discrimination ($\mathbf{\Delta^{13}C}$) can be made use as a selection criterion for yield under water stress as reported by Adiredjo *et al*, (2014). In C_3 plants like rice, it can be used as an indicator of water use efficiency and can be exploited by breeders for selecting drought-tolerant plants (Cattivelli *et al.*, 2008).

Wright *et al,* (1994) reported water use efficiency or crop plants always coincide with decreased CID values of the genotypes. A similar trend was noticed in the genotypes, N-22 recorded the least CID value 21.84 ($\Delta^{13}C(mil^{-1})$ whereas the

highest was seen from Ptb 35 with 23.49 ($\Delta^{13}C$)(mil^{-1}). A similar kind of results were reported by Gao, 2018. The CID of rice genotypes Qishanzhan, Akihikari and Bendao was found to be decreasing with the increase in severity with which drought stress was imposed. The value of CID for the three rice genotypes were in rage of 20.5, 21 and 24.7 under water stress condition. This decrease in CID is coincided with the WUE of the rice genotypes

5.2 EFFECT OF WATER STRESS ON ROOT PARAMETERS:

Deeper rooting is a complex trait governed by the angle of root growth and maximum length of the root (Abe and Morita, 1994). Though shallow rooting favours the acquisition of phosphorus from the superficial layers of soil, deeper rooting becomes beneficial in extracting the water from deeper layers of subsoil when subjected to water stress. In the present study, a water stress of 50% FC throughout the growth period had shown a significant increase in the rooting behaviour of rice genotypes at the booting stage. The mean difference in root among rice genotypes was 13.02%.

Ingram *et al*, (1994) reported a greater root length density below 20/30 cm when rice crops were grown under rainfed lowland scenario. At 100% FC genotypes, N 22, Ptb 29 and Ptb 30 recorded highest root length. Under 50% FC genotypes N 22, Ptb 29 and Ptb 30 under study followed the same trend as reported by Ingram *et al,* (1994) i.e., increased their rooting depth at 50% FC, whereas genotypes Ptb 35, Ptb 39 and Ptb 57 restricted their root growth under stress due to the hard compaction of soil and inability of roots to penetrate deep into it. Renukha *et al,* (2013) also reported a significant variation in root length at genotypic and treatment level. From their results, tolerant genotypes like NLR 33671 exhibited the highest root length followed by NLR 3010.

Ganapathy, *et al,* (2010) reported a positive correlation between root volume, grain yield and dry matter accumulation under stress. Drought resistance of the plant can be improved by improving the thickness of the root making the

roots of capable of increasing in root length to extract the water from subsoil layers (Yogameenakshi *et al*, 2004). Increased in root volume directly makes root capable of mining to greater depths in search of water. This similar trend was noticed in the study where genotypes N 22, Ptb 29 and Ptb 30 had recorded higher root volume under 100% FC also shown a significant increase in root length as well as volume under 50% FC condition.

There is a significant increase in root dry weight among the tolerant genotypes, a mean increase of 25.9% increase was noticed compared with 100% FC. This increased root dry weight was associated with better water potential in leaves and partitioning of assimilates more towards the root. Kavitha, (2014) reported a similar significant difference for root dry weight among the genotypes and a progressive increase in dry weight was seen up to 60DAS. Sridhara *et al,* (2012) stated that higher root length, volume, weight and number were recorded under aerobic condition compared to puddling.

Plant under stress conditions often exhibits phenotypic plasticity to overcome the negative effects of the stress (Kathiresan *et al,* 2006). Xu., *et al* (2015) from their experiments on drought tolerant and susceptible genotypes, IRAT109 and Zhenshan97B reported a significant increase in R/S ratio among genotypes under drought stress. Tolerant genotype ZS97 had shown an increase of 50% whose results are on par with the results shown by Ptb 29 in the study, whereas susceptible genotype IRAT had shown an increase of 41% is on par with the results of Ptb 57, whereas decreased root-shoot ratio among the susceptible genotypes reveals the inability of genotypes to partition the assimilates under a given stressful situation.

Turner, (2001) specific root length (SRL) are positively correlated with increased crop productivity under water stress. Kato *et al.* (2009) reported from the experiments on Akihikari and IRAT109 rice varieties under aerobic and flooded condition there is a significant difference among the genotypes as well as the treatments for specific root length. Similarly in the study genotypes as Ptb 29 and

Ptb 30 had shown a significant difference for specific root length under different water regimes, whereas Ptb 35, Ptb 39 and Ptb 57 genotypes haven't shown any significant difference under 100% and 50% FC.

Kadam *et al,* (2015) from his experiments on rice genotypes reported that the tolerant genotype N-22 had shown lower leaf weight ratio of 16% under stress. These findings were in line with the genotypes N 22, Ptb 29 and Ptb 30 under study. Stem weight ratio of N-22 and IR 64 were found to be increasing significantly under stress condition, a similar strategy was shown by a rice genotype PTB 57 with an increase of 14.3%. Root weight ratio was found to be highest in N 22 under 100% FC condition, whereas in the genotypes, N 22, Ptb 29 and Ptb 30 exhibited a significant increase in root weight ratio under 50% FC, whereas Ptb 35, Ptb 39 and Ptb 57 genotypes had shown an increasing trend which is not significant.

The photosynthetic productivity of a crop mainly depends on leaf area, chlorophyll content and gaseous exchange of the leaf. In present study leaf area in present study showed a mean reduction of 43.33%. However genotypes like N-22 haven't shown much reduction in yield due to increased thickness of leaf as reported from specific leaf area and this increased leaf thickness offers better chlorophyll content and gaseous exchange. These findings are in line with Chauhan *et al,* (1996) who reported a 54.3% reduction in leaf area when plants were imposed with water stress.

5.3 EFFECT OF WATER STRESS ON BIOCHEMICAL PARAMETERS:

The starch accumulation in roots found to be high at booting stage then starts decreasing as the grain starts to develop. This stored starch in roots serves as a driving force for grain development and shows variations in accumulation in tolerant and susceptible genotypes under water stress. In the present study, there is an increase in starch among the genotypes N 22, Ptb 29 and Ptb 30 by an increase of 43.8% compared with 100% FC, whereas Ptb 35, Ptb 39 and Ptb 57 genotypes haven't showed any significant increase in starch content under 50% FC.

These findings are in line with Singh *et al,* (2013) where drought susceptible rice genotypes BPT-5204 and Saita exhibited least increase in starch content in their roots under water stress whereas tolerant genotypes like N-22 had shown a significant increase in starch.

Xu *et al.* (2015) reported a similar trend in starch accumulation under water stress in rice genotypes ZS97 and IRAT109. Under water stress among the genotypes a sharp increase in root starch content in ZS97 by 133% compared to control, and had no effect in case of IRAT109.

Protein profiling of tolerant and susceptible genotype roots were done using SDS-PAGE to study up-regulation and down-regulation of proteins. Among the tolerant and susceptible genotypes, there is a differential accumulation of proteins. Genotypes, N 22, Ptb 29 and Ptb 30 under both the treatments exhibited a protein band of 35kDa, whereas a novel protein band was noticed in N-22 under stress with 45kDa. These results were in line with Singh *et al,* (2013) who observed a similar band profiling, from their studies on N-22 and Ratna. They reported the presence of a novel protein band of 39±2 kDa only in N-22 but not in any other drought susceptible genotype i.e., Ratna.

5.4 EFFECT OF WATER STRESS ON ROOT ANATOMICAL PARAMETERS:

Root, the principle organ and the entry point for water and mineral nutrients shows several modifications when exposed to severe water stress. These modifications at phenotypic level includes both architectural and anatomical. The regulation of water entry into plant system strongly depends on the anatomy of plant root and the responses of root anatomy are diverse. The anatomical features of root includes, diameter of root determining the penetrating capacity of root, xylem number and diameter determining axial conductivity of water, sclerenchyma and aerenchymatous tissue determining radial conductivity of water (Sibounheuang *et al.,* 2006). All these modifications were found to be useful traits for extraction of

water from deeper layers of soil and preventing the loss of water from plant (Yambao *et al.,* 1992).

Root diameter variations occurs as a result of change in number and size or width of cortical cells in stele diameter. Root diameter was found to be closely associated with penetrating ability of root. Qian Cai *et al.* (2017) reported increase in root diameters under mild and severe water stress treatments than under irrigated condition, especially during the late growing season. Kadam *et al,* (2015) also reported a similar kind of results in rice genotypes 'IR64', Apo, and 'N-22'. There is a significant reduction in root diameter under stress condition in susceptible genotypes 'IR64' where as the tolerant genotypes haven't shown decrease but an increase in root diameter by Apo and N-22

The present study revealed a similar kind of results. Under 100% FC genotypes N 22 and Ptb 29 had recorded higher root diameter. Under 50 % FC these genotypes i.e., N 22 and Ptb 29 had shown an increase in root diameter whereas genotypes Ptb 30, Ptb 35 and Ptb 39 neither shown a significant decrease nor increase.

Stele is the central portion of the root that is found to have conducting tissues i.e., xylem and phloem. Stele diameter was reported to be better indicator of root penetration ability compared with root diameter (Chimungu *et al,* 2015). Kadam, *et al.* (2015) reported that there is no significant difference in stele diameter at root shoot junction under both control and water stress condition, but the tolerant rice genotypes 'N-22' exhibited a higher diameter of stele at 6 cm from the root apex. Similar results were noticed from the present study. The stele diameter was found increasing significantly by 31.67% i.e., from 0.367mm in 100% FC to 0.431mm under 50% FC condition.

Under moisture deficit condition the efficiency in water uptake would be greatly influenced by root anatomical phenes associated with axial and radial conductance of water (Lynch, *et al.,* 2014). Xylem vessel traits mainly number,

diameter, and area affect the axial conductance of water whereas the radial conductance of water is affected by cortical traits and the presence of suberized cell layers. Gowda *et al,* (2011) reported that the presence of larger xylem vessels and thick roots are associated with improved drought tolerance in aerobic rice. A similar trend was noticed in the present study where the six genotypes N 22, Ptb 29, Ptb 30, Ptb 35, Ptb 39 and Ptb 57 had shown an overall increase in late metaxylem number, diameter and early metaxylem number by 14.01%, 16.66% and 15.10% under 50% FC respectively.

Knodo *et al.* (2000) reported that presence of thickened sclerenchymatous will always be associated with offering structural support to roots when they were exposed to adverse soil conditions such as drought. In the present study genotypes had shown two way trend in sclerenchymatous tissue width, increase is associated with conferring structural support whereas decrease in thickness is associated with increase the permeability in water uptake.

Aerenchyma tissue formation occurs rapidly in roots of grasses when they are exposed to anoxic condition to facilitate gaseous exchange (Jackson and Armstrong, 1999), whereas Jackson *et al.,* (1985) reported the rapid formation on aerenchyma even in well aerated environments. The results of the present study were in line with both the cases, genotypes like N-22 and Ptb 29 had shown an increase in width of aerenchyma under stress to reduce the cost of metabolic energy needed for cell maintaince, whereas genotypes like Ptb 30 had shown reduction in aerenchyma as the conditions are aerobic.

Kadam *et al,* (2015) reported that stele diameter to root diameter ratio was strongly and influenced under water stress condition. Among the rice genotypes they studied i.e., N-22, IR 64, and Apo they noticed an increasing trend with stele diameter to root diameter was noticed in all the three genotypes under water deficit conditions. A similar trend was noticed in tolerant rice genotypes of present study where the stele to root diameter showed a mean increase of 19.4%.

5.5 EFFECT OF WATER STRESS ON GROWTH AND YIELD PARAMETERS:

Under water stress conditions the non-availability of water reduces the turgor pressure which influences the cell division and cell elongation activities of plant and resulting in reduction in plant height. Plant height was reported to be reduced as a response to water stress irrespective of genotypes. In the present study all the genotypes had shown a significant reduction in plant height. There is a mean reduction in plant height by a mean value of 14.44%.

The highest plant height under stress condition was recorded from 'N-22' showing a reduction 16.42% than control whereas least plant height was noticed in susceptible genotypes like Ptb 35, Ptb 39 and Ptb 57. These findings are in line with that of Singh *et al,* (2018) reported a low reduction in N-22 plant height by 17.56% whereas least plant height from susceptible Swarna Sub 1.

Fukai *et al,* (1999) reported that phase change from vegetative to reproductive will be greatly influenced by water stress condition. Under drought condition susceptible genotypes were expected to flower early whereas tolerant genotypes maintains more or less similar to normal condition. In the present study days to 50% flowering showed a two way trend among the genotypes under study. Genotypes like Ptb 35 and Ptb 39 had shown a significant reduction in days to flowering whereas genotypes N 22, Ptb 29 and Ptb 30 had shown same duration as control. Singh *et al,* (2108) reported a similar results, showing less reduction in days to flowering under tolerant genotypes i.e., N-22 by 9.65% similar kinds of results were seen in study. Whereas susceptible genotypes Ptb 35 and Ptb 59 had shown a significant reduction in days to flowering.

Under water stress condition due to reduction in growth and photosynthetic process in plant the number of tiller and productive tiller per plant were greatly reduced (Quampah *et al,* 2011). In the present study there is a reduction in tiller and productive tiller number among all the genotypes ranging less in tolerant (Ptb 29, Ptb 30 and N 22) to more in susceptible genotypes (Ptb 39 and Ptb 35). Similar results were noticed from Singh *et al.* (2018) where tolerant genotypes N-22

showed the least reduction in tiller number whereas susceptible genotypes Swarna sub 1 had shown highest reduction in tiller number.

Boonjung and Fukai, (1996) reported a reduction in grain number, seed setting rate i.e., fertility percentage, and grain yield when rice panicle were exposed to water stress. Zaman *et al.* (2018) reported the variations in yield components associated with panicle formation and maturation under water stress in two genotypes aerobic rice variety MA1 and lowland rice variety MR253. Upon incidence of stress both the varieties reported a reduction in panicle number, 100 grain weight, grain yield. A similar kind of results were noticed in the present study where a reduction of panicle length, 1000 grain weight and gran yield among the genotypes was noticed. The mean reduction of panicle length in the study was 15.44%, grain yield 26.43%, spikelet fertility percentage 11.82% and 1000 grain weight by 4.5% under stress.

5.6 GENOTYPING OF TOLERANT AND SUSCEPTIBLE GENOTYPES USING *DRO1* SPECIFIC MARKERS AND DROUGHT RELATED SSR, EST-SSR.

The genotypes under study were screened with all the available *DEEPER ROOTING QTL* specific markers and other drought related SSR and EST-SSR available. Among the various markers studied, RM 518 (171 bp) produced polymorphism between tolerant and susceptible genotypes. The RM 518 marker produced a product size of ~ 180 bp in tolerant genotypes whereas in susceptible genotypes it produced a product size of ~ 171 bp. Mohammadi *et al.*(2013) reported the location of RM 518 in rice chromosome number 4 QTL associated with water use efficiency (Figure 31).

Veeraghattapu *et al.* (2015) reported trough in silico approach that the QTLs flanked by markers RM 518 - RM 261 associated with CID in rice.

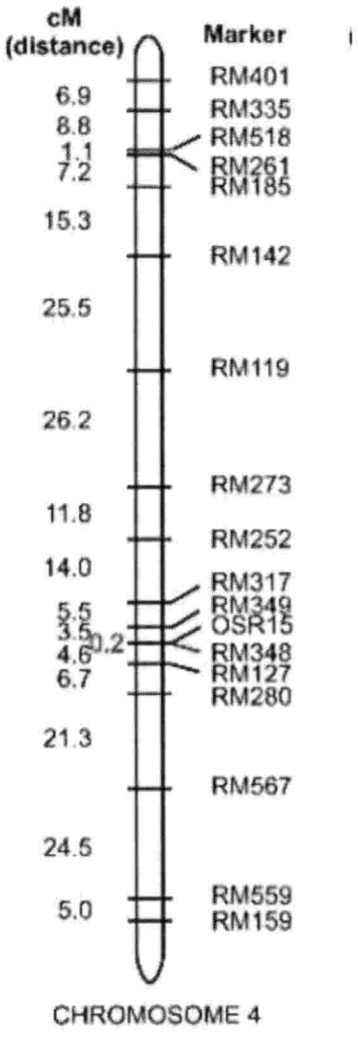
Mohammadi *et al.* (2013) reported three QTLs associated with total spikelet per plant were present on chromosome 4, 7 and 9, collectively accounting for a total phenotypic variation of 27%. The major effect QTL, qTSP4.1s, flanked by SSR markers RM 551 and RM 518 on rice chromosome 4.

Prince *et al.* (2015) reported a region C20 on rice chromosome 4 found to be linked with SPAD (leaf chlorophyll content) under water stress condition. The same QTL was reported to have 36.8% of the phenotypic variation of biomass under managed stress environments.

5.7 EXPRESSION ANALYSIS OF DROUGHT RELATED GENE - RT PCR

A differential expression of genes was noticed among tolerant genotype Ptb 30 and susceptible Ptb 35. RT-PCR results show that tolerant genotype produced a product size of 190 bp whereas susceptible 180 bp. The sequenced product of 190 bp tolerant genotypes show that the gene that showed differential expression is LRR receptor-like serine/threonine-protein kinase RCH1 (LOC4335004. Transcriptome profile from NCBI-Blast in flower buds, flowers, flag leaves and roots sampled before flowering and after flowering, milk grains and mature seeds in rice shows higher levels of expression from roots before flowering which coincides with our study results. Universal protein resource shows that the expression of gene LRR receptor-like serine/threonine-protein kinase RCH1 (LOC4335004) is associated with regulation of root meristem growth.

Figure 31. Chromosome 4 of rice showing the position of marker RM 514 (Lang et al. 2013)



145

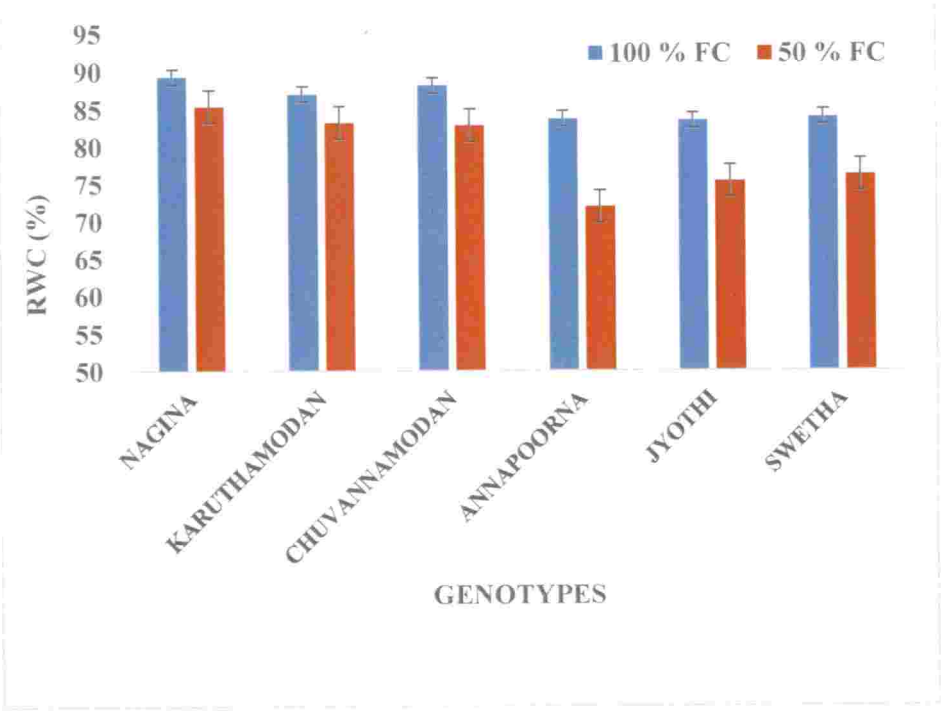


Figure 1: Variation in relative water content (%) of rice genotypes at booting stage under 100% FC and 50% FC.

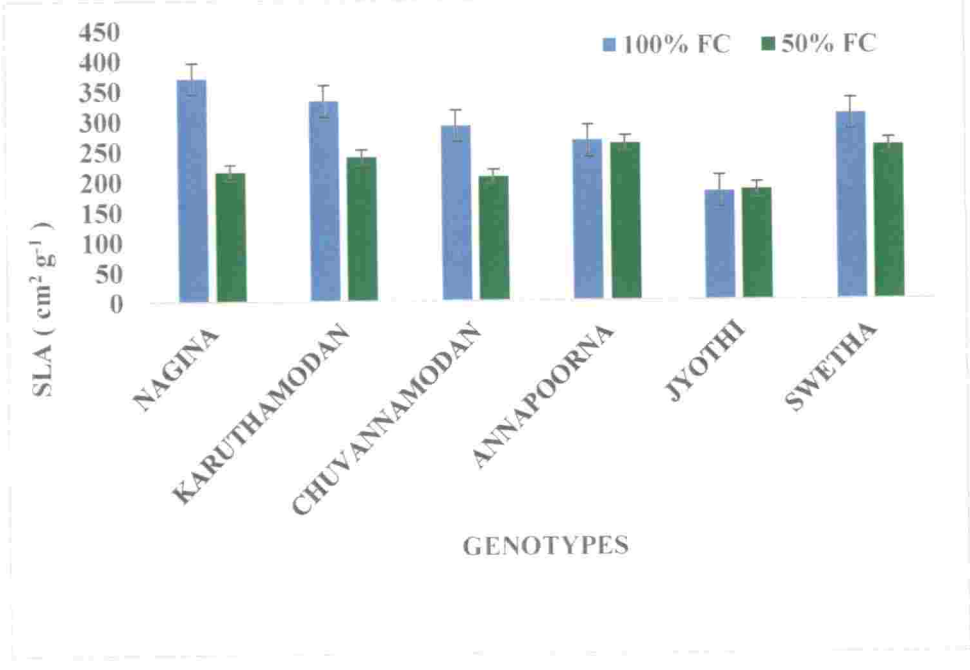


Figure 2: Variation in specific leaf area (cm^2 g^{-1}) of rice genotypes at booting stage under 100% FC and 50% FC.

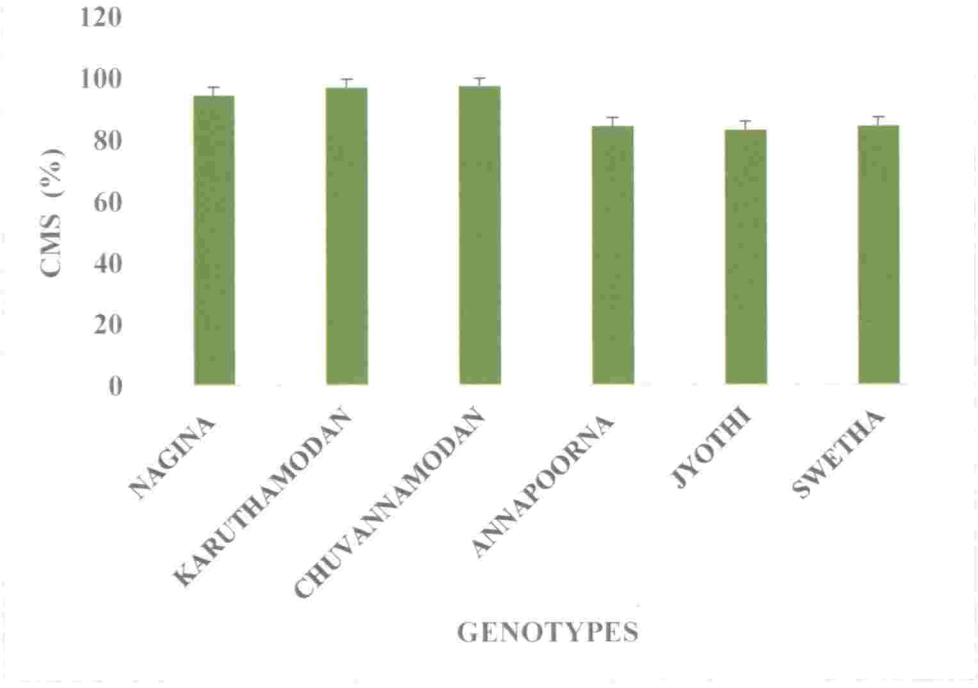


Figure 3: Variation in cell membrane stability index (%) of rice genotypes at booting stage under 100% FC and 50% FC.

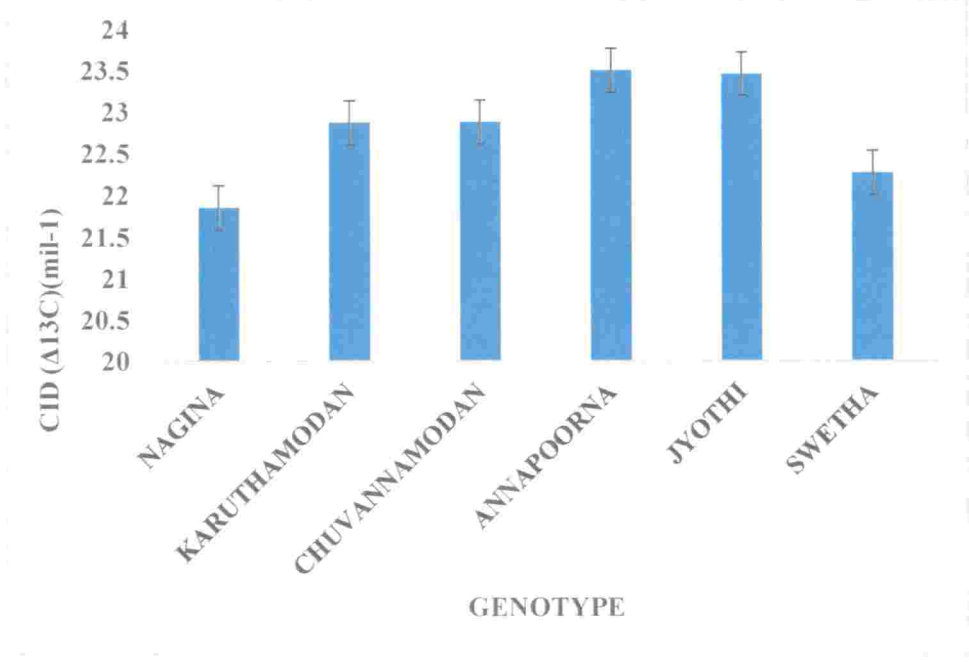


Figure 4: Variation in carbon isotope discrimination ($\Delta^{13}C$)(mil^{-1}) of rice genotypes at booting stage under 100% FC and 50% FC.

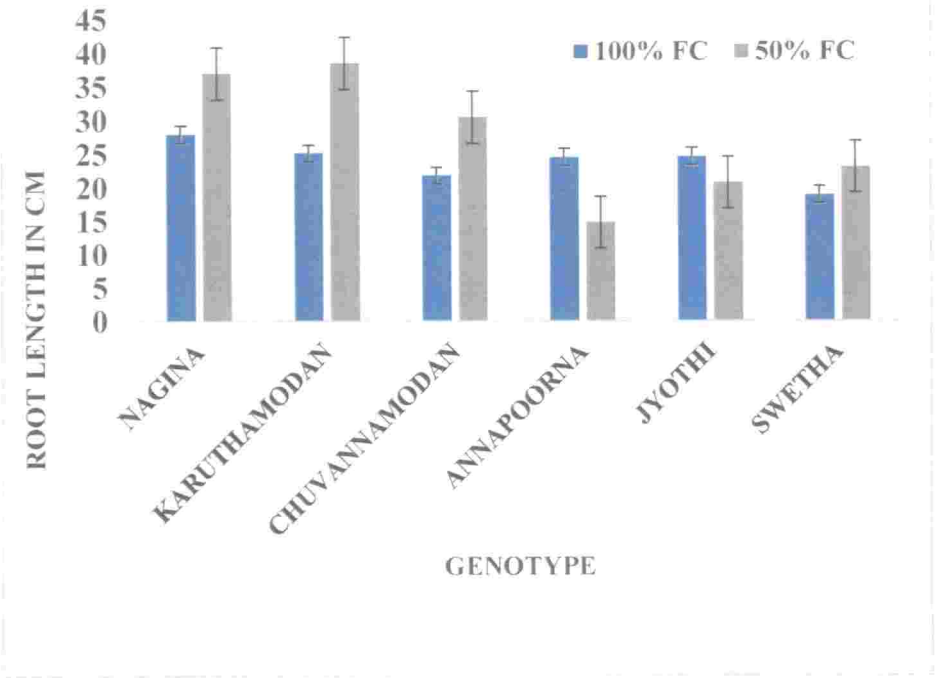


Figure 5: Variation in Root length (cm) of genotypes at booting stage under 100% & 50% FC.

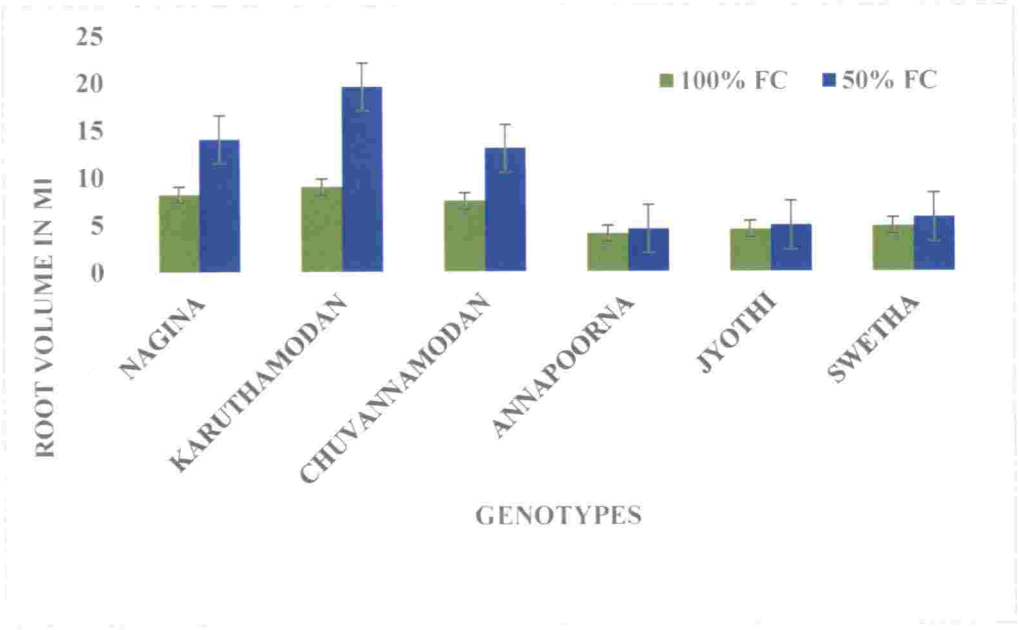


Figure 6: Variation in Root volume (mL) of genotypes at booting stage under 100% & 50% FC.

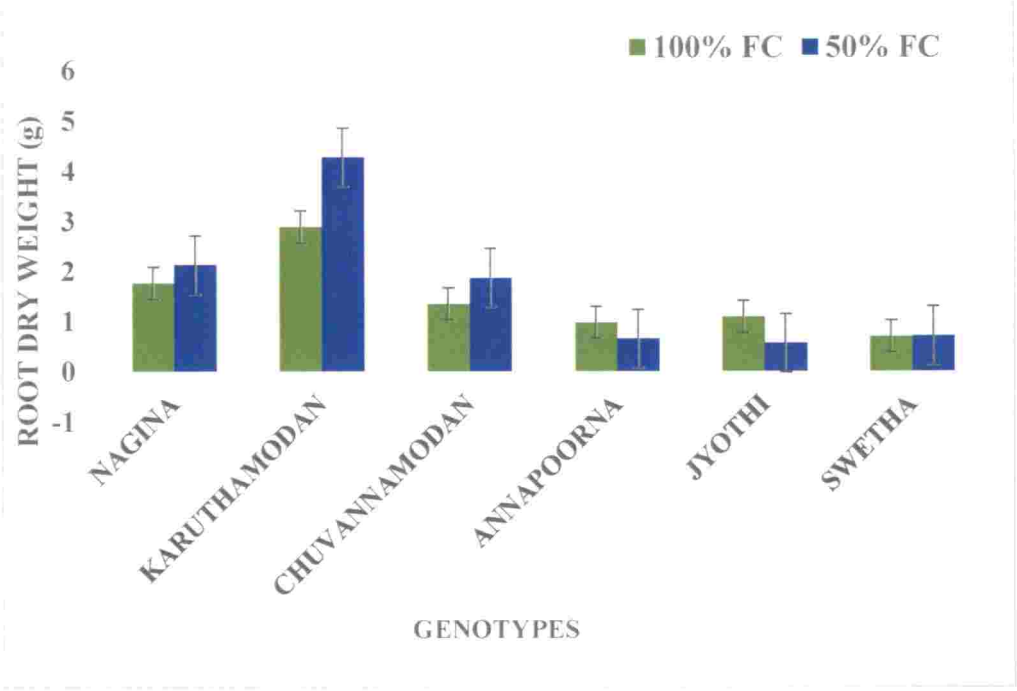


Figure 7: Variation in Root dry weight (g) of genotypes at booting stage under 100% & 50% FC.

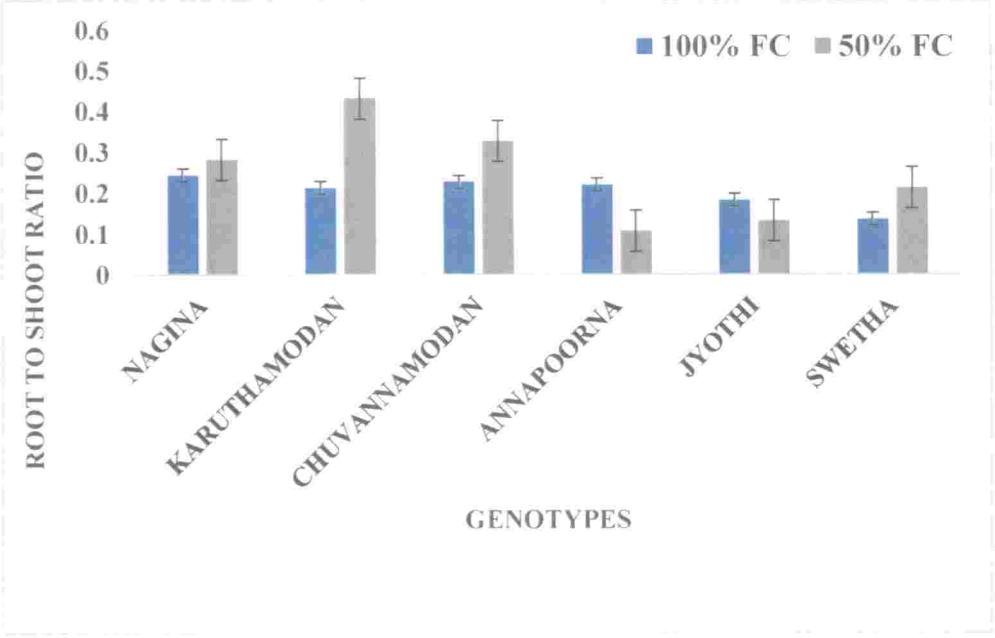


Figure 8. Variation in Root shoot ratio of genotypes at booting stage under 100% & 50% FC.

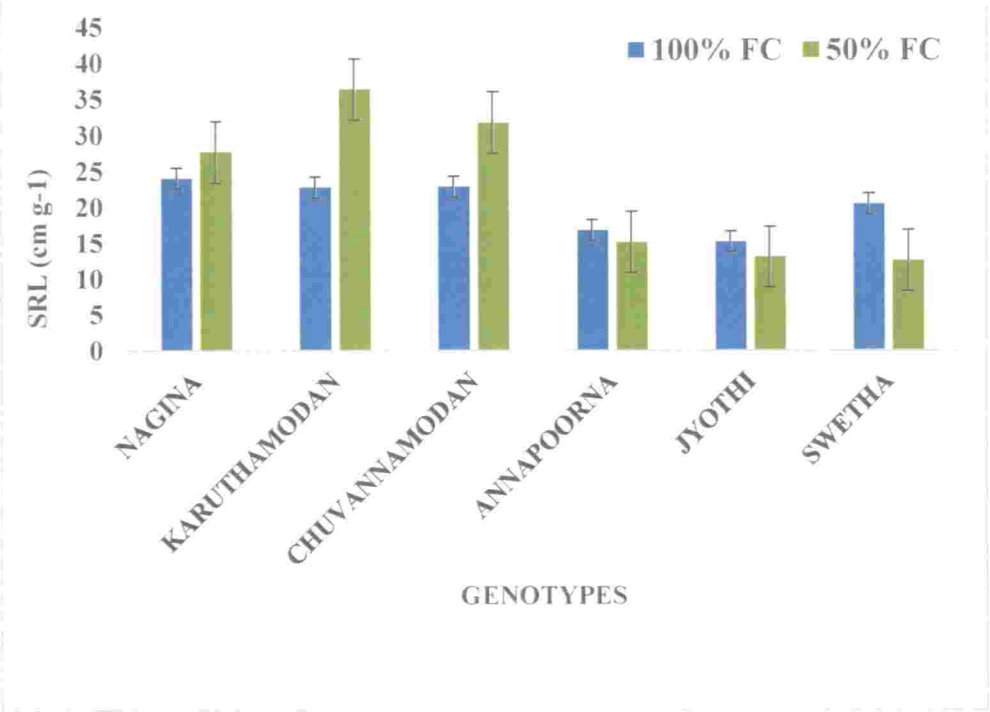


Figure 9: Variation in Specific root length (cm g^{-1}) of genotypes at booting stage under 100% & 50% FC.

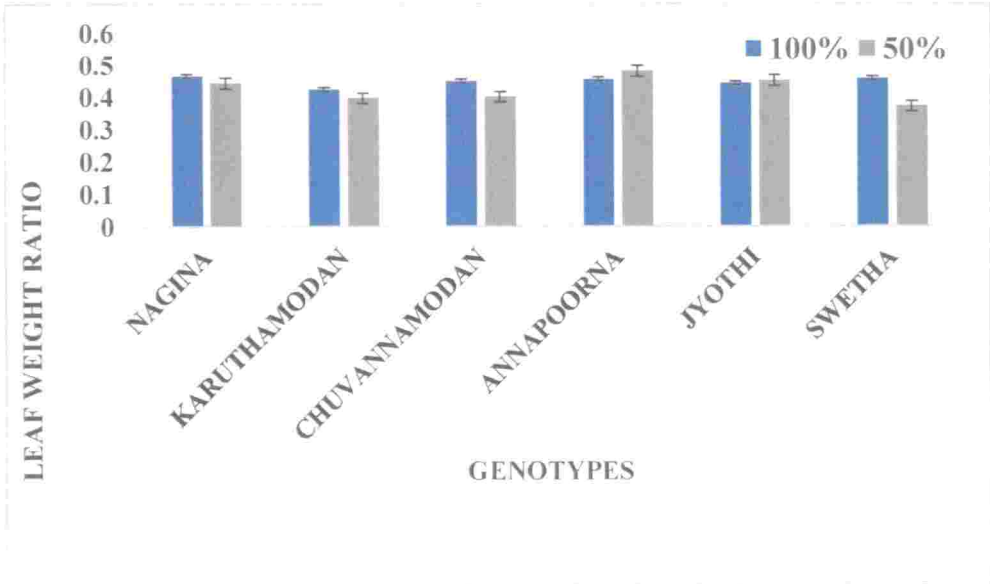


Figure 10: Variation in leaf weight ratio of genotypes at booting stage under 100% & 50% FC.

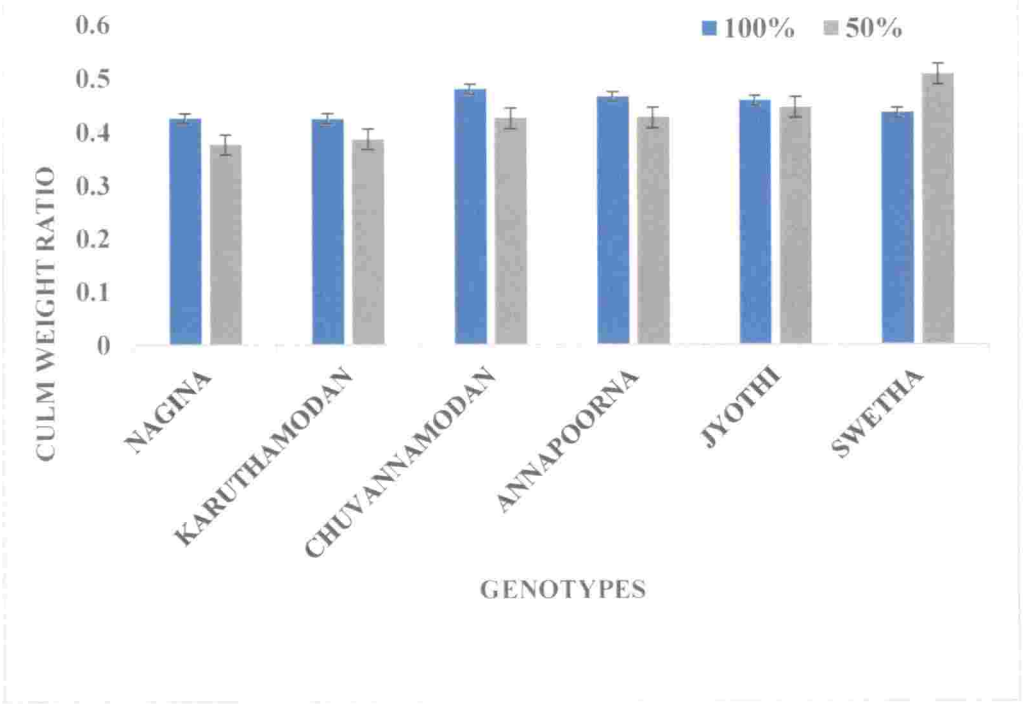


Figure 11. Variation in culm weight ratio of genotypes at booting stage under 100% & 50% FC.

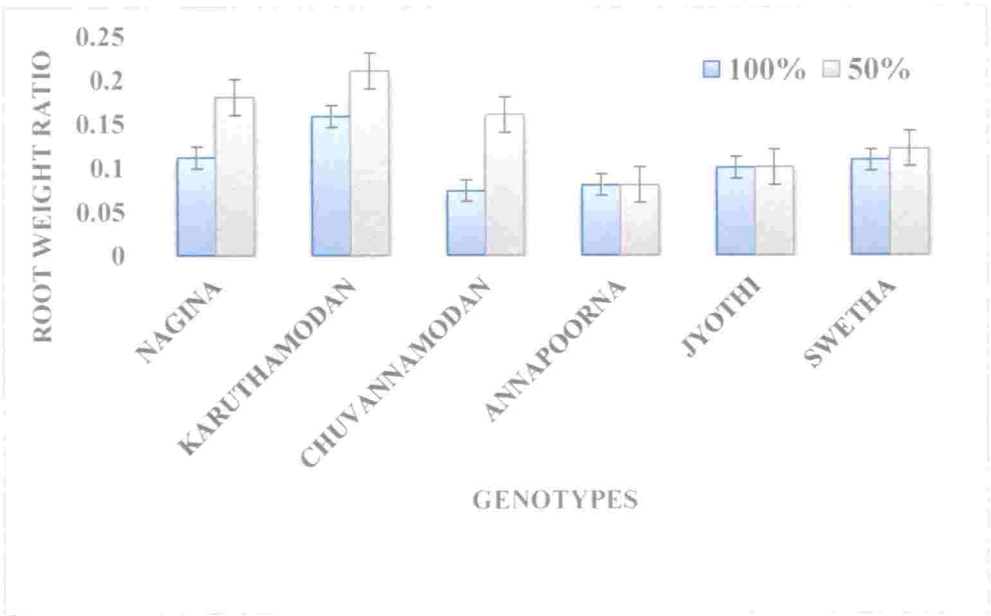


Figure 12. Variation in root weight ratio of genotypes at booting stage under 100% & 50% FC.

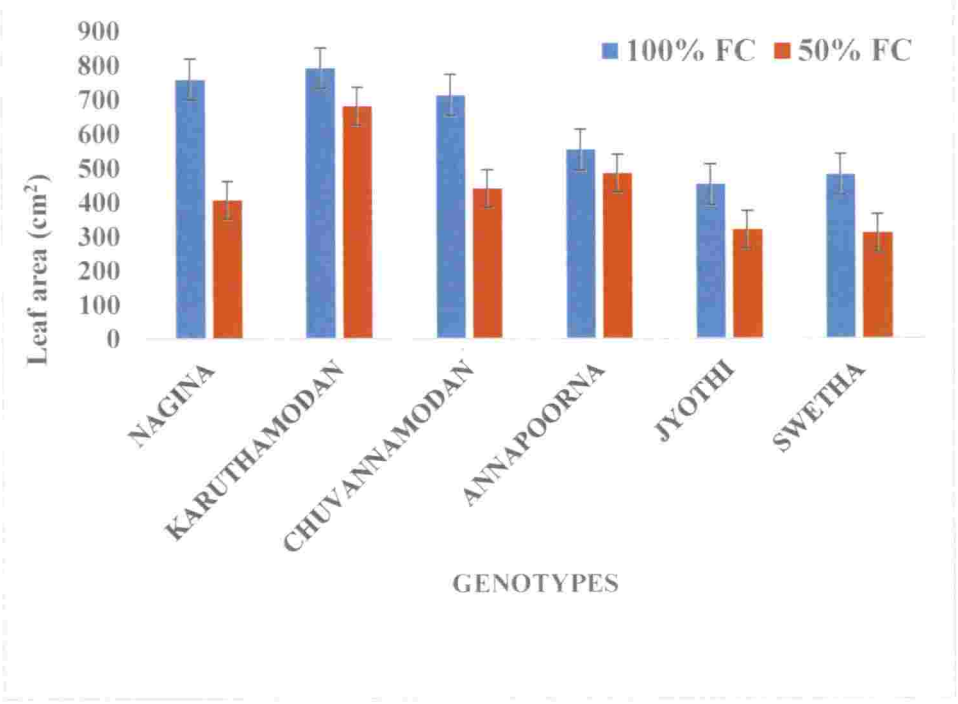


Figure 13: Variation in Leaf area (cm^2) of genotypes at booting stage under 100% & 50% FC.

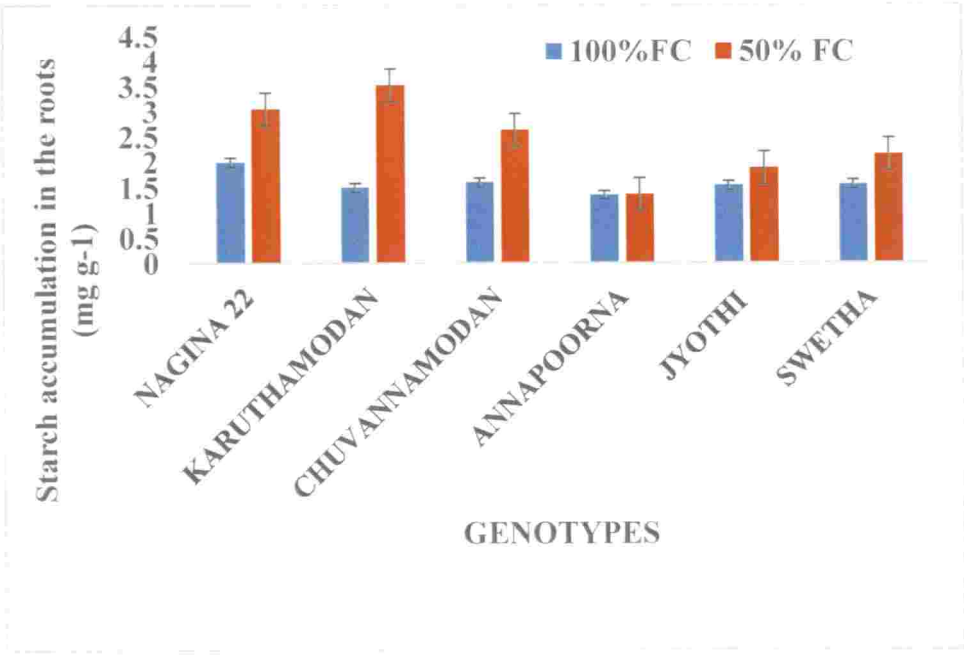


Figure 14. Variation in Starch accumulation in the roots (mg g^{-1}) of genotypes at booting stage under 100% & 50% FC.

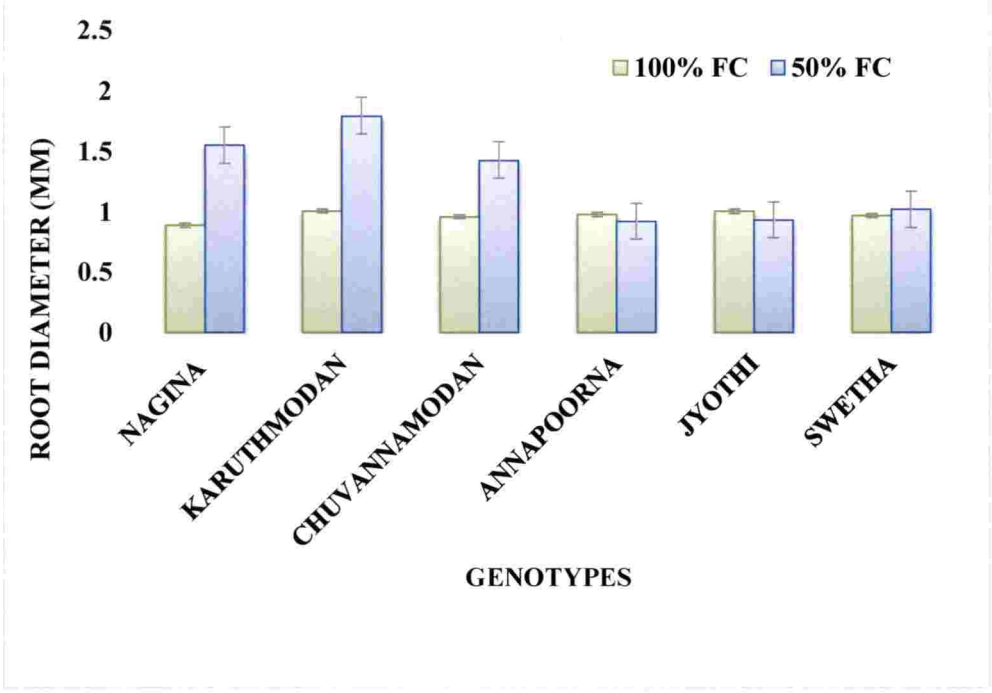


Figure 15. Variation in Root diameter (mm) of genotypes at booting stage under 100% & 50% FC

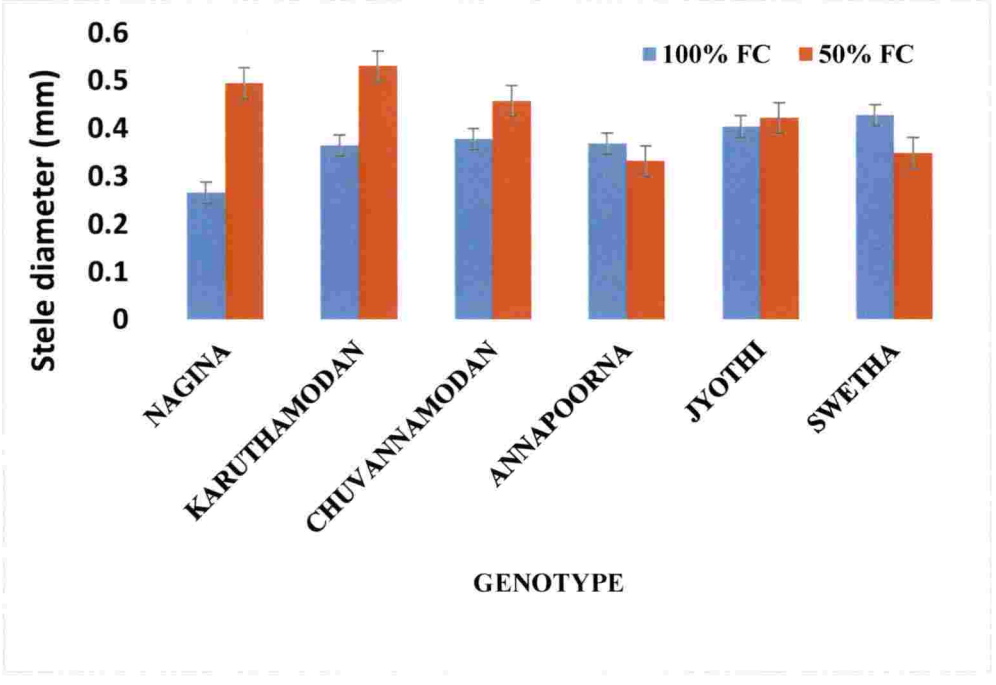


Figure 16. Variation in Stele diameter (mm) of genotypes at booting stage under 100% & 50% FC.

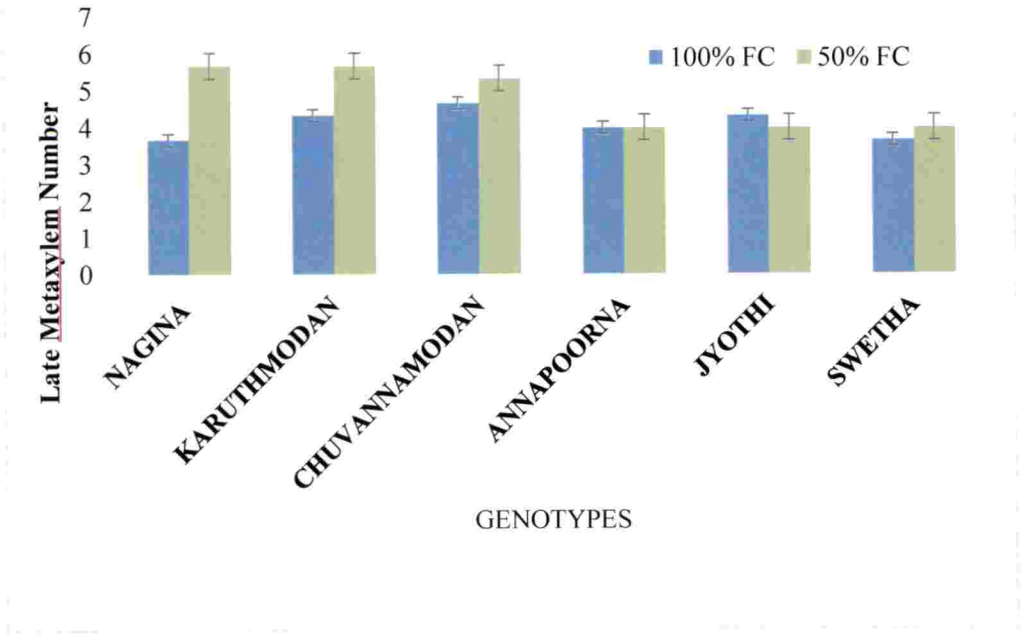


Figure 17: Variation in Late Metaxylem Number of genotypes at booting stage under 100% & 50% FC.

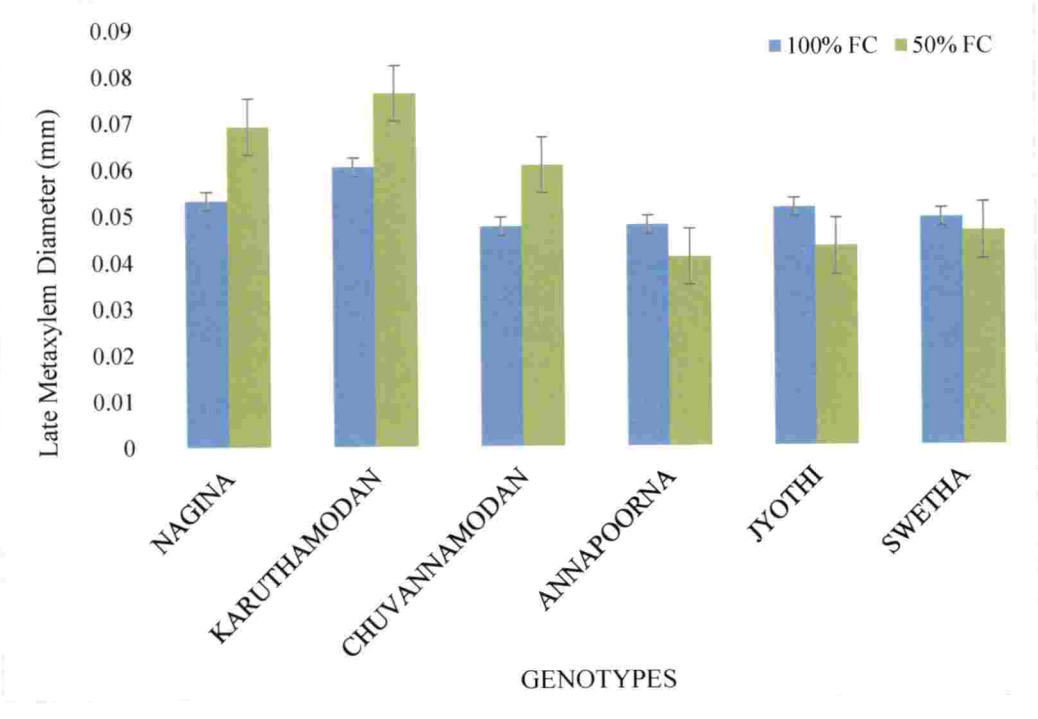


Figure 18: Variation in Late Metaxylem Diameter (mm) of genotypes at booting stage under 100% & 50% FC.

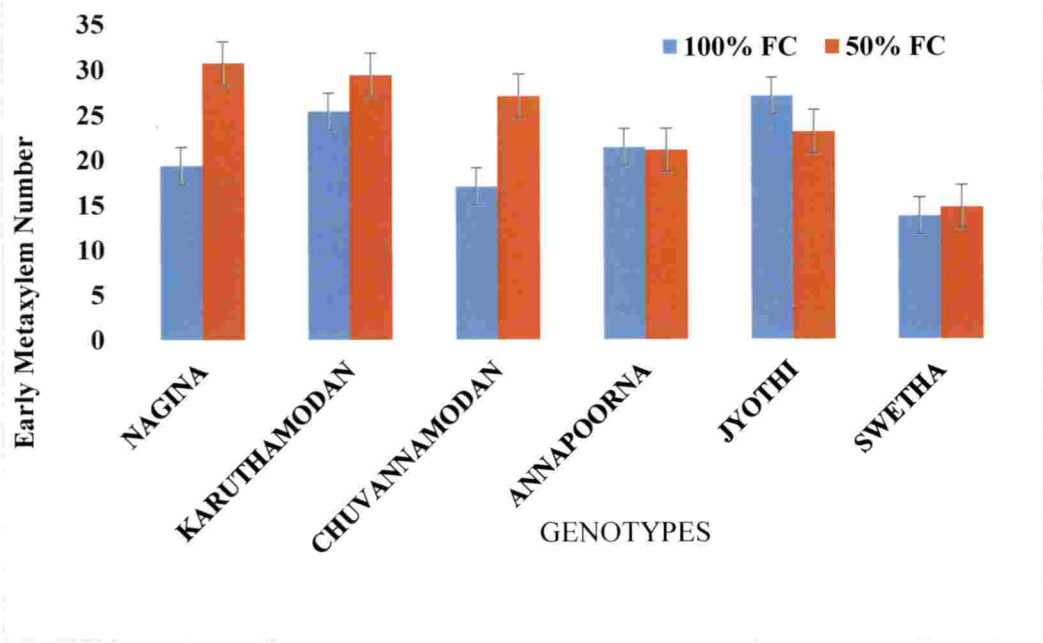


Figure 19: Variation in Early Metaxylem Number of genotypes at booting stage under 100% & 50% FC.

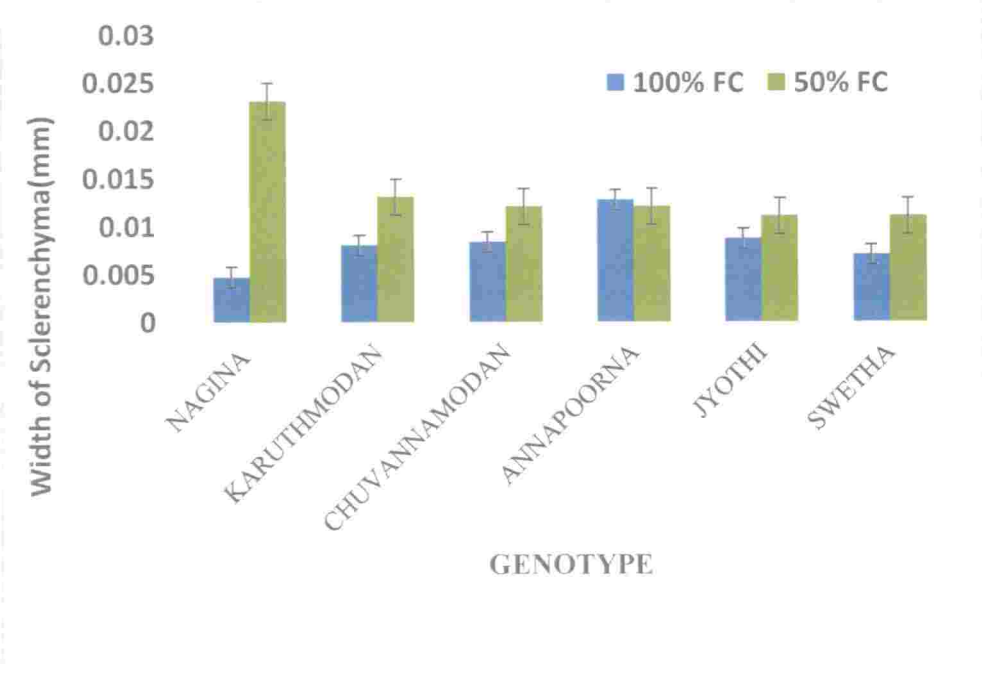


Figure 20: Variation in Width of Sclerenchyma (mm) of genotypes at booting stage under 100% & 50% FC.

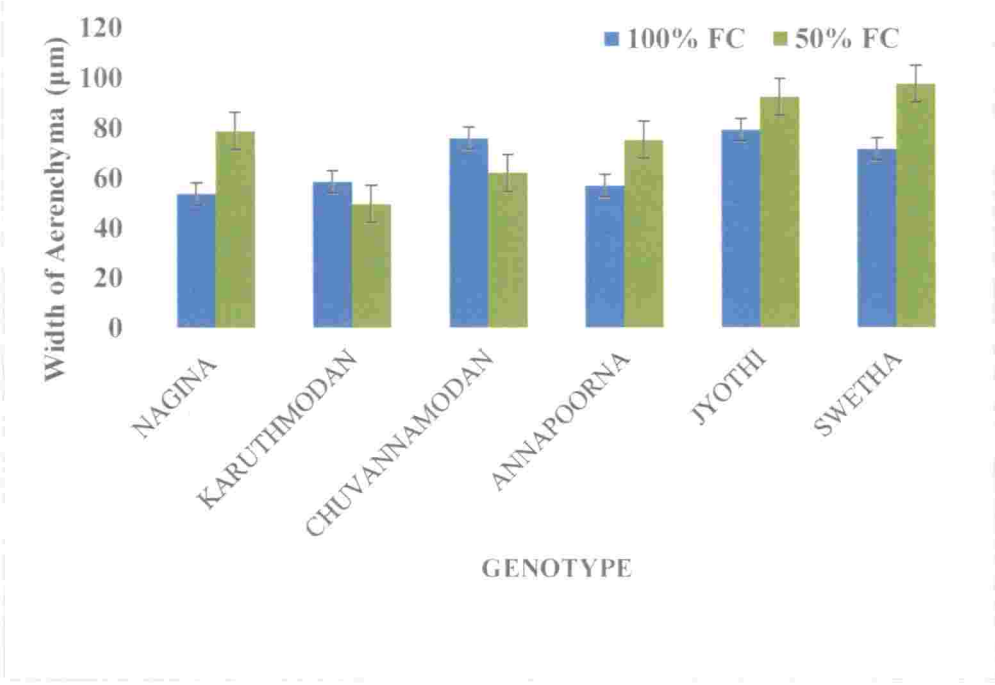


Figure 21: Variation in Width of Aerenchyma (µm) of genotypes at booting stage under 100% & 50% FC.

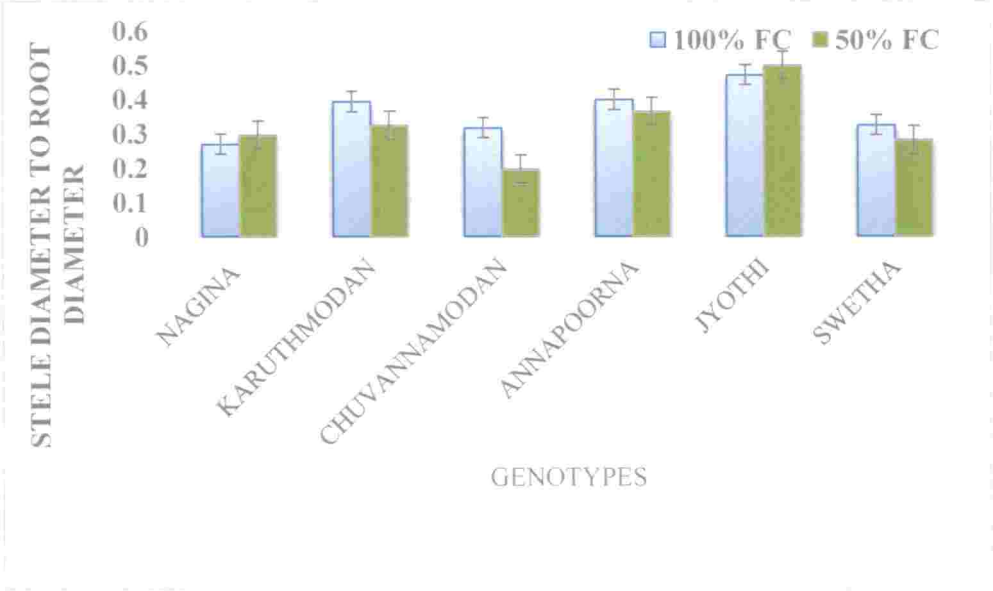


Figure 22: Variation in Stele diameter to root diameter of genotypes at booting stage under 100% & 50% FC.

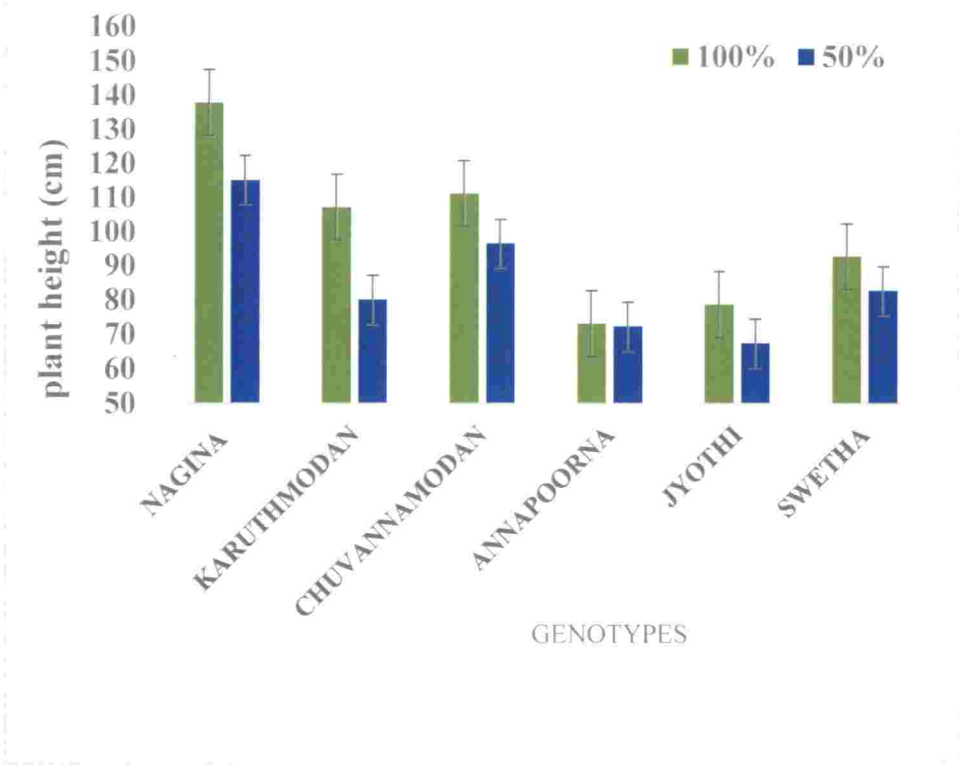


Figure 23. Variation in plant height (cm) of genotypes at booting stage under 100% & 50% FC.

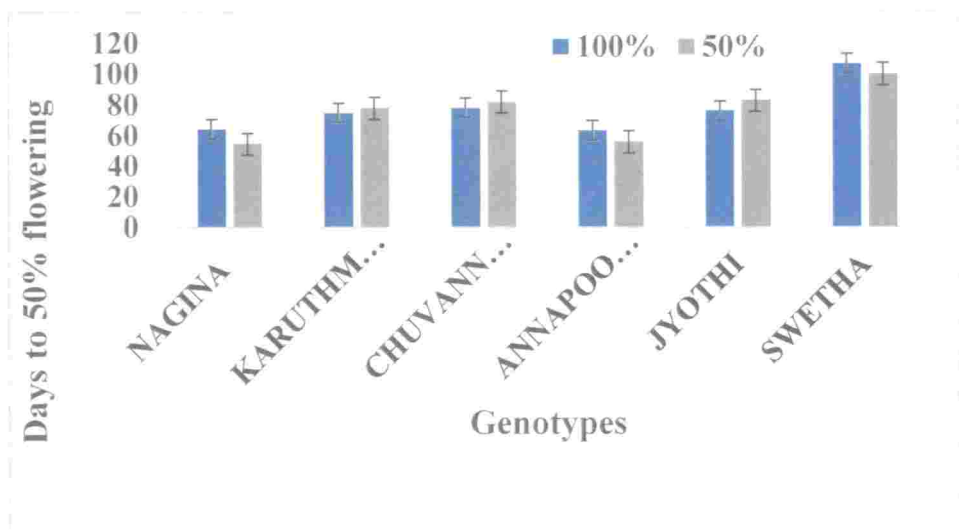


Figure 24. Variation in days to 50% flowering of genotypes at booting stage under 100% & 50% FC.

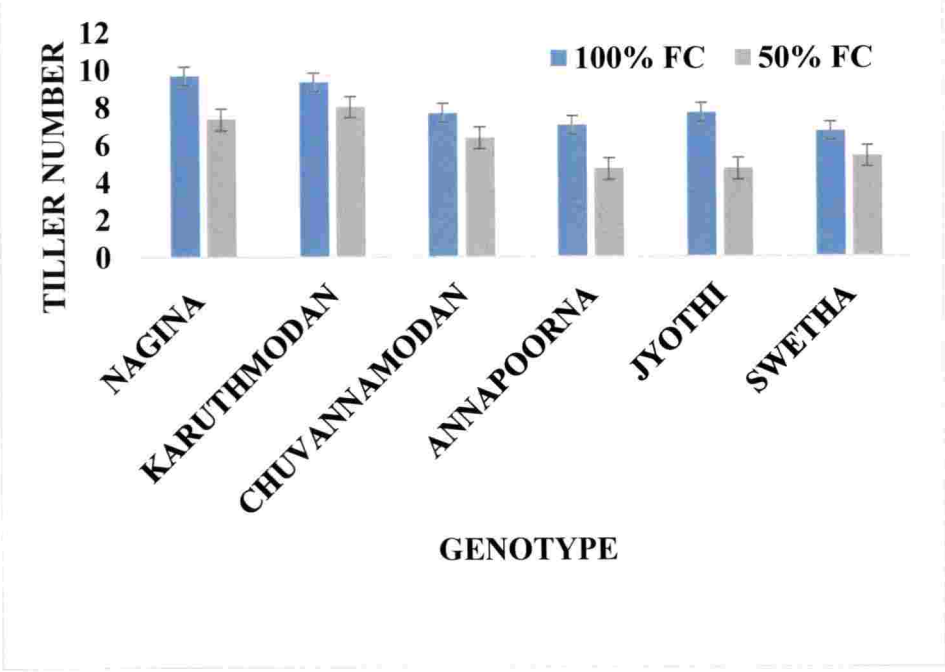


Figure 25. Variation in tiller number of genotypes at booting stage under 100% & 50% FC.

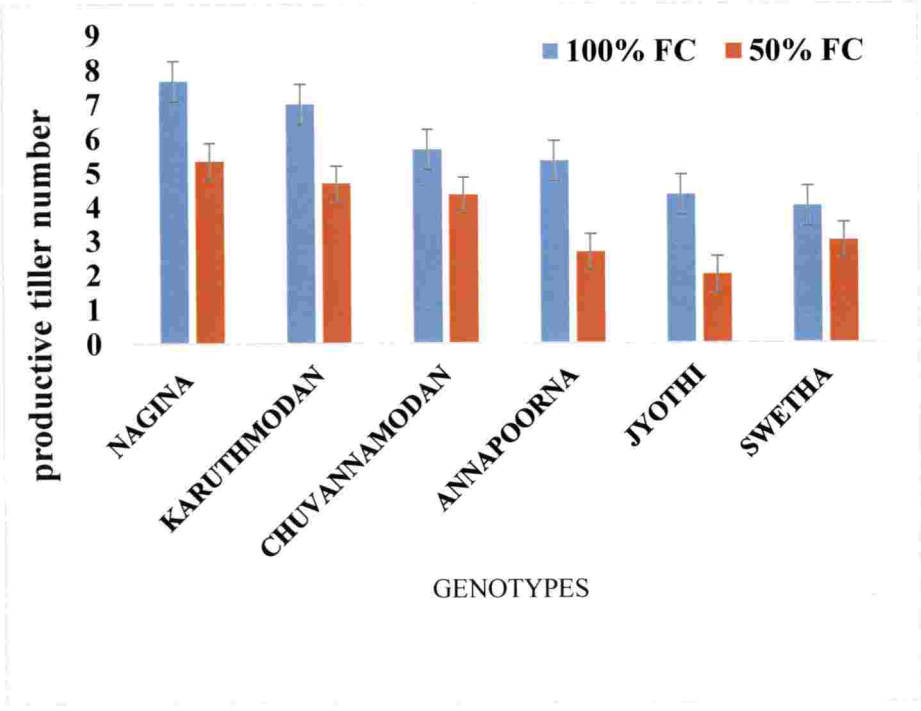


Figure 26. Variation in productive tiller number of genotypes at booting stage under 100% & 50% FC.

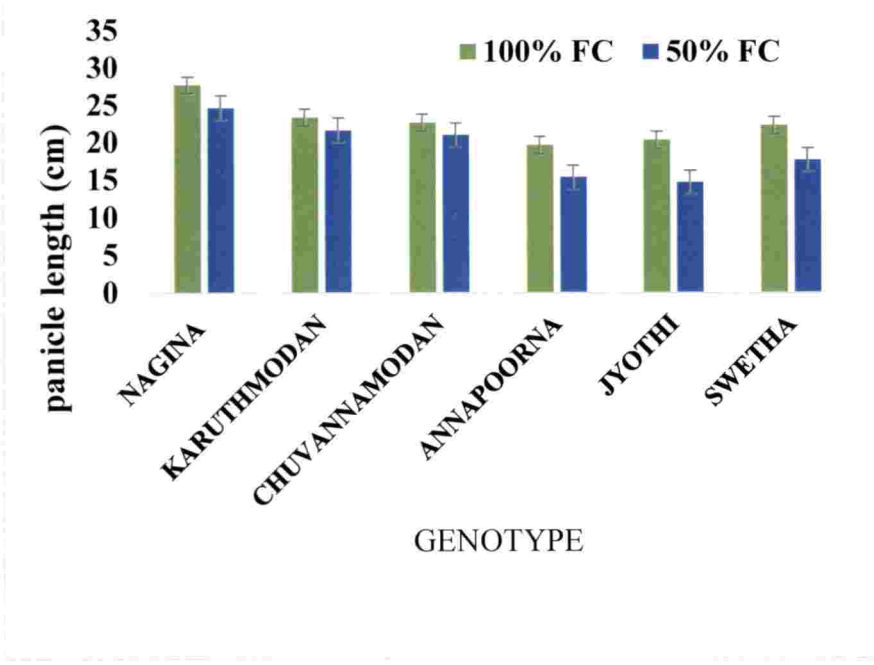


Figure 27. Variation in panicle length (cm) of genotypes at booting stage under 100% & 50% FC.

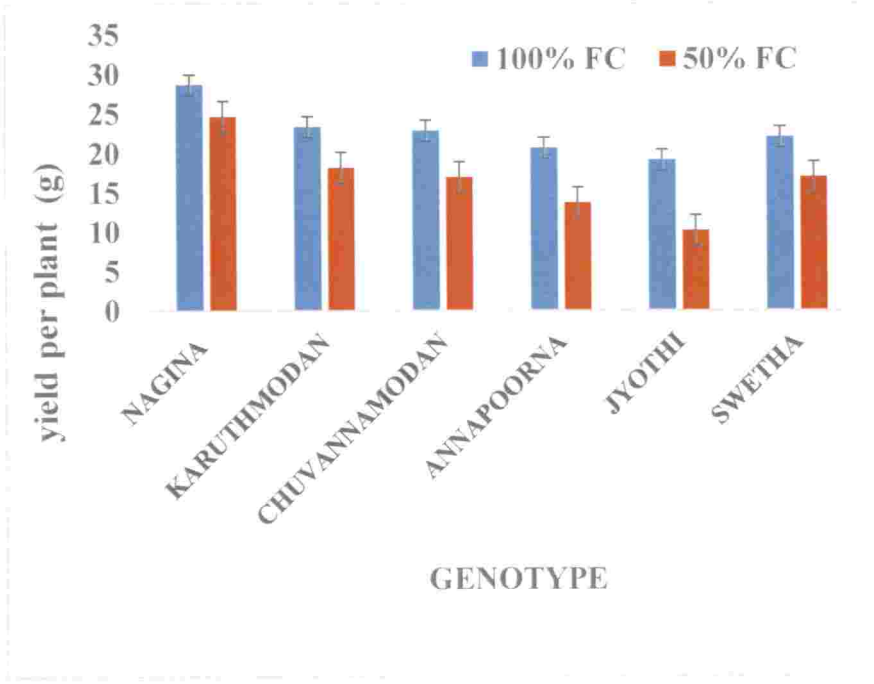


Figure 28. Variation in yield per plant of genotypes at booting stage under 100% & 50% FC

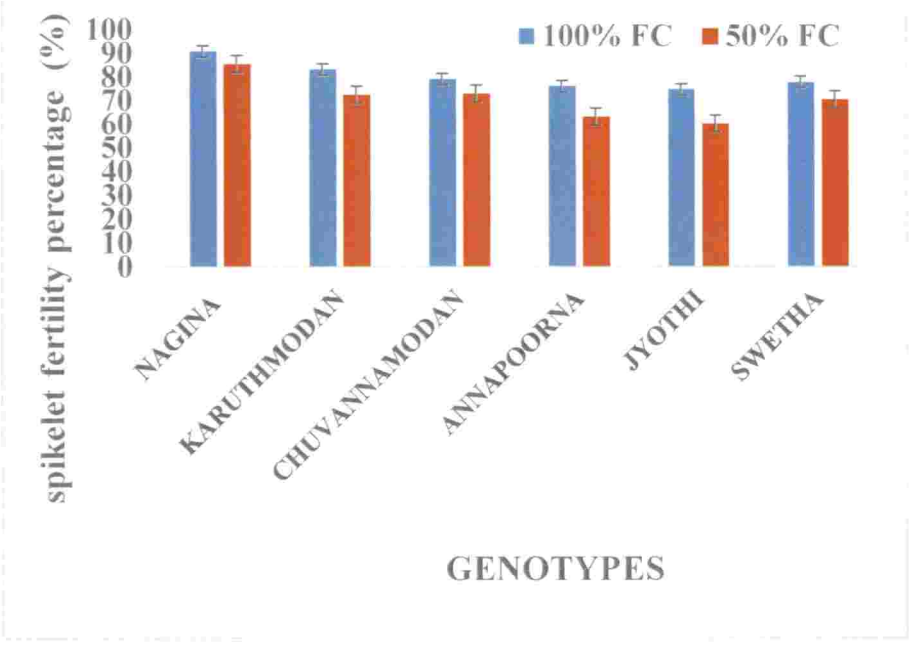


Figure 29. Variation in spikelet fertility percentage of genotypes under 100% & 50% FC.

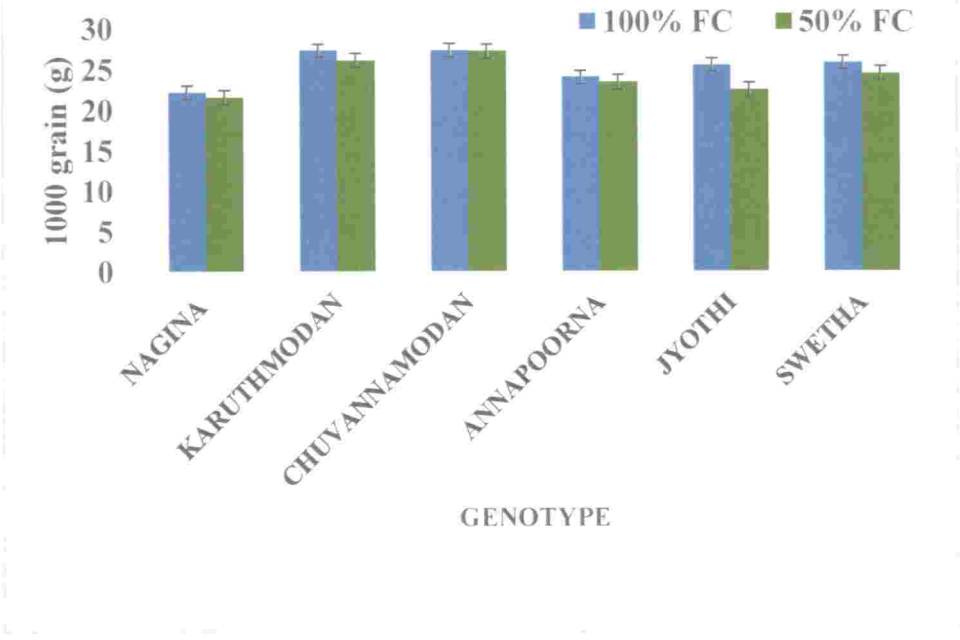


Figure 30. Variation in 1000 grain (g) weight of genotypes under 100% & 50% FC

Summary

6. SUMMARY

The salient findings of present investigation to the adaptive plasticity in root-shoot morphology and physiology, root anatomical plasticity under water stress in selected rice genotypes and molecular characterization using root specific genes are summarized in this chapter:

- A set of six varieties were evaluated in first experiment to study physio-morphological traits and anatomical plasticity of roots at 50% FC soil moisture.
- In first experiment physio morphological and biochemical parameters were evaluated and found a significant variation among the genotypes under control and stress conditions.
- Genotypes N 22, Ptb 29 and Ptb 30 found to maintain higher relative water content and cell membrane stability index even under depleting soil moisture.
- Specific leaf area found to reduce under stress conditions among genotypes N 22, Ptb 29 and Ptb 30 which curtails excess loss of water under stress condition.
- Root length, root dry weight, root volume and root to shoot ratio were found to be significantly increasing under control and stress conditions in genotypes N 22, Ptb 29, Ptb 30 and Ptb 57.
- Under stress condition there is differential accumulation of biomass in plant system. Under water stress condition more assimilates were diverted towards root system increasing the root weight ratio than culm and leaf weight ratio.
- Leaf area was found to be decreasing with stress in genotypes as an adaptation to curtail transpiration.
- An increase in starch content in roots was noticed among the genotypes under water stress condition.
- Protein profiling of rice roots revealed a upregualtion of protein of size 48kDa under water stress in N 22.

- In the second experiment anatomical plasticity of rice roots were studied at 100% FC and 50% FC soil moisture.
- Root diameter increased significantly among the genotypes N 22, Ptb 29 and Ptb 30 under water stress whereas rest of genotypes were left unaffected.
- Stele diameter associated with conducting tissues was found to be increasing in genotypes.
- Xylem tissue associated with axial transport of water increased significantly among the genotypes under stress.
- Width of sclerenchyma associated with prevention of radial loss of water increased under stress in genotypes like N 22, Ptb 29 and Ptb 30.
- There is a two way behaviour among the width of aerenchyma under stress in genotypes N 22 and Ptb 30, showing the reduction in maintenance cost.
- Growth and yield parameters found to be significantly reducing under stress condition compared with control.
- Width of sclerenchyma, Plant height, PTN, PL had shown a positive correlation with yield under stress condition.
- RWC, LMXN, LMXD, SDTRD, Tiller number, CMS, Root length, Root volume, Root shoot ratio, SRL, RWR, Starch content, Plant height were also found positively correlated with yield by contributing towards productive tiller number.
- Screening of genotypes with *DRO1* specific markers and other drought related SSR and EST-SSR revealed that all the *DRO1* specific markers were found to be monomorphic for tolerant and susceptible genotypes whereas an EST-SSR RM 518 found to be polymorphic.
- The genomic region flanked by this markers was found on chromosome 4 of rice and was reported to be associated with water use efficiency of rice.
- Expression studies results shows a differential accumulation of serine/threonine-protein kinase RCH1 (LOC4335004) which is found to be associated with the regulation of growth in root apical meristem in Ptb 30 and Ptb 35.

- From the experiment it can be stated as the genotypes N 22, Ptb 29 and Ptb 30 were found to be performing better and regarded as tolerant genotypes under 50% FC compared to genotypes Ptb 35, Ptb 39 and Ptb 57 which had shown a reduction in performance under stress were regarded as susceptible genotypes towards drought.
- The genotypes that were identified and evaluated for drought tolerance can be used a donor plants in breeding programs to improve drought tolerance in rice.
- RM 518 can be used to distinguish tolerant and susceptible genotypes of rice for drought tolerance.

Future line of work:

The root morphological and anatomical traits that were significantly varying under water stress can be made use in regular breeding program to develop drought tolerant cultivars.

A further investigation on anatomical plasticity reveals hidden knowledge of how root traits are helpful in conferring tolerance towards drought.

Karuthamodan (Ptb 29) can be used as a donor parent and more number of QTL's associated with water use efficiency can be identified.

References

7. REFERENCES

Abe, J. and Morita, S. 1994. Growth direction of nodal roots in rice: its variation and contribution to root system formation. *Plant and Soil,* 165, 333-337.

Abramoff, M.D., Magalhaes, P.J. and Ram, S.J. 2004. Image processing with Image J. Biophotonics International.11:36-42

Adiredjo, A.L., Navaud, O., Muños, S., Langlade, N.B., Lamaze, T. and Grieu, P., 2014. Genetic control of water use efficiency and leaf carbon isotope discrimination in sunflower (Helianthus annuus L.) subjected to two drought scenarios. *PloS one*, *9*(7), p.e101218.

Ahmadikhah, A. and Marufinia, A., 2016. Effect of reduced plant height on drought tolerance in rice. *3 Biotech*, *6*(2), p.221.

Akram, M. 2011. Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agricultural Research,* 36, 455-468.

Ali, Q., Ahsan, M., Tahir, M. H. N., Elahi, M., Farooq, J. and Waseem, M. 2011. Gene Expression and Functional Genomic Approach for abiotic stress tolerance in different crop species. *IJAVMS,* 5, 221-248.

Araki, H., Morita, S., Tatsumi, J. and Iijima, M. 2002. Physiol-morphological analysis on axile root growth in upland rice. *Plant production science,* 5, 286-293.

Ashkani, S., Rafii, M. Y., Shabanimofrad, M., Miah, G., Sahebi, M., Azizi, P., Tanweer, F. A., Akhtar, M. S. and Nasehi, A. 2015. Molecular breeding strategy and challenges towards improvement of blast disease resistance in rice crop. *Frontiers in plant science,* 6, 886.

Azhiri-Sigari, T., Yamauchi, A., Kamoshita, A. and Wade, L. J. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. II. Root growth. Plant Prod. Sci. 3: 180-188

Babu, R. C., Nguyen, B. D., Chamarerk, V., Shanmugasundaram, P., Chezhian, P., Jeyaprakash, P., Ganesh, S., Palchamy, A., Sadasivam, S. and Sarkarung, S. 2015. Genetic analysis of drought resistance in rice by molecular markers. *Crop Science,* 43, 1457-1469.

Barker, R., Dawe, D., Tuong, T., Bhuiyan, S. and Guerra, L. 1999. The outlook for water resources in the year 2020: challenges for research on water management in rice production. *Southeast Asia,* 1, 1-5.

Basu, S., Ramegowda, V., Kumar, A. and Pereira, A. Plant adaptation to drought stress [version 1; peer review: 3 approved] F1000Research 2016, 5(F1000 Faculty Rev):1554.

Beena, R. 2005. Studies on physio-morphological traits and genetic markers associated with drought response in rice (*Oryza sativa* L.). *PhD (Crop physiology) thesis, Tamil Nadu Agricultural University, Coimbatore, India.*

Begg, J.E. and Turner, N.C., 1976. Crop water deficits. In *Advances in agronomy* (Vol. 28, pp. 161-217). Academic Press.

Bengough, A.G., McKenzie, B.M., Hallett, P.D. and Valentine, T.A., 2011. Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *Journal of experimental botany*, *62*(1), pp.59-68.

Bhargava, S. and Sawant, K. 2013. Drought stress adaptation: metabolic adjustment and regulation of gene expression. *Plant Breeding,* 132, 21-32.

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

Blum, A., Klueva, N., and Nguyen, H. T. 2001. Wheat cellular thermo-tolerance is related to yield under heat stress. *Euphytica*117: 117-123.

Blum, A., Zhang, J. and Nguyen, H. 1999. Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. *Field Crops Research,* 64, 287-291.

Boonjung, H. and Fukai, S. 1996. Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. *Field Crops Research,* 48, 47-55.

Boopathi, N. M., Swapnashri, G., Kavitha, P., Sathish, S., Nithya, R., Ratnam, W. and Kumar, A. 2012. Introgression, Evaluation and Bulked Segregant Analysis of Major Yield QTL under Water Stress into Indigenous Elite Line for Low Water. *Rice Science,* 19.

Boutraa, T., Akhkha, A., Al-Shoaibi, A. A. and Alhejeli, A. M. 2010. Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (Triticum durum) grown in Saudi Arabia. *Journal of Taibah University for Science,* 3, 39-48.

Bramley, H., Turner, N.C., Turner, D.W. and Tyerman, S.D., 2009. Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. *Plant physiology*, *150*(1), pp.348-364.

Cabangon, R., Tuong, T. P. and Abdullah, N. 2002. Comparing water input and water productivity of transplanted and direct-seeded rice production systems. *Agricultural Water Management,* 57, 11-31.

Cai, Q., Zhang, Y., Sun, Z., Zheng, J., Bai, W., Zhang, Y., Liu, Y., Feng, L., Chen, F. and Zhang, Z. 2017. Morphological plasticity of root growth under mild water stress increases water use efficiency without reducing yield in maize. *Biogeosciences,* 14, 3851.

Cattivelli, L., Rizza, F., Badeck, F.W., Mazzucotelli, E., Mastrangelo, A.M., Francia, E., Marè, C., Tondelli, A. and Stanca, A.M., 2008. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crops Research*, *105*(1-2), pp.1-14.

Chandler, P. M. and Robertson, M. 1994. Gene expression regulated by abscisic acid and its relation to stress tolerance. *Annual review of plant biology,* 45, 113-141.

Chaudhary, P., Godara, S., Cheeran, A.N. and Chaudhari, A.K., 2012. Fast and accurate method for leaf area measurement. *International journal of computer applications*, *49*(9), pp.22-25.

Chauhan, B. S. and Abugho, S. B. 2013. Effect of water stress on the growth and development of Amaranthus spinosus, Leptochloa chinensis, and rice. *American Journal of Plant Sciences,* 4, 989.

Chauhan, B.S. and Johnson, D.E., 2010. Responses of rice flatsedge (Cyperus iria) and barnyardgrass (Echinochloa crus-galli) to rice interference. *Weed science*, *58*(3), pp.204-208.

Chen, X., Cho, Y. and McCouch, S. 2002. Sequence divergence of rice microsatellites in Oryza and other plant species. *Molecular Genetics and Genomics,* 268, 331-343.

Chimungu, J. G., Loades, K. W. and Lynch, J. P. 2015. Root anatomical phenes predict root penetration ability and biomechanical properties in maize (Zea mays). *Journal of Experimental Botany,* 66, 3151-3162.

Clark, L., Cope, R., Whalley, W., Barraclough, P. and Wade, L. 2002. Root penetration of strong soil in rainfed lowland rice: comparison of laboratory screens with field performance. *Field Crops Research,* 76, 189-198.

Close, T. J., Kortt, A. A. and Chandler, P. M. 1989. A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. *Plant molecular biology,* 13, 95-108.

Coburn, J. R., Temnykh, S. V., Paul, E. and McCOUCH, S. R. 2002. Design and application of microsatellite marker panels for semiautomated genotyping of rice (*Oryza sativa* L.). *Crop Science,* 42, 2092-2099.

Cole, C. T. 2003. Genetic variation in rare and common plants. *Annual Review of Ecology, Evolution, and Systematics,* 34, 213-237.

Collard, B. C., Jahufer, M., Brouwer, J. and Pang, E. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica,* 142, 169-196.

Cook, A., Marriott, C., Seel, W. and Mullins, C. 1997. Does the uniform packing of sand in a cylinder provide a uniform penetration resistance? *Plant and soil,* 190, 279-287.

Cornelissen, J., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D., Reich, P. B., Ter Steege, H., Morgan, H. and Van Der Heijden, M. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian journal of Botany,* 51, 335-380.

Courtois, B., Shen, L., Petalcorin, W., Carandang, S., Mauleon, R. and Li, Z. 2003. Locating QTLs controlling constitutive root traits in the rice population IAC 165× Co39. *Euphytica,* 134, 335-345.

Cregan, P., Jarvik, T., Bush, A., Shoemaker, R., Lark, K., Kahler, A., Kaya, N., VanToai, T., Lohnes, D. and Chung, J. 1999. An integrated genetic linkage map of the soybean genome. *Crop Science,* 39, 1464-1490.

De Micco, V. and Aronne, G., 2012. Morpho-anatomical traits for plant adaptation to drought. In *Plant responses to drought stress* (pp. 37-61). Springer, Berlin, Heidelberg.

Degenkolbe, T., Do, P. T., Zuther, E., Repsilber, D., Walther, D., Hincha, D. K. and Köhl, K. I. 2009. Expression profiling of rice cultivars differing in their tolerance to long-term drought stress. *Plant molecular biology,* 69, 133-153.

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

Deshmukh, V., Mankar, S., Muthukumar, C., Divahar, P., Bharathi, A., Helen Baby, T., Rajurkar, A., Reena, S., Poornima, R., Senthivel, S. and Chandra Babu, R., 2017. Genome-wide consistent molecular markers associated with phenology, plant production and root traits in diverse rice (Oryza sativa L.) accessions under drought in rainfed target populations of environment. *Curr. Sci.*

Devi, K. R., Sudhakar, P. and Sivasankar, A. 2013. Screening of paddy genotypes for high water use efficiency and yield components. *BIOINFOLET-A Quarterly Journal of Life Sciences,* 10, 214-224.

Dingkuhn, M., Tivet, F., Siband, P.-L., Asch, F., Audebert, A. and Sow, A. Varietal differences in specific leaf area: a common physiological determinant of tillering ability and early growth vigour, 2001. IRRI.

Directorate of Economics & Statistics. Department of agriculture, cooperation and farmers welfare, ministry of agriculture and farmers welfare , Govt of India. (2018) (https://eands.dacnet.nic.in/)

Ehleringer, J. R., Hall, A. E. and Farquhar, G. D. 1993. *Stable isotopes and plant carbon-water relations*, Academic Press San Diego.

Engelaar, W.M.H.G., Van Bruggen, M.W., Van den Hoek, W.P.M., Huyser, M.A.H. and Blom, C.W.P.M., 1993. Root porosities and radial oxygen losses of Rumex and Plantago species as influenced by soil pore diameter and soil aeration. *New Phytologist*, *125*(3), pp.565-574.

Evans, L.T. 1998. Feeding the ten billion: plants and population growth. Cambridge University Press, Cambridge, UK, 247 p.

Farquhar, G. D. 1983. On the nature of carbon isotope discrimination in C4 species. *Functional Plant Biology,* 10, 205-226.

Fitter, A. 2002. Characteristics and functions of root systems. *Plant roots.* CRC Press.

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., Mueller, N. D., O'Connell, C., Ray, D. K. and West, P. C. 2011. Solutions for a cultivated planet. *Nature,* 478, 337.

Fontenelli, J.V., da Silva, T.J.E.A.U., Bonfim-Silva, E.M. and de Freitas Sousa, H.H.E., 2016. Soil moisture maintenance methods in cultivation in a greenhouse. *African Journal of Agricultural Research*, *11*(5), pp.317-323.

Fukai, S. and Cooper, M., 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crops Research*, *40*(2), pp.67-86.

Fukai, S., Pantuwan, G., Jongdee, B. and Cooper, M., 1999. Screening for drought resistance in rainfed lowland rice. *Field Crops Research*, *64*(1-2), pp.61-74.

Ganapathy, S., Ganesh, S., Shanmugasundaram, P. and Babu, R. C. 2010. Studies on root traits for drought tolerance in rice (*Oryza sativa* L.) under controlled (PVC pipes) condition. *Electronic Journal of Plant Breeding,* 1, 1016-1020.

Gao, Q., Sun, J., Tong, H., Wang, W., Zhang, Y., Zhang, G., Chen, W. 2018. *Evaluation of rice drought stress response using carbon isotope*

*discrimination. Plant Physiology and Biochemistry, 132, 80–88.*doi:10.1016/j.plaphy.2018.08.030.

Gaspar, T., Franck, T., Bisbis, B., Kevers, C., Jouve, L., Hausman, J.-F. and Dommes, J. 2002. Concepts in plant stress physiology. Application to plant tissue cultures. *Plant Growth Regulation,* 37, 263-285.

Gowda, V. R., Henry, A., Yamauchi, A., Shashidhar, H. and Serraj, R. 2011. Root biology and genetic improvement for drought avoidance in rice. *Field Crops Research,* 122, 1-13.

Greenland, D.J. 1997. The sustainability of rice farming. CAB International, Wallingford, UK, 273 p.

GRiSP (Global Rice Science Partnership). 2013. Rice almanac, 4th edition. Los Baños (Philippines): International Rice Research Institute. 283

Gunn, S., Farrar, J., Collis, B. and Nason, M. 1999. Specific leaf area in barley: individual leaves versus whole plants. *The New Phytologist,* 143, 45-51.

Guo, D., Xia, M., Wei, X., Chang, W., Liu, Y. and Wang, Z., 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist*, *180*(3), pp.673-683.

Gupta, P. K. and Varshney, R. K. 2004. Cereal genomics: an overview. *Cereal Genomics.* Springer.

Hamrick, J. 1989. Isozymes and the analysis of genetic structure in plant populations. *Isozymes in plant biology.* Springer.

Hanamaratti, N.G. and Salimath, P.M., 2012. Association of flowering delay under stress and drought tolerance in upland rice (Oryza sativa L.). *International Journal of Forestry and Crop Improvement*, *3*(2), pp.99-104.

Henry, A., Cal, A. J., Batoto, T. C., Torres, R. O. and Serraj, R. 2012. Root attributes affecting water uptake of rice (*Oryza sativa*) under drought. *Journal of experimental botany,* 63, 4751-4763.

Henry, A., Cal, A.J., Batoto, T.C., Torres, R.O. and Serraj, R., 2012. Root attributes affecting water uptake of rice (Oryza sativa) under drought. *Journal of experimental botany*, *63*(13), pp.4751-4763.

Hirt, H. and Shinozaki, K. 2003. *Plant responses to abiotic stress*, Springer Science & Business Media.

Ingram, K., Bueno, F., Namuco, O., Yambao, E. and Beyrouty, C. 1994. Rice root traits for drought resistance and their genetic variation.

Jackson, M. and Armstrong, W. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology,* 1, 274-287.

Jackson, M. B., Fenning, T. M. and Jenkins, W. 1985. Aerenchyma (gas-space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. *Journal of Experimental Botany,* 36, 1566-1572.

Jain, S. M., Brar, D. S. and Ahloowalia, B. 2010. *Molecular techniques in crop improvement*, Springer.

Jongdee, B., Fukai, S. and Cooper, M. Genotypic variation for grain yield of rice under water-deficit conditions. Proceedings of 9th Australian Agronomy Conference, Wagga Wagga, 1998. 403-406.

Kadam, N. N., Yin, X., Bindraban, P. S., Struik, P. C. and Jagadish, K. S. 2015. Does morphological and anatomical plasticity during the vegetative stage make wheat more tolerant of water deficit stress than rice? *Plant physiology,* 167, 1389-1401.

Kamoshita, A., Babu, R. C., Boopathi, N. M. and Fukai, S. 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field crops research,* 109, 1-23.

Kamoshita, A., Zhang, J., Siopongco, J., Sarkarung, S., Nguyen, H. and Wade, L. 2002. Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions. *Crop Science,* 42, 255-265.

Kanagaraj, P., Prince, K.S.J., Sheeba, J.A., Biji, K.R., Paul, S.B., Senthil, A. and Babu, R.C., 2010. Microsatellite markers linked to drought resistance in rice (Oryza sativa L.). *Current Science*, pp.836-839.

Kanbar, A. and Shashidhar, H. 2011. Participatory selection assisted by DNA markers for enhanced drought resistance and productivity in rice (*Oryza sativa* L.). *Euphytica,* 178, 137-150.

Kanbar, A. and Shashidhar, H.E., 2011. Participatory selection assisted by DNA markers for enhanced drought resistance and productivity in rice (Oryza sativa L.). *Euphytica*, *178*(1), pp.137-150.

Kang, S.-Y. and Morita, S. 1998. Comparative analysis of root and shoot growth between Tongil and Japonica type rice. *Korean J. Crop Sci,* 43, 161-167.

Kataria, N. and Singh, N. 2013. Amelioration of water stress by potassium fertilizer in chickpea (Cicer arietinum L.). J. Trop. Plant Physiol., 5: 22-32

Kathiresan, A., Lafitte, H., Chen, J., Mansueto, L., Bruskiewich, R. and Bennett, J. 2006. Gene expression microarrays and their application in drought stress research. *Field Crops Research,* 97, 101-110.

Kato, Y., Okami, M. and Katsura, K. 2009. Yield potential and water use efficiency of aerobic rice (*Oryza sativa* L.) in Japan. *Field crops research,* 113, 328-334.

Kavitha, K. 2014. *Evaluation of Rice Genotypes for Growth, Drought Tolerance and Yield Under Aerobic Method of Cultivation.* ACHARYA NG RANGA AGRICULTURAL UNIVERSITY.

Kebriyaee, D., Kordrostami, M., Rezadoost, M. H. and Lahiji, H. S. 2012. QTL analysis of agronomic traits in rice using SSR and AFLP markers. *Notulae Scientia Biologicae,* 4, 116-123.

Khush, G. S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. *Plant molecular biology,* 59, 1-6.

Kirkegaard, J., Lilley, J., Howe, G. and Graham, J. 2007. Impact of subsoil water use on wheat yield. *Australian Journal of Agricultural Research,* 58, 303-315.

Kondo, M., Aguilar, A., Abe, J. and Morita, S. 2000. Anatomy of nodal roots in tropical upland and lowland rice varieties. *Plant production science,* 3, 437-445.

Kumar, R. and Kujur, R. 2003. Role of secondary traits in improving the drought tolerance during flowering stage in rice. *Indian journal of plant physiology,* 8, 236-240.

Kumar, R. and Kujur, R., 2003. Role of secondary traits in improving the drought tolerance during flowering stage in rice. *Indian journal of plant physiology*, *8*(3), pp.236-240.

Kumar, S., Dwivedi SK, Singh SS, Jha SK, Lekshmy S, Elanchezhian R, Bhatt BP (2014). Identification of drought tolerant rice genotype by analysing drought tolerance indices and morpho-physiological traits. SABRAO Journal of Breeding and Genetics 46:217-230.

Kumar, S., Dwivedi, S. K., Singh, S. S., Bhatt, B. P., Mehta, P., Elanchezhian, R. Singh, O. N. 2014. Morpho-physiological traits associated with

reproductive stage drought tolerance of rice (Oryza sativa L.) genotypes under rain-fed condition of eastern Indo-Gangetic Plain. Indian Journal of Plant Physiology, 19(2), 87–93.

Kundur, P., Sanjay, R., Shashidhar, H. and Gowda, V. 2015. Study of rice (*Oryza sativa* L.) Root anatomy under aerobic and waterlogged conditions. *Int J Appl Pure Sci Agric,* 1, 18-26.

Kvet, J., Ondok, J. P., Necas, J., and Jarvis, P. G. 1971. Methods of growth analysis. Pages 343–391 *in* Sestak, Z., Catsky, J., and Jarvis,P. G., eds. *Plant Photosynthetic Production. Manual of Methods*. W. Junk, The Hague.

Lafitte, R., Blum, A. and Atlin, G. 2003. Using secondary traits to help identify drought-tolerant genotypes. *Breeding rice for drought-prone environments*, 37-48.

Li Y.Y., Sperry J.S., and Shao M., 2009. Hydraulic conductance and vulnerability to cavitation in corn (Zea mays L.) hybrids of differing drought resistance. Environ. Exp. Botany, 66(2), 341-346.

Li Z, Mu P, Li C, Zhang H, Li Z, Gao Y. 2005. QTL mapping of root traits in a doubled haploid population from a cross between upland and lowland japonica rice in three environments. Theor Appl Genet; 110: 1244-52.

Lilley, J., Ludlow, M., McCouch, S. and O'toole, J. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. *Journal of Experimental Botany,* 47, 1427-1436.

Lisar, S. Y., Motafakkerazad, R. and Hossain, M. M. 2012. Water Stress in Plants: Causes, Effects and Responses. Water Stress, Prof. Ismail Md. Mofizur Rahman (Ed.), ISBN: 978-953-307-963-9. *Tech. doi,* 10, 39363.

Lugojan, C. and Ciulca, S. 2011. Evaluation of relative water content in winter wheat. *Journal of Horticulture, Forestry and Biotechnology,* 15, 173-7.

Lynch, J. P., Chimungu, J. G. and Brown, K. M. 2014. Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. *Journal of Experimental Botany,* 65, 6155-6166.

Mahalakshmi, V. and Bidinger, F. 1985. Water stress and time of floral initiation in pearl millet. *The Journal of Agricultural Science,* 105, 437-445.

Mallik, A. and Govindaswamy, T. 1962. The drought problems of India in relation to agriculture. *Ann. Arid Zone,* 31, 106-113.

Mambani, B. and Lal, R. 1983. Response of upland rice varieties to drought stress. *Plant and Soil,* 73, 73-94.

Manful, J. T., Graham-Acquaah, S. 2016. African rice (Oryza glaberrima): a brief history and its growing importance in current rice breeding efforts. In: Wrigley C, Corke H, Seetharaman K, Faubion J, editors. Encyclopedia of food grains. 2nd ed. Oxford, UK: Academic Press. p 140–146.

McCouch, S. R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., Huang, N., Ishii, T. and Blair, M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. *Oryza: From Molecule to Plant.* Springer.

McCouch, S. R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R.F. Jellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein. 2002. Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). DNA Res., 9: 199-204-7.

Mohammadi, R., Mendioro, M. S., Diaz, G. Q., Gregorio, G. B. and Singh, R. K. 2013. Mapping quantitative trait loci associated with yield and yield components under reproductive stage salinity stress in rice (*Oryza sativa* L.). *Journal of genetics,* 92, 433-443.

Mostajeran, A. and Rahimi-Eichi, V. 2008. Drought stress effects on root anatomical characteristics of rice cultivars (*Oryza sativa* L.). *Pakistan Journal of Biological Sciences,* 11, 2173-2183.

Mu, P., Li, Z., Li, C., Zhang, H., Wu, C., Li, C. and Wang, X. 2003. QTL mapping of the root traits and their correlation analysis with drought resistance using DH lines from paddy and upland rice cross. *Chinese Science Bulletin,* 48, 2718-2724.

Mumbani, B., Lal, R. 1983. Response of upland rice varieties to drought stress. II. Screening rice varieties by means of variable moisture regimes along a toposequence. Plant and Soil 73:73–94.

Muthurajan, R., Shobbar, Z.-S., Jagadish, S., Bruskiewich, R., Ismail, A., Leung, H. and Bennett, J. 2011. Physiological and proteomic responses of rice peduncles to drought stress. *Molecular biotechnology,* 48, 173-182.

Nathan, K. K. 2000. Characteristics of Drought in Kerala, India. Drought. *Net. News* 12(1): 1-5.

Nguyen, H. T., Babu, R. C. and Blum, A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Science,* 37, 1426-1434.

Nguyen, T., Klueva, N., Chamareck, V., Aarti, A., Magpantay, G., Millena, A., Pathan, M. and Nguyen, H. 2004. Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Molecular Genetics and Genomics,* 272, 35-46.

O'Toole, J. and Moya, T. 1978. Genotypic Variation in Maintenance of Leaf Water Potential in Rice 1. *Crop Science,* 18, 873-876.

Pantuwan, G., Fukai, S., Copper, M., Rajatasereekul and S,O'Toole, J. C. 2000 .Field screening for drought resistance in increased lowland rice production in the Mekong region.Proceedings of international workshop , 69-77.

Parfitt, J. M. B., Concenço, G., Downing, K., Larue, J. and da Silva, J. T. 2017. Rice growth under water stress levels imposedat distinct developmental stages. *Embrapa Clima Temperado-Artigo em periódico indexado (ALICE)*.

Peng, S., Bouman, B., Visperas, R. M., Castañeda, A., Nie, L. and Park, H.-K. 2006. Comparison between aerobic and flooded rice in the tropics: agronomic performance in an eight-season experiment. *Field Crops Research,* 96, 252-259.

Perez, C. M., Palmiano, E. P., Baun, L. C. and Juliano, B. O. 1971. Starch metabolism in the leaf sheaths and culm of rice. *Plant Physiology,* 47, 404-408.

Phillips, R. L. and Vasil, I. K. 2013. *DNA-based markers in plants*, Springer Science & Business Media.

Pitman, W.D., Holte, B.E. Conrad and E.C. Bashaw, 1983. Histological differences in moisture stressed and non-stressed kleingrass forage. Crop Sci., 23: 793-795.

Prasad, S., Kumar, A., Kumar, A., Mishra, V., Kumar, K., Dwivedi, R., Kumar, A., Ram, C., Nandan, R., Singh, P. and D. Dwivedi, K.2019. Effect of drought stress at reproductive stage of rice (Oryza sativa L.) genotypes. Bull. Env. Pharmacol. Life Sci., Vol 8 [3]

Premachandra, G. S., Saneoka, H., & Ogata, S. (1990). Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soyabean. The Journal of Agricultural Science, 115(01), 63.

Price, A. H., Townend, J., Jones, M. P., Audebert, A. and Courtois, B. 2002.

Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. *Plant molecular biology,* 48, 683-695.

Prince, S. J., Beena, R., Gomez, S. M., Senthivel, S. and Babu, R. C. 2015. Mapping consistent rice (*Oryza sativa* L.) yield QTLs under drought stress in target rainfed environments. *Rice,* 8, 25.

Purushothaman, R., Zaman-Allah, M., Mallikarjuna, N., Pannirselvam, R., Krishnamurthy, L., Lakkegowda, C. and Gowda, L. 2013. Root Anatomical Traits and Their Possible Contribution to Drought Tolerance in Grain Legumes. Plant Prod. Sci. 16(1):1-8.

Quampah, A., Ren-Ming, W., Shamsi, I. H., Jilani, G., Zhang, Q., Hua, S. and Xu, H. 2011. Improving water productivity by potassium application in various rice genotypes. *International Journal of Agriculture and Biology,* 13.

Raman, C. and Rao, Y. 1981. Blocking highs over Asia and monsoon droughts over India. *Nature,* 289, 271.

Rao, K. M., Raghavendra, A. and Reddy, K. J. 2006. *Physiology and molecular biology of stress tolerance in plants*, Springer Science & Business Media.

Ray, D.K., Ramankutty, N., Mueller, N.D., West, P.C. and Foley, J.A., 2012. Recent patterns of crop yield growth and stagnation. *Nature communications*, *3*, p.1293.

Rejeth, 2017, Identification of microsatellite markers associated with root traits for drought tolerance in rice. M. Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 64p.

Rich, S. M. and Watt, M. 2013. Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. *Journal of experimental botany,* 64, 1193-1208.

Richards, R., Rebetzke, G., Condon, A. and Van Herwaarden, A. 2002. Breeding

opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop science,* 42, 111-121.

Sadasivam, S., 1996. *Biochemical methods*. New age international.

Sagari, R. R. 2006. "India's Livestock Economy," Seminar 564.

Samra, J. S. 2004. Review and analysis of drought monitoring, declaration and management in India. Working Paper 84. International Water Management Institute, Colombo, Sri Lanka.

Sandhu, N. 2013. *Molecular and physiological studies to identify traits enhancing rice cultivars adaptation to aerobic conditions for sustainable rice production in water short situations.* CCSHAU.

Sarwar, J. M., Nozulaidi, B. N. M., Khairi, B. C. L. M. and Mohd, K. Y. 2013. Effects of water stress on rice production: bioavailability of potassium in soil. *Journal of Stress Physiology & Biochemistry,* 9.

Sarwar, J.M., Nozulaidi, B.N.M., Khairi, B.C.L.M. and Mohd, K.Y., 2013. Effects of water stress on rice production: bioavailability of potassium in soil. *Journal of Stress Physiology & Biochemistry*, *9*(2).

Senthilkumar, M., Sadashiva, A. and Laxmanan, V. 2017. Impact of water stress on root architecture in tomato (Solanum lycopersicum Mill).

Serraj, R., McNally, K. L., Slamet-Loedin, I., Kohli, A., Haefele, S. M., Atlin, G. and Kumar, A. 2011. Drought resistance improvement in rice: an integrated genetic and resource management strategy. *Plant Production Science,* 14, 1-14.

Sharp, R. and Davies, W. 1985. Root growth and water uptake by maize plants in drying soil. *Journal of Experimental Botany,* 36, 1441-1456.

Sibounheuang, V., Basnayake, J. and Fukai, S., 2006. Genotypic consistency in the expression of leaf water potential in rice (Oryza sativa L.). *Field Crops Research*, *97*(2-3), pp.142-154.

Sinclair, T. and Ludlow, M. 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. *Functional Plant Biology,* 12**,** 213-217.

Singh, A., Mall, A., Singh, P. and Verma, O. 2010. Interrelationship of genetics parameters for quantitative and physiological traits in rice under irrigated and drought conditions. *ORYZA-An International Journal on Rice,* 47**,** 142-147.

Singh, A., Shamim, M. and Singh, K. 2013. Genotypic variation in root anatomy, starch accumulation, and protein induction in upland rice (*Oryza sativa*) varieties under water stress. *Agricultural Research,* 2**,** 24-30.

Singh, A.K., Mall, A.K., Singh, P.K. and Verma, O.P., 2010. Interrelationship of genetics parameters for quantitative and physiological traits in rice under irrigated and drought conditions. *Oryza-An International Journal on Rice*, *47*(2), pp.142-147.

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

Singh, S., Prasad, S., Yadav, V., Kumar, A. and Jaiswal, B., 2018. Effect of drought stress on yield and yield components of rice (Oryza sativa L.) genotypes. *Int. J. Curr. Microbiol. Appl. Sci*, *7*, pp.2752-2759.

Slatyer, R.O.1955, Studies of the water relations of crop plants grown under natural rainfall in northern Australia. Australian Journal of Agriculture Research, 61: 365-377.

Sridhara, C., Shashidhar, H., Gurumurthy, K. and Ramachandrappa, B. 2012. Effect of genotypes and method of establishment on root traits, growth and yield of aerobic rice. *Agricultural Science Digest,* 32.

This, D., Comstock, J., Courtois, B., Xu, Y., Ahmadi, N., Vonhof, W. M., Fleet, C., Setter, T. and McCouch, S. 2010. Genetic analysis of water use efficiency in rice (*Oryza sativa* L.) at the leaf level. *Rice,* 3, 72.

Thumma, B. R., Naidu, B. P., Chandra, A., Cameron, D. F., Bahnisch, L. M. and Liu, C. 2001. Identification of causal relationships among traits related to drought resistance in Stylosanthes scabra using QTL analysis. *Journal of Experimental Botany,* 52, 203-214.

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

Tuong, T. and Bouman, B. 2003. Rice production in water-scarce environments. *Water productivity in agriculture: Limits and opportunities for improvement,* 1, 13-42.

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

Turner, N. C., Wright, G. C. and Siddique, K. 2001. Adaptation of grain legumes (pulses) to water-limited environments.

Uga, Y., Okuno, K. and Yano, M. 2011. Dro1, a major QTL involved in deep rooting of rice under upland field conditions. *Journal of experimental botany,* 62, 2485-2494.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K. and Kanno, N. 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions.

Nature genetics, 45, 1097.

Van der Weele, C.M., Spollen, W.G., Sharp, R.E. and Baskin, T.I., 2000. Growth of Arabidopsis thaliana seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of Experimental Botany*, *51*(350), pp.1555-1562.

Van der Werf, A., Visser, A., Schieving, F. and Lambers, H. 1993. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast-and slow-growing species. *Functional Ecology*, 63-74.

Varshney, R. K., Korzun, V. and Börner, A. 2004. Molecular maps in cereals: methodology and progress. *Cereal genomics.* Springer.

Vassileva, V., Demirevska, K., Simova-Stoilova, L., Petrova, T., Tsenov, N. and Feller, U. 2012. Long-term field drought affects leaf protein pattern and chloroplast ultrastructure of winter wheat in a cultivar-specific manner. *Journal of agronomy and crop science,* 198, 104-117.

Veeraghattapu, R., Patil, S., Deborah, D.A.K., Kiranamayi, S.L. and Vemireddy, L.N., 2015. Identification and Functional Characterization of Genes Underlying Major QTL for Water use Efficiency in Rice using in silico approach. *Vegetos-An International Journal of Plant Research*, *28*(2), pp.203-207.

Virgona, J., Hubick, K., Rawson, H., Farquhar, G. and Downes, R. 1990. Genotypic variation in transpiration efficiency, carbon-isotype discrimination and carbon allocation during early growth in sunflower. *Functional Plant Biology,* 17, 207-214.

Waseem, M., Ali, A., Tahir, M., Nadeem, M.A., Ayub, M., Tanveer, A., Ahmad, R. and Hussain, M., 2011. Mechanism of drought tolerance in plant and its

management through different methods. *Continental Journal of Agricultural Science*, *5*(1), pp.10-25.

Weerathaworn, P., Soldati, A. and Stamp, P. 1992. Anatomy of seedling roots of tropical maize (Zea mays L.) cultivars at low water supply. *Journal of Experimental Botany,* 43, 1015-1021.

Wilson, J. B. 1988. A review of evidence on the control of shoot: root ratio, in relation to models. *Annals of botany,* 61, 433-449.

Wright, G., Rao, R. and Farquhar, G. 1994. Water-use efficiency and carbon isotope discrimination in peanut under water deficit conditions. *Crop Science,* 34, 92-97.

Xu, W., Cui, K., Xu, A., Nie, L., Huang, J. and Peng, S. 2015. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. *Acta physiologiae plantarum,* 37, 9.

Xue, Q., Soundararajan, M., Weiss, A., Arkebauer, T. J. and Baenziger, P. S. 2002. Genotypic variation of gas exchange parameters and carbon isotope discrimination in winter wheat. *Journal of plant physiology,* 159, 891-898.

Yamaguchi-Shinozaki, K., Shinozaki, K. 2006. Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. Annu. Rev. Plant Biol. 2006. 57:781–803.

Yambao, E. B., Ingram, K. T. and Real, J. G. 1992. Root xylem influence on the water relations and drought resistance of rice. *Journal of Experimental Botany,* 43, 925-932.

Yeo, A. 1998. Predicting the interaction between the effects of salinity and climate change on crop plants. *Scientia Horticulturae,* 78, 159-174.

Yogameenakshi, P., Nadarajan, N. and Anbumalarmathi, J. 2004. Correlation and

path analysis on yield and drought tolerant attributes in rice (*Oryza sativa* L.) under drought stress. *Oryza,* 41, 68-70.

Yoshida, S. and Hasegawa, S. 1982. The rice root system: its development and function. *Drought resistance in crops with emphasis on rice,* 10, 97-134.

Zaman, N. K., Abdullah, M. Y., Othman, S. and Zaman, N. K. 2018. Growth and physiological performance of aerobic and lowland rice as affected by water stress at selected growth stages. *Rice Science,* 25, 82-93.

Zhang, G., Zhang, M., Zhao, Z., Ren, Y., Li, Q. and Wang, W. 2017. Wheat TaPUB1 modulates plant drought stress resistance by improving antioxidant capability. *Scientific reports,* 7, 7549.

Zhang, J. 2011. China's success in increasing per capita food production. *Journal of Experimental Botany,* 62, 3707-3711.

Zhu, J., Brown, K.M. and Lynch, J.P., 2010. Root cortical aerenchyma improves the drought tolerance of maize (Zea mays L.). *Plant, cell & environment*, *33*(5), pp.740-749.

Zubaer, M., Chowdhury, A., Islam, M., Ahmed, T. and Hasan, M. 2007. Effects of water stress on growth and yield attributes of aman rice genotypes. *International Journal of Sustainable Crop Production,* 2, 25-30.

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

Abstract

PHYSIOLOGICAL AND ANATOMICAL PLASTICITY OF ROOT TRAITS UNDER WATER STRESS AND MOLECULAR CHARACTERIZATION USING ROOT SPECIFIC GENES IN RICE (*Oryza sativa* L.)

by

Chennamsetti Lakshmi Naga Manikanta

(2017-11-100)

Abstract of the thesis
Submitted in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2019

The study entitled "Physiological and anatomical plasticity of root traits under water stress and molecular characterization using root specific genes in rice (*Oryza sativa* L.)" was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani during October – January, 2017-19 with the objective to quantify the adaptive plasticity in root-shoot morphology, physiology and root anatomical plasticity under water stress in selected rice genotypes and molecular characterization using root specific genes.

The extent of variation for various physiological, biochemical and anatomical characters were assessed as an indicator of water stress from six selected genotypes collected from RARS Pattambi and N-22 from IIRR, Hyderabad. Plants were maintained under 100% and 50% field capacity (FC) soil moisture in a rain out shelter. A set of five replications were maintained and observations were made at booting stage on root, physiological, biochemical and anatomical parameters and significant variations for these traits were noticed for tolerant and susceptible genotypes.

The study revealed that parameters such as relative water content (RWC) (%), specific leaf area ($cm^2\ g^{-1}$), and cell membrane stability index (%) were found to be decreasing but not significant under stress condition whereas root parameters, biochemical and biomass partitioning were found to be increasing among the tolerant and susceptible genotypes. The highest RWC was recorded from N-22 (85.37%) under stress condition whereas least from Ptb 35 (71.96%). N-22 showed the highest reduction in specific leaf area with 219.9 $cm^2\ g^{-1}$ whereas Ptb 39 showed an increasing trend in specific leaf area by 1.5% with 183.73 $cm^2\ g^{-1}$ under stress. Cell membrane stability index (%) was highest in Ptb 30 (97.10%) under stress whereas least was recorded from Ptb 39 (83.11%). Carbon isotope discrimination ($\Delta^{13}C$)(mil^{-1}) was least for N-22 (21.84) ($\Delta^{13}C$)(mil^{-1}) and highest in Ptb 39 (23.49) ($\Delta^{13}C$)(mil^{-1}) at panicle initiation.

Study on root parameters of tolerant and susceptible genotypes at two FC levels exhibited significant variation among root parameters. Root length was

highest in Ptb 29 (38.46 cm) and least in Ptb 35 (20.66 cm) under water stress. Among the genotypes Ptb 29 was found to be performing better for other root characters viz., root volume, root dry weight, root/shoot ratio and specific root length whereas least performance was noticed from susceptible genotypes Ptb 35 and Ptb 39. A significant differences in biomass partitioning was noticed among the genotypes, for characters such as leaf weight ratio, stem weight ratio and root weight ratio. Under stress root weight ratio was highest in Ptb 29 (0.21) and lowest in Ptb 35 (0.106).

Anatomical studies revealed significant effects at both genotype and treatment levels. Tolerant genotypes were found to be more responsive under water stress for anatomical traits. N-22 and Ptb 29 exhibited an increase in root diameter (1.55mm and 1.796 mm), stele diameter (0.49 and 0.31 mm), late metaxylem number and late metaxylem diameter (5.6, 0.069 mm and 5.6, 0.076 mm respectively. Early metaxylem number found to be increasing in tolerant genotypes N-22 (30.66) whereas susceptible genotypes exhibited declining trend. Sclerenchymatous tissue was found to be highest in N-22 (0.024mm) whereas Ptb 35 a susceptible genotype exhibited lowest value for sclerenchyma with 0.012 mm.

Yield attributes were found to vary significantly among genotypes. Spikelet fertility percentage and yield per plant was highest in N-22 with 85.66% and 24.66 g respectively. 1000 grain weight was highest in Ptb 30 (27.23 g) and lowest in Ptb 39 (22.5 g).

Genotyping of the selected tolerant and susceptible rice genotypes using available *DEEPER ROOTING* QTL specific primers and other available drought specific SSR primers was done from seedlings raised in a petri dish. It was found that *DRO1* specific microsatellite markers did not exhibited polymorphism among tolerant and susceptible genotypes but another drought related SSR primer RM 518 showed polymorphism for tolerant and susceptible genotypes.

Expression studies were done between one tolerant and one susceptible genotypes i.e., Ptb 29 and Ptb 35 with *DEEPER ROOTING* QTL specific primers

and EST- SSR RM 518. Results of expression studies using RM 518 exhibited differential expression under 100% FC and 50% FC condition and also among the genotypes Ptb 29 and Ptb 35.

Significant variation was observed for physio-morphological and yield components among rice genotypes under 100% FC and water 50% FC conditions. Genotypes with better root traits such as root length, root shoot ratio and root anatomical plasticity exhibited more tolerance towards drought. The tolerant genotypes i.e., N-22. Ptb 29 and Ptb 30 can be used as donor plants in breeding programs for trait introgression for developing drought tolerant cultivars. Microsatellite marker RM 518 which could distinguish drought tolerant and susceptible genotype can be used for marker assisted selection for drought tolerance in rice. A differential expression of drought related genes was seen in tolerant and susceptible genotypes under water stress condition.