DIFFERENTIAL CELL WALL RESPONSES TO SALINITY AND DROUGHT IN RICE (*Oryza sativa* L.)

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Affectionately Dedicated to

My Family

&

My Dear Ones

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU – 560 065

CERTIFICATE

This is to certify that the thesis entitled **"DIFFERENTIAL CELL WALL RESPONSES TO SALINITY AND DROUGHT IN RICE (*Oryza sativa* L.)"** submitted by **Mr. BILLY CHERIAN, I.D. No. PALB 4236**, for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) IN PLANT BIOTECHNOLOGY** to the University of Agricultural Sciences, GKVK, Bangalore, is a *bonafide* record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

Bangalore
June, 2016

(H. V. VIJAYAKUMAR SWAMY)
Major advisor

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June 2016 ***(Billy Cherian)***

"DIFFERENTIAL CELL WALL RESPONSES TO SALINITY AND DROUGHT IN RICE (*Oryza sativa* L.)"

Billy Cherian

ABSTRACT

Plant response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular, and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants. The effect of drought on rice plants considerably varies with genotypes, developmental stages, and degree and duration of drought stress. A better understanding of the complex physiological mechanisms underlying drought response is important to improve rice yields under water-limited environments. Salinity on the other hand will create osmotic stress to the plant creating a physiological drought. The present study compares the effect of salinity and drought on five different varieties varying in their tolerance level towards these stresses. Chosen five varieties were grown in pipes at Department of Biotechnology, UAS, GKVK, Bangalore, during *kharif* 2016. ARB 6 which is an aerobic rice variety showed drought stress tolerance because of its longer roots, effective suberization and high passage cell density. Pokkali which is saline tolerant variety undergone extensive suberization in its root cells to cope up with the ionic stress. Kalanamak expressed saline tolerance by keeping its ionic concentration in cell sap low and moderate suberization and it performed well in moderate drought. Performance of Jaya and IR 20 in both salinity and drought stress was not promising.

July, 2016
UAS, GKVK, Bengaluru-65

Signature of major advisor
(H. V. Vijayakumar Swamy)

ಭತ್ತದಲ್ಲಿ(*ಒರಿಜ ಸಟೈವ* ಎಲ್.) ಲವಣತ್ವ ಹಾಗೂ ಬರ ಪರಿಸ್ಥಿತಿಗೆ ಕೋಶ ಗೋಡೆಯ ಬೇಧನಾತ್ಮಕ ಪ್ರತಿಕ್ರಿಯೆ

ಬಿಲ್ಲಿ ಚೆರಿಯನ್

ಪ್ರಬಂಧ ಅಮೂರ್ತ

ಬರ ಪರಿಸ್ಥಿತಿ ವಿರುದ್ಧ ಸಸ್ಯಗಳ ಪ್ರತಿಕ್ರಿಯೆಯು ಒಂದು ಜಟಿಲ ಜೈವಿಕ ಪ್ರತಿಕ್ರಿಯೆಯಾಗಿದ್ದು ಹಲವಾರು ಶಾರೀರಿಕ, ಕೊಶೀಯ ಮತ್ತು ಆಣ್ವಿಕ ಬದಲಾವಣೆಗಳನ್ನು ಒಳಗೊಂಡಿರುತ್ತದೆ. ಆ ಸಸ್ಯಗಳಲ್ಲಿ ಬರಪರಿಸ್ಥಿತಿಯ ಪ್ರತಿಕ್ರಿಯೆಯಲ್ಲಿ ಅನೇಕ ಅನುವಂಶಿಕ ಧಾತುಗಳ ಪಾತ್ರ ಮಹತ್ವದ್ದಾಗಿರುತ್ತದೆ. ಬರ ಪರಿಸ್ಥಿತಿಯ ಪ್ರತಿಕ್ರಿಯೆಯು ಜೀನೋಟೈಪ್ ಗಳ ಬೆಳವಣಿಗೆಯ ಹಂತ, ಬರ ಒತ್ತಡ ಮಟ್ಟ ಹಾಗೂ ಅವಧಿಯನ್ನು ಅಧಾರಿಸಿರುತ್ತದೆ. ಈಗಿರುವ ಬರ ಪರಿಸ್ಥಿತಿಯಲ್ಲಿ ಭತ್ತದ ಇಳುವರಿ ಅಭಿವೃದ್ಧಿಗಾಗಿ ಇಂತಹ ಜಟಿಲ ಶಾರೀರಿಕ ಪ್ರಕ್ರಿಯೆಯನ್ನು ಪೂರ್ಣವಾಗಿ ತಿಳಿದುಕೊಳ್ಳುವ ಅವಶ್ಯಕತೆ ಇರುತ್ತದೆ. ಈ ಲವಣತ್ವವು ಸಹ ಪೊರೆತೂರ್ಪು ಒತ್ತಡವನ್ನು ಹೆಚ್ಚಿಸುವುದರ ಜೊತೆಗೆ ಶಾರೀರಿಕ ಬರದ ಒತ್ತಡವನ್ನು ಹೆಚ್ಚಿಸುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ 5 ವಿವಿಧ ಭತ್ತದ ತಳಿಗಳನ್ನು ಉಪಯೋಗಿಸಿ ಲವಣತ್ವ ಮತ್ತು ಬರದ ಒತ್ತಡಗಳ ವಿರುದ್ಧ ಈ ತಳಿಗಳ ಪ್ರಕ್ರಿಯೆಯನ್ನು ಹೋಲಿಕೆ ಮಾಡಲಾಗಿದೆ. ಆಯ್ಕೆ ಮಾಡಿದ 5 ತಳಿಗಳನ್ನು ಬೆಂಗಳೂರು ಕೃಷಿ ವಿಶ್ವ ವಿದ್ಯಾನಿಲಯ, ಗಾ.ಕೃ.ವಿ.ಕೆ. ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗದ ತಾಕುಗಳಲ್ಲಿ ಪಿವಿಸಿ ನಳಿಕೆಗಳನ್ನು ಉಪಯೋಗಿಸಿ ಬೆಳೆಯಲಾಯಿತು. ಏ ಆರ್ ಬಿ 6 ಒಂದು ಏರೋಬಿಕ್ ಭತ್ತದ ತಳಿಯಾಗಿದ್ದು ಗಮನಾರ್ಹ ರೀತಿಯಲ್ಲಿ ಬರದ ಒತ್ತಡವನ್ನು ಸಹಿಸುವ ಸಾಮರ್ಥ್ಯವನ್ನು ಹೊಂದಿದೆ. ಏ ಆರ್ ಬಿ 6 ನ ಈ ಸಾಮರ್ಥ್ಯಕ್ಕೆ ಕಾರಣ ಈ ತಳಿಯು ಉದ್ದನೆಯ ಬೇರುಗಳಲ್ಲಿ ಸುಬೇರಿನ್ ಅಂಶದ ಶೇಕರಣೆ ಮತ್ತು ಉತ್ತಮ ಕೋಶ ಮಾರ್ಗ ಸಾಂದ್ರತೆಯಾಗಿರುತ್ತದೆ. ಪೊಕಾಳಿಯು ಲವಣತ್ವವನ್ನು ಸಹಿಸುವ ಶಕ್ತಿಯನ್ನು ತೀವ್ರವಾದ ಸುಬೇರಿನ್ ಅಂಶದ ಶೇಕರಣೆಯಿಂದ ಪಡೆದುಕೊಂಡಿದೆ. ಇದೇ ರೀತಿ ಕಾಲನಮಕ್ ತಳಿಯು ತನ್ನ ಕೋಶದ ಜೀವರಸದಲ್ಲಿ ಅಯಾನಿನ ಪ್ರಮಾಣವನ್ನು ಕಡಿಮೆ ಇರಿಸಿ ಹಾಗೂ ಸುಬೇರಿನ್ನಿನ ಅಂಶವನ್ನು ಕೋಶಗೋಡೆಯಲ್ಲಿ ಹೆಚ್ಚಿಸಿ ಲವಣತ್ವವನ್ನು ಮಧ್ಯಮ ಮಟ್ಟದಲ್ಲಿ ಸಹಿಸಿಕೊಳ್ಳುವ ಸಾಮರ್ಥ್ಯವನ್ನು ಹೊಂದಿದೆ. ಆದರೆ ಜಯ ಮತ್ತು ಐಆರ್ 20 ತಳಿಗಳಲ್ಲಿ ಯಾವುದೇ ರೀತಿಯ ಗಮನಾರ್ಹ ಸಹಿಷ್ಣು ಶಕ್ತಿಯು ಕಂಡು ಬಂದಿಲ್ಲ.

ಜುಲೈ ೨೦೧೬

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ — ಪ್ರಧಾನ ಸಲಹೆಗಾರರು

ಗಾ.ಕೃ.ವಿ.ಕೆ. ಬೆಂಗಳೂರು — (ಪೆಚ್.ವಿ. ವಿಜಯಕುಮಾರಸ್ವಾಮಿ)

Differential Cell Wall Responses to Salinity in Rice

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Introduction

- For the past couple of decades soil salinity and water stress are causing considerable loss in yield and productivity in rice.
- Rice, one of the most important crops globally, is particularly affected since it is relatively salt-sensitive.
- Excessive Na^+ ion is toxic and impart ionic and osmotic stress.
- Environmental factors such as drought, salt stress and growth conditions increases the formation of apoplastic barriers in roots.
- The cultivar Pokkali is salinity tolerant and is to have more extensive hydrophobic barriers in its roots than does IR20, a more sensitive cultivar.
- Barriers located in the root endodermis and exodermis prevent the direct entry of external fluid into the stele.
- Exposing plants to a moderate stress of 100 mM NaCl resulted in deposition of additional hydrophobic aliphatic suberin.
- It is known that in the case of rice, these barriers are bypassed by most of the Na^+ that enters the shoot.

Objective

- Differences in biochemical composition of sap exuded from the varieties at the peak vegetative stage on being subjected to stress.

Materials & Methods

- **Plant materials:** Four rice genotypes differing in their tolerance towards salinity are selected and grown in PVC pipes.

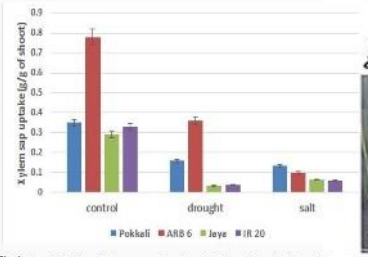
	Genotypes	Salt tolerance
1	**Pokkali**	Highly tolerant costal rice
2	**ARB 6**	Susceptible aerobic rice, exhibit drought tolerance
3	**IR 20**	Highly susceptible high yielding variety
4	**Jaya**	Mild tolerant high yielding variety

- 30 days after germination plants are treated with NaCl solution (50mM, 100mM, 150mM concentration) for seven days continuously.
- On 37th day shoots were cut off by razor blades at distances of 40–70 mm from their base.
- The cut ends were wrapped with a blotting paper and kept overnight to absorb the exuding sap.
- Next day blotting papers were removed and gently rocked in double distilled water in a conical flask to remove the sap from the paper for analysis.
- Sap containing solution was used for the analysis of Na^+ & K^+ using flame photometry.
- Drought condition is induced by withholding water for three to seven days.

Results

Sl. No		50 mM		100 mM		150 mM	
		Na^+	K^+	Na^+	K^+	Na^+	K^+
1	Pokkali	48	36	81	73	109	89
2	ARB 6	61	45	115	93	138	122
3	Jaya	71	52	108	89	125	99
4	IR 20	68	47	105	87	131	107

Table 1: Na^+ and K^+ concentration in xylem sap at different concentrations of NaCl



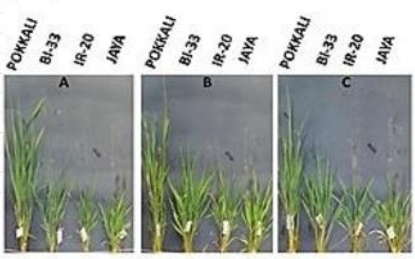


Fig 1: Quantification of xylem sap uptake in control, drought and salt condition.

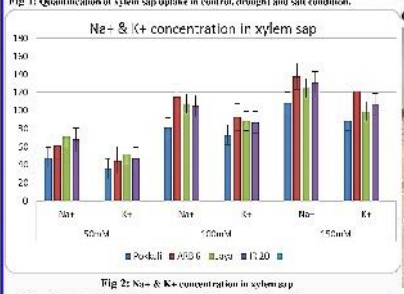
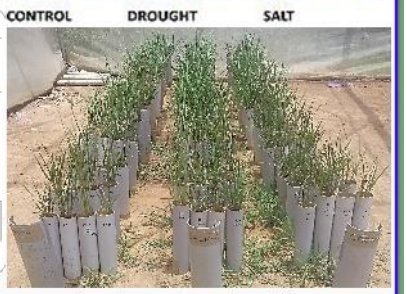


Fig 2: Na^+ & K^+ concentration in xylem sap



- ✓ In fig 1 ARB 6 show high level of xylem sap uptake compared to other genotypes due to high passage cell density, this peculiar property of ARB 6 owes to its water stress tolerance.
- ✓ In fig 2 saline tolerant genotype Pokkali shows less amount of Na^+ in xylem sap which indicates its ability to block and compartmentalize the toxic concentration of Na^+ ions.
- ✓ Since the sap uptake of ARB 6 is higher the concentration of Na^+ & K^+ in its xylem sap will be comparatively higher.

Discussion

- ARB 6 has very high xylem sap uptake in control plants, and its ability to maintain high uptake even under drought stress contributes to its survival under drought stress.
- ARB 6 has very long roots that extend almost vertically downwards in order to tap into water reserves deep within the soil.
- Pokkali is very successful in adding osmolytes to the xylem sap under saline stress and also in restricting Na^+ entry into the sap, thus maintaining reasonable xylem flow under saline conditions without subjecting the shoot to Na^+ loading.
- High amount of waxy barrier formation in Pokkali will check the amount of toxic ions into the stelelar region.
- Due to higher passage cell density ARB 6 had slightly elevated concentration of Na^+ and K^+ in its xylem sap.

Summary

- Stresses in the form of drought and salinity induce the deposition of hydrophobic aliphatic suberin in rice roots.
- Due to its deep rooting character and high passage cell density ARB 6 shows high tolerance to drought condition, hence it is suitable for aerobic system of cultivation.
- Pokkali has innate ability to thrive in saline conditions, combining the unique abilities of these peculiar genotypes will produce elite lines with salinity and drought tolerance combined.

Advisory committee

Chairperson: Dr. H. V. Vijayakumar Swamy
Members : Dr. H. E. Shashidhar
Dr. Veena S. Anil
Dr. M. K. Mathew

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I INTRODUCTION

The most abundant component of probably all life forms on earth is water. Since it is not only the essential solvent for nutrients and cell constituents but also involved in a great portion of chemical reactions, maintaining the water content is vital. Organisms of most taxonomic kingdoms form a cell wall surrounding the cell, which increases resistance against mechanical stress, while also supporting the water and ion and via these the pressure homoeostasis, a process which can become a high proportion of the cell's energy consumption, even in aquatic life forms.

The colonization of terrestrial habitats by plants, starting about 480 million years ago (Kenrick and Crane, 1997), required substantial adaptations of the outermost cell layers to the aerial environment, which is characterized by a strong negative water potential. The needs of light capturing and at the same time protection against radiation, of water and nutrient uptake as well as prevention of dehydration without loss of gas exchange, increased selection pressure and led to the evolution of tissues and organs of distinct functionality.

Nearly all multi-cellular organisms are separated from the environment by an outermost layer of tightly connected cells, the epidermis. Peptide polymers, such as keratin in the skin cells of mammals, birds and reptiles, or polysaccharides like chitin, forming the exoskeleton of arthropods in combination with proteins or minerals, or the cell wall of fungi together with glucans, provide mechanical strength. These polymers in combination with solvent extractable lipids also protect living organisms against uncontrolled water loss and cell damage by radiation.

All above-ground organs of plants in the primary developmental state are equipped with an epidermis which is covered by a lipophilic incrustation of 0.1–10 µm thickness. This cuticle is comprised of waxes, mainly aliphatic with lesser amounts of aromatic components, embedded and deposited atop a polyester matrix, composed of aliphatic compounds and glycerol (Nawrath *et al*., 2013).

Rice (*Oryza sativa* L.), a cereal of high economic and social value, is used as a staple food by more than half of world's population. It is the only cereal which is solely produced for human consumption. According to FAO, India constitutes 2.28 % of the total land area and 20.97 % of the total irrigated agriculture area but contributes 21.61 % to the production of milled rice and 21.73 % of paddy. The productivity of rice in India is 3.52 t/ha while the overall productivity of rice in the world is 4.4 t/ha (Anon. 2012). Thus, there is a need to increase productivity in all rice habitats by mainly rain-fed lowland habitat, which constitutes almost 79 % of the total land area.

Salinity is an environmental factor that greatly affects plant growth and development and is a major constraint for crop production. This stress is complex and causes a number of determinant effects. Among them ionic and water constraints constitute the most important. The water constraint even called osmotic pressure is characterized by difficulties to absorb water. The ionic constraint interferes with the

uptake of nutrients, and causes direct toxicity due to the ions Na^+ and Cl^-. Salinity also interferes with the structure of the soil, causes an indirect stress and increases the sensitivity to diverse biotic stresses (Araya *et al*., 1991). To limit the effect of salt in plant productivity, amelioration and utilization of salt-affected soils are needed. Two approaches are used to solve the problem: by using technical approaches (water and soil management) and biological approaches.

Plant productivity is severely threatened by enhanced salinity. Actually 800 Mha of land throughout the world are salt-affected, either by salinity (397 Mha) or the associated conditions of sodicity (434 Mha) (Anon. 2005). Salinity is generally defined as the presence of excessive amounts of soluble salt that hinders or affects the normal functions needs for plant growth. It is measured in terms of electric conductivity (ECe), or of the exchangeable Na^+ percentage (ESP) or with the Na^+ absorption ratio (SAR) and pH of saturated soil paste extract. Therefore, saline soils are those with ECe more than 4 dSm^{-1} equivalent to 40 mM NaCl, ESP less than 15 % and a pH below 8.5 (Abrol, 1986; Szabolcs, 1994).

Among species, rice (*Oryza sativa*) may play a major role because of its role as 2^{nd} most consumed cereal in the world, and on the other hand its capacity to survive a long submerging time. Since the genome of rice became completely sequenced, rice is increasingly becoming the model plant for cereals. In addition, the establishment of the rice-mutant database with at present approximately 40,000 independent lines is another important reason for the selection of rice as model plant. To create rice tolerant lines capable to grow and minimize the toxicity effects induced by salt stress and capable to improve the productivity, it is necessary to identify the molecular mechanisms involved in the tolerance or the sensitivity of plants to salt.

When submitted to salt stress, plants perceive first the constraint as drought stress. Due the low water potential in the medium, plants cannot absorb water and tend to lose its water. This constitutes the osmotic constraint or water deficit of salinity. As consequence, plant growth is inhibited because the plant ability to take up water is reduced, and growth becomes slower. According to Song *et al*. (2005), the reduction of germination observed by the three species of the halophyte *Sueda* is due to the osmotic and ionic toxicity. Germination of the species is improved, when the salinity is alleviated. Therefore, to survive in conditions of osmotic potential, plants must either limit water loss by regulation of its transpiration or proceed to the readjustment of its osmotic potential.

In India, area under rice cultivation remained stagnant and even declined in the recent years due to water availability. Drought reduces yield by 15–50 per cent depending on the stress intensity and crop growth period at which the stress occurs in rice (Srividhya *et al*., 2011). Eastern India, comprising Jharkhand, Orissa, and Chhattisgarh alone accounts loss of about 40 per cent of the total rice production due to severe drought (Pandey and Bhandari, 2009). Developing high yielding and drought resistant varieties for rainfed area is priority for improving rainfed rice production.

Plant response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular, and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants (Zhang *et al*., 2012). The effect of drought on rice plants considerably varies with genotypes, developmental stages, and degree and duration of drought stress (Wang *et al*., 2011). A better understanding of the complex physiological mechanisms underlying drought response is important to improve rice yields under water-limited environments.

Roots, the hidden half of a plant, are important for numerous functions including water and nutrient uptake that make it difficult to overlook their importance to plant productivity (MacMillan *et al*., 2006). Root traits are key component in rice plant adaptation to drought stress (Courtois *et al*., 2009). Root traits related to drought response are complex and controlled by many genes, each with a small genetic effect (Sharma *et al*., 2011). Selection and breeding for desirable root traits associated with drought tolerance have been practiced in rice and the differential response of rice genotypes to drought has been related to root system characters (Steele *et al*., 2006; Kanbar and Shashidhar, 2010). Among the root morphological traits, maximum root length, root dry weight, root volume, root to shoot weight and length ratios are associated with drought tolerance in upland rice (O'Toole and Soemartono, 1981; Yoshida and Hasegawa, 1982; Babu *et al*., 2001; Kanbar *et al*., 2009).

The effect of water stress may vary with the variety, degree and duration of water stress and the growth stage of the rice crop. Water requirement is low at the seedling stage. Unless there is severe water stress, the effect during this stage could be recovered. Water stress during vegetative stage reduces plant height, tiller number and leaf area. Three days drought around critical period may reduce yield causing high percentage of sterility.

In drought-stressed lowland rice, the implications of apoplastic root barrier formation are complex since the soil at the start of the season is flooded, then fluctuates or steadily decreases based on rainfall patterns. Hose *et al*. (2001) concluded that the extent and rate of casparian band and suberin lamella formation depend on environmental conditions (drought, hypoxia, salt, heavy metal, and nutrient availability). Typically, a greater degree of casparian band and suberin lamella development resulted in less water permeability of the root.

The symplastic and transcellular components together are usually considered as the cell-to-cell pathway. Along the cell-to-cell path, water transport may be regulated in terms of an expression and activation of aquaporin's and development of suberin lamellae. The water conductivity of the apoplastic path is decreased by the deposition of suberin in casparian bands. Apoplastic barriers are composed of lignin and suberin, among that aliphatic suberin rather than lignin and aromatic suberin have pronounced effect. Environmental factors such as drought, salt stress and growth conditions increases the formation of apoplastic barriers in roots (Schreiber *et. al*. 2005).

The cultivar Pokkali is salinity tolerant and is to have more extensive hydrophobic barriers in its roots than does IR20, a more sensitive cultivar. These located in the root endodermis and exodermis prevent the direct entry of external fluid into the stele. However, it is known that in the case of rice, these barriers are bypassed by most of the Na+ that enters the shoot (Krishnamurthy *et al*. 2009)

Krishnamurthy *et al*. (2011) point out that: Exposing plants to a moderate stress of 100 mM NaCl resulted in deposition of additional hydrophobic aliphatic suberin in Pokkali and IR 20. Pokkali roots have a lower permeability to water than those of IR20. Conditioning plants with 100 mM NaCl effectively reduced Na+ accumulation in the shoot and improved survival of the plants when they were subsequently subjected to a lethal stress of 200 mM NaCl.

Present study was undertaken with the following objectives to screen the rice genotypes which are drought and salinity tolerant by analyzing the xylem sap and root tissues.

1. Quantifying the difference in the extent of suberization under aerobic, drought and salinity conditions using contrasting genotypes.
2. Differences in biochemical composition of sap exuded from the varieties at the peak vegetative stage on subjected to stress.

II REVIEW OF LITERATURE

Work done earlier in the chosen areas of research is summarized below under the following headings.

2.1 Rice cultivation and salt condition
- 2.1.1 Soil salinity and its impact on food supply
- 2.1.2 The effects of salinity on plants with an emphasis on rice
- 2.1.3 Effect of NaCl in inducing salinity
- 2.1.4 Factors affecting Na^+ tolerance in rice
- 2.1.5 Reducing Na^+ uptake to the shoots
- 2.1.6 Compartmentation of Na^+ within and between cells

2.2 Rice cultivation and drought condition
- 2.2.1 Response of rice towards drought stress
  - 2.2.1.1 Drought Resistance
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- 2.2.2 Drought tolerance traits
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  - 2.2.2.2 Dehydration avoidance

2.3 Root traits under abiotic stress
- 2.3.1 Root dimensions
- 2.3.2 Deep root system
- 2.3.3 Maximum root length

2.4 Suberin formation under abiotic stress
- 2.4.1 Suberin lamelle
- 2.4.2 Apoplastic bypass flow

2.5 Xylem sap evaluation of stressed plants

Rice is the world's most important food crop and a primary source of food for more than half of the world's population. India is considered to be one of the primary centers of origin of rice (*Oryza sativa ssp. indica*) in the world. Rice is a major staple food cereal, and next to wheat, is extensively cultivated across all regions.

2.1 Rice cultivation and salt condition

Rice is one of the most suitable crops for saline soils although it is usually considered moderately sensitive to salinity. Saline soils are usually under waterlogged condition; other crops could not grow in these areas except rice. Salt tolerance is

generally a sustained growth of the plant in the soil environment impregnated with NaCl or other salt combinations (Shimose, 1963).

Earlier studies conducted under controlled conditions reported that salt injury in rice plant is caused by both osmotic imbalance and accumulation of chloride (CI^-) ions (Pearson *et al.,* 1966). Later studies, however, indicated that injury is due to the excessive sodium (Na^+) uptake, and chloride, being essentially a neutral anion, is tolerated over a wide range of concentrations. The disruptive effect of Na^+ and its interference with the role of cytoplasmic K^+ preempted CI^- toxicity. Moreover, Na-K imbalance adversely affects grain yield (Akbar *et al.,* 1972). The typical mechanism of salinity tolerance in rice is the exclusion or reduction of Na^+ uptake and increased absorption of K^+ to maintain a good Na-K balance in the shoot (Korkor and Abdel-Aal, 1974).

The detection of salinity induced injuries, however, are very complex even under controlled conditions (Kaddah *et al.,* 1975). Moreover, it requires expensive and time-consuming tissue analysis. The visual symptoms of salt stress may still be the most appropriate for mass screening. Salt injury starts with reduction in effective leaf area. The oldest leaves start to roll then die, followed by the next older, and so on. Finally, the survivors have the old leaves losing vitality with the youngest remaining green (Maas and Hoffman, 1977).

Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during reproductive stage, and then becomes increasingly more tolerant at maturity (Clarkson and Hanson, 1980). However, some studies reported that at flowering, rice is not sensitive to salinity (Devitt *et al.,* 1981). Hence, to know the response of the rice plant to salinity as a whole, it is imperative that the effects be observed in all the various stages of its development, that is at early seedling, vegetative and reproductive stages (Mori and Kinoshita, 1987).

2.1.1 Soil salinity and its impact on food supply

Most naturally salt-affected soils (primary salinisation) are caused by the weathering of parental rocks (Flowers and Yeo, 1995). However, these areas are not particularly important to agriculture as they are mostly coastal salt marshes and inland deserts; the major problem for agriculture is secondary salinisation caused by irrigation and forest clearance (O'Farrell *et al.,* 1997). Excessive irrigation, beyond that required for plant growth, raises the water table and carries salts that have accumulated in the soil to the surface. When the water evaporates, the salts dissolved in the water are left behind in the soil, thus increasing salinity.

Approximately 1500 Mha of land are cultivated without irrigation and of this only about 2 % is salt affected, whereas of the 230 Mha of irrigated land about 20 % is thought to be affected by salinisation. Although irrigated land covers only 15 % of total cultivated land of the world, it is the irrigated land that is the most productive in world agriculture by producing one third of the world's food (Flowers and Flowers, 2005). Approximately 10 Mha of irrigated land worldwide are abandoned every year due to secondary

salinization. Moreover, it has been anticipated that the sea level will rise by 1.0 m in the next 500 years because of the melting of the Antarctic ice sheet as a result of global climate change. This rising sea level will adversely affect land used in coastal areas, increase saline soils and consequently affect food production. Thus, salinity could seriously threaten to food supply for the world population (Munns and Tester, 2008).

2.1.2 The effects of salinity on plants with an emphasis on rice

Yeo *et al.* (1991) reported that an addition of 50 mM NaCl to the culture solution stopped leaf elongation of rice genotypes IR 2153 and Pokkali for 20 min, after which the elongation rate resumed to the non-salinised control rate by 24 h. It was hypothesised that stopping leaf elongation was due to a limitation of water supply to the growing zone caused by salinisation. A similar result was observed in barley (*Hordeum vulgare* L.) leaves under salt stress. A study in barley, bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.) by Munns *et al.* (1995) indicated that genotypes differing in salt tolerance did not differ in the osmotic effect of salinity.

Generally, the effects of salinity on plants are associated with osmotic effects, ionic effects and nutritional imbalance (Tester and Davenport 2003; Fricke *et al.* 2004). The osmotic effect is induced by the effect of NaCl outside the plant. Raising the external concentration of NaCl reduces the ability of plants to take up water by decreasing the external water potential; this can lead to the reduction of plant growth (Munns, 1993, 2002; Fricke, 2004a; Fricke *et al.*, 2004).

Fricke (2004b) and Fricke *et al.* (2004, 2006) reported that leaf elongation velocity (LEV) of barley leaf 3 was zero or close to zero within 10 min of the addition of 100 mM NaCl to the culture solution. This rapid cessation in growth of the leaf under saline stress was due to the reduction of xylem water potential in the elongation zone, generated by NaCl in the culture solution. Then the LEV recovered to about 40-50 % of the original value between 20 and 30 min as a result of a decrease in the transpiration and stomatal conductivity caused by an increase (by 3-6 times compared with non salinised plants) in leaf abscisic acid (ABA), leading to an increase in the xylem water potential and growth resumption (Flowers and Flowers, 2005; Rengasamy, 2006; Qadir *et al.*, 2008; Shabala and Cuin, 2008).

2.1.3 Effect of NaCl in inducing salinity

Yeo and Flowers (1983) found that the activities of nitrate reductase and malate dehydrogenase extracted from leaves of rice IR2153 were inhibited by 50 % when Na^+ concentration was 150 and 265 mM, respectively. They have also reported that there was no evidence of enzymatic adaptation to function specifically under saline conditions in rice as the activities of those enzymes from both control and salt-stressed plants (50 mM NaCl for 8 d) were inhibited more than 50 % at Na^+ concentrations in excess of 200 mM. Also, enzymes extracted from salt-tolerant halophyte, *Suaeda maritima* L. were not more tolerant to Na^+ than those extracted from salt-sensitive glycophyte, *Pisum sativum* L. (Flowers 1972; Flowers *et al.*, 1977).

The ionic effect is induced by the effect of NaCl within the plant. When NaCl taken up via the transpiration stream exceeds the ability of cells to compartmentalise it in the vacuole, excessive Na^+ and Cl^- rapidly builds up in the cytoplasm and inhibits enzyme activity (Munns, 2002; Tester and Davenport, 2003; Munns and Tester, 2008). Although the concentration at which Na^+ in cytoplasm becomes toxic is not well defined, *in vitro* studies showed that most enzymes started to be inhibited at a concentration above 100 mM NaCl or about 0.5 mmol/gDW (Munns and Tester, 2008).

2.1.4 Factors affecting Na^+ tolerance in rice

The mechanisms employed by plants in general to tolerate salinity have been frequently reviewed (Flowers *et al.*, 1977; Greenway and Munns, 1980). For rice, salinity tolerance is a complex issue, which includes restriction of entry of Na^+ into the root xylem, reducing Na^+ uptake to the shoots, leaf-to-leaf distribution, compartmentation of Na^+ within and between cells and plant vigour (Yeo and Flowers, 1984a, 1986; Yeo *et al.* 1990; Yeo, 1992; Flowers *et al.*, 2000; Munns, 2002; Munns *et al.*, 2002; Tester and Davenport 2003; Flowers and Colmer, 2008; Munns and Tester, 2008; Plett and Moller, 2010; Zhang *et al.,* 2010).

2.1.5 Reducing Na^+ uptake to the shoots

Na^+ and other solutes reach the shoots of rice, as in other plants, by way of the transpiration stream (Yeo and Flowers, 1984b; Yeo *et al.*, 1987). Therefore, reducing the transpiration rate should lower the net transport of Na^+ to the shoot. An ability to control transpiration has been proposed as an important mechanism for plants growing in saline conditions and the ability to control transpiration can be expressed as either an absolute transpiration volume or the ratio between growth and transpiration, namely water-use efficiency, WUE (Flowers *et al.*, 1988; Yeo *et al*., 1992). In a study on the effect of salinity (50 mM NaCl for 7 d) on WUE of seven rice genotypes (Pokkali, Bhura Rata, Amber, IR2153, IR4630, IR36 and IR28), a positive correlation between WUE and salinity resistance was observed: the greater the WUE, the greater the resistance to salinity. Consistently, Yadav *et al.* (1996) reported that a low Na^+ transporting line of rice cv. IR36 had a higher WUE than a high Na^+ transporting line.

2.1.6 Compartmentation of Na^+ within and between cells

Once Na^+ has entered the cells, it has to be compartmentalised in the vacuoles in order to maintain low cytoplasmic Na^+ concentrations and a high K^+/Na^+ ratio (Flowers *et al.*, 1977; Fricke *et al.*, 1996). Na^+ in the cytoplasm is toxic to the cells because it will inhibit enzyme activities and interrupt metabolic processes by competing with K^+ for the binding sites. The compartmentation of Na^+ from the cytoplasm into the vacuole is mediated by the vacuolar Na^+/H^+ antiporter (NHX) that is driven by the proton gradient generated by the vacuolar H^+ translocating enzymes, H^+ ATPase and H^+ PPiase. The compartmentation of Na^+ into the vacuole reduces the deleterious effects of Na^+ in the cytoplasm, creates an osmotic potential for driving water into the cells and thus extends survival (Blumwald *et al.*, 2000).

Verma *et al.* (2007) found that over-expression of *PgNHX1* from a halophytic plant, *Pennisetum glaucum*, increased salt tolerance in transgenic rice plants (PB1) which were able to complete their life cycle and produce seeds at 150 mM NaCl, a salt concentration that the wild-type plants could not endure.

2.2 Rice cultivation and drought condition

Worldwide, about 80 Mha of irrigated lowland provide 75 % and about 60 Mha of rainfed lowlands supply about 20 % of the rice production. Rice needs more water compared to other crops, on an average about 2,500 liters of water is needed to produce 1 kg of rough rice. Irrigated rice receives an estimated 34-43 % of the total world's irrigation water. Worldwide water for agriculture is becoming increasingly scarce, day by day due to uncertain and uneven rainfall distribution patterns, shrinking groundwater resources, increasing level of salts in soil solution and diverting the fresh water resources to competing urban and industrial uses (Hsiao, 1982). In the coming future, water availability may be more affected due to ongoing changes in global climate. Because of semi-aquatic ancestry, rice is extremely sensitive to water shortage.

Drought is the major constraint to rice production in rainfed areas across Asia. Drought can be simply defined as reduction in yield due to shortage of water. Throughout the world, about 34 per cent (~54 Mha) of the total land under rice cultivation is under rainfed condition (Maclean *et al.*, 2002). Asia occupies 32.1 per cent rainfed low land rice of the total rice area which currently averages production of 2.3 tonnes per hectare (Tuong and Bouman, 2003). Drought is the most devastating among abiotic stresses and it depresses yield by 15-50 per cent depending on the vigor and period of stress in rice. The global reduction in rice production due to drought averages 18 million tonnes annually (O'Toole, 2004).

Drought is an abiotic stress that limits productivity in both rainfed and upland ecosystems. In Asia, severe and minor drought occurs in East and Northeast India, East China, Central Myanmar, Thailand. In the eastern states of India viz., Jharkhand, Orissa and Chhattisgarh, 40 per cent of the total rice production is lost in severe droughts valued at $650 million (Pandey *et al.*, 2005) and poorest rice farmers are most affected in these areas. In rainfed areas, high yielding semi-dwarf rice varieties are not widely grown because of their poor adaptability to more stressful rainfed conditions (Pandey and Bhandari, 2008). Rice is sensitive to drought stress during reproductive growth and even moderate stress can result in drastic reduction in grain yield (Venuprasad *et al.*, 2007).

Drought risk reduces productivity even in non-drought years, because farmers avoid applying fertilizer and other inputs for fear of losing crop, becoming mired in a cycle of low productivity, poverty and food insecurity (Venuprasad *et al.*, 2008). Drought mitigation, through development of drought resistant varieties with higher yields suitable for water-limiting environments will be a key to improve rice production and ensure food security to 3 billion people in Asia. The development of such varieties requires a good knowledge of the physiological mechanisms and the genetic control of the traits contributing in drought resistance (Bouman, 2009).

2.2.1 Response of rice towards drought stress

2.2.1.1 Drought Resistance

Drought resistance refers to the ability of a crop to produce its economic product with minimum loss in a water-deficit environment relative to the water constraint free management. Drought resistance is a complex trait whose expression depends on action and interaction of different morphological, physiological and biochemical characteristics (Mitra, 2001). Rice crop responds to drought condition by stomatal closure, leaf rolling, enhanced root growth, enhanced ABA production etc., to minimize water deficit (Price *et al.,* 2002a). Physiological studies suggest that drought resistance in rice mainly depends on following components.

1) Ability of roots to exploit deep soil water to provide for evaporative transpirational demand.
2) The capacity of osmotic adjustment, which allows the plant to maintain turgor and protect meristem from desiccation.
3) Ability to control and reduce water loss (Nguyen *et al.*, 2004).

In field crop production, survival alone is not sufficient during a drought; the crop needs to produce a reasonable yield for subsistence requirements. Rice plant has several mechanisms to adapt under drought condition. Responses of plants to cope up drought situation are drought escape, drought avoidance, drought tolerance and drought recovery (Zhu *et al*., 2008; Matsukura *et al.*, 2010; Lata and Prasad, 2011). Besides these, a complex network of stress, signaling and regulation of gene expression exists in rice crops responding and adapting to the stresses. The stress signals are perceived through diverse known and unknown sensors and transduced by various signaling components to various physiological and metabolic responses to adapt to the stresses (Singh *et al.*, 2012).

2.2.1.2 Drought Escape

Drought escape is defined as the ability of a plant to complete its life cycle before serious soil water deficits develop. This mechanism involves rapid phenological development, developmental plasticity (variation in duration of growth period depending on the extent of water deficit) and remobilization of pre-anthesis assimilates to the grain (Turner, 1979). In drought-prone upland areas of eastern India and Bangladesh, drought escape is an important mechanism that allows rice to produce grain despite limited water availability (Khush *et al.,* 1997; Bernier *et al.,* 2008).

2.2.1.3 Drought Avoidance

Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture. Rice varieties which cope with drought using their root systems to maintain their plant water status comes under drought avoidance category. Such varieties therefore minimize the yield losses caused by drought (Blum *et al.,* 1989; Samson *et al.,* 2002). Mechanisms for improving water uptake, storing it in plant cell and reducing water loss confer drought avoidance (Wang *et al.*,

2006). The ability to access water at depth through the possession of an effective rooting system in lower soil layers is considered a crucially important drought avoidance mechanism in upland rice. Rice varieties which avoid drought usually have deep, coarse roots with a high ability of branching and penetration, higher root to shoot ratio, elasticity in leaf rolling, early stomatal closure and high cuticular resistance (Singh *et al.*, 2010).

2.2.1.4 Drought Tolerance

Drought tolerance is the ability of a plant to live, grow and reproduce satisfactorily with limited water supply or under periodic conditions of water deficit (Turner, 1979). Rice varieties differ greatly in their tolerance to drought. It is a quantitative trait, with complex phenotype and genetic control (McWilliam, 1989). Genetically, drought tolerance of rice is a complex trait under polygenic control and involves complex morpho-physiological mechanisms (Li and Xu, 2007), such as maintenance of turgor through osmotic adjustment, increased elasticity in cell, decreased cell size and desiccation tolerance by protoplasmic resistance (Sullivan and Ross, 1979). The responses of plants to tissue water deficit determine their level of drought tolerance (Mitra, 2001) and the traits associated with such phenomena are considered as secondary traits. Secondary traits used for selection purpose are mainly the root traits, osmotic adjustment and traits governing maintenance of plant water potential like relative water content (Babu *et al.*, 2001; Kato *et al.*, 2006).

Roots play the principal role for nutrient and water uptake in plants so that the understanding of interactions between root function and drought tolerance becomes quite important for the global food security (Gowda *et al.*, 2011). Molecular breeding based on the collection of drought tolerant cultivars from all over the world and the comprehension of the drought-resistance mechanisms will help in finding genes from drought adaptive germplasm (Yu *et al.*, 2012).

2.2.1.5 Drought Recovery

Recovery of a genotype from drought is related to its ability to retain green leaves during that period and lines with good leaf retention can supply more assimilate to the developing panicle during subsequent recovery. This in turn results in production of a large number of spikelets (Malabuyoc and Aragon, 1985). Drought recovery is an important mechanism in early period of crop development. Some genotypes are able to produce more tillers upon relief of drought and these tillers are productive if the remaining growing season is long enough to complete grain filling. Rice population subjected to drying soil condition shows difference in response to stress and genotypes differ in their recovery upon dehydration (Lilley and Fukai, 1994; Fukai and Cooper, 1995). Drought resistance in terms of recovery responses is most likely dependent mainly on one or more of the following components:

(i) Moderated water use through reduced leaf area and shorter growth duration.

(ii) Ability of the roots to exploit deep soil moisture to provide for evapo-transpirational demand.

(iii) Increased capacity for osmotic adjustment (OA) allows to retain turgor and protect meristem from extreme desiccation.

(iv) Control over non-stomatal water loss from leaves (Nguyen *et al.*, 1997).

The overall goal of improving drought resistance is to develop new rice genotypes that can potentially double yield under drought stress in rainfed environments.

2.2.2 Drought tolerance traits

2.2.2.1 Osmotic adjustment

Osmotic adjustment (OA) is an adaptive process in response to adverse growing conditions such as drought. During OA solutes accumulate in cells and decrease the osmotic potential as water stress develops (Steponkus *et al.,* 1982; Hsiao *et al.,* 1984; Turner *et al.,* 1986). Osmotic adjustment is a major cellular stress-responsive and adaptive dehydration avoidance trait and is important in many crop plants because it has been repeatedly linked to yield under drought stress (Tangpremsri *et al.,* 1991). It has been demonstrated that OA has two major functions in plant production under drought stress it enables leaf turgor maintenance for the same leaf water potential thus supporting stomatal conductance under lower leaf water status (Fukai, 1999; Ali *et al.,* 1999; Zhang *et al.,* 2001; Sellin, 2001) and it improves root capacity for water uptake (Babu *et al.,* 2003).

OA capacity is an important shoot-related component of drought tolerance in crops (Chimenti *et al.,* 2006). OA delays leaf rolling, tissue death and leaf senescence under water stress in rice and (Blum, 2009) has been shown to enhance grain yield under water-limited conditions in several other crops. Despite our understanding of the role of putative traits in drought resistance these traits are rarely selected for crop improvement programs because phenotypic selection for most root traits and OA is difficult and labor intensive. However, a yield benefit due to OA is yet to be demonstrated in rice (Farooq *et al.,* 2009, Blum, 2011).

2.2.2.2 Dehydration avoidance

Effective drought resistance in crop plants is largely achieved by dehydration avoidance. Dehydration tolerance that involves the survival of plants under extreme dehydration is less important in crop production under water limited supply (Fukai and Cooper, 1995). Dehydration tolerance is important in seed embryos, resurrection plants and certain desert vegetation. It is also important for freezing tolerance and winter survival. Dehydration avoidance traits conserve plant water status and turgor through the effective use of water. Genotypic variation for dehydration tolerance capacity in rice is large (Lilley and Ludlow, 1996; Babu *et al.,* 2001). During terminal stress dehydration tolerance may allow plants to maintain metabolic activity for longer time and to translocate more stored assimilates to the grain. So, if breeding approaches increase dehydration tolerance of rice then it could be possible to increase or at least stabilize the yield of rainfed rice (Blum, 2005, Blum, 2011).

2.3 Root traits under abiotic stress

Root system is a complex three dimensional structure that provide functions central to plant fitness such as water and nutrient acquisition, also act as storage organs and synthesizers of plant growth regulators. Rice is characterized by a shallow root system compared with other cereal crops. It has been hypothesized that coarse roots have a direct role in drought resistance because larger diameter roots are related to penetration ability. Chang *et al.* (1972) compared root traits of several upland and lowland varieties and found that drought resistance was associated with coarse, long roots, dense root system and high root to shoot ratio.

2.3.1 Root dimensions

Among the several factors contributing to enhanced drought resistance root characters are believed to be vital components in the mechanisms of dehydration postponement since they contribute to regulation of plant growth, extraction of water and nutrients from deeper unexplored soil layers (Nicou *et al.*, 1970; Yoshida and Hasegawa, 1982; O'Toole, 1982; Armenta-Soto *et al.*, 1983; Ekanayake *et al.*, 1985; O'Toole and Bland, 1987). Significant genetic variation exists among different rice cultivars for root morphological traits such as root diameter (O'Toole and Bland, 1987; Ingram *et al.*, 1994), root depth (Nemoto *et al.*, 1998), root pulling force (Price *et al.*, 1997a), root to shoot ratio (Thanh *et al.*, 1999; Ali *et al.*, 2000; Babu *et al.*, 2001), root number, root growth plasticity (Price *et al.*, 2002; Toorchi *et al.*, 2006) and root penetration ability (Kato *et al.*, 2007; Clark *et al.*, 2008).

2.3.2 Deep root system

Deep root system is thought to enable plants to avoid drought stress by absorbing water from deep soil layers and concluded that the root length density in deeper soil was one of the factors that determined drought resistance in rice (Yoshida and Hasegawa, 1982). Root length density and root thickness are used to characterize root system development of rice cultivars (O'Toole and Bland, 1987). Deep rooting is a complex trait that combines the effects of the root growth angle and root length in seminal and nodal roots of cereal crops (Price and Courtois, 1999). Typical upland rice cultivars have deeper rooting than lowland cultivars (Araki *et al.*, 2002). The deep root system in upland rice may contribute greatly to its drought resistance through enhanced water uptake (Allah *et al.*, 2010).

2.3.3 Maximum root length

Maximum root length is the most important character for plant standing. The longer the roots are better the plants standing and this is important in shaping plant characters like panicle length and panicle number (Subbarao *et al.*, 2000), which in turn will enhance the grain output. In fact, many crop varieties with an insufficient root length density below 50 cm soil depth were shown not to use the sub-soil water to meet their transpiration demand (Passioura, 2002; Kashiwagi *et al.*, 2006).

2.4 Suberin formation under abiotic stress

The Casparian band is a wall modification that appears electron-dense in a transmission electron microscope. This is due to the incrustation of lignin and, to a lesser extent, suberin into the framework of the primary wall (Bonnett, 1968). The plasma membrane tightly adheres to the wall so that, upon treatment with a hypertonic solution, band plasmolysis occurs in endodermal cells. In most plants, Casparian bands mature within 10 mm of the root tip. They rarely occupy more than 1/3–1/2 of the anticlinal walls. Because endodermal Casparian bands are such a consistent feature of roots, they can be expected to have at least one essential function. This is generally understood to be prevention of the apoplastic passage of ions from the cortex to the stele (Robards *et al*., 1973).

The evidence that Casparian bands are, in fact, effective barriers to apoplastic ion movement is widespread and convincing, beginning with the classic work of de Rufz de Lavison in 1910. Since then, the endodermal Casparian band has been shown to block the free apoplastic passage of various ions, heavy metals, and fluorescent dyes (Nagahashi *et al.,* 1974; Singh and Jacobson, 1977). The consequence of this blockage is that in a healthy root any ion not in the symplast is prevented from entering the root stele and, thus, the shoot system. The proteins (pumps, carriers, and channels) in the plasmalemmae of the root epidermal and cortical cells are the arbiters of which ions will enter the symplast and move into the stele (Moon *et al.,* 1986; Peterson *et al.,* 1981; Peterson, 1987).

The second (and much less studied) control point for determining which ions are delivered to the shoot is the movement from the symplast to the apoplast in the stele. This step is a critical part of the sequence that keeps the concentration of ions low in the root core symplast, setting up the gradient that favors centripetal movement by diffusion through plasmodesmata from the epidermal and cortical cell cytoplasms (Schreiber *et al.,* 1994; Schreiber, 1996). Once the ions have been released into the cell walls of the endodermis, pericycle and stelar parenchyma, they are free to diffuse into the lumena of the tracheary elements (tracheids or vessel members) and so be carried to the shoot in the transpiration stream. It is assumed that diffusion into the tracheary elements occurs mainly by way of the (nonlignified) pits (Cholewa, 2000; Bucking *et al.,* 2002).

2.4.1 Suberin lamelle

Plants have developed various strategies and mechanisms to withstand different environmental stresses. Both anatomical and physiological adaptations aid growth under unfavourable conditions (Perumalla and Peterson, 1986). The root system is particularly affected by abiotic stresses because it is in direct contact with the soil environment. Proper performance of a root relies on Casparian bands and suberin lamellae present in the root endo- and exodermis (Reinhardt and Rost, 1995). Casparian bands, deposited in anticlinal walls, are characterized by the impregnation of the primary wall pores with lignin and suberin (Schreiber *et al.,* 1999). Suberin lamellae are deposited on the inner face of the primary cell walls as secondary wall modifications.

A major function of the Casparian band is to block the non-selective apoplastic bypass flow of water and ions into the stele (Ma and Peterson 2003). Suberin lamellae, completely covering the primary cell walls, impede the passage of water and ions through the plasma membrane into the root stele. It may be noted that Casparian bands are not totally impermeable to water, or even to ions (Ranathunge *et al.,* 2005). Not much work has been done to understand the deposition and function of these apoplastic barriers in response to salt stress in rice.

Attempts have been made to understand the structure and chemical composition of these specialized cell walls in other plants. In a comparative study of rice with corn, authors have shown that rice exhibits lower apoplastic water permeability than corn roots which correlated with multi-fold greater amounts of suberin in rice compared to corn (Schreiber *et al.,* 2005). Only a few attempts have been made to understand how the chemical composition of apoplastic barriers changes in response to environmental stresses. It was shown that castor plants (*Ricinus communis* L.) reinforce their apoplastic transport barriers in roots in response to NaCl stress (Schreiber *et al.* 2005), while development of Casparian bands was observed in response to salinity in maize and cotton. It has also been shown that the barrier to radial oxygen loss and to Fe^{2+} uptake in rice is increased by exposure of the roots to organic acids and sulphide (Armstrong and Armstrong, 2001). Not much is known about the response of apoplastic barriers to various stresses in rice.

2.4.2 Apoplastic bypass flow

Since bypass flow is reported to be the major pathway of Na+ entry into the shoots in rice (Yeo *et al.,* 1988), characterization of the apoplastic forming barriers in the roots assumes additional importance. When grown in soil, most plants have been shown to develop enhanced Casparian bands which may play an important role in regulating the uptake of water and nutrient ions (Perumalla *et al.,* 1990) but the chemical characterization and a detailed study comparing hydroponically grown and soil-grown plants is lacking in any crop plant.

Suberin is a biopolymer consisting of an aliphatic and an aromatic domain with the aliphatic component being the major contributor to its barrier properties (Schreiber *et al.,* 1999). However, their expression and regulation under stress conditions have not been extensively characterized.

2.5 Xylem sap evaluation of stressed plants

Salt tolerance in plants is physiologically and genetically complex resulting in a wide range of adaptations in different plants. Among crop plants the yield potential of rice has been found to be especially sensitive to soil salinity. The observed intraspecific variations in salt sensitivity among rice varieties could result both from known adaptations at the whole plant level (Yeo and Flowers, 1982) and from variations in less well-understood mechanisms at the cellular level. Indeed, tolerance to relatively high shoot NaCl in the tolerant cultivar, Pokkali and in rice calli adapted to salt (Flowers *et al.,* 1985) suggests that rice cells are capable of mounting a salt-tolerance response at the cellular level.

High salinity disturbs intracellular ion homeostasis, leading to membrane damage, metabolic inactivation and secondary effects that ultimately result in cell death. Sodium toxicity is primarily a cytosolic event but cellular adaptive responses to salt stress in plants are complex and remain poorly understood. Testing the hypothesis that cytosolic NaCl is a determinant of cell survival requires direct, non-invasive measurements of ion activities in the cytosol of living plant cells, which are technically challenging. However, a role for ion transport systems has been established by other approaches such as mutational analysis (Zhu, 2002), salt-induced enhancement of activity/expression and overexpression studies of genes/ proteins that could be involved in plant salt-tolerance responses (Flowers, 1972).

III MATERIAL AND METHODS

The details of the materials used, methods adopted and statistical tools used for analysis, in the different experiments are presented in objective wise.

1. Quantifying the difference in the extent of suberization under aerobic, drought and salinity conditions using contrasting genotypes.
2. Differences in biochemical composition of sap exuded from the varieties at the peak vegetative stage on subjected to stress.

3.1 Experiment I

Quantifying the difference in the extent of suberization under aerobic, drought and salinity conditions using contrasting genotypes.

3.1.1 Field Experiment – March 2016

3.1.1.1 Plant Material

The materials used for the study was rice accessions and land races known for their salinity tolerance and drought resistance. The genotypes represent subspecies of *Oryza sativa* (Table 1).

Table 1: Rice genotypes used for the root study

Sl. No.	Genotype	Sub species	Origin	History
1	ARB 6	Indica	India	Improved
2	Pokkali	Indica	India	Traditional
3	Jaya	Indica	India	Improved
4	IR-20	Indica	IRRI	Improved
5	Kalanamak	Indica	India	Traditional

3.1.1.2 Experimental Site

The experiments were carried out during the month of March 2016 at aerobic rice field of Department of Plant Biotechnology, University of Agricultural Sciences, GKVK campus, Bangalore, India located at the latitude of 12^0 58' North; longitude 77^0 35' East and altitude of 930 meters above mean sea level. The annual rainfall ranges from 679.1 mm to 888.9 mm. Climatic conditions at the experimental site during the corresponding months are presented in Figure 1. Physico- chemical properties of soil at experimental site are presented in Table 2.

Table 2: Soil properties of experimental site used for root study

Parameters	Value
Particle size distribution	
Sand (%)	78.0
Silt (%)	8.8
Clay (%)	13.2
Textural class	Sandy loam
pH	6.1
Electrical conductivity (dS m^{-1})	0.22
Organic carbon (g Kg^{-1})	7.4
Cation exchange capacity (cmol Kg^{-1})	9.1

3.1.1.3 Experimental method

Selected genotypes were grown in PVC pipes with dimensions of 7.5 cm diameter and 60 cm length. The experiments were laid out in two factor randomized complete block design (RCBD) with 4 replications at the aerobic rice field. Two sets of pipes were laid, one for saline condition and another for drought condition. Pipes were placed in pits and strengthened with soil around it. The pipes were filled with top surface soil and FYM in the ratio of 4:1. Fertilizers were not applied; vermi-compost was used at the time of pipe filling. Seeds were directly seeded in each pipes and after the germination only one seedling was allowed to grow. The plants were well watered upto 30 days of growth.

From the 31st day onwards treatment started. Saline condition plants were treated with sodium chloride (NaCl) solutions of 100 mM, 150 mM and 200 mM concentrations for the next 7 days continuously. Salt treatment was done at morning time and at evening time controlled irrigation with pure water was done for the treated pipes. Drought condition plants were of 3 sets and water withholding was done for 3 days, 5 days and 7 days respectively from the 31st day onwards. Control plants were maintained for each of the conditions used and treated with regular supply of pure water.

3.1.1.4 Method of root sampling and measurement

Pipes at the pits were carefully removed and soaked in water overnight to loosen the soil. The next day, roots were cleaned thoroughly and carefully using a sieve and fine jet of water. The cleaned roots were collected for recording observations and further analysis.

Plate 1: Experimental layout of pipes for the study

Plate 2: Plants in pipes soon after transplanting

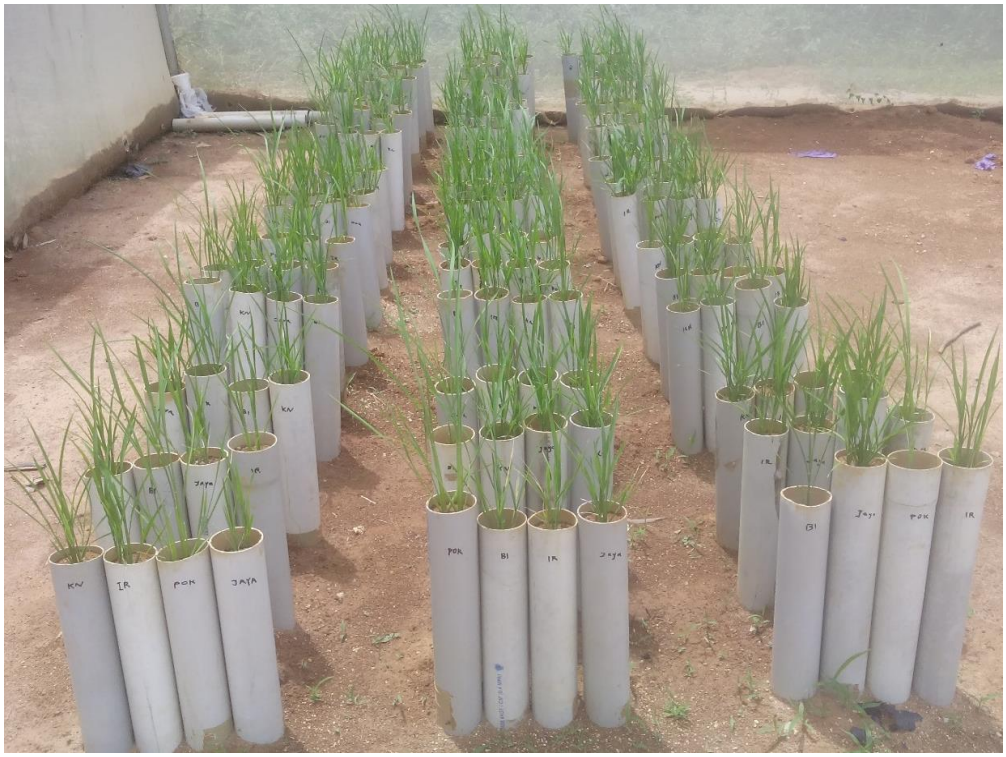


Plate 3: Plants of 30 days old in pipes just before the treatments

Plate 4: Plants after 7 days of treatments

3.1.1.5 Processing of collected roots

Before processing root length and root number were recorded. Processing of root was done in a tray of water. Individual roots were separated, lateral roots and fine root hairs were removed. A fine brush was used to remove the attached dirt from the roots. Healthy roots were selected and separated to another tray with distilled water. Cleaned and processed roots were divided into upper, middle and lower portions and separately stored in falcon tubes with distilled water.

3.1.1.6 Root slicing for microscopy

Stored roots were transferred to a petriplate with distilled water, root hairs were removed and cut into 2 cm pieces for convenience. Roots were chopped into 200µm pieces by Mc ILWAIN Tissue Chopper (The Mickle Laboratory Engineering Co. Ltd, UK). Chopped tissues were collected in distilled water with a 0 size camel paint brush. It was stored in 15 ml falcon tubes and used for microscopic studies.

3.1.1.7 Tissue staining and microscopy

Chopped tissues were spread on a thin glass slide, excess water was removed by an insulin syringe. Tissues were stained with 2 % phloroglucinol for an hour. Tissues were washed multiple times to remove excess of stain. A drop of 50 % glycerol is added to tissues and cover slip is placed on it. Prepared tissues were observed Olympus BX51 microscope (Olympus life sciences, Japan) in brightfield and clear images were taken using camera (DP70 Digital *Camera* System, Olympus life sciences, Japan).

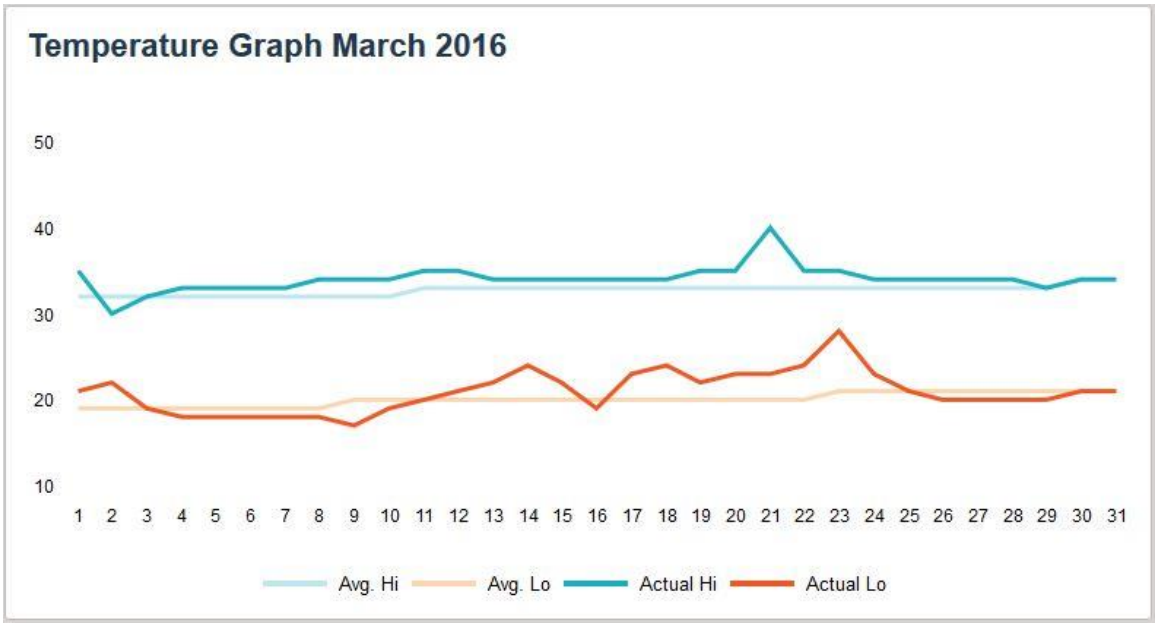


Fig. 1(a): Temperature variation at the month of March 2016

	Actual Temperature	Normal Temperature	Variation
High	34	33	
Low	21	20	
Average	27	26	+1
Precipitation	77 mm	3 mm	+74 mm
Snow	0 CM		

Fig. 1(b): Average weather parameters during March 2016

Fig. 1: Weather parameters at the experimental site during pipe experiment

3.2 Experiment II

Differences in biochemical composition of sap exuded from the varieties at the peak vegetative stage on subjected to stress.

3.2.1 Xylem sap collection

After 7 days of continuous salinity and drought treatment plants were prepared for xylem sap collection. For xylem sap collection shoots was cut off using razor blades at distance of 40–70 mm from their base. The cut portion was covered with pre-weighed blotting paper, a piece of plastic film was used to wrap over the blotting paper and fixed tightly with rubber band. The setup was kept undisturbed for overnight, next morning blotting paper was collected and change in weight was recorded.

3.2.2 Xylem sap extraction from blotting paper

Xylem sap absorbed blotting paper was soaked in 40 ml of double distilled water and gently rocked in a conical flask. The resulting solution was filtered and stored for flame photometric estimation of Sodium and Potassium ions.

3.2.3 Flame photometric evaluation of xylem sap

Systronics flame photometer 128 (Systronics, Ahmedabad, India) was used for the estimation of Sodium and Potassium in xylem sap samples. Flame photometer was switched on and calibrated using 100 ppm, 50ppm and 0 ppm of sodium and potassium solutions respectively. Samples were aspirated and reading in ppm (parts per million) was noted. After 15 samples reading machine was recalibrated for precision.

3.2.4 Observations recorded

3.2.4.1 Root length (cm)

Root length was measured from the base of the plant (collar region) to the tip of the longest root after treatment.

Plate 5: Pictorial representation of xylem sap collection from plants

Plate 6: Pictorial representation root washing

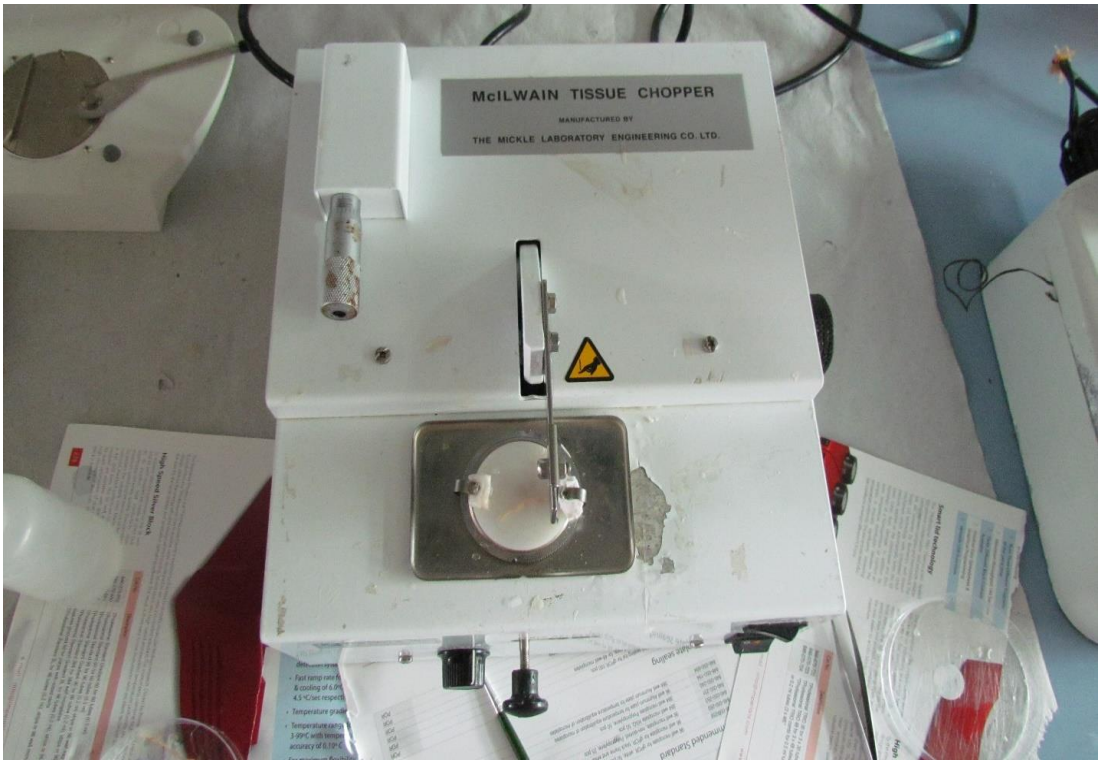


Plate 7: MCILWAIN tissue chopper

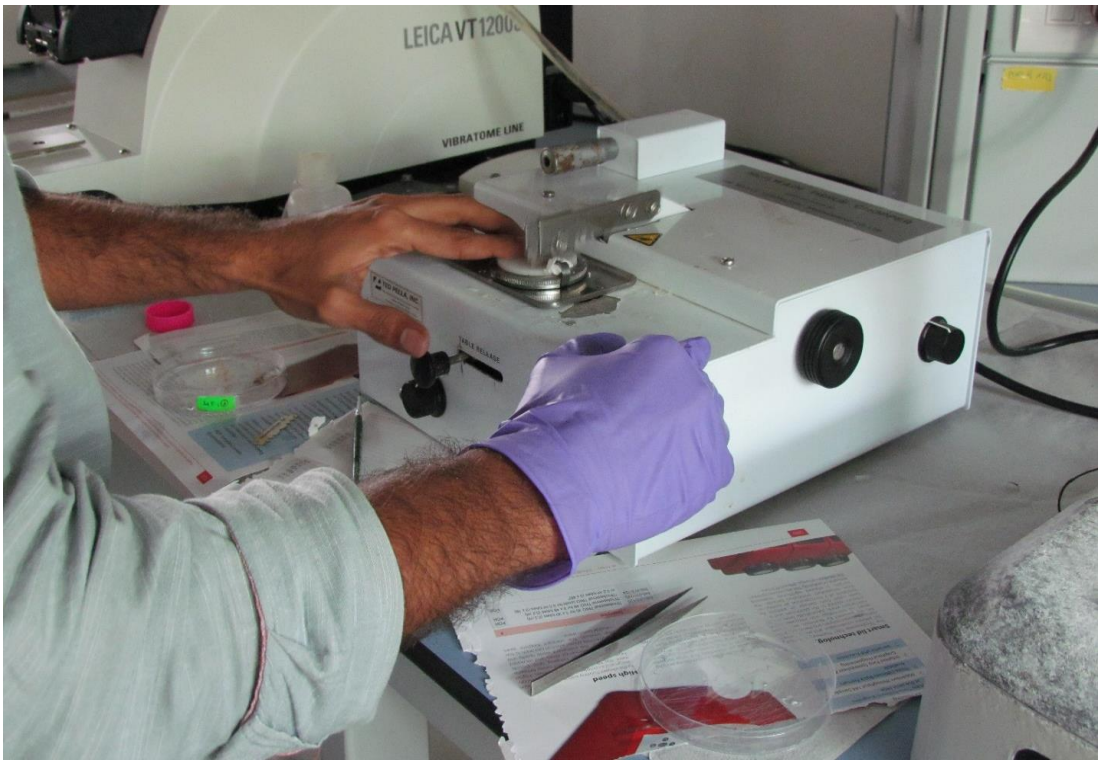


Plate 8: Chopping of plant roots for microscopy with tissue chopper

Plate 9: Xylem sap analysis of flame photometer for Na^+ and K^+

Plate 10: Microscopy of root section by Olympus BX 51

3.2.4.2 Root number

Number of roots per plant were counted at the base of the root system manually after the treatment.

3.2.4.3 Number of tillers

Number of tillers per plant was counted after the treatment.

3.2.4.4 Total number of leaves

Number of leaves in each plant was counted after the treatment.

3.2.4.5 Shoot length (cm)

Shoot length was measured for each plant after treatment.

3.2.4.6 Total xylem sap uptake (mg)

The weight difference in the xylem sap collected blotting paper was recorded as the amount of xylem sap uptake.

3.3 Statistical Analysis

The observations recorded in respect of all the above quantitative traits were subjected to the following standard statistical analysis.

3.3.1 Descriptive statistics

The following descriptive statistics were estimated as per Sunderaraj *et al.* (1972).

3.3.1.1 Mean

Mean is the sum of all observations in the sample divided by the number of observations.

3.3.1.2 Range

Range is the minimum and maximum values of the observations in the sample.

3.3.1.3 Standard error

It is measured of uncontrolled variation present in a sample which is estimated by dividing the standard deviation by the square root of number of observations in the sample and is denoted by SE.

3.3.1.4 Variance

Variance is defined as the average of squared deviations of individual observations from the mean or it is the square of the standard deviations. It is expressed as the sum of squares of the deviations of all observations of a sample from its mean and divided by (n-1), where n is the number of observations.

3.3.2 Correlation Studies

To estimate the degree of association between the traits studied, phenotypic correlation was computed by using the formula given by Webber and Moorthy (1952).

$$r_p = \frac{COV\ (X, Y)}{[V(X).\ V\ (Y)]^{1/2}}$$

Wherein,

r_p = phenotypic correlation co-efficient.
COV (X, Y) = Phenotypic covariance.
V(X) and V(Y) = Phenotypic variances of the traits X and Y

3.3.4 Analysis of Variance

The analysis of variance for different characters was carried out using mean data in order to assess the genetic variability among genotypes as given by Gomez and Gomez (1976). Analysis of variance was done by using software SAS version 9.4.

ANOVA for Two Factorial Randomized Complete Block Design (RCBD)

Source of variation	Degrees of freedom	Mean sum of squares	Expected M. S. S
Replication	(r-1)	SSR	MSR
Treatment	ab - 1	SST	MST
Genotype (A)	a - 1	SSA	MSA
Treatment (B)	b - 1	SSB	MSB
A x B	(a-1)(b-1)	SSAB	MSAB
Error	(r-1)(ab-1)	SSE	MSE
Total	rab - 1	SSTD	

Where,

r= number of replications
a= number of treatments (genotypes)
b= number of treatments given

IV EXPERIMENTAL RESULTS

The results obtained from the present investigations are presented under the following subheadings:

4.1 Genotypic variations of root traits under drought and salinity stress

Variations observed in the root characters are described below.

4.1.1 Root Length (RL, cm)

Comparing the means of RL across the genotype ARB 6 possessed the maximum root length (50.5) and Jaya possessed the least RL (39.5) under the control condition. In the drought condition also ARB 6 maintains the maximum root length (56.72) with a considerable increase compared to the control condition. Although Pokkali is a saline tolerant variety it showed considerable increase in RL (51.52) compared to its control (48.62). Jaya and IR 20 show similar RL s in drought stress, showing a slight increase from control. Kalanamak showed the least RL (34.89), it exhibited a considerable decrease in RL compared to control (42.1).

Pokkali with a RL of 46.96 maintained lengthiest root in salinity stress although a slight decrease from control condition (48.62) was observed. Considerable decrease in RL was observed for Jaya which possess least RL (33.34) compared to its control (39.5). Kalanamak (46.42) followed by ARB 6 (44.14) and IR 20 (41.5) comes in between.

4.1.2 Root Number (RN, cm)

Jaya is the leader in RN with a mean value of 53.75 followed by ARB 6 (47), Pokkali (44.25), Kalanamak (38.75) and least RN is observed for IR 20 (25) in the control condition. RN increased slightly for ARB 6 (49.5) and it leads in the drought condition followed by Pokkali (46), Jaya (45.33), Kalanamak (26.83) and IR 20 (19.58). RN drastically decreased for Jaya, considerable decrease was also observed in Kalanamak and IR 20. Interestingly Pokkali showed increase in root number in drought condition.

Pokkali maintained higher RN (51.58) in salinity stress followed by Kalanamak (45.16), Jaya (40.5) and ARB 6 (33.41). IR 20 was having least RN (30.41), but recorded an increase compared to the control condition. RN of ARB 6 and Jaya considerably decreased from the control condition. In salinity condition it is observed that Pokkali, Kalanamak and IR 20 are exhibiting increased RN.

4.2 Genotypic variations of Shoot traits under drought and salinity stress

Variations observed in the shoot traits are described below.

4.2.1 Number of Tillers (NT)

In control condition Kalanamak possessed higher NT with the observed mean value of 3 followed by Jaya and ARB 6 (2.75), Pokkali (2.5) and IR 20 (2.25).

In drought stress NT increased considerably for Jaya (3.33) where as a slight increase in NT was observed in ARB 6, Pokkali, IR 20. Kalanamak showed a slight decrease in the mean NT.

In salinity stress NT increased for every genotype expect Kalanamak, which show no change in NT. Mean NT is highest for ARB 6 (3.33) in salinity followed by Pokkali (3.25), Kalanamak (3), Jaya (2.91) and lowest was observed for IR 20 (2.33).

4.2.2 Total Number of Leaves (TNL)

In control condition Pokkali and Jaya with mean TNL of 4 stand first followed by ARB 6 (3.75), Kalanamak and IR 20 (3.25).

In drought condition ARB 6 and Pokkali maintain a higher mean TNL of 4, followed by Kalanamak (3.58), Jaya (2.91) and least was observed for IR 20 (2.5).

ARB 6 (4.08) maintained the higher TNL in salinity stress followed by Pokkali and Jaya (3.58), Kalanamak (3.33). The least TNL in salinity stress was observed in IR 20.

4.2.3 Shoot Length (SL, cm)

All plants were healthy in control condition whereas excessive rolling of leaves was observed in plants under drought condition. Pokkali possessed longest shoot in both control (43) and drought (45.33) conditions followed by Kalanamak, ARB 6, Jaya and IR-20. SL of ARB 6, Kalanamak and IR 20 decreased with drought. IR 20 (26.33) possessed the shortest SL in drought stress.

Shoots of the plants started to die in ARB 6, IR-20 and Jaya where as in Pokkali and Kalanamak shoot was in better condition when subjected to salt stress. In saline stress also Pokkali maintained the highest SL of 38.5, although a considerable decrease was observed from that of control. Least SL of 20.75 was observed in Jaya, where in the control SL was 26.25.

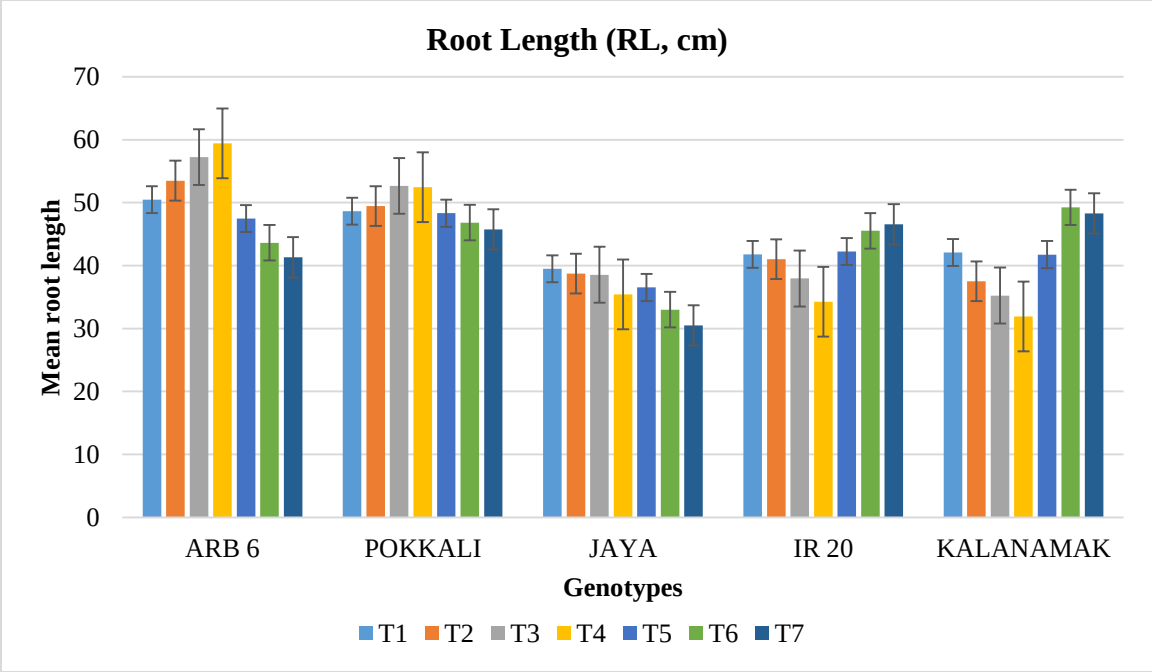
4.3 Genotypic variations of Passage Cell Number (PCN)

Passage cell number was found to having more variation among the genotypes.

4.3.1 Passage Cell Number-Endodermis (PCN-En)

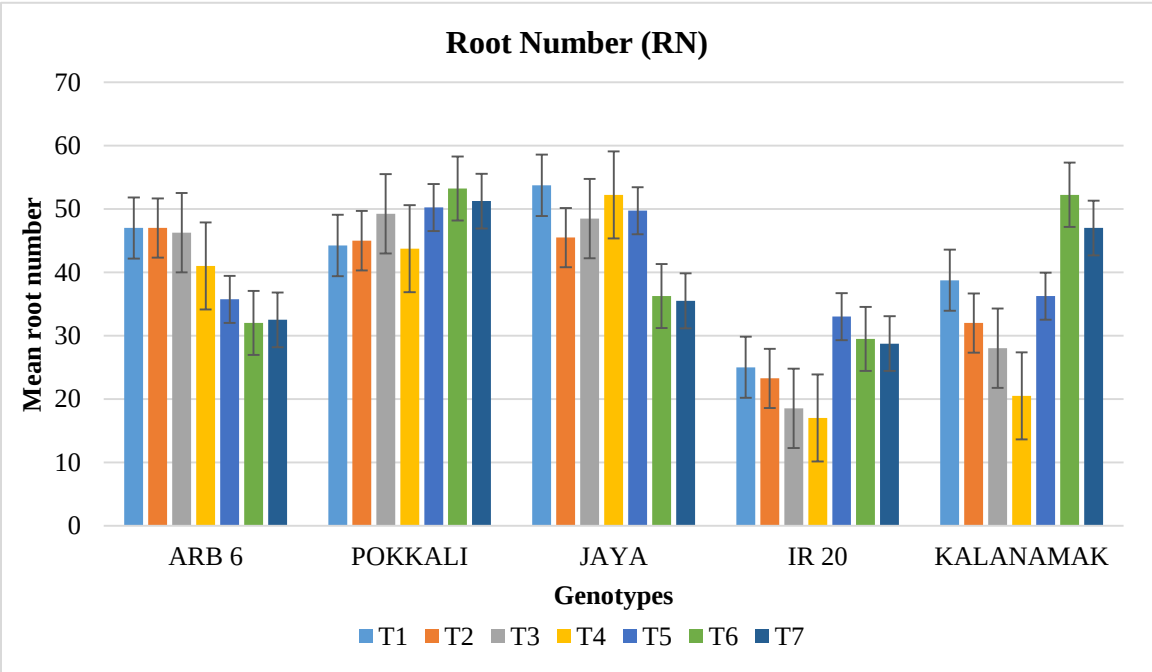
The result of PCN shows that in control condition maximum PCN was observed in ARB 6 followed by Pokkali, Kalanamak Jaya and IR 20 at the endodermal walls.

In drought stress PCN in endodermis drastically increased for ARB 6 and Pokkali with a mean value of 14.5 and 11.91 compared to 5.5 and 4.75 in control condition. PCN for Kalanamak also increased from 4 to 7.16 in the drought condition. A slight increase in PCN was observed in IR 20, whereas Jaya did not show any change.



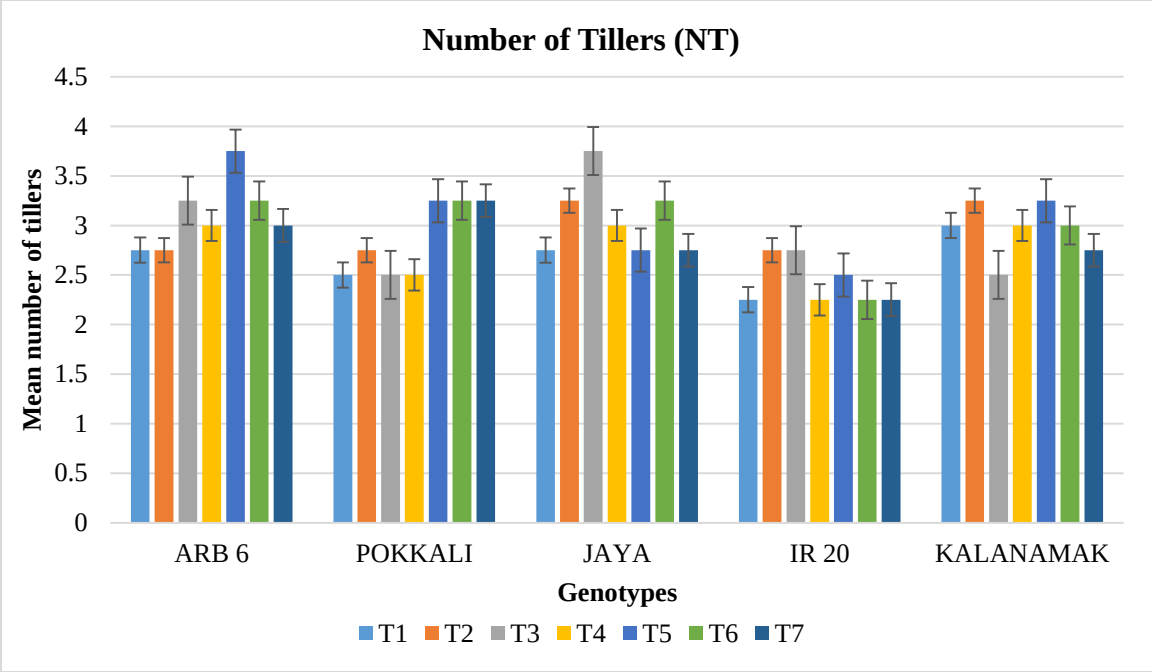
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 2: Mean value for Root Length of genotypes observed after treatment



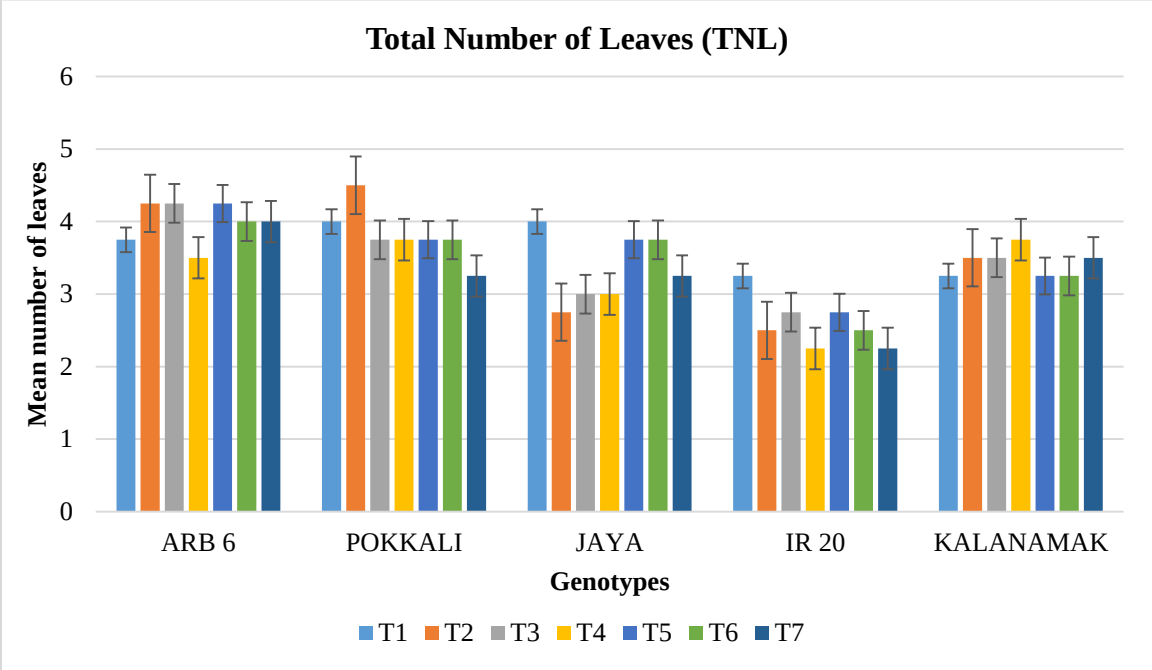
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 3: Mean value for Root Number of genotypes observed after treatment



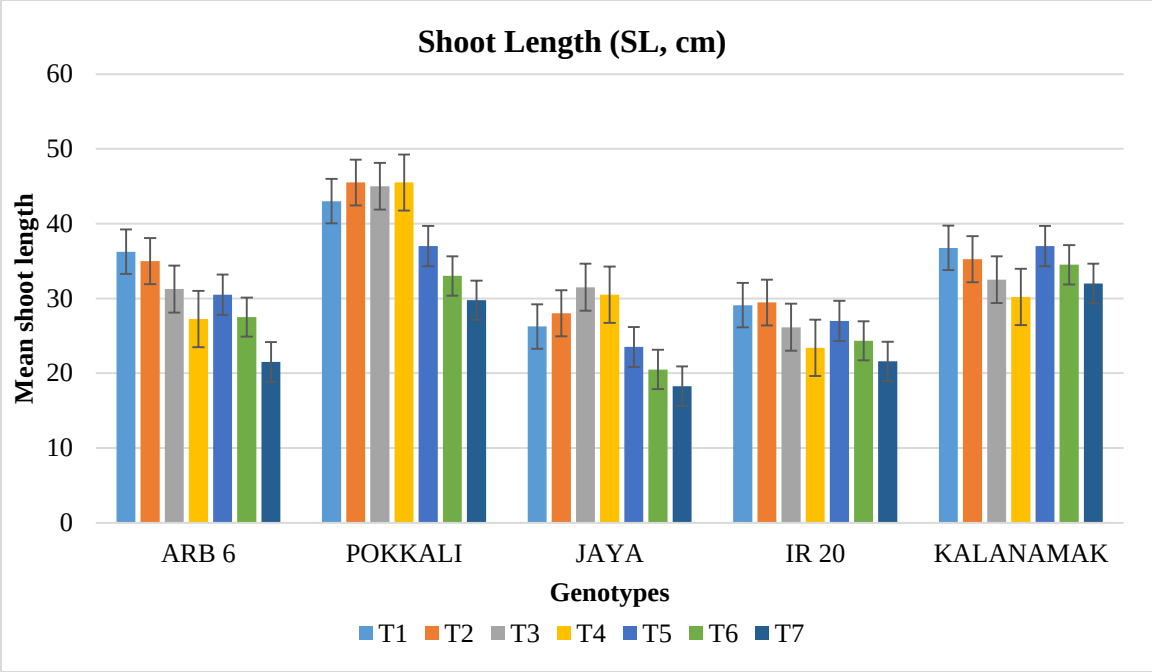
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 4: Mean value for Number of Tillers for genotypes observed after treatment



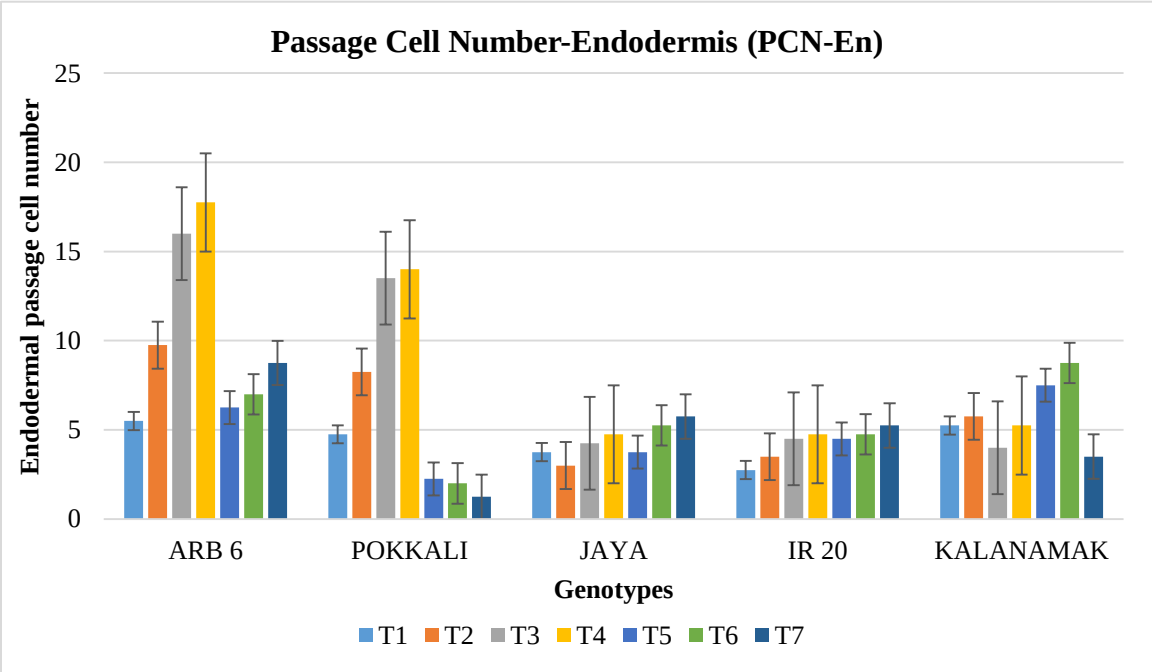
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 5: Mean value for Total Number of Leaves in genotypes observed after treatment



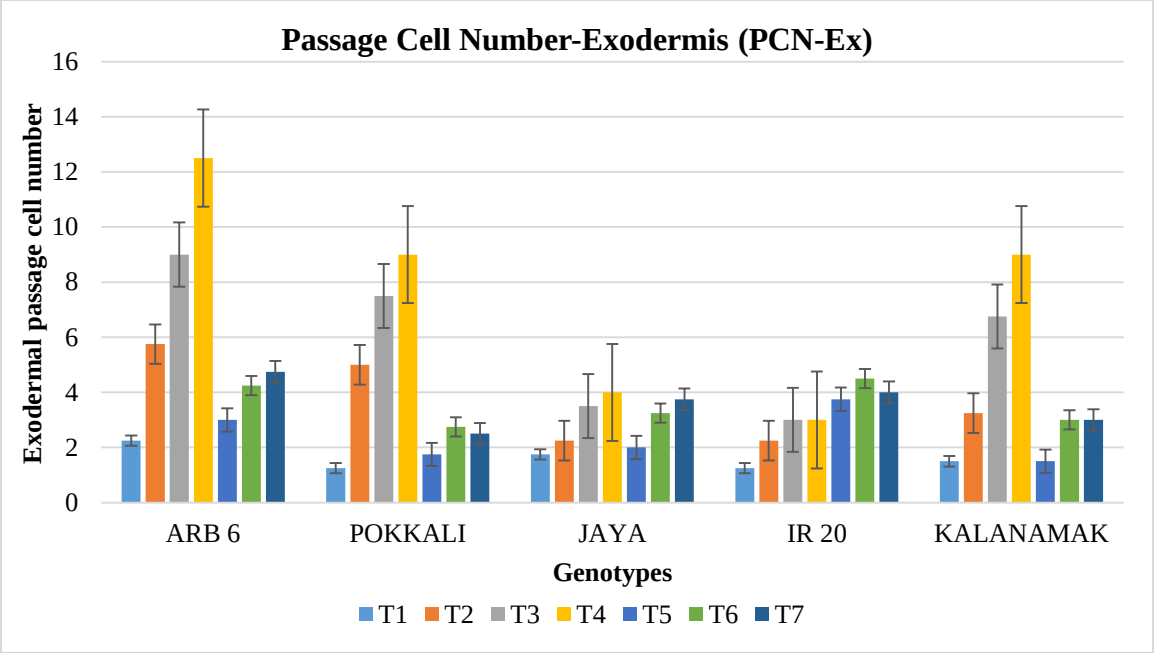
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 6: Mean value for Shoot Length of genotypes observed after treatment



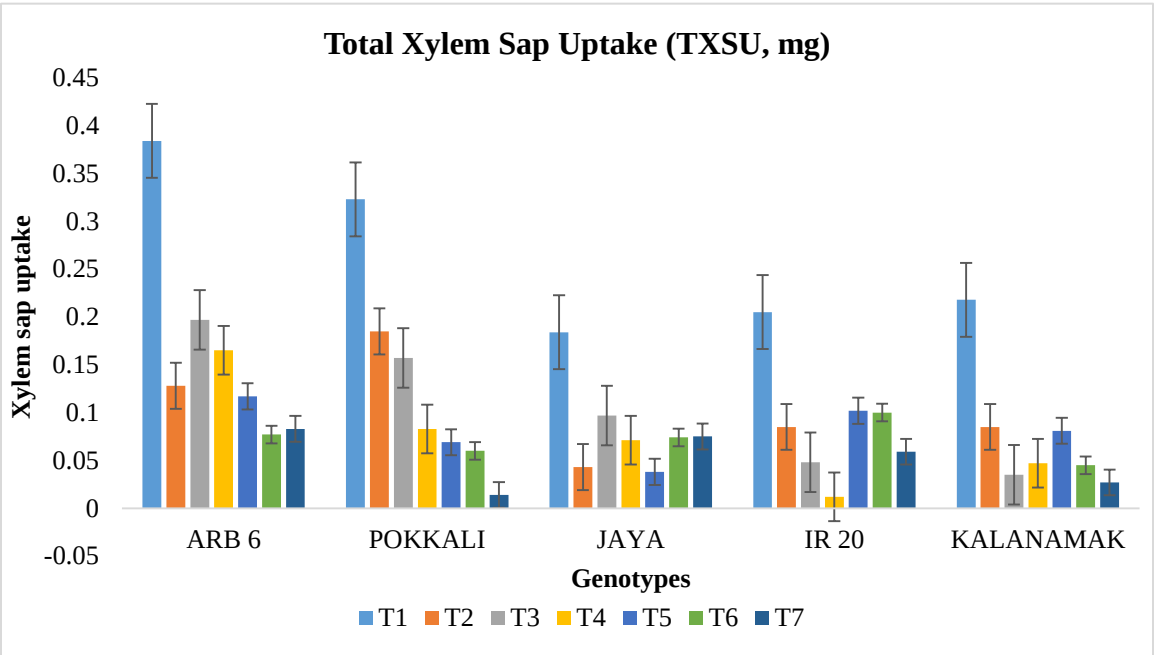
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 7: Mean value for Passage Cell Number-Endodermis in genotypes observed after treatment



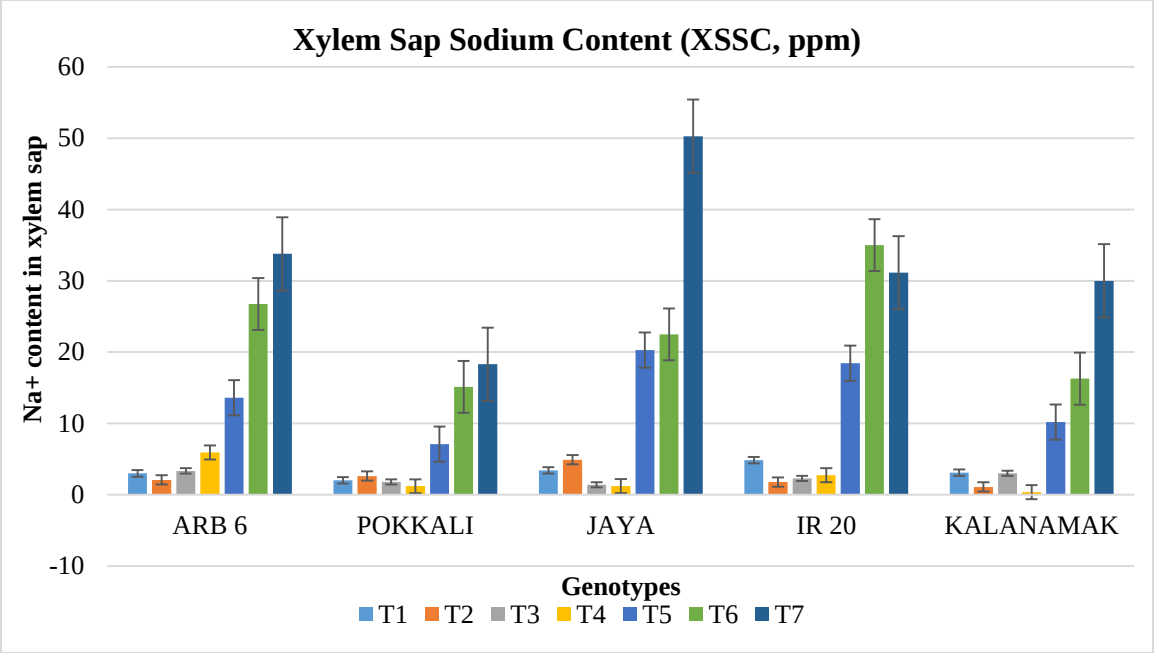
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 8: Mean value for Passage Cell Number-Exodermis in genotypes observed after treatment



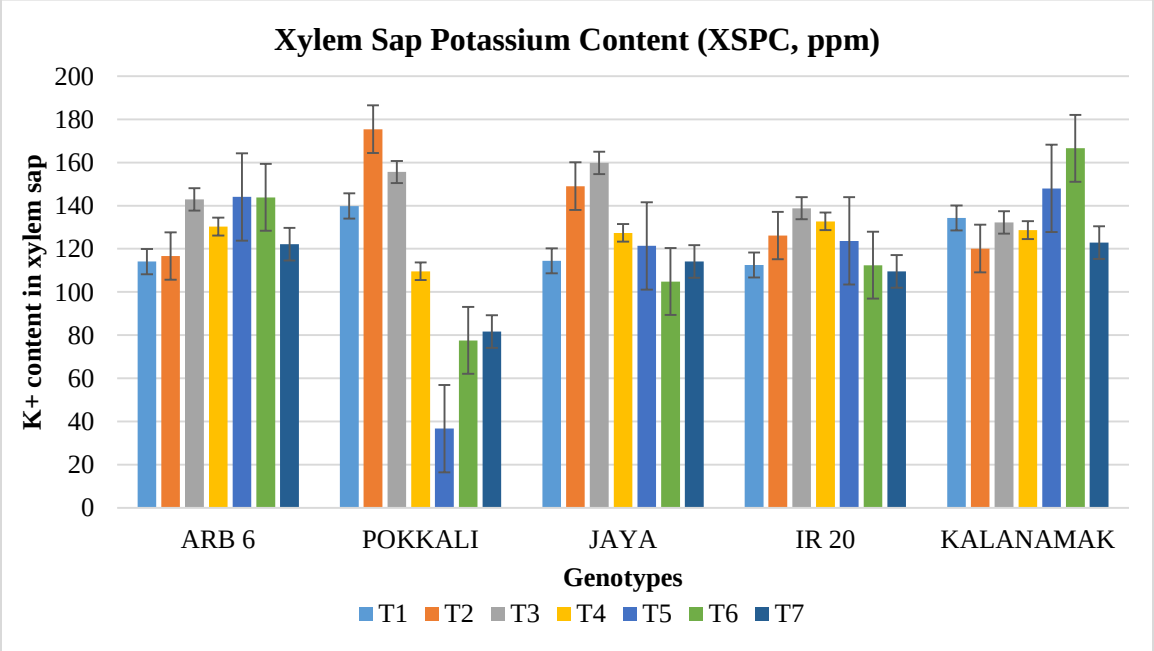
T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 9: Mean value for Total Xylem Sap Uptake in genotypes observed after treatment



T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 10: Mean value for Xylem Sap Sodium Content in genotypes observed after treatment



T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]
T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Fig. 11: Mean value for Xylem Sap Potassium Content in genotypes observed after treatment

In salinity stress also ARB 6 possessed high PCN, where as in the case of Pokkali PCN decreased dramatically. In Pokkali PCN decreased to 1.88 from 4.75 of control condition. Kalanamak also show decreased PCN compared to control condition. Considerable changes were not observed in Jaya and IR 20.

4.3.2 Passage Cell Number-Exodermis (PCN-Ex)

Exodermal PCN is less compared to endodermal, in exodermis also ARB 6 have more PCN.

In drought stress considerable increase in PCN was observed in all genotypes. ARB 6 leads in the PCN in drought condition followed by Pokkali, Kalanamak, Jaya and IR 20.

In saline stress ARB 6 and IR 20 possessed the higher PCN followed by Jaya, Kalanamak and Pokkali have the least PCN in exodermis.

4.4 Genotypic variation in xylem sap amount and content

4.4.1 Total Xylem Sap Uptake (TXSU, mg)

TXSU was considerably higher in the case of ARB 6 followed by Pokkali, Kalanamak, IR 20 and Jaya.

In drought stress also ARB 6 had high xylem sap uptake, but considerable reduction was their compared to control condition. All other genotypes follow same pattern as they all had reduced xylem sap uptake at drought. The least was IR 20 with 0.048 compared to 0.205 in control condition.

In salinity stress ARB 6 uptake more xylem sap and Pokkali was noted with the least xylem sap uptake. Considerable reduction in TXSU was observed in salinity stress. Kalanamak also showed a close range of TXSU with that of Pokkali.

4.4.2 Xylem Sap Sodium Content (XSSC, ppm)

In control condition IR 20 showed higher Na^+ content in xylem sap followed by Jaya, Kalanamak, Pokkali and ARB 6.

In drought stress Na^+ content was higher for the ARB 6 xylem sap and least Na^+ content was observed in the xylem sap of Pokkali. Kalanamak show similarity with Pokkali in the Na^+ in xylem sap.

In saline stress Jaya had higher Na^+ content in xylem sap followed by IR20, ARB 6, Kalanamak and Pokkali. Pokkali showed the least Na^+ content in xylem sap.

4.4.3 Xylem Sap Potassium Content (XSPC)

In control condition K^+ content was more in xylem sap of Pokkali followed by Kalanamak, Jaya, ARB 6 and IR 20.

In drought stress Pokkali had more K^+ content and Kalanamak had the least. K+ content of Pokkali, Jaya, IR 20 increased in drought stress compared to control condition.

In salinity stress Kalanamak showed high K^+ content and Pokkali showed least. Other genotypes did not show much variation from the control condition.

4.5 Analysis of variance for observed quantitative traits

The mean sum of squares for variation in 9 morpho-anatomical traits of 5 rice genotypes are presented in the Table 3. Highly significant differences were observed in the genotype x treatment factor mean sum of squares across the varieties for traits like RL, SN, NT, TNL, PCN, XSSC, TXSU reflecting the presence of variability for these traits, but XSPC was not found to be significant. Genotypic factor mean sum of squares are showing significance for traits like RL, SN, NT, TNL, PCN, XSSC, TXSU, here also XSPC was not found to be significant. Mean sum of squares for seven treatments applied were found to be significant for RL, SL, RN, PCN, XSSC and TXSU, but son significant to the NT, TNL and XSPC. Mean sum of squares for genotype x treatment interaction was found to be significant for traits like RL, SN, RN, TNL, PCN, XSSC and TXSU, but non-significant for NT and XSPC.

4.6 Phenotypic correlations study for traits observed after treatments

An attempt was made to understand the phenotypic correlation co-efficient of different quantitative traits in the genotypes studied during the experiment. The results of correlation analysis are presented in Table 4, 5, 6, 7, 8, 9 and 10.

4.6.1 Root Length

Root Length significantly correlated positively with Total Xylem Sap Uptake (0.995) at 1 % value in well-watered control (T1) condition. No other traits were significant with the Root Length for this condition. In 3 days of water withholding condition (T2) root length was found to be significant positively with the passage cell number (0.914) at 5 % error value. In T2 no other correlations were significant with the root length. A significant positive correlation was observed for root length with Total Xylem Sap Uptake (0.965) at 1 % error value in 5 days of water withholding condition (T3). In T3 Passage Cell Number (0.896) also correlated significantly with Root Length at 5 % error value. Root length showed no significant correlation with any other characters in 7 days of water withholding (T4). In every saline condition (100 mM, 150mM, 200Mm) Root Length was not correlated significantly with any other traits.

4.6.2 Shoot Length

In well-watered condition shoot was not significantly correlated to any other traits. In 3 days of water withholding Shoot Length was found to be significant positively at 5 % error value with Total Number of Leaves (0.885) and Total Xylem Sap Uptake (0.934). In 5 days of water withholding condition no significant correlation was found for Shoot Length with other traits. In 7 days of water withholding treatment Shoot Length was significantly correlated negatively with the Xylem Sap Potassium Content (-0.99).

Table 3: ANOVA for root, shoot and extent of suberization in the selected genotypes

Source of Variation	df	Mean sum of square								
		RL	**SL**	**RN**	**NT**	**TNL**	**PCN**	**XSSC**	**XSPC**	**TXSU**
Replication	3	4.25	2.30	16.86	0.32	0.24	9.78**	40.27*	25.41	0.002
Treatment	34	209.65**	195.49**	465.49**	0.62**	1.40**	165.41**	881.93**	2684.43	0.028**
Genotype (A)	4	1036.24**	1019.57**	2350.4**	2.10**	7.94**	369.60**	537.97**	2552.63	0.039**
Treatment (B)	6	12.93**	260.89**	106.60**	0.50	0.51	400.07**	3992.76**	3127.95	0.101**
A * B	24	121.07**	41.8**	241.06**	0.41	0.54*	72.72**	161.55**	2595.52	0.007*
Error	102	3.87	1.34	27.78	0.33	0.31	1.97	12.92	938.59	0.004
CD @ 1 %		0.53	0.31	1.42	0.16	0.15	0.38	0.97	8.25	0.017
CD @ 5 %		0.70	0.41	1.88	0.21	0.20	0.50	1.28	10.92	0.022
CV		4.50	3.71	13.47	20.02	16.31	13.90	29.56	24.43	56.94

**: Significant at 1 %
*: Significant at 5 %

RL: Root Length
SL: Shoot Length
RN: Root Number
NT: Number of Tillers
TNL: Total Number of Leaves
PCN: Passage Cell Number
XSSC: Xylem Sap Sodium Content
XSPC: Xylem Sap Potassium Content
TSXU: Total Xylem Sap Uptake

Table 4: Phenotypic correlation co-efficient for root anatomical characters and general traits in well-watered condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.738	0.188	-0.036	0.333	0.783	-0.578	0.214	0.995**
SL	1	0.064	0.147	0.157	0.474	-0.787	0.809	0.695
RN		1	0.568	0.824	0.656	-0.648	0.061	0.252
NT			1	.029	0.489	-0.443	0.261	0.027
TNL				1	0.490	-0.628	0.086	0.355
PCN					1	-0.657	0.058	0.837
XSSC						1	-0.724	-0.577
XSPC							1	0.160

**: Correlation is significant at 1 %.

Table 5: Phenotypic correlation co-efficient for root anatomical characters and general traits in 3 days of water withholding condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.560	0.572	-0.758	0.778	0.914*	-0.111	0.120	0.760
SL	1	0.371	-0.400	0.885*	0.729	-0.295	0.549	0.934*
RN		1	0.017	0.618	0.573	0.558	0.422	0.323
NT			1	-0.387	-0.556	0.315	-0.107	-0.698
TNL				1	0.943*	-0.252	0.286	0.863
PCN					1	-0.311	0.062	0.814
XSSC						1	0.511	-0.339
XSPC							1	0.456
TXSU								1

*: Correlation is significant at 5 %.

RL: Root Length SL: Shoot Length RN: Root Number
NT: Number of Tillers TNL: Total Number of Leaves PCN: Passage Cell Number
XSSC: Xylem Sap Sodium Content
XSPC: Xylem Sap Potassium Content
TSXU: Total Xylem Sap Uptake

Table 6: Phenotypic correlation co-efficient for root anatomical characters and general traits in 5 days of water withholding condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.489	0.636	0.035	0.832	0.896*	0.262	0.272	0.965**
SL	1	0.630	-0.355	0.468	0.542	-0.300	0.485	0.469
RN		1	0.468	0.550	0.519	-0.297	0.780	0.787
NT			1	-0.106	-0.216	-0.282	0.536	0.271
TNL				1	0.977**	0.582	-0.054	0.776
PCN					1	0.526	-0.026	0.810
XSSC						1	-0.806	0.115
XSPC							1	0.460
TXSU								1

*: Correlation is significant at 5 %.
**: Correlation is significant at 1 %.

Table 7: Phenotypic correlation co-efficient for root anatomical characters and general traits in 7 days of water withholding condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.370	0.482	0.087	0.442	0.868	0.077	-0.395	0.870
SL	1	0.480	-0.088	0.636	0.342	-0.461	-0.99**	0.151
RN		1	0.414	0.314	0.248	-0.241	-0.446	0.595
NT			1	0.555	0.338	-0.700	0.216	0.547
TNL				1	0.740	-0.838	-0.547	0.530
PCN					1	-0.290	-0.318	0.846
XSSC						1	0.335	-0.172
XSPC							1	-0.118
TXSU								1

**: Correlation is significant at 1 %.
RL: Root Length SL: Shoot Length RN: Root Number
NT: Number of Tillers TNL: Total Number of Leaves PCN: Passage Cell Number
XSSC: Xylem Sap Sodium Content
XSPC: Xylem Sap Potassium Content
TSXU: Total Xylem Sap Uptake

Table 8: Phenotypic correlation co-efficient for root anatomical characters and general traits in 100 mM NaCl treatment condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.642	-0.115	0.670	0.358	0.102	-0.736	-0.433	0.607
SL	1	0.003	0.563	0.075	-0.514	-0.978**	-0.351	0.184
RN		1	-0.062	0.409	-0.692	-0.132	-0.697	-0.834
NT			1	0.765	0.093	-0.627	0.053	0.401
TNL				1	0.064	-0.224	-0.106	-0.041
PCN					1	0.510	0.590	0.732
XSSC						1	0.482	-0.158
XSPC							1	0.346
TXSU								1

**: Correlation is significant at 1 %.

Table 9: Phenotypic correlation co-efficient for root anatomical characters and general traits in 150 mM NaCl treatment condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.845	0.466	-0.299	-0.344	-0.348	-0.175	0.318	-0.317
SL	1	0.826	0.209	0.093	-0.622	-0.667	0.258	-0.750
RN		1	0.421	0.221	-0.904*	-0.926*	-0.032	-0.894*
NT			1	0.967**	-0.187	-0.720	-0.046	-0.627
TNL				1	0.013	-0.541	-0.076	-0.439
PCN					1	0.765	0.368	0.642
XSSC						1	0.024	0.943*
XSPC							1	-0.303
TXSU								1

**: Correlation is significant at 1 %.
*: Correlation is significant at 5 %.
RL: Root Length SL: Shoot Length RN: Root Number
NT: Number of Tillers TNL: Total Number of Leaves PCN: Passage Cell Number
XSSC: Xylem Sap Sodium Content
XSPC: Xylem Sap Potassium Content
TSXU: Total Xylem Sap Uptake

Table 10: Phenotypic correlation co-efficient for root anatomical characters and general traits in 200 mM NaCl treatment condition for the selected genotypes

	SL	RN	NT	TNL	PCN	XSSC	XSPC	TXSU
RL	0.850	0.345	-0.068	-0.239	-0.460	-0.342	-0.177	-0.650
SL	1	0.766	0.344	0.067	-0.715	-0.725	-0.273	-0.858
RN		1	0.671	0.172	-0.861	-0.851	-0.512	-0.873
NT			1	0.599	-0.246	-0.894*	-0.438	-0.320
TNL				1	0.320	-0.514	0.456	0.259
PCN					1	0.501	0.587	0.962**
XSSC						1	0.398	0.621
XSPC							1	0.607
TXSU								1

**: Correlation is significant at 1 %.
*: Correlation is significant at 5 %.
RL: Root Length SL: Shoot Length RN: Root Number
NT: Number of Tillers TNL: Total Number of Leaves PCN: Passage Cell Number
XSSC: Xylem Sap Sodium Content
XSPC: Xylem Sap Potassium Content
TSXU: Total Xylem Sap Uptake

In 100 mM NaCl treatment Shoot Length showed significant negative correlation to Xylem Sap Sodium Content (-0.978), in further treatments no significant correlation was found for Shoot Length.

4.6.3 Root Number

Root Number show no significant correlation in well-watered control, 3, 5 and 7 days of water withholding, 100 and 200 mM of NaCl treatments. It showed significant negative correlation with Passage Cell Number (-0.904), Xylem Sap Sodium Content (-0.926) and Total Xylem Sap Uptake (-0.894) in 150 mM NaCl treatment.

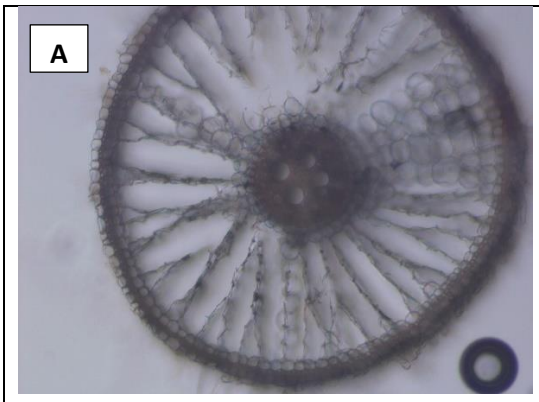
4.6.4 Number of Tillers

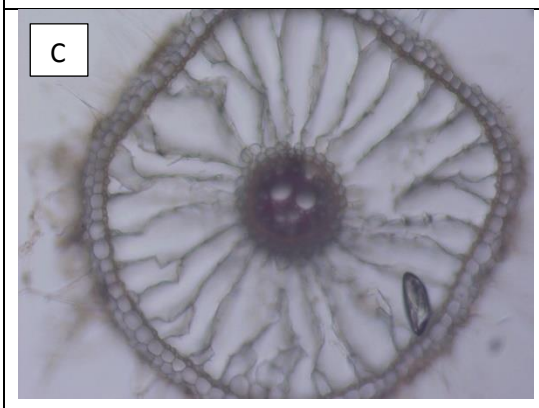
Number of Tillers show significant positive correlation in 150 mM NaCl treatment with Total Number of Leaves (0.967) at 5 % error value and significant negative correlation in 200 mM NaCl treatment with Xylem Sap Sodium Content (-0.894) at 1 % error value. No other traits in the whole treatments were significant with the Number of Tillers.

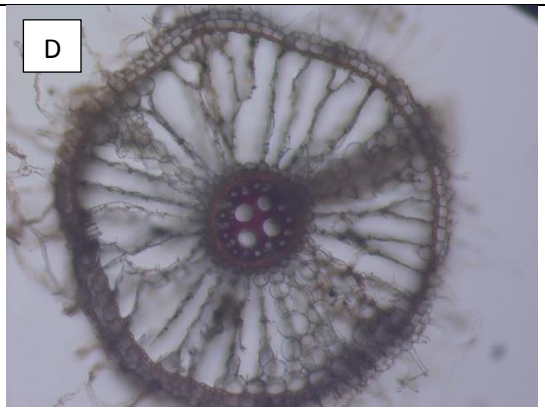
4.7 Genotypic variation in the suberization pattern observed by microscopic study

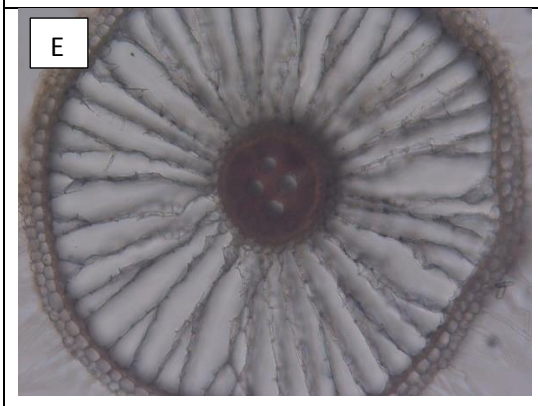
Result of the microscopic observation of root sections shows that under control (well-watered) condition the percentage of suberization in exodermis was more in ARB 6 followed by Pokkali, Kalanamak, Jaya and IR-20 the least. However, Pokkali and ARB 6 had highest deposition of suberin in endodermis followed by Kalanamak, Jaya and IR-20. The level of suberization was observed to reduce significantly under drought both in exodermis and endodermis. In exodermis of Pokkali, suberization had considerably reduced, but the reduction was more drastic in ARB 6 followed by Kalanamak, Jaya and IR-20. The endodermal suberin levels in Pokkali and ARB 6 were almost same, whereas, the Suberization had reduced in endodermis of Jaya and IR-20.

The level of suberization was observed to increase under salt stress in Pokkali and Kalanamak both in exodermis and endodermis. However, in ARB 6, IR-20 and Jaya the extent of suberization increased in exodermis with slight or no change in endodermal suberin percentage.









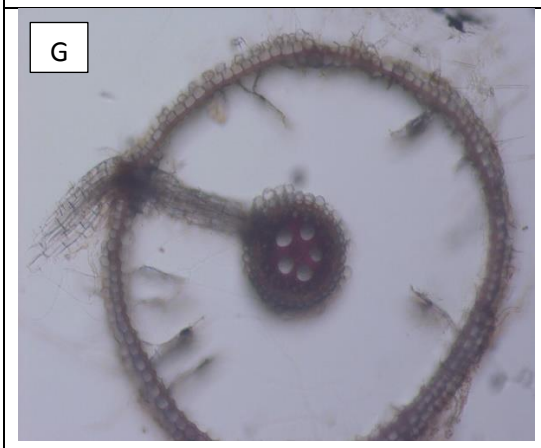


Plate 11: Root sections ARB 6

A: Well watered condition

B: 3 days of water withholding

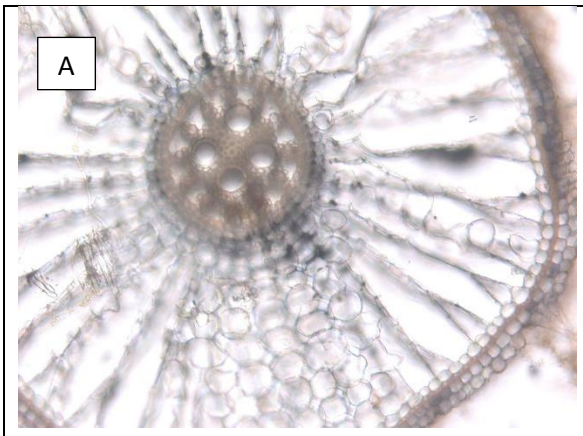
C: 5 days of water withholding

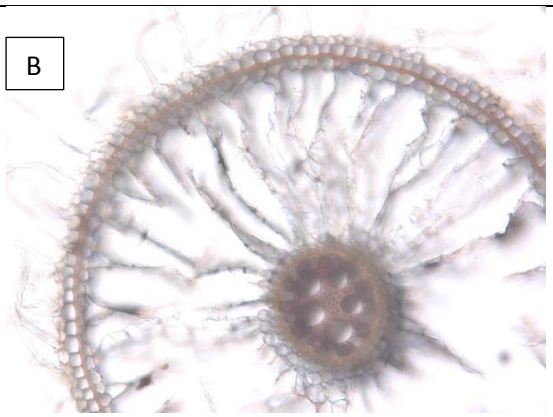
D: 7 days of water withholding

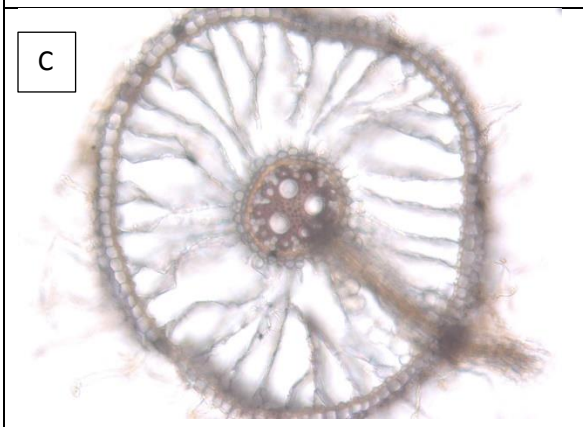
E: 100 mM NaCl treatment

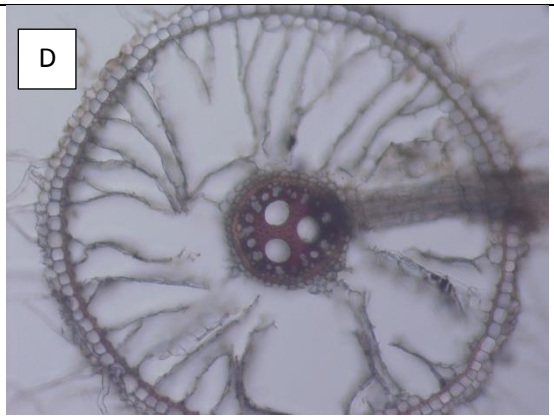
F: 150 mM NaCl treatment

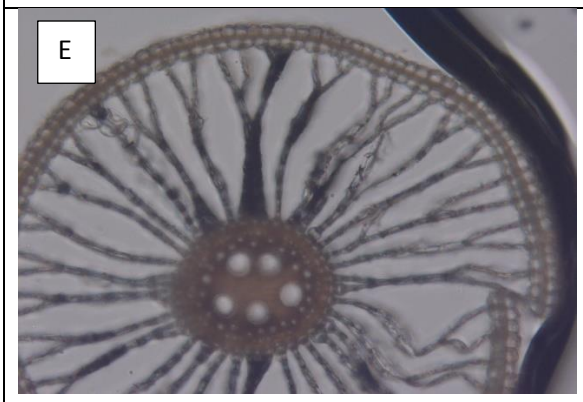
G: 200 mM NaCl treatment











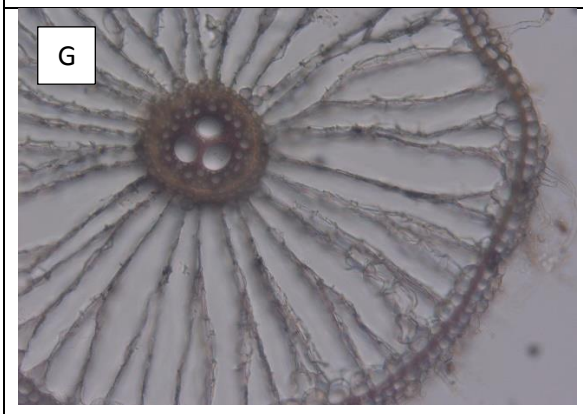


Plate 12: Root sections IR 20

A: Well watered condition

B: 3 days of water withholding

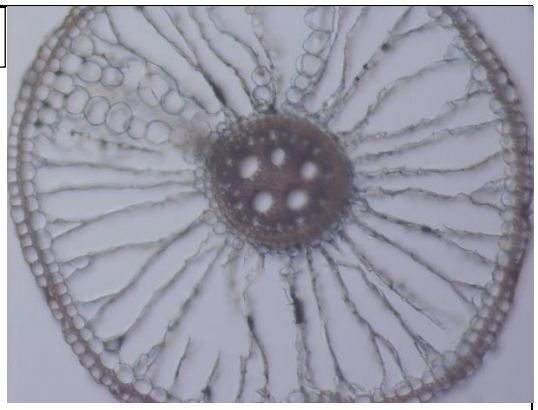
C: 5 days of water withholding

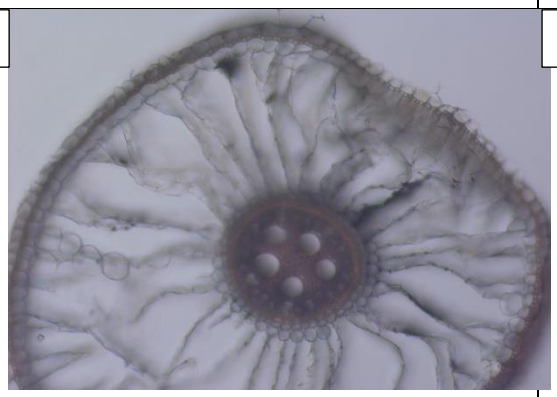
D: 7 days of water withholding

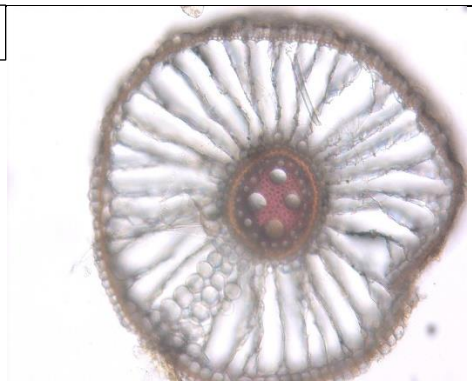
E: 100 mM NaCl treatment

F: 150 mM NaCl treatment

G: 200 mM NaCl treatment







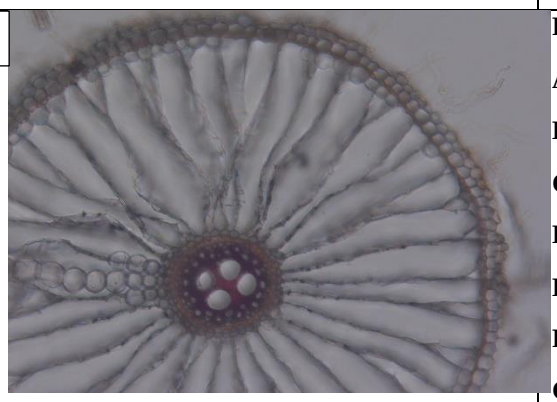


Plate 13: Root sections Jaya

A: Well watered condition

B: 3 days of water withholding

C: 5 days of water withholding

D: 7 days of water withholding

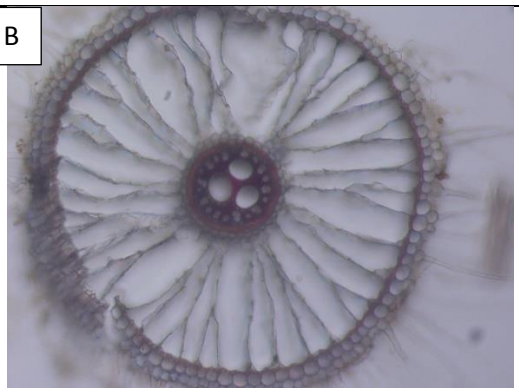
E: 100 mM NaCl treatment

F: 150 mM NaCl treatment

G: 200 mM NaCl treatment

A

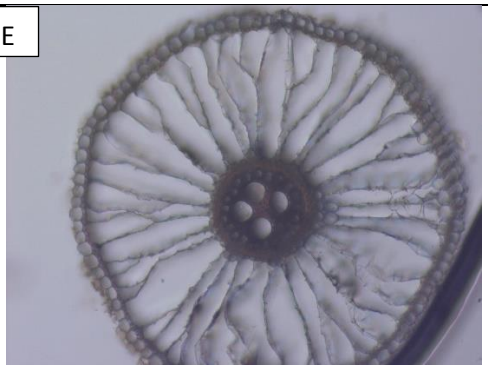
B



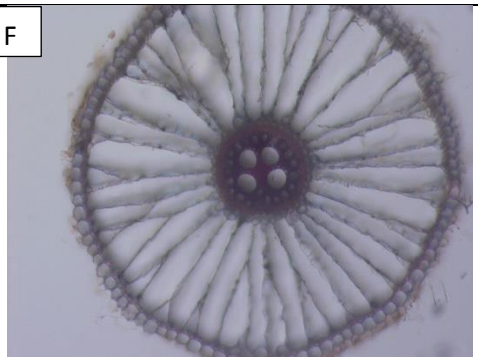
C

D

E



F



G

Plate 14: Root sections Kalanamak

A: Well watered condition

B: 3 days of water withholding

C: 5 days of water withholding

D: 7 days of water withholding

E: 100 mM NaCl treatment

F: 150 mM NaCl treatment

G: 200 mM NaCl treatment

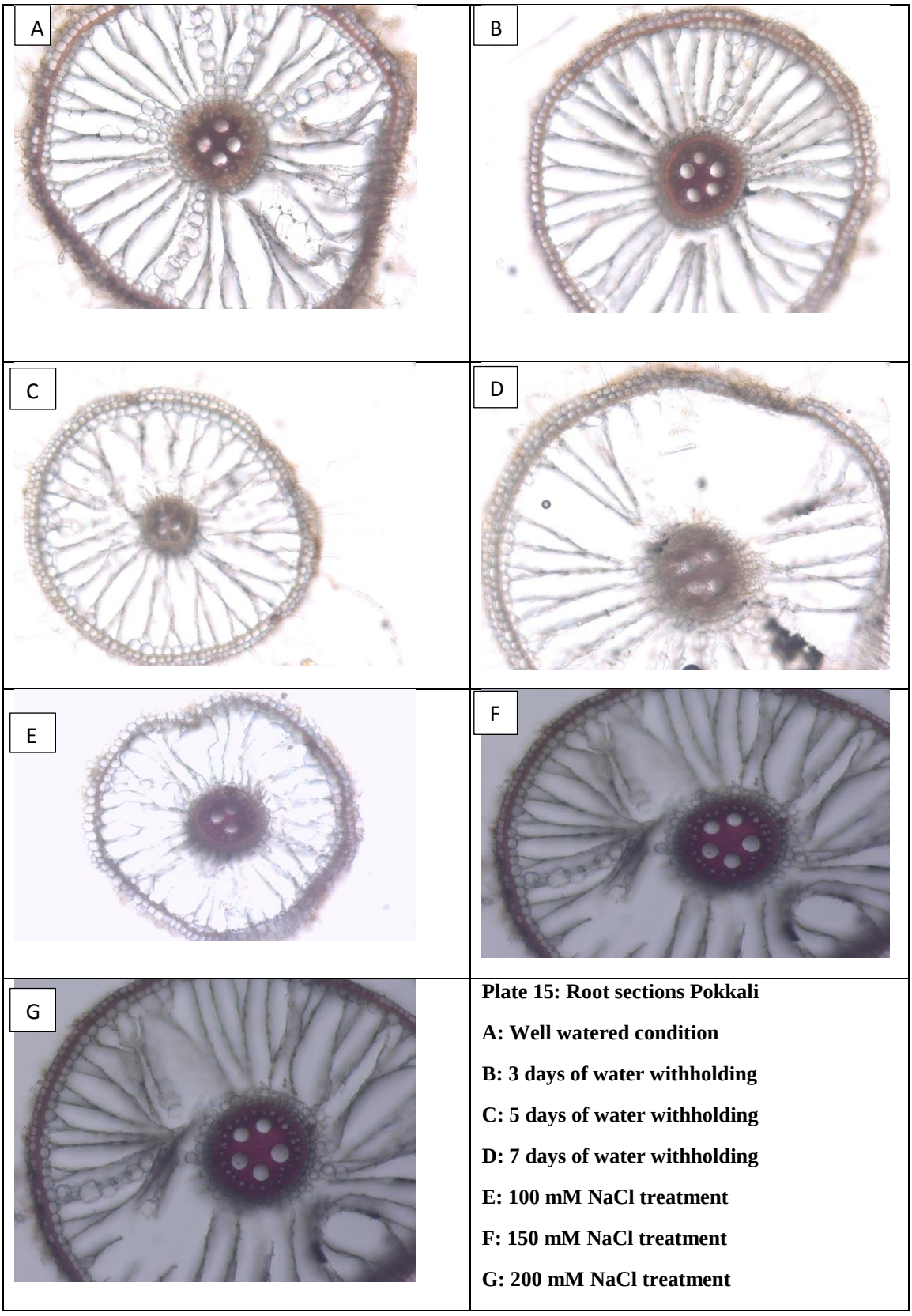


Plate 15: Root sections Pokkali

A: Well watered condition

B: 3 days of water withholding

C: 5 days of water withholding

D: 7 days of water withholding

E: 100 mM NaCl treatment

F: 150 mM NaCl treatment

G: 200 mM NaCl treatment

V DISCUSSION

Rice (*Oryza sativa* L.) is a dietary staple of more than half of the world and 65% of the Indian population. Rice is the only crop which is cultivated and adapted to a wide range of ecosystems, majority of the rice is cultivated in semi-aquatic conditions in many parts of the world but in India, of the 44 Mha of total rice area, 33% is grown in rainfed low lands and 15% in uplands. Mostly in upland areas farmers take up the practice of irrigation to cope with soil water deficit but salts which come along with irrigation water eventually get accumulated and leads to high salt build-up in the soil. All plants tolerate salinity up to a certain threshold level without any yield reduction, after which an increase in salinity level significantly reduces yield. Although, in rainfed lands drought is considered as the major limitation for high crop yield, growing rice is also a challenge in uplands, where salinity is major production constraint.

Since rice requires more water to grow properly, therefore increasing drought stress would threaten the sustainability of these plants. In order to avoid the situation, breeders have come up with a new concept of aerobic rice, where water requirements of plant are reduced at genetic level. Aerobic rice is called so because its roots grow in aerobic condition, as its seeds are directly sown in non-puddled soil with no water logging required. Recent studies have shown aerobic rice to perform excellently in terms of high grain yield under severe drought stress.

5.1 Genotypic variations in root and shoot traits

Significant increase in root length in ARB 6 (drought tolerant variety) was observed under drought stress compared to control. Although, Pokkali and Kalanamak salinity tolerance, they also showed an increase in root length under drought condition. IR-20 showed slight decrease, whereas, Jaya showed no change in root length in drought condition. ARB 6 possessed the longest roots of all in drought condition which suggests its efficiency in water mining, hence, explaining its tolerance to water deficit condition, followed by Pokkali, Kalanamak, Jaya and IR-20 in the order.

Jaya possessed maximum number of roots in control condition, followed by ARB 6, Kalanamak, Pokkali and IR-20. Drought was found to trigger an increase in the number of roots in all the five varieties of rice, compared to their root number in control. The data suggests that all five varieties of rice responded differently to drought stress. ARB 6 performed well as it possessed longest roots and showed deep root growing pattern in drought. Surprisingly, Pokkali (salt tolerant variety) also had long roots in drought. However, Kalanamak, Jaya and IR-20 showed no change and possessed almost same root lengths as that in control condition.

All plants were healthy in control condition whereas excessive rolling of leaves was observed in plants under drought condition. Pokkali possessed longest shoot in both control and drought conditions followed by Kalanamak, ARB 6, IR-20 and Jaya. No significant difference was observed in terms of tiller number in Pokkali under control and drought condition, but the number of tillers increased in Kalanamak, ARB 6, Jaya and IR-20 in drought stress. The number of leaves increased in Pokkali and ARB 6 but

decreased in IR-20 and Jaya. Passage cell number along with the root length increased drastically in the case of ARB 6 and Pokkali.

5.2 Analysis of variance

The phenotypic variation manifested by the genotype has two components namely genotypic and environmental. Analysis of variation of all the 9 characters revealed that significant differences are there among the means of all genotypes for root length, shoot length, root number, number of tillers, total number of leaves, passage cell number, xylem sap sodium content and total xylem sap uptake. Evident variations can be observed in this study because of the selection of contrasting varieties for their responses towards salinity and drought. This indicated more scope for selection for these genotypes in future generations. The differences observed in these traits could be attributed to variations present in genotypes as well as the environmental factors.

5.3 Correlation study

Correlation coefficient analysis helps to determine the nature, direction (negative or positive) and magnitude of relationship between any two characters. The study of relationship of different traits has great importance in the indirect selection of a complex trait associated with a simple trait.

The positive correlation of root length with passage cell number and total xylem sap uptake directly indicate that as the length of root increases xylem sap uptake increases. Xylem sap uptake indicates the movement of water from soil to plant shoot. In the drought condition where plants tend to form suberization, number of passage cell have a significant role in maintaining the level of xylem sap uptake. According to Mc-Donough and Gauch (1959) the root systems of drought resistant plants are characterized by wide variety of apparent adaptations. These responded to such predominant soil conditions as the duration of soil dryness and the depth that is normally wet. Plants become adapted to dry conditions mainly by developing an extensive root system rather that structural modification of the roots. So root length is a trait which will help plants to increase the drought tolerance. Considering these facts, the variety ARB 6 which have higher root length in the drought condition will perform well with more uptake of water for the evapotranspirational requirements.

The positive correlation of shoot length to the total xylem sap uptake can be justified by the fact that with increased availability of water the biomass can be increased. This can be supported by the positive relation between shoot length and the total number of tillers. In the case of Pokkali which show the maximum shoot length this can be understood. Shoot length also show a negative relation to the sodium content, which may be due the compartmentalization and exclusion of Na^+ ions in the shoot. Pokkali has been well known for its salt resistance, and that mechanism works with the extent of suberization along with the exclusion and compartmentalization.

In the higher saline stress root number shows negative correlation with passage cell number, total xylem sap uptake and xylem sap sodium content. It is so evident from the data obtained that Pokkali which possess the highest root number in salinity is

tolerant to salt because of this phenomenon. And it is understood that if total xylem sap uptake decreases the sodium content also will decrease.

Number of tillers showing positive correlation with number of leaves and negative correlation with xylem sap sodium content. as number of tillers increases the number of leaves also increases, it's a fact that does not need much explanation. It may be due the process of equal distribution of sodium ion in shoots, the negative correlation of sodium content to the number of tillers is observed.

In the drought condition we can see a positive correlation between total number of leaves to number of passage cells. This can be explained by the fact that increase in passage cell number in turn increase the water uptake, which will directly aid to the increase in biomass.

In higher saline stress positive correlation can be observed between passage cell number and total xylem sap uptake. Saline susceptible varieties will have more passage cell even in the high saline stress, which will in turn leads to higher inflow of xylem sap. The inability to reduce the number of passage cell in case of salinity can be a reason for the susceptibility of the varieties which respond poorly to salt stress.

Sodium content in xylem sap show positive correlation with total xylem sap uptake in higher saline stress. It is due to the fact that more amount of inflow of water will increase the uptake of more Na^+ ions into the plant parts.

5.4 Varietal difference in xylem sap uptake

The extent of xylem sap uptake was significantly high in well-watered condition in all four varieties compared to drought. In drought, the sap uptake was significantly more in ARB 6 and interestingly followed by Pokkali, Kalanamak and least in Jaya and IR-20. Furthermore, it was noticed that ARB 6 always took up more xylem sap in control as well as drought condition compared to other three varieties. In correlation analysis it is shown that increased xylem sap uptake in the case of saline stress will cause in increased content of Na^+ ions in the plants. Due to the mechanism of Na/K pump the increased Na^+ ion content will reduce the K^+ ions in the plants. Xylem sap uptake is also related to number of roots in the drought condition.

Increased xylem sap uptake in drought condition is good for plants, in this study the increased number of passage cells in the case of ARB 6 helps it to be the drought tolerant variety. Root length, root number and passage cell number alone may not be the reason for the drought tolerance.

The case is different in the salinity condition where the plant should reduce the xylem sap uptake. Pokkali is the best example for that, in this study it shows drastic reduction in the passage cell number in the salinity stress. It is to be noted that the same Pokkali have more number of passage cells in well-watered and drought condition. When subjected to salt stress extensive suberization happens and passage cells are blocked in Pokkali.

5.5 Varietal difference in suberization pattern

Suberization decreased for ARB 6, Pokkali and Kalanamak considerably on the drought stress, they also possess long roots which will help to take up more water from down layers of soil. Number of passage cells increased drastically for ARB 6 followed by Pokkali and Kalanamak, which allows the inflow of water to stellar region. In saline stress Pokkali showed higher degree of suberization with less number of passage cell followed by Kalanamak. ARB 6, Jaya and IR 20 possessed less suberization compared to Pokkali. In saline stress, passage cell number did not considerably decreased in ARB 6, which will be harmful for the plant in higher saline conditions.

Although suberization is common and seen in most of the epidermal and endodermal cells, its deposition in the passage cells aids to the salinity tolerance in the varieties studied. For the varieties which lack extensive suberization in the case of salinity will susceptible ones and in drought viceversa.

VI SUMMARY

The results obtained from various experiments conducted with the objectives viz; (i) quantifying the difference in the extent of suberization under aerobic, drought and salinity conditions using contrasting genotypes and (ii) differences in biochemical composition of sap exuded from the varieties at the peak vegetative stage on subjected to stress are briefly summarized in this chapter.

- Five different genotypes named ARB 6, Pokkali, Kalanamak, IR 20, Jaya which differ in their responses towards salinity and drought were used for the study.
- Aerobic rice variety ARB 6 showed high tolerance to drought stress and exhibited tolerance to mild saline stress, but marginal or no tolerance towards the higher salinity stress.
- ARB 6 had higher number of passage cells which enhances it water uptake capacity, in stress conditions.
- Extent of suberization in ARB 6 was found to be low in drought condition and increased in saline condition.
- Pokkali, a well-known saline tolerant variety performed well in all the saline stress applied, it also exhibited moderate tolerance to drought stress but showed wilting symptoms at higher drought stress.
- Passage cell number of Pokkali decreased drastically in salinity stress safeguarding the plant from the ill effects of saline toxicity.
- Kalanamak, a traditional rice variety was found to be tolerant to salinity in this study, it almost stands just below with Pokkali in salinity tolerance but higher drought stress affected it like all other genotypes expect ARB 6.
- Passage cell number of Kalanamak also decreased significantly in saline stress like in the case of Pokkali.
- Jaya showed mild tolerance to both drought and salinity stress, it is observed that Jaya had maximum number of roots in control condition but in salinity stress it reduced drastically.
- IR 20 was least tolerant to both drought and salinity stress and in higher degrees of both treatments it showed wilting symptoms.
- In correlation study it is evident that Root Length and Passage Cell Number positively correlate with the Total Xylem Sap Uptake.
- In the saline treatments Number of Roots have a positive correlation with the Xylem Sap Sodium Content.
- In analysis of variance, Total Xylem Sap Uptake was found to be highly significant across all the factors considered.

FUTURE LINE OF WORK

1. Kalanamak has to be further evaluated by molecular analysis for its salinity tolerance ability.
2. An improved variety can be hypothesized considering the high drought tolerance ability of ARB 6 and high saline tolerant ability of Pokkali and Kalanamak.

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APPENDIX-I

Phloroglucinol-HCl staining protocol

Stain preparation: There are various procedures to make up the staining solution but commonly it is prepared as a saturated solution of phloroglucinol in 20 % hydrochloric acid. The hydrochloric acid used is about 2 N. Be sure to handle the solution with care. Wear gloves. Prepare this solution in the fume hood. First dissolve phloroglucinol (about 2.0 g) in 80 ml of 20 % ethanol solution and then add 20 ml of concentrated HCl (12 N) to it.

Staining the root sections

1. Transfer freehand sections into holder chambers and stain sections in 2 % (w/v) Phloroglucinol-HCl for 1 hour. (use 0 size paint brush for transferring sections, Pasteur pipette for staining for adding dye)
2. Rinse by passing holders through several changes of distilled water; blot excess water from holders after each transfer. (insulin syringe is used to remove excess water)
3. Transfer sections to slides and mount in the 50 % (v/v) glycerine solution.

APPENDIX-II

Mean performance of genotypes for different traits

Number of Tillers

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	2.75	2.5	2.75	2.25	3
T2	2.75	2.75	3.25	2.75	3.25
T3	3.25	2.5	3.75	2.75	2.5
T4	3	2.5	3	2.25	3
T5	3.75	3.25	2.75	2.5	3.25
T6	3.25	3.25	3.25	2.25	3
T7	3	3.25	2.75	2.25	2.75

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Total Number of Leaves

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	3.75	4	4	3.25	3.25
T2	4.25	4.5	2.75	2.5	3.5
T3	4.25	3.75	3	2.75	3.5
T4	3.5	3.75	3	2.25	3.75
T5	4.25	3.75	3.75	2.75	3.25
T6	4	3.75	3.75	2.5	3.25
T7	4	3.25	3.25	2.25	3.5

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Shoot Length (cm)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	36.25	43	26.25	29.1	36.75
T2	35	45.5	28	29.45	35.25
T3	31.25	45	31.5	26.15	32.5
T4	27.25	45.5	30.5	23.4	30.2
T5	30.5	37	23.5	26.975	37
T6	27.5	33	20.5	24.325	34.5
T7	21.5	29.75	18.25	21.575	32

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Root Length (cm)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	50.5	48.625	39.5	41.775	42.1
T2	53.5	49.475	38.75	41	37.5
T3	57.25	52.65	38.55	37.95	35.25
T4	59.425	52.45	35.45	34.275	31.925
T5	47.475	48.325	36.525	42.25	41.75
T6	43.625	46.825	33	45.525	49.25
T7	41.325	45.75	30.5	46.575	48.275

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Root Number

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	47	44.25	53.75	25	38.75
T2	47	45	45.5	23.25	32
T3	46.25	49.25	48.5	18.5	28
T4	41	43.75	52.25	17	20.5
T5	35.75	50.25	49.75	33	36.25
T6	32	53.25	36.25	29.5	52.25
T7	32.5	51.25	35.5	28.75	47

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Passage Cell Number (Endodermis)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	5.5	4.75	3.75	2.75	5.25
T2	9.75	8.25	3	3.5	5.75
T3	16	13.5	4.25	4.5	4
T4	17.75	14	4.75	4.75	5.25
T5	6.25	2.25	3.75	4.5	7.5
T6	7	2	5.25	4.75	8.75
T7	8.75	1.25	5.75	5.25	3.5

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Passage Cell Number (Exodermis)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	2.25	1.25	1.75	1.25	1.5
T2	5.75	5	2.25	2.25	3.25
T3	9	7.5	3.5	3	6.75
T4	12.5	9	4	3	9
T5	3	1.75	2	3.75	1.5
T6	4.25	2.75	3.25	4.5	3
T7	4.75	2.5	3.75	4	3

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Xylem Sap Sodium Content (ppm)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	3	2.025	3.425	4.85	3.1
T2	2.09	2.625	4.915	1.8	1.1
T3	3.35	1.79	1.4	2.29	3.025
T4	5.925	1.2	1.225	2.765	0.383
T5	13.6	7.108	20.275	18.45	10.175
T6	26.74	15.115	22.475	34.99	16.275
T7	33.775	18.308	50.275	31.15	30

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Xylem Sap Potassium Content (ppm)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	114.05	139.845	114.375	112.475	134.25
T2	116.658	175.408	149.025	126.145	120.125
T3	142.875	155.59	159.8	138.808	132.225
T4	130.308	109.6	127.375	132.713	128.625
T5	144.05	36.658	121.325	123.665	148
T6	143.825	77.533	104.85	112.415	166.575
T7	122.125	81.64	114.1	109.55	122.85

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl

Total Xylem Sap Uptake (gm)

	ARB 6	POKKALI	JAYA	IR 20	KALANAMAK
T1	0.384	0.323	0.184	0.205	0.218
T2	0.128	0.185	0.043	0.085	0.085
T3	0.197	0.157	0.097	0.048	0.035
T4	0.165	0.083	0.071	0.012	0.047
T5	0.117	0.069	0.038	0.102	0.081
T6	0.077	0.06	0.074	0.1	0.045
T7	0.083	0.014	0.075	0.059	0.027

T1: Control, T2: 3 DWW, T3: 5 DWW, T4: 7 DWW: [DWW (Days of Water Withholding)]

T5: 100 mM NaCl, T6: 150 mM NaCl, T7: 200 mM NaCl