Differential salt tolerance mechanism in rice (*Oryza sativa* L.) at early seedling and reproductive stages

A

Thesis submitted to the

Odisha University of Agriculture and Technology

In partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

(Plant Physiology)

By

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COLLEGE OF AGRICULTURE

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BHUBANESWAR-751003, ODISHA

2023

ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

BHUBANESWAR, ODISHA

2023

CERTIFICATE- I

This is to certify that the thesis entitled **"Differential salt tolerance mechanism in rice (*Oryza sativa* L.) at early seedling and reproductive stages"** submitted in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE (PLANT PHYSIOLOGY)** to the Odisha University of Agriculture and Technology is a faithful record of *bona fide* and original research work carried out by **Ankita Mohanty, Adm. No. 18123M01** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

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ACKNOWLEDGEMENT

Thanks to the Almighty God for giving me this opportunity to express my heartfelt gratitude to all the dedicated people whose moral support and kind co-operation encouraged me during the course of investigation.

I am extremely delighted and feel blessed to have worked under the guidance of excellent pursuing and ever helpful personality, chairman of my advisory committee, Dr. K. Chakraborty, Senior Scientist, Crop Physiology and Biochemistry Division, ICAR-NRRI, Cuttack. His valuable and timeless suggestion, constant inspiration, affectionate dealings, constructive criticism and advice helped me at every stage. His innovative ideas, meticulous nature and close monitoring of the research work helped me to complete the manuscript with perfection. My sense of obligation is negligible and unquantifiable in respect of his energy and time spent on this endeavor. It was a life time opportunity to work with him, to know about an experimental man in work and to gather valuable knowledge and experience during the course of my investigation. I am grateful for his articulate comments, transcendent recommendations, and unwavering encouragement, all of which contributed to a pleasant learning environment. I could not have imagined having a better mentor for my Ph.D. study.

It is my pleasure to express my deepest sense of gratitude and indebtedness to Dr. K. C. Samal, Professor and Head, Department of Plant physiology, OUAT for his kind words, immense patronage and encouragement in the study period and it is my golden opportunity to have him as the member of my Advisory Committee.

My special obligation and gratefulness goes to Dr. K. Chattopadhyay, Priincipal Scientist, Crop Improvement Division ICAR-NRRI, Cuttack for his concern and magnanimity in providing timely co-operation in all my formalities. His help and advice are always invaluable.

I am hereby extending my deep sense of gratitude to Dr. R. K. Panda, Assistant Professor, Department of Plant physiology, and Dr. M. Satapathy, Professor Department of Agronomy, for their guidance and unceasing help throughout the period of study, research, and completion of this thesis.

I shall be failing in my duty if I do not express my obligation to Dr. Rajkumari Bhol for her noble advice, incessant inspiration, co-operation, immense help and prudent suggestion during the preparatory stage of the manuscript.

I also wish to place a record of my sincere and heartiest thank to seniors and juniors for their constant encouragement and valuable help throughout my Ph.D. degree programme.

Words fall short to express my feelings for my beloved friends Nandini, Ritesh, Anwesa, Chinmayee and Baby Rani and my lab mates, Dr. Priyanka Jena, Subhankar Mondal, Sambit Mohapatra, Debasis Mohapatra, Jitendra Senapati, Banamali Behera and Chandan Bhoi, who in one way or the other rendered their selfless assistance and well wishes that kept me in good spirit and made my post-graduation period a memorable one.

At last but not the least I bow down my head before my parents Mr. Bidyut Kumar Mohanty and Late Mrs. Saswati Mohanty and all my family members with all reverence and veneration for their blissful holy blessing, unfathomable love, incessant inspiration, solicitous contrivance and selfless sacrifice and dedication for which I have reached to this stage of my career.

Finally, I am thankful to the Almighty God for his heavenly blessings which enabled me to achieve this seemingly invincible task.

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ABBREVIATIONS

%	:	Percentage
^{0}C	:	Degree celsius
µg	:	Microgram
µg L^{-1}	:	Microgram per liter
µL	:	Microliter
g	:	Gram
mg	:	Miligram
mg/g	:	Miligram per gram
ha	:	Hectare
ha^{-1}	:	Per hectare
RH	:	Relative humidity
SE(m)±	:	Standard error mean
CD	:	Critical difference
viz.,	:	Namely
SES	:	Standard Evaluation System
mM	:	milimole
mM/L	:	Milimole per liter
SSI	:	Stress Susceptibility Index
YSI	:	Yield Stability Index
Fv/Fm	:	Variable fluorescence/Maximum fluorescence
Y(NO)	:	Non regulated energy dissipation
ppm	:	Parts per million
SOD	:	Super oxide dismutase
CAX	:	Catalase
POX	:	Peroxidase
NaCl	:	Sodium Chloride
EC	:	Electrical Conductivity
No.	:	Numbers

ABSTRACT

In the last few decades, severe crop loss has been witnessed due to many environmental constraints and salinity is one of the major factors. Across the globe, around 0.8 billion hectares of land are affected due to salinization and this figure is further expected to increase due to a rise in mean sea level and faulty irrigation management in agricultural land. Rice (*Oryza sativa* L.) is one of the major food grains, consumed by half of the world's population, and provides about 30-80% of the daily required calories. Rice is relatively salt-sensitive in its early seedling and reproductive stages. However significant stage-specific variability exists amongst the genotypes at different growth stages. So in this present investigation, ten different rice genotypes having a differential tolerance response to salinity stress at seedling and reproductive stages were evaluated to identify the prevailing mechanistic differences between these two stages. Initially, 10 genotypes with differential responses to salinity were evaluated based on their performance related to morpho-physiological attributes, tissue ion concentration (Na^+ and K^+), and yield-related traits under salinity at both the stages in *kharif* and *rabi* of 2020 and 2021 in the net house of ICAR-NRRI, Cuttack. In the early seedling stage, the plants were grown hydroponically and imposed to salt stress (12 dS m^{-1}) at the 3-4 leaf stage. While in the reproductive stage, plants were grown in the modified pot culture method (4:1 Soil:Stone) and subjected to salt stress (8 dS m^{-1}) at the booting stage till harvesting. In the seedling stage, the most tolerant genotype was FL478 followed by AC41585 and AC39416A, while Rashpanjor, CSR27, Binadhan 8, and Luna Suvarna were categorized under the moderately tolerant group. Sadri and Sabita were susceptible and IR29 was the most susceptible genotype in response to 12 dS m^{-1} of salt stress at the seedling stage. On the other hand in the reproductive stage, Rashpanjor and AC41585 were found to be the two most tolerant genotypes followed by AC39416A, while the FL478 become moderately tolerant to prolonged salt stress of 8 dS m^{-1} at the reproductive stage. From the initial ten, we took four rice (AC41585, FL478, Rashpanjor, and IR29) genotypes of having stage-specific variations in salt-tolerance ability and studied traits related to osmotic adjustment, ROS balance, and ionic regulations towards salt stress at both seedling and reproductive stages. Based on the ion accumulation pattern at the seedling stage both FL478 and AC41585 were shown to be preferential transporter of K^+ for maintaining comparatively lower Na^+/K^+ ratios in leaf tissues. Along with the tissue ion concentration, gene expression profiles of different Na^+/H^+ transporters, high-affinity K^+ transporters, and H^+-pumps indicated stringent ion exclusion behaviour of FL478 and AC41585, which perfectly resembles the phenotypic manifestation of salt tolerance at the seedling stage. But, even with such dominating ion exclusion, FL478 failed to show the expected level of tolerance at the reproductive stage, while AC41585 and Rashpanjor could. So to identify the additional factor(s) which might help a few genotypes to tolerate salt stress at the reproductive stage more efficiently than others, we have studied the contribution of tissue tolerance traits in these four genotypes. Interestingly we found the highest tissue tolerance (measured in terms of LC_{50} score) in IR29 followed by Rashpanjor and AC41585, while it was the least in FL478. Besides, tissue tolerance assay, upregulation in the *OsNHX1*, and vascular H^+- pumps indicated effective sequestration of excess Na^+ in leaf tissues of IR29, Rashpanjor, and AC41585. So we conclude that only ion exclusion, without much contribution of tissue tolerance, could be enough for seedling stage tolerance, however, may not be sufficient for the reproductive stage tolerance. Rather a fine balance between ion exclusion and tissue tolerance is crucial for prolonged salt tolerance at the reproductive stage.

INTRODUCTION

In the past few decades, the changing climatic scenarios have altered the natural ecosystem and accelerated the conditions that turned into more unfavourable for plantgrowth. Many abiotic stresses have negatively affected grain production and threatened food security worldwide. Amongst them, soil salinity is one of the major reasons that affects about 50% of the irrigated land (Fita *et al*., 2015). Rise in mean sea level, faulty irrigation and poor drainage lead to the intrusion of salt into the agricultural land. Across the globe, around one-third of irrigated land and about 20%was affected by salinity (Munns, 2005). Out of that, approximately 6.73 million hectares of irrigated land are salt degraded in India alone(Sharma and Singh, 2015).Rice is one of the most important cultivated food grains, feeds more than half of the world's population and provides about 50-80% of the daily calories requirement (Khush, 2005). As compared to other stages of the crop growth cycle, the early seedling and booting stages are two most vulnerable stages in rice (Ahmadizadeh *et al*., 2016). It can endure a salt stress of about 3-4 dS m^{-1}, but anything more than that has been associated with substantial yield loss. (Gao *et al*., 2007). Plant development and physiology are hampered by soil salinity in two ways.After being exposed to salt stress, plants first experience osmotic stress due to the presence of excess solutes outside the root zone, which preventing the plants to draw water through osmotic force (Uddin *et al*., 2016; Chakraborty *et al*., 2016a). Subsequently, the ionic stress takes over, due to the high build up of noxious Na^+, which competes with K^+ uptake and mobility inside the cell and interferes with several metabolic pathways(Munns and Tester, 2008).It also severely disturbs the essential metabolic and physiological processes andleads to a severe loss ofchlorophyll and hampered plant biomass accumulation and other growth factors (Ismail *et al*., 2007; Baker, 2008). The cellular osmotic and ionic imbalance leads to partial stomatal closure to conserve the water status of the plant which reduces the supply ofCO_2required for photosynthesis. Impairment of the photo-chemical apparatus causes reduction in quantum efficiency ofPSII and increased energy dissipation during the photochemical process, which are two major causes of deterioration in the photosynthesisrate (Gilmore *et al*., 1996; Strasser *et al*., 2000). Due to the accumulation of photo-reducing power,a cascade of biochemical reactions is taking place inside the cell which produced harmful reactive oxygen species (ROS) in chloroplast and mitochondria due to the leakage in the electron transport chain under stress (Yamane *et al*., 2009). Higher concentrations of

ROS ($O_2^{\bullet-}$, H_2O_2,$^1O_2$and $OH^{\bullet-}$) in the cell mainly caused the denaturation of proteins, destruction of DNA, and peroxidation of lipids(Mittler *et al*., 2010). Besides, salinity is restricting growth and development severely due to the drop in photosynthesis, reduction in stomatal conductance and accumulation of toxic ions in the transpiration stream, which is considered to be one of the major factors in restricting growth during the seedling stage. Not only in the seedling stage but in the reproductive stage also significant loss in yield as well as in yield-related attributes like tiller number, panicle length, spikelet number per panicle and grain filling is evident under salt stress (Rao *et al*.,2013; Zeng *et al*.,2009).

Hence to overcome oxidative damage, cellular detoxification is essential. For that plant has a well-developed array of antioxidant enzymesto scavenge the harmful reactive oxygen species that evolved during the period of stress (Vaidyanthan *et al*., 2003). Enzymes like superoxide dismutase (SOD; EC1.15.1.1), Catalase (CAT; EC 1.11.1.6), Peroxidase (POX;EC 1.11.1.7), and some non-enzyme antioxidants like ascorbate and reduced glutathione play a major role in ROS scavenging process (Apse and Blumward, 2002). SOD is the major scavenger of $O_2^{\bullet-}$, whereas, both CAT and POX decomposed the H_2O_2 into H_2O. However superior ROS scavenging system along with the production of some compatible solutes stabilizes the cellular function under salinity (Hasegawa *et al*., 2000). Many osmoprotectants like proline, trehalose, and glycine betaine were found to maintain favourable osmotic potential between cells and stabilizethe cellular osmotic potential(Shivakumar *et al*., 2001). Some of the previous studies revealed the presence of a higher amount of glycine betaine in the leaf might protect the photosynthetic apparatus (fluidity of thylakoid membrane) under drought and salt stresses (Wang *et al*., 2010; Chakraborty and Sairam, 2018).

Apart from osmotic stress hyperaccumulation of ions creates ionic toxicity in the later phase.Excess ion accumulation accounts for two major components- (i) Na^+ toxicity and (ii) K^+ retention in cells. Through the passive flow large amount of Na^+ is transported from the root-soil interface by both symplastic and apoplastic pathways. High Na^+ in the transpiration stream sometimes hampers the K^+ uptake due to their chemical and ionic similarities (Chakraborty *et al*., 2018) and high competence between these ions restricts the binding site for K^+ in different biophysical processes and reduces the potential binding under salinity. Eventually, the excess build-up of Na^+ ions in

coordination with poor retention of K^+ triggers programmed cell death events in plants (Chakraborty *et al*., 2016b). To encounter the ill effects of salinity, plants employed different adaptive strategies to survive the adversity and maintain cellular homeostasis. Along with this to minimize the load of the excess amount of Na^+ from the xylem, plant system has adopted different strategies like Na^+ exclusion, K^+ retention, and excess Na^+ sequestration (vacuolar sequestration) (Munns and Tester, 2008). Ion exclusion is one of the mechanisms that is directly associated with the retrieval of excess Na^+ from the xylem to the soil for maintaining a low Na^+/K^+ ratio in the growing tissues. Whereas, the retention of an ample amount of K^+ and sequestration of excess Na^+ in the vacuole was also essential in maintaining ionic homeostasis (Munns and Teaster 2008).

In the case of ionic exclusion,SOS (salt excessively sensitive) pathway is crucial for maintaining ionic homeostasis. SOS1 is a key plasma membrane Na^+/H^+ antiporter preliminarily associated with Na^+ ion exclusion during salt stress. SOS1 functions in association with SOS2 and SOS3 complex which all together extrude Na^+ ions out of the cell at the expense of ATP molecules (Shi *et al*., 2000; Zhu, 2001; Quintero *et al*., 2002). That is why the higher activityof proton pumps such plasma membrane (PM) bound ATPase activities is associated with the active pumping of Na^+ out of the cell against the concentration gradient or into physiologically inert apoplastic spaces of the cell (Mansour, 2014). In addition, HKT (High-affinity K^+ transporter) family transporters are also playingessential role in the selective transport of ions and balancing ionic homeostasis(Shabala *et al*., 2015; Hamamoto *et al*., 2015; Zhang *et al*., 2017).The majority of the class I family of HKT transporters, such as *HKT1.1* and *HKT1.5* function by retrieving Na^+ back from the xylem sap to restrict upward movement of $Na^+$and preventing to reach photosynthetically active tissue. Specifically,*HKT1.5* (*SKC1/HKT 8*) is an exclusive player located in the xylem parenchyma and regulates the entry of Na^+ ions into the xylem (Ren *et al*., 2005). Whereas, the class II family of HKT transporters is more specific in transporting K^+ rather than restricriting Na^+ (Lan *et al*., 2010; Horie *et al*., 2011). For instance, *HKT2.4*significantly helps in maintaining a higher K^+ level during salinity and is more likely a K^+ channel/transporter than a Na^+/K^+ antiporter. Despite the stringent ion exclusion mechanism, some noxious Na^+ still manages to get intothe metabolically active tissues and impair their normal functioning (Yokoi *et al*., 2002). These excess ions need to be stored either in the vacuole or in the apoplastic region to limit it below

the cytotoxic threshold level (Blumwald, 2000; Wang *et al*., 2012; Bonales-Alatorre *et al*., 2013).K^+ retention in the metabolically active tissues is another extremely important mechanism used by rice and other crops in addition to Na^+ exclusion and sequestration (Munns and Tester, 2008; Wu *et al.*, 2013, 2015; Chakraborty *et al*., 2016).Munns*et al*. (2012) reported that in the seedling stage of rice, K^+-specific transporters such AKTs and HAKs effectively maintain cellular homeostasis. More specifically, *OsHAK1* and *OsHAK5* transporters were crucial for K^+ acquisition, transportation, and maintenance of a high K^+/Na^+ ratio in rice under salinity stress (Chen *et al*., 2015).

It is well known that ion exclusion is a robust mechanism that effectively extrudes excess Na^+ ions from the cytosol to the external medium. This mechanism is crucial for maintaining a low Na^+ ion content in the upper plant tissue. During seedling stage salt stress, ion exclusion is one of the promising strategies for some of the genotypes like Nona Bokra and FL478, where hyper action of *OsHKT1.5* leads to Na^+-unloading from the xylem stream (Ren *et al*., 2005). Bonilla *et al*. (2002) reported that *Saltol*QTL identified for seedling stage salttolerance contributes in maintainance of lower Na^+/K^+ ratio in the shoot. But some wild accessions and landraces of rice exhibited considerable salttolerance despite showing high upward transport of Na^+. All these genotypes were found to have a relatively high tissue tolerance capacity which helps them tosequester excess amount of Na^+ in the vacuole inorder to protect their photosynthetic pigment system (Prusty *et al*., 2018; Chakraborty *et al*., 2020).

Unlike the seedling stage, the scenario becomes even grimmer if salt stress occurs at the reproductive stage. The panicle development and grain filling is directly affected due to the tremendous pressure of external Na^+ for a prolonged period (Moradi *et al*., 2003; Singh and Flower, 2010). For maintaining optimum yield, strong ionic regulation and restricted movement of Na^+ into the photosynthetically active flag leaf is most essential (Lisa *et al*., 2011; Razzaque *et al*., 2017). Yet, as ion exclusion requires a lot of energyit might have a negative effecton crop yield during prolonged salt exposure in reproductive stage. Some earlier studies reported that FL478 (an exclusive Na^+ excluder) relies heavily on the active pumping of Na^+ out of the cell via different proton pumps, but on the contrary, some other genotypes were able to balance the process of tissue tolerance and ion exclusion to achieve tolerance at seedling stage with lesser energy expenditure (Munns *et al*., 2019; Chakraborty *et al*., 2020).

So, we hypothesized that the mechanism of salttolerance in rice may not bethe same at the early seedling and reproductive stages. At the seedling stage, strict ionic regulation may be able to offer acceptable tolerance, but it may not be equally effective in providing field-level salt-tolerance in the reproductive stage. For this, in our investigation,we took ten rice genotypes of having differential salt tolerance abilities at both the early seedling and reproductive stages. Based on different morpho-physiological and biochemical assessments four distinct rice genotypes were selected with stage-specific variations in salt-tolerance abilityand studied their physiological and molecular responses towards salt stress at both stages to understand the influence of these traits on salinity tolerance in rice at the early seedling and reproductive stages.

In light of the above hypothesis the present Ph.D. investigation is carried out on the topic entitled "Differential salt tolerance mechanism in rice (*Oryza sativa* L.) at early seedling and reproductive stages" with the following objectives

Objectives

1. To evaluate and characterize rice genotypes showing salinity tolerance at early seedling and reproductive stages

2. To elucidate salt-tolerance mechanism individually at early seedling and reproductive stages

3. To identify the commonalities and differences in salt-tolerance mechanism at early seedling and reproductive stages in selected rice genotypes

REVIEW OF LITERATURE

The present study entitled "Differential salt tolerance mechanism in rice (*Oryza sativa* L.) at early seedling and reproductive stages" was carried out at ICAR-National Rice Research Institute, Cuttack, Odisha during *kharif* and *rabi* 2020 and 2021. The review of literature from the previous research work pertaining to present investigations is presented hereunder.

2.1. Effects of salt stress on morphology of plants at early seedling stage and flowering stage

Fifty mMNaCl stress caused significant decline in panicle weight, panicle length, primary branches/panicle, filled seeds/panicle, unfilled seeds/panicle, filled seeds/plant, unfilled seeds/ plant, total seeds/panicle, total seed weight/panicle, 1000-seed weight and total seed weight/plant which was probably linked to reduce viability of pollen under stress condition, thus resulting failure of seed set (Abdullah *et al*., 2001).

Munns (2002) reported that, magnitude of salt induced yield losses might be affected by many physiological, biochemical factors at different stages of rice plants. Three major factor might be the excessive Na^+ uptake and translocation in the growing plant part and its subsequent distribution in different vegetative and floral parts especially in leaves where it causes leaf mortality thereby reduces transportation of total assimilates to the growing region

Ali *et al*. (2004) demonstrated adverse effect of salt stress on 18 advance rice genotypes under 8.5 dS $m^{-1}$of salinity stress. The plants were artificially salinized up to 90 days after transplanting. Significant reduction in yield, flag leaf area fertility percentage, number of productive tillers and panicle length and number of primary branches per panicle was reduced. Highest reduction in yield was recorded in DM-38-88 and NR-1 followed by others. Pigment content was also hampered in all the genotypes as compared to the stressed plants.

Pattanagul and Thitisakasakul (2008) were evaluated 3 different rice genotypes at seedling stage on the basis of their differential response to salinity at seedling stage at four different levels of salinity (0, 50, 100 and 150 mM of NaCl) for 9 days. Out of

three, the root and shoot growth of Pokkali (tolerant) cultivar was least affected as compared to KhaoDawk Mali 105 and Luang Anan under highest level of salinity stress. But significant loss in dry matter accumulation was observed in all the genotypes under stress as compared to their control plants. The relative waster content of Pokkali was dropped from 90 to 77% under stress, whereas, relative water content (RWC) was dropped for other two genotypes more than Pokkali in all conditions of salt stress.

Aisha *et al.* (2014) conducted an experiment where they revealed that salinity level of 50mM and 70mM cause significant (about 50%) reduction in seeding fresh weight on exposure for 1-2 weeks. This effect became more pronounced with the duration even in low level of salinity. Higher reduction of ≥ 70% was observed in 70mM salt stress l. About 70% of yield loss was observed in 70mM salinity level applied at reproductive stage. More than 50% spikelet sterility and about 80% of grain weight was lost in higher level of salinity. It was also observed that the reduction in panicle number and panicle weight positively co related with yield loss.

Moradi and Ismail *et al*. (2007) observed that, there was clear cut difference in biomass production and plant growth in both early seedling stage and reproductive stage in rice. About 18% and 15% decrement in plant biomass was observed in IR651 and IR632 (tolerant) in seedling stage and 46% in case of IR 29. Similar kind of results were observed in reproductive stage also

Rao *et al*. (2008), reported that salinity negatively affected the floret fertility in rice at reproductive stage, which ultimately leads to spikelet sterility and poor seed set. Up to 50% reduction in 1000 seed weight and yield was recorded in susceptible genotypes.

Ebrahimi *et al*. (2011) evaluated the yield and yield related attributes of popular Iranian local rice variety Hashemi under different levels of salinity (0,2,4 and 8 dS m^{-1}) at four different stages viz; tillering, panicle formation, heading and ripening. The yield decreased significantly ($P< 0.01$) as compared to control plants and was maximum under higher level of salinity. 1000 seed weight and grain filling percentage also dropped drastically, when the plants were imposed to salinity at flowering and grain filling phase.

Golizadeh and Navabpour (2011) reported severe reduction in root and shoot length, plant biomass, leaf area, older leaf rolling and chlorosis after 6 weeks of 8dS m^{-1} salt stress at the seedling stage in rice. The tolerant genotypes Gharib and Shahpasand scored 3 according to SES score, whereas IR28 and IR29 were considered susceptible with ascore of 9 at seedling stage salt stress.

Mohammad (2011) experimented salt stress 200mM NaCl in rice variety Tarornazmon for 14 days. They reported that a decrement of in root (54%) and shoot length (71%) was observed as compared to control. Fresh weight and dry weight also reduced by 95% and 75% respectively after 14 days of stress.

Rahman *et al*. (2015) evaluated the performance of four different rice genotypes (Binadhan-8, PBRC-37, NERICA-1 and NERICA-10) under four different levels of salinity (control, 6,9 and 12 dS m^{-1}) of salt stress to understand the ill effect of salinity on the flag leaf chlorophyll content, yield and yield attributing parameters. Sharp and significant reduction in plant height, total tillers per hill, panicles per hill, numbers of filled grains and grain weight with increasing salinity level except panicle length. The adverse effect was more with increase in salinity level in all the genotypes but was lowest in NERICA-1 as compared to other three.

When subjected to 0.6% and 1.2% of salt stress, 10 different rice genotypes, showed severe decline in root and shoot biomass and survivability under high Na^+ concentration after 35 days of salinity treatment. The plant biomass was highly suppressed under 1.2% of salt stress rather than 0.6% in vegetative stage (Usatov and Pavel, 2016).

Sen *et al*. (2017) conducted an experiment to study the effect of salinity at both seedling and reproductive stage with 10 rice lines including salt tolerant genotype pokkali and susceptible variety (IR29) as control. At vegetative stage the salinity screening was done with five different salinity concentrations (0, 4, 6, 8 and 10 dS m^{-1}) and reproductive stage 8 dS m^{-1} of salt stress was applied. The increased level of salt stress at seedling stage cause severe retardation in growth and almost all of leaves were drying and dead completely (score 7 and 9). Plants of IR29 and OM7347 were dead at the electrical conductivity (EC) 10 dS/m, whereas Pokkali (the tolerant variety) and IR93350 were evaluated at score 5. During reproductive stage salinity stress caused the

reduction of overall vigour of rice lines/varieties especially in the pollen germination, fertilization and grain yield in rice.

Mouhamad *et al*. (2017) investigated three genotypes of IRRI rice (IR71999-3R-3-2-2B-1-1, IR71829-3R-82-1-1and IR63731-1-3-3-2) and two varieties of rice (Anber and Jasmine) were grown hydroponically and given salinity stress (1.2, 4, 8 and 10 dS m^{-1}). They reported reductions in rice plant production (yield) reached more than 30% while the dry matter weight was decreased by 24% in all varieties of rice at higher concentration of salinity.

10 genotypes along salt tolerant (Pokkali) and susceptible (IR29) were evaluated for salinity tolerance at both seedling and flowering stage by Sen-Huang *et al*. (2017). In seedling stage the genotypes given a SES score according to IIRI. All the genotypes except Pokkali and OM7347 scored 7 and 9 respectively and almost dead under EC 10 dS m^{-1}. About 80-90% decrement in dry matter accumulation was observed in susceptible genotype IR29. Significant decrement in root and shoot length was observed in all the genotypes was recorded as compared to the controlled plant. The growth was hampered in reproductive stage as well. Pollen viability, filled grain percentage and yield were highly declined under 8 dS m^{-1} of salt stress at reproductive stage.

Zhang *et al*. (2018) studied the impact of salinity stress on the germination and seedling growth of two weedy (wild) rice cultivar (*JYGY-1 and JYFN-4*) and two cultivated rice genotype (Nipponbare and 9311). They raised the plants in hydroponics solution for 2 weeks and imposed the salinity stress of 150 mM. Adverse effect of salt stress was seen after 3 days of the stress period in 2 weeks old seedling and it became drastic after 7 days of imposition of stress. Shoot length was significantly decreased in case of all the genotypes under salt stress as compared to the control plants.

The study of Chakraborty *et al*. (2019) revealed that, imposition of 8 dS m^{-1} of salt stress at booting stage caused more than 50% of reduction in yield in susceptible genotype (Sabita) but < 10% in case of tolerant (Pokkali). The susceptible genotype showed highest Yield Susceptibility Index (Yield-SSI) along with 55% spikelet sterility under prolong period of stress.

Four rice genotypes (FL478, Kamini, AC847 and IR29) were hydroponically grown and evaluated for salinity tolerance (EC 12 dS m^{-1}) at early seedling stage by Chakraborty *et al.* (2020). After a week of imposition of stress, the plants were scored on the basis of their Visual Salt Injury protocol by IIRI. IR29 was highly susceptible and was scored 9. It was observed that both root and shoot biomass in IR29 was drastically reduced under salt stress followed by AC847 and Kamini. Salinity also hampered the root, shoot length and relative water content (RWC) in all the genotypes as compared to the control plants.

2.2. Effect of salt stress on photosynthetic pigments, photosynthetic efficiency and chlorophyll fluorescence

Net photosynthesis and transpiration was highly affected in early seedling stage under salt stress. Salt stress did not affect the whole plant initially but the older leaves where the excess Na^+ accumulated was recorded with least rate of photosynthesis. There was a negative correlation between the Na^+ concentration and the net photosynthesis rate and the stomatal conductance was almost dropped to half of its initial under salt stress. Salinity also affected the stomatal aperture but the CO_2 concentration was remained unchanged in rice (Yeo and Flower, 1985)

Mishra *et al*. (1997) studied two rice varieties, Jaya (susceptible) and Damodar (resistant) to evaluate the salinity tolerance in 25day plant. It was observed that, Damodar had an increment in pigment and total protein content under stress however in Jaya the total chlorophyll content was reduced under stress. But in case of 15 days old plant, both the chlorophyll and carotinoid content was reduced under salt stress in both the cultivars.

Salinity evaluation of four rice genotypes i.e. two tolerant (Nona Bokra and Pokkali) and two susceptible (IR29 and IR8) revealed that with increment in NaCl content in leaves, the photosynthetic activity of PS I and PS II and electron transport were reduced more in sensitive cultivars as compared to tolerant. There was more reduction in PS II efficiency than PS I and gradual increment in non-photochemical quenching in susceptible genotypes as compared to tolerant (Tiwari *et al*., 1997).

Dionisio-Sese and Tobita (2000) studied the impact of 6 dS m^{-1} and 12 dS m^{-1} of salt stress on chlorophyll fluorescence and photosynthetic efficiency of four

cultivated rice genotypes. They found substantial reduction in carbon assimilation rate under salt stress in all the cultivars. However they found that, the Fv/Fm (maximum quantum yielding efficiency of PS II) was unchanged for Pokkali but the overall or actual efficiency of photochemical energy conversion (actual quantum yield Fv/Fm) declined with increasing salinity in all cultivars except Pokkali. The study revealed a decline in photosynthetic efficiency and enhanced non photochemical quenching in susceptible genotypes.

Sultana *et al*. (1999) conducted an experiment to evaluate salinity tolerance at the reproductive phase for *Oryza sativa* L. cv. Koshihikari, moderately salt resistant variety. The plants were irrigated with saline water (0, 25, 50, 100, and 200 mMNaCl) at a volume of 1.5 times that of the soil. Rate of photosynthesis was measured after 15days of imposition of stress. Drastic reduction was observed in the total chlorophyll and carotenoid content. NaCl stress led to a significant loss inpN,g_s and E in stressed plants as compared to the control plants. Net photosynthesis was hampered more in flowering stage than milking stage. About 50% drop in rate of pN was observed under stress. Similarly, about 35% of g_s (gas exchange) were dropped under stress in flowering stage.

According to Moradi and Ismail (2007) response to salt stress (12 dS m^{-1}) in both seedling stage and reproductive stage were similar, where rate of photosynthesis, stomatal conductance and transpiration rate was decreased more in susceptible genotype IR29 (48%). Chlorophyll fluorescence after 7 days of imposition of salt stress in seedling stage had no significant effect on quantum yield of PSII, where progressive increment in qN (Non-photochemical quenching) and ETR decreased progressively. However in reproductive stage dramatic change in qN was observed in all genotypes but it was highest in IR29 and salinity tolerant genotype IR651 there was minimum reduction in Φ_{PSII} under salt stress.

A salt stress of 200mM caused reduction in both chlorophyll a (33%) and chlorophyll b (41%) content after 14days of imposition of stress. Rate of photosynthesis was hampered due to decline in efficiency of PS II (Fv/Fm), photon yield and non-photochemical quenching which leads to reduction in growth (Mohammad, 2011).

Hao *et al*. (2012), investigated to understand the effect of salt stress on chlorophyll (Chl) content and fluorescence of salt-tolerate rice (*Oryza sativa* L.), of three different genotypes, V11, V12 and V13 and they were subjected to salinity levels of 0, 4, 8 $gpot^{-1}$ (S0, S1, S2) for 75 days. The total chlorophyll content and chla initially increased initially of three rice cultivars. F_0 (initial fluorescence) and F_m (maximum fluorescence) decreased, whereas the maximum quantum efficiency of PSII (F_v/F_m) increased gradually. Non-photochemical quenching NPQ in V11 and V12 decreased slightly, whereas NPQ in V13 were the opposite. There was no significant difference between three salt treatments and rice cultivars for Chl (a), Chl (b), Chl (a+b), F_m, ETR and NPQ. Leaf chlorophyll fluorescence response of different rice genotypes under stress was different and significant difference was found in high salt stress.

Studies of Sarkar *et al*. (2013) depicted that the chlorophyll content and photosynthetic rate varied greatly across the genotypes (FR13A, Rashpanjor, Pokkali and IR42) after 7 days of imposition of stress in seedling stage. In tolerant lines the chlorophyll content did not degrade up to 5 days of salt stress but in IR42 (66%) and FR13A (61%) the decrement was very rapid and high. The decrement shown the similar rate and was highest for IR42 after 7 days of imposition of salt stress. The chlorophyll fluorescence parameters like F_o, F_v, F_v/F_m and overall performance index (PIABS) changed with time in salt-treated plants compared to the control plants. Fv/Fm values were very lower for susceptible genotypes and almost constant for tolerant one (Pokkali).

Two contrasting rice genotype (Pokkali-tolerant and IR29-susceptible) when imposed to 13.25 dS m^{-1} of salt stress at booting stage, the sensitive variety (IR29) had 84% drop in total chlorophyll content, which was8 times more than the tolerant (Pokkali). The degradation of cholorophyll content directly affects the chlorophyll fluorescence parameters. A sharp decline in F_v/F_m value (20.8%) indicated the reduction in quantum yield capacity of PSII in case of IR29. Whereas, in Pokkali was able to stabilise the efficiency of PSII and *p*N (net photosynthesis), g_s (stomatal conductance)and *E* (rate of transpiration) of the flag leaf even under salt stress (Zeng and Shanon *et al*., 2000).

Total chlorophyll content decreased with increasein salt stress in all rice varieties in 4 dSm^{-1}, Pokkali and MR211 consisted of comparatively higher amounts of

total chlorophyll. MR211, Pokkali, and M232 showed lesser reductions, while severe reductions were observed in IR20, BRRI dhan29, and MR219 due to salt stresses at 8 and 12 dSm^{-1}. The chlorophyll a/b ratio varied significantly with salinity levels but there was no specific trend was found (Hakim *et al*., 2014).

The chlorophyll content and chlorophyll fluorescence of 42 different rice varieties were recorded under different level of salinity (Control, 6 dS m^{-1} and 12 dS m^{-1}) at seedling stage after 14 days of imposition of stress. FL478, Hassani, Shahpasand, Gharib and Nemat showed signs of tolerance and had significant higher amount of chlorophyll under salt stress. Under normal conditions, Fv/Fm was recorded in the range of 0.75–0.80 for all the rice varieties. Under the salt stress conditions, this ratio was reduced about 7–19.69% in all the varieties except some tolerant genotypes (Kordrostami *et al*., 2017).

Nounjan *et al*. (2018) was carried out an experiment to find out the physiological response of four rice genotype (CSSL8-94, CSSL8-95, KhawDawk Mali 105-sensitive and Pokkali- tolerant) under salt stress of 150 mM NaCl. After 9 days of imposition of salt stress significant decrement in total chlorophyll content and net photosynthetic rate were recorded in all the genotypes except for Pokkali. However the PSII efficiency (F_v/F_m value) was not that much affected even under stress plants.

Impact of salt stress on chlorophyll content and chlorophyll fluorescence in reproductive stage was analysed by Chakraborty *et al*. (2019). Where there was no significant difference was found in initial and maximum fluorescence in flag leaf after 2 weeks of imposition of salt stress in tolerant and moderately tolerant genotypes. In case of sensitive genotype (Sabita) the drop in values of both initial (F_0) and maximum fluorescence (F_m) was very much significant. However there were no remarkable changes observed in Fv/F_m values under saline treatment.

Gerona *et al*. (2019) reported that, estimated the chlorophyll concentration of first, second and third leaf from the top of 6 different rice genotypes (IR686, Sadri, CSR28, IR670, Rc222 and Pokkali) at flowering stage under 10 dS m^{-1} of salt stress. On an average, the chlorophyll concentration was decreased in all the genotypes and in all the leaves but more than 50% in case of susceptible genotypes. While in tolerant

ones (Pokkali) showed relatively smaller corresponding reductions of 21%, 28% and 40%.

Pattanagul and Thitisakasakul (2008) reported severe loss in photosynthesis rate and growth of young seedlings of two varieties of rice (Pokkali-tolerant and KhaoDawkmali 105- sensitive). Rice was grown for 14 days and there was slight reduction in net photosynthesis in both the genotypes. But in KMDL more than 50% of stomatal conductance and transpiration rate was hampered as compared to control under stress. PSII efficiency was highly compromised under stress in both of the genotypes as compared to normally grown plants.

2.3. Effect of salt stress on the tissue ion (Na^+ and K^+) concentration and Na^+/K^+ ratio

Dionisio-Sese and Tobita (2000), studied the impact of 6 dS m^{-1} and 12 dS m^{-1} of salt stress on four contrasting rice genotypes (Hitombore, Bankat, IR29-susceptible check and Pokkali-tolerant check). Na^+/K^+ ratio was effectively in all the genotypes as compared to control except for Pokkali in both the level of stress. Susceptible genotype IR29 had highest leaf Na^+/K^+ ratio in 12 dS m^{-1} of salt stress followed by Hitombore. Tolerant genotype (Pokkali) was able to sustain the stress by minimising the Na^+ uptake through the period of stress.

Moradi and Ismail (2007) conducted an experiment by taking three contrasting rice genotypes (IR651, IR632 and IR29). Evaluation for salt tolerance was done in both seedling and reproductive stage. Significant increment in Na^+ content was at both the stages as compared to the control plants. Susceptible genotypes were observed to have lower K^+/Na^+ ratio in its leaf tissues. Similarly in reproductive stage, IR29 had lowest K^+/Na^+ as compared to other genotypes.

Salinity stress of 8dS m^{-1} was negatively correlated with Na^+ and positively correlated with K^+ concentration. The increase in Na^+ and decrease in K^+ concentration resulted in increase in Na^+/K^+ ratio (Gholizadeh and Navabpour, 2011). Increased level of Na^+ and elevated level of Na^+/K^+ ratio in response to salinity was negatively correlated with the level of tolerance. On the other hand the K^+ retention ability in the shoots of the genotypes at early seedling stage was positively correlated with the growth rate and biomass accumulation even under stress condition. The lowest leaf of

the tolerant genotypes were observed to have highest Na^+/K^+ ratio as compared to the growing tip of the plants.

One hundred and six different rice genotypes were subjected to 12 dS m^{-1} of salt stress at early seedling stage in hydroponics solution. Salinity had significantly altered the tissue Na^+, K^+, Na^+/ K^+ in all the genotypes including the tolerant check (Pokkali) as compared to control plant. Susceptible genotypes had high Na^+ content and significantly low content of K^+ in leaf tissues. Na^+/ K^+ ratio had increased with increased susceptibility (Kanawapee *et al.*, 2012).

Sarkar *et al.* (2013) reported that increase in Na^+/ K^+ ratio was accelerated under salinity stress. Absorbance of excessive Na^+ and less K^+ retention in the actively growing tissues were one of the major criteria of susceptibility, observed in IR29 in seedling stage. About 200% increments in the ratio of Na^+/ K^+ were observed under 12 dS m^1 of salt stress as compared to tolerant genotype (Pokkali).

Influence of two different levels of salinity (6 dS m^{-1} and 12 dS m^{-1}) was evaluated by Kordrostami *et al*. (2017) for 42 different rice varieties at seedling stage and the Na^+, K^+ and Na^+/K^+ ratio was estimated after 14 days of imposition of stress. The association analysis and the correlation study of Na^+/K^+ ratio with tolerance score, plant growth and chlorophyll content were highly negatively correlated.

Zhang *et al*. (2018), studied the impact of salinity stress on the germination and seedling growth of two weedy (wild) rice cultivar (*JYGY-1 and JYFN-4*) and two cultivated rice genotype (*Nipponbare and 9311)*. They raised the plants in hydroponics solution for 2 weeks and imposed the salinity stress of 150 mM. After 7 days of salt treatment they observed remarkable change in Na^+/ K^+ ratio in both root and shoot of all the treated plants. Under stress significant increment of Na^+ content was observed in both root and shoot of susceptible genotype, while it was much lower in case of tolerant *JYGY-1*. Similarly, the K^+ content in the tissues of *JYGY-1* (tolerant) was highest amongst all the genotypes.

Gerona *et al*. (2019), measured the Na^+, K^+ content and Na^+/K^+ ratio from the first, second and third leaves from the top and their corresponding leaf sheaths and reproductive parts (main stalk and branches with spikelet) in 6 contrasting rice genotypes at reproductive stage. The study showed significant high Na^+ accumulation

in both vegetative and reproductive part in case of susceptible genotypes. Higher accumulation of Na^+ in the older leaf and lesser accumulation were observed in tolerant genotypes.

Tabassum *et al*. (2021) evaluated 12 genotypes under three different level of salt stress of 0, 8 and12 dS m^1 in seedling stage and for reproductive stage at 4 and 6 dS m^1.This study mentioned susceptible genotypes were having highest Na^+/K^+ in the shoot and the tolerant genotypes maintained low Na^+/K^+ in the leaf as compared to the susceptible one.

Yong *et al*. (2022) conducted a greenhouse trial to evaluate the salinity tolerance in seedling stage in six wild species, one cultivated rice (IR64) and one land race (Pokkali). High variability was noticed in Na^+ and K^+ fluxes across the leaves of stressed plants. In controlled condition net Na^+ flux was zero, while in salinity, IR29 had about 150 nmol $m^{-2}\,s^{-1}$. Similarly the K^+ flux was drastically shifted to negative value except for *O. coarctata* under salinity. A strong positive correlation was also noticed in shift of Na^+ and K^+ fluxes and growth parameters.

2.4. Effect of salt stress on water status of the plant

Relative water content (RWC) used as one of the important tools for screening of sixteen rice genotypes subjected to 6 dS m^{-1} of salt stress up to 2 weeks. The genotypes with less salinity injury symptoms were seen to having high RWC. Cultivars with medium RWC had less salinity injury score. RWC trait was strongly and directly correlated with low Na^+/K^+ ratio and salt injury score. Therefore, RWC was considered as an effective, cheap and important tool for salinity tolerant in rice genotypes (Suriya-arunoj *et al*., 2004).

Two contrasting rice PR-115 and Super-7 were evaluated under water stress on the basis of different morphological conditions and different physiological condition (electrolyte leakage, osmotic potential RWC and MDA content). Tolerant genotype with high RWC was seen to maintain low electrolyte leakage, low MDA enhancement and low non-photochemical quenching (Khan *et al*., 2017)

Salt stress (100mM NaCl) was imposed to 7 days old rice plants (Ashfal, Benapol, Jamainaru, Gunshi, Mohini and BRRIdhan 29 and Pokkali) and significant

decline in relative water content (RWC) was observed as compared to their respective control plants. Least (6.6%) decline was observed in Pokkali but more than 10% drop in RWC was observed in rest of the genotypes (Polash *et al*., 2018).

Chakraborty *et al*. (2020), evaluated 4 genotypes for understanding the salt tolerance genotypes in seedling stage under 12 dS m^{-1} of NaCl stress. After 7 days of imposing of salt stress plants were evaluated on the basis of different morpho-physiological characters like shoot and root length, plant dry weight, chlorophyll content, tissue Na^+ and K^+ content, relative water content (RWC) and leaf water potential. Tolerant genotype (FL478) was able to maintain high water content and water potential inside the leaf tissues, while in susceptible (IR29) salt stress highly affected leaf water potential.

2.5. Effect of salt stress on production of compatible osmolytes

Salinity reduces the growth of plant through osmotic effects, reduces the ability of plants to take up water and this causes reduction in growth. There may be salt specific effects. If excessive amount of salt enters the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence, and reduces the photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002)

Proline content in flag leaf at the time of flowering was increased significantly in susceptible genotype IR29 (about 369%) and least in IR651 (35%) under 12 ds m^{-1} of salt stress. However, in the control plant highest proline concentration was observed in IR651 (Moradi and Ismail 2007)

Chutipaijit *et al*. (2009) did comparative studies of differential response to salinity in four varieties of *indica* rice . Proline accumulation relative water content and MDA content response were studied under 100mM of salt stress during early seeding stage. RWC of tolerant genotypes (KDML 105 and SY) was decreased by0.5% but in susceptible lines it exceeded 1%. The tolerant lines had less membrane damage (lipid peroxidation; 7.94-19.26%) and enhanced proline level ((56.56-78.56%) than sensitive genotypes.

Hakim *et al.* (2014) studied the proline accumulation in leaves under 4,8 and 12 dS m^{-1} of salt stress in six different rice genotypes along with one tolerant (Pokkali and one susceptible check. Initially the proline content did not significantly increase under 4 dS m^{-1} of salt stress but the content was gradually increased with increase in level of salinity.

Nguyen *et al*. (2021) studied relationship associated with rapid proline accumulation and salinity tolerance in Australian wild rice *Oryza australiensis*. 150mM of NaCl stress was imposed for about 14 days of *O. australiensis*, cultivated *O. Sativa* group, Nipponbare and pokkali and the results shown there was a rapid accumulation free proline in *O. australiensis* and suddenly lowers down the osmotic potential of the plant tissue. Both Pokkali and *O. australiensis* was able to maintain leaf water potential and cell membrane integrity after exposure to salt stress while Nipponbare was failed to do so.

Rahman *et al*. (2016) conducted experiment where150 mMNaCl salt stress induced severe oxidative damage in 12 days old rice cultivar (BRRI dhan47). Leaf water potential was decreased in the plants under salt stress. However increment in proline content eventually increased osmotic potential under stress. 6. Exogenous application of Mn eliminated the osmotic stress and reduced the proline production in stress induced plants.

Abdallaha *et al.* (2016) also reported elevated level of trehalose content in the seeds of two rice genotypes (Giza 177 and Giza 178) increased the soluble sugar content and the activities of SOD, CAT and POX with the increased level of salinity.

Nounjan *et al*. (2018) was carried out an experiment to find out the physiological response of four rice genotype (CSSL8-94, CSSL8-95, KhawDawk Mali 105-sensitive check and Pokkali- tolerant check) under salt stress of 150 mMNaCl. A drastic increment in proline content was observed in all the four genotypes. However, it was highest in CSSL8-94 followed by Pokkali, which signified the better osmotic adjustment of tolerant lines under salt stress. Accumulation of sugars was also observed in all the genotypes under stress. About 30% increment in soluble sugar was observed in treated Pokkali plants as compared to controlled plants.

An extremely significant influence of salt stress (6 dS m^{-1}) on the increase of proline and glycine betaine in rice seedlings were detected in mutant rice as compared to the wid type. The proline accumulation was notably high in mutant than wild type under 100 mMNaCl. The increment in glycine betaine and proline content in the shoot of mutant reflects the higher osmotic protection against the salt stress. Trehalose is a natural non reducing sugar accumulated in high amount in the stem of mutant genotype under salt stress (Forough *et al.*, 2019).

2.6. Effect of salinity on ROS production, membrane damage and enzymatic scavenging system

Vaidyanath *et al.*, 2003 reported elevation in lipid peroxidation and H_2O_2 content in leaf tissues of two contrasting genotypes (Pokkali and Pusa Basmati 1) under varying concentration of salt stress of 100, 150, 200, 250 and 300 mMNaCl. In response to salt stress tolerant genotype had high increment in catalase, proline and ascorbate activity in both shoot and root tissues which positively correlated with the lesser membrane damage as compared to the susceptible genotype.

Salinity enhanced the oxidative damage and ROS scavenging enzyme activity in two contrasting rice cultivars (Pokkali- tolerant and IR28- susceptible). MDA content rapidly increased in root tissue of susceptible genotype IR28, while Pokkali had no symptoms of salt injury after 120 mM of salt stress. Basal level of CAT and SOD activity was higher than IR28. However the activity of POX was highly induced in IR28 than Pokkali after a week of imposing salt stress (Demiaral and Turkan *et al.*, 2005).

Salt stress imposed at both seedling stage and reproductive stage in three contrasting rice cultivars (IR651, IR632 and IR29) was shown high degree of oxidative damage of 6 and 12 dS m^{-1} (at seedling stage) and 6 dS m^{-1} at reproductive stage. However the MDA content and proline content was increased in all the genotypes but more at higher level of salinity irrespective of any growth stage. But elevation in activity of ROS scavenging enzymes (SOD, APX and GR) was high in IR632 (tolerant) and was least in IR29 (susceptible). However, the activity of SOD and GR was greatly induced in 12 dS m^{-1} of salt stress as compared to 6 dS m^{-1} (Moradi and Ismail, 2007).

Kumara *et al*. (2009) studied the antioxidant enzyme activity in three different rice cultivars Panvel-3 (tolerant), Kalarata (moderately tolerant) and Karjat-3 (sensitive) under 0 and 300 mM of NaCl stress. In sensitive cultivars (Karjat-3 and Kalarata) both SOD and GR activity was reduced under stress, but induced in Panvel-3. Similarly higher activity of both POX and CAT was observed in the stressed Panvel-3 plants. Salinity caused several disturbances in maintaining the plants osmotic potential and produced several reactive oxygen species. Over production of ROS hampers the nutritional deficiency and hampers the physiological functions (Munns and Tester 2008).

According to Umed *et al*. (2008) almost 2-3 folds of increment in H_2O_2 and TBARS content under 100 and 200 mM of salt stress with respect to the control plants in response to salinity stress in all the genotypes.

According to Gill and Tuteja (2010), plants produce some amount of reactive oxygen species (ROS) under normal condition during photo respiration in mitochondria, chloroplast, peroxisome and plasma membranes. Besides several environmental cues has been creating unfavourable situation and hindered the availability of ample amount of CO_2which affects the carbohydrate fixation and contributes to excessive ROS production in chloroplast.

Gill *et al*. (2015) Revealed that, induction in three main categories of Superoxide dismutase (SOD; EC 1.15.1.1) Cu/Zn-SOD, Fe-SOD, and Mn-SOD, which leads the frontline defence in the antioxidant defence system by dismutating O^{2-} into H_2O_2 and reducing the possibility of OH^- formation

Rahman *et al*. (2016), were hydroponically grown 12 days old rice cultivar (BRRI dhan47) and subjected to 150mM of NaCl salt stress and studied the ROS generation and both enzymatic and non-enzymatic scavenging cascade under salt stress. Exposure to salt stress significantly increased the SOD, APX and GPX activities with the increasing duration of salt stress as compared to the control plants. While the CAT activity was declined with increase in duration of salt stress in the stressed seedlings.

According to Mehla *et al*. (2017), Catalase (CAT; EC 1.11.1.6) is a tetrameric heme-containing enzyme highly induced under abiotic stress for ROS detoxification, which converts 26 million H_2O_2 molecules into H_2O in 1 minute.

Forough *et al.* (2019) assessed the response of Hashemi rice genotype and its mutant to salinity stress of 100 mM of NaCl stress. Accumulation of high amount of H_2O_2 and increased lipoxygenase activity in the shoot of wild type as compared to mutant was the result of more oxidative stress in plant as compared to its control plants.

Jovanovic *et al.* (2018) revealed that another member of anti oxidant scavenging enzyme, Peroxidase (EC. 1.11.1.7) mainly oxidizes PhOH for producing phenoxyl radical (PhO^-) more commonly referred to Q_A, where H_2O_2 accepts electron and is converted to H_2O.

According to Nounjan *et al.* (2018), four rice genotypes (CSSL8-94, CSSL8-95, KhawDawk Mali 105-sensitive check and Pokkali- tolerant check) when imposed to 150 mM of NaCl stress showed elevated level of electrolyte leakage (about 95%) in salt sensitive cultivar KhawDawk Mali 105 as compared to rest three after 9 days of imposing salt stress. However the MDA content was lowest in tolerant genotype Pokkali and highest in sensitive KhawDawk Mali 105 (42%) as compared to control plants.

In tomato plants over accumulation of O_2^- (157%) and H_2O_2 (176%) increased the cellular damage with high MDA (94%) content and electrolyte (EL) by about 158% under 100mM of NaCl-induced salt stress (Ahanger *et al.*, 2020).

Three weeks old rice plant (*Oryza sativa* L. cv. Nipponbare) was subjected to 200 mM of NaCl stress and the oxidative stress was measured in the apical and basal region of the leaves. NBT content, an indicator of O_2^{-1} generation, H_2O_2 damage and MDA production was incredibly high in the apical region after 12 hrs of stress and even more after 24-72 hrs. After 48 hrs of stress MDA and H_2O_2 content was also significantly increased and caused membrane damage. In response to the oxidative damage SOD activity was slightly increased in the apical region. However the activity of H_2O_2 scavenging enzyme APX and CAT was not differ significantly from control plants but the activity was decreased after 48 hrs of stress (Yamane *et al.*, 2009).

2.7. Molecular mechanism of salt tolerance

According to the reports of Matsumoto and Chung (1988), in the root tissues of barley high activity of vacuolar Na^+/H^+ antiporter was functioned with H^+ driving force provided by the H^+ pimps located in the vacuolar tissues in response to NaCl stress.

Niu *et al*. (1993) studied the salt stress induced regulation in H^+-ATPase gene expression in glycophyte tobacco (*Nicotiana tabacum* 1. var Wisconsin 38) and the halophyte *Atriplex nummularia* L.). Results of the study showed that, the mRNA transcripts of H^+-ATPase was more pronounced in the roots of halophyte than glycophyte in response to NaCl stress. After 8 hours of exposure to salt stress (400mM), H^+-electrochemical gradient was induced several folds in order to maintain the ion homeostasis for salt adaptation in halophyte.

Shi *et al*. (2000) examined the tissue specific pattern of gene expression and sub cellular localization of SOS1 in Arabidopsis. The results suggested that, at the root-soil interface, SOS1 would act extruding the excess of Na^+ ions from root epidermal cells. In addition, analysis of the Na^+ root-shoot partition in the *sos1* mutant under different saline regimes indicated that SOS1 also participated in the redistribution of Na^+ between roots and shoot in a complex manner.

Blumward (2000) reported that considerable amount of Na^+ sequestered inside the vacuole by a Na^+/H^+ antiporter, which was energised by tonoplast proton pumps and excess Na^+ was driven inside by the electrochemical gradient generated across the vacuolar membrane by V-ATPase and V-Ppase.

Guo *et al.* (2012) hypothesised the role of *SOS1* involving in regulating K^+ and Na^+ co transport in a fuctional model. They reported that plasma membrane Na^+/K^+ antiporter (SOS1) was involved in Na^+ efflux and K^+ uptake for transport in *Puccinellia tenuiflora (PtSOS1)*. The expression of *PtSOS1* was observed in different concentration (25-150mM of NaCl) in root tissues. Significant correlation was observed in Selective transport and activity of *PtSOS1*.

Palmgren (2001) reported that plasma membrane proton pumps are mostly associated with the SOS family genes. To prevent accumulation of excess Na^+ retrieval to physiologically inactive cytosolic space is very much essential in order to achieve salt tolerance. For removal of sodium ions out of the cell, plasma membrane specific Na^+/H^+ pumps were actively generating the electrochemical proton gradient across the PM. These pumps remain active in normal condition but the upregulation under stress situation might provide better adaptability under salt stress

According to Maser *et al*. (2002) Na^+ transporter, *AtHKT1* was mostly expressed in the root stele and vascular culture. *athkt1* null plants were observed to have lower accumulation of Na^+ levels in roots and the disruption produced higher level of Na^+ in the shoot. Wild-type *AtHKT1* effectively regulates root/shoot Na^+ distribution and restricts the upward transport of Na^+ to leaves.

Studies of expression in xenopus oocytes by Berthomieu *et al*. (2004) indicated that *AtHKT1* was involved in Na^+ recirculation from shoots to roots, probably by mediating Na^+ loading into the phloem sap in shoots and unloading in roots. The recirculation was probably due to removal of large amounts of Na^+ from the shoot and playing a crucial role in plant tolerance to salt tolerance.

Golldack *et al*. (2003) have studied the cDNA transcript analysis of *OsAKT1* (homologous to inward-rectifying potassium channels of the AKT/KAT subfamily), located in the indica rice (*Oryza sativa* L.). High transcript abundance was found in the root tissues as compared to leaves. Differential salt tolerant rice (Pokkali, BK and IR29) lines were evaluated in150mM NaCl salt stress for 48 hours, suppression of *OsAKT1* transcripts in salt sensitive (IR29) leads to increase (5-10 folds) accumulation of Na^+, while in BK or Pokkali. The results of the above studies revealed that *OsAKT1*improves the overall tolerance in response to salinity.

Fuchs *et al*. (2005) has conducted patch clamp study on rice root protoplast to identify an inward rectifying K^+ channel. They were able to characterize *OsAKT1*, voltage dependant inward rectifying K^+ channel has been regulated by extra cellular Ca^{2+} ion in 4 day old rice seedlings under salt stress. It was found homologous to the Arabidopsis root inward rectifier (*AtAKT1*) and mRNA transcript of AKT1 was decreased in root protoplast in response to salt stress.

Ren *et al*. (2005) reported that *SKC1* (HKT1.5) was preferentially expressed in the xylem parenchyma cell. *SKC1* (*HKT 1.5*) is preferentially expressed in stem parenchyma and selectively transport Na^+ under salt stress Physiological analysis of *SKC1* was involved in regulating the K^+/Na^+ ratio in the shoot tissues and potential tool for salinity tolerance.

Martinez-Atienza *et al*., (2007) have identified and represented the functional characterisation of a rice plasma membrane Na^+/H^+ antiporter, a functional homologue

of *AtSOS, OsSOS1* actively reducing the cellular Na^+ level. It was also evident that Arabidopsis protein kinase SOS2 and Ca^{+2} – dependant activator SOS3 were also associated in the SOS salt tolerance pathway of cereals and monocots.

Fakuda *et al*. (2011) cloned vacuolar Na^+/H^+ antiporter gene (OsNHX1) and identified four NHX-type antiporter genes (OsNHX2, OsNHX3, OsNHX4 and OsNHX5) from rice (*Oryza sativa*). The results of their study suggested that, the major role of this gene family was to compartmentalized the Na^+ inside the vacuole of cytoplasm.

Horie *et al*. (2011), characterized two Na^+ insensitive K^+ transporters (*OsHAK2* and *OsHAK5* isolated from *Oryza sativa* cv. Nipponbare) by using bacterial expression system. When it was expressed in an *E. coli,* the results of the experiment showed that,*OsHAK5* was functioned as Na^+ insensitive K^+ transporters in the presence of large amount of extracellular Na^+, and selective for K^+ transport, while *OsHAK2* was sensitive to extracellular Na^+ and exhibit stronger affinity towards Na^+ than K^+. Apart from that, expression of *OsHAK5* in cultured-tobacco BY2 (*Nicotiana tabacum* cv. Bright Yellow 2) cells enhanced the accumulation of K^+ but not Na^+ in the cells during salt stress and conferred increased salt tolerance to the cells. It also confirmed the plasma membrane localized *OsHAK5* can be used as one of the important tool to enhance the salt tolerance in plants.

The study of Sassi *et al*. (2012) identified a new functional type in K^+ and Na^+ permeable HKT transporter subfamily *HKT2.4* displayed high permeability to K^+ as compared to Na^+ under low external Na^+ condition. In planta expression and physiological characterization of K^+ uptake by HAK5 was confirmed by the studies of Yang *et al.* (2014). Cellular and tissue localization of *OsHAK5* of the KT/HAK/KUP family in rice (*Oryza sativa*) was analyzed by using both *OsHAK5* knockout mutants and overexpression lines in three genetic backgrounds. The study revealed that K^+ influx and transport from the root to aerial parts was severely impaired by knockout of *OsHAK5* but the overexpression of the same improves both the transportation and influx in to the root cells. The overall results of the experiment revealed that *OsHAK5* plays a major role in K^+ acquisition by roots faced with low external K^+ and in K^+ upward transport from roots to shoots in K^+-deficient rice plants.

Chen *et al.* (2015) reported *OsHAK1*, belongs to the KT/KUP/HAK gene family mostly expressed in the steles of root and bundle sheath cells of shoot apical meristem tissues and up-regulated in under K^+ deficiency or salt stress. The study revealed that over expression of *OsHAK1* in rice increased the K^+/Na^+ ratio.

Wang *et al.* (2016) stated that HKT 1 family member *OsHKT 1.1* binds with *OsMYBc* transcription factor, expressed mostly in the phloem of leaf blades of rice (*Oryza sativa*) and plays an important role in Na^+ accumulation in the shoot portion and check further upward movement of Na^+.

Ahmad *et al*. (2016) characterized the main K^+ uptake channel, the K^+ inward rectifying channel AKT1 was done by with both loss and overexpression of *OsAKT1* in rice. Higher external Na^+ concentration in growth media suggested a strong correlation of AKT1 in Na^+ uptake in such conditions. The overall data suggested that overexpression of *OsAKT1* improved osmotic tolerance.

High affinity K^+ transport *OsHAK1* was reported to have major roles in K^+ retention and translocation in rice under osmotic and water stress as well. The transgenic lines with over expression of *OsHAK1*, was seen to have higher membrane stability and better growth rate than mutants (Chen *et al.*, 2015).

Another member of HKT1 family *HKT1.5* plays a major role in maintaining lower Na^+/K^+ ratio in the leaf tissues. Data observed from the real time PCR, microarray and RNA sequencing showed the preferential expression in the shoot portions rather than the root. The differential expression of HKT1.5 in tolerant was due to four amino acid substitution similar to halophytes. The resultant alteration in pore rigidity increases the likelihood of Na^+ transport from xylem sap to parenchyma and further to soil and this could probably help the tolerant plants to sustain the salt stress (Shohan *et al*., 2019).

Mahi *et al*. (2019), studied the salt tolerance response in a sos1-loss-function mutant of rice and the response was correlated with the Na^+ loading in the xylem. The result shows acute Na^+ sensitivity in the root of the *sos1* mutant. In the wild type mutant severe down regulation of salt stress related genes were also observed. Thus, this study suggested the Na^+/ H^+ exchanger SOS1 is one of the major players in

regulating the salt tolerance in rice by controlling the upward transport of Na^+ in the xylem stream.

Musavizadeh *et al*. (2021), studied two *OsAKT*and three *OsKAT* family genes for understanding their physiochemical properties associated with salt tolerance and characterization was done on the basis of protein structure, evolution, duplication, in silico gene expression, and protein–protein interactions. Based on the real-time PCR results revealed that under salt stress *OsAKTs* and *OsKATs* are highly induced in root and shoot tissues of rice cultivars and *OsKAT1* was identified as one of the key gene involved in the rice response to salt stress.

Farooq *et al.* (2021), investigated the role of nine high-affinity K^+ transporter (HKT) encoding Na^+-K^+ symporter five *OsNHX* Na^+/H^+ antiporters, and *OsSOS1* Na^+/K^+ antiporter genes. The results revealed that Na^+/H^+ antiporters (*OsNHX* and *OsSOS1*) was related to maintain Na^+/K^+ homeostasis in different rice genotypes under salt stress. High *OsNHX* and *OsSOS1* regulated Na^+ inside the cells effectively. In tolerant (Nagdong and Pokkali) genotypes higher gene expression was observed as compared to susceptible (Cheongcheong and IR28).

Solis *et al*. (2022) deciphered the role of NHX family genes in different cultivated and wild rice species. The study revealed that NHX1 and SOS1/NHX7confered contribution of tissue tolerance nature crucial in wild rice. Major function of NHX family genes was to accumulate and restrict the Na^+ in the mesophyll cells if wild rice *O. alta*, *O. latifolia*, and *O. coarctata*.

MATERIALS AND METHODS

This chapter explicates the comprehensive background of studied genotypes, the experimental approach as well as the techniques and methodology used throughout the whole experiment. This present study entitled "Differential salt tolerance mechanism in rice (*Oryza sativa* L.) at early seedling and reproductive stages" was conducted to understand the underlying mechanisms of differential salt tolerance response of ten different rice genotypes at both stages. The plants were grown in the net house of the Crop Physiology and Biochemistry division of ICAR-NRRI, Cuttack (85°55' 48''E–85°56'48''E and 20°26'35''N–20°27'20''N).

3.1. Details of experimental materials and conditions

Initially, 175 rice germplasm were collected from different coastal areas of India and screened for understanding their differential salt tolerance response to salinity by Chattopadhyay *et al*. (2018) at NRRI, Cuttack. Out of that, a panel of ten genotypes was selected including one susceptible (IR29) and one tolerant (FL478) checks for further investigation. Seed materials used for this study were collected from the ICAR-NRRI gene bank. At first, all ten genotypes were evaluated separately in both the early seedling and reproductive stages for two consecutive *kharif* and *rabi* seasons of 2020 and 2021. In both the stages the plants were evaluated on the basis of different morpho-physiological parameters and in reproductive, along with morpho-physiological parameters some yield-related attributes were also taken into account. Based on their performance in both the stages and in both seasons, four genotypes with stage-specific variations in salt-tolerance ability were selected for further studies to elucidate the underlying differential tolerance mechanisms.

Table 1. Brief description of the selected genotypes used in the experiment

Genotypes	Duration (Days)	Characteristics
FL478	90-100	A salt-tolerant recombinant inbred line was derived from Pokkali (Landrace) × IR29. It is an international salt-tolerant check at the seedling stage
IR29	90-100	A released variety from IRRI (International Rice Research Institute, Manila, Philippines) used as a susceptible check in salinity studies
AC41585	115–120	Pokkali type cultivar collected from the brackish waterlogged region of Kerala
Sadri	90-100	An Iranian landrace imported from IRRI, moderately tolerant to the salinity stress at the seedling stage, but tolerant to reproductive stage stress
AC39416A	115–120	Pokkali type cultivar collected from the brackish waterlogged region of Kerala
Rashpanjor (IC 575321)	145-155	A landrace collected from the coastal saline rice-growing areas of Odisha and WB
CSR27	110-120	A salinity and sodicity tolerant cultivar released from CSSRI, Karnal, Haryana
Binadhan 8	120-130	Medium stature salt-tolerant cultivar, originally released for cultivation in saline areas of Bangladesh
Luna Suvarna	140-150	A released salt tolerant cultivar from NRRI, Cuttack developed for the saline coastal area of Odisha
Sabita	150-160	A late maturing cultivar released by NRRI, Cuttack for RSL ecology and used as a susceptible check for reproductive stage salt stress

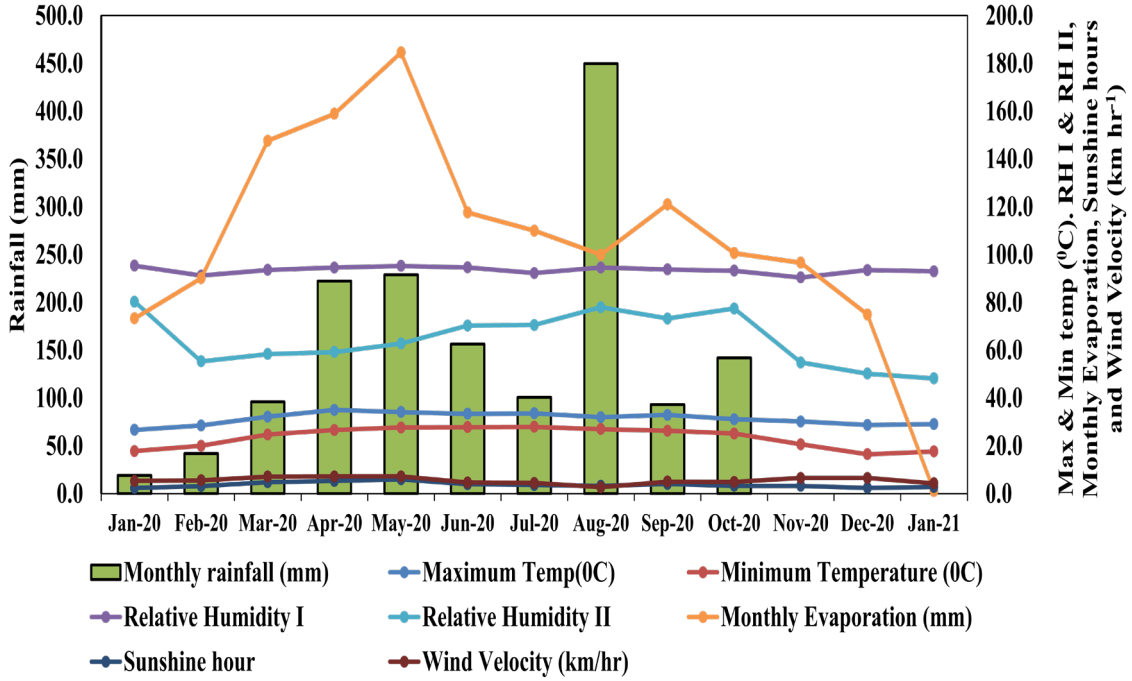


Figure 1. Meteorological parameters during crop growth period -2020

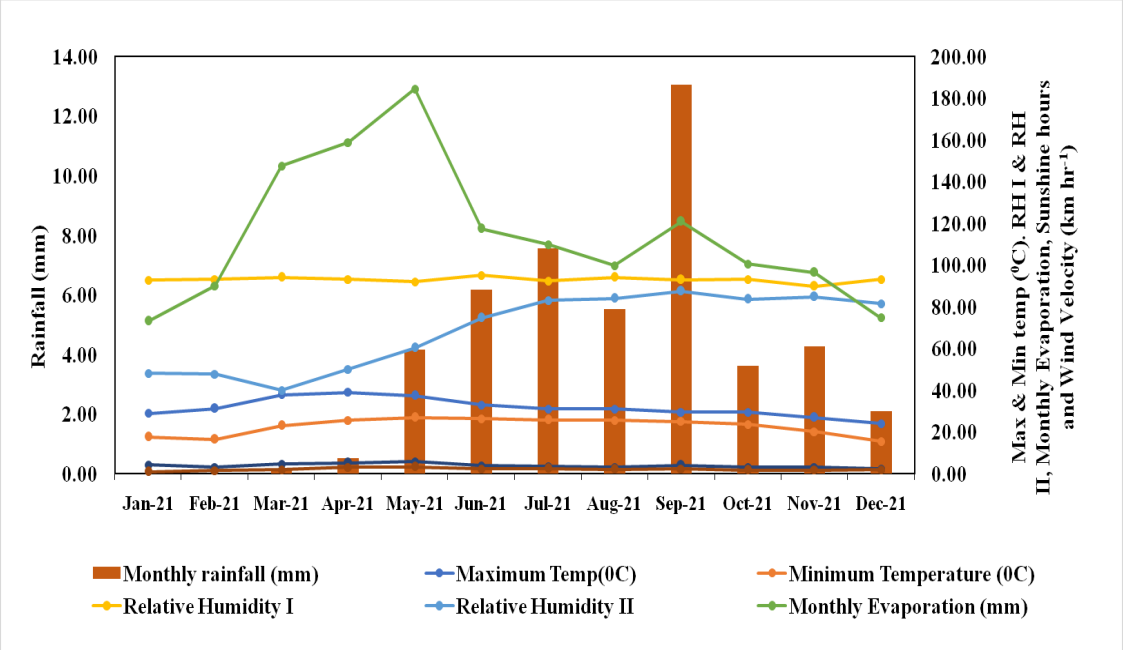


Figure 2. Meteorological parameters during crop growth period -2021

3.2. Experimental setup for evaluation of salinity tolerance at the early seedling stage

The assessment of salt tolerance at the early seedling stage was done for two seasons i.e. *rabi* 2020 and 2021. The whole experiment was conducted in the net house of Crop Physiology and Biochemistry Division of ICAR-NRRI, Cuttack in Factorial CRD experimental design, where one factor was treatment (control and stress) and another one was genotype. To break the dormancy the seeds were preheated at 45°C for 5 days. For better germination, the seeds were surface sterilized with 70% ethanol and repeatedly washed with distilled water. They were then placed on moistened paper within petri plates for 2 days in the dark for better germination. The pre-germinated seeds were planted into the floating 10x10 styrofoam panel after germination, one seed in each hole. The styrofoams were placed on the trays filled with Yosidha nutrient solution (Gregorio *et al*., 1997). The plants were allowed to grow normally until they reached the 3-4 leaf stage, with the pH of 5.0 being periodically maintained. One set of plants was imposed to salt stress EC (electrical conductance) 6 dS m^{-1} for 2 days to avoid the immediate shock of salt stress, and then they were subjected to salt stress of 12 dS m^{-1}. Another set of plants was grown normally in the Yosidha solution. The plants were kept under salt stress until most of the IR29 genotype (Susceptible check) got a Visual Salt Injury score of 9 (SES score, IRRI).

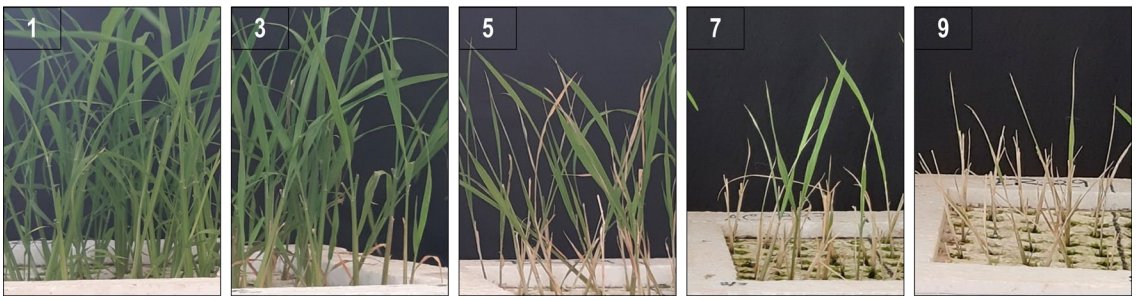
3.2.1. Evaluation of morphological parameters at the seedling stage

3.2.1.1. Visual Salt Injury (VSI) and standard evaluation score at the early seedling stage

In response to salt stress at early seedling stage, all the 10 genotypes were given an SES score by following the standard protocol developed by IRRI (Gregario *et al*., 1997). On a scale of 1 to 9, the stressed plants were rated based on the visible salt damage. A plant with no visible signs of damage was given a score of 1 and was considered to be extremely tolerant, while a very susceptible plant with significant damage to the stalk and total chlorosis of the leaves was given a score of 9. The tolerant, moderately tolerant, and susceptible genotypes were given the intermediate SES scores of 3, 5, and 7 respectively. The SES scoring was done for all the hydroponics trays under both controlled and stressed conditions individually.

3.2.1.2. Total plant biomass (g)

For evaluation of total plant biomass at the early seedling stage, three plants from each of the genotypes were collected from the hydroponics solution after IR29 attained an SES score of 9. Samples in three replicates were kept in the oven at 80°C for 7-8 days until the samples were completely dried. After complete drying, the weight of the individual plant was taken with the help of a weighing machine and the mean was expressed in terms of mg.



SES Score	Damage symptoms	Tolerant/Susceptible
1	Normal growth and no symptoms of leaf rolling	Highly tolerant
3	Few leaves showed leaf rolling on tips	Tolerant
5	Retardation of growth was associated with leaf rolling	Moderately tolerant
7	Growth completely stopped. Most leaves were rolled. Some plants are dying	Susceptible
9	Majority plants died	Highly susceptible

Figure 3. Image showing SES Score and Visual Salt Injury (VSI) in plants in response to salt stress at the early seedling stage

3.2.1.3. Root and shoot length (cm)

Both root and shoot length was measured at the early seedling stage for each genotype × treatment combination. Three plants from each combination were taken out after IR29 attained an SES score of 9. The shoot and root were separated manually and the length was measured with the help of a scale and expressed in centimetres (cm).

3.3. Experimental setup for evaluation of salinity tolerance at the reproductive stage

In *kharif* 2020 and 2021, the same panel of genotypes was evaluated for differential salt tolerance response in the reproductive stage. The experiment was performed by using the standard procedure as previously described in Chattopadhyay *et al*. (2018) and the experimental design was Factorial CRD. The seeds were soaked

overnight and allowed to germinate by placing them on moistened filter paper kept inside the petri dish. The sprouted seeds were transferred to the small earthen pots containing soil. The plants were allowed to grow for about 4 weeks until they attained desirable vigour for transplanting into specialized pots. The plants were transplanted into specialized perforated pots (12-inch diameter) containing a mixture of soil (80%) and gravel (20%). In these pots, gravels of three different sizes were arranged according to their size. Gravels of 10-15 mm diameter (large) were placed at the bottom of the pot and above that gravels of 6-8 mm (medium) size are placed and the smallest gravels (2-3 mm) were placed at the top. Above the layers of gravel, a thin layer of fine sand of about 2-3 cm was placed and the rest of the pot was filled with well-grounded and sieved soil (texture sandy loam and pH 6.7). The experiment was conducted following the Completely Randomized Design (CRD) with three replications. All the plants were grown under normal conditions until they reached the booting stage. As the genotypes belonged to different maturity groups, they came to flower at different time points. Just before the flowering i.e. at the booting stage, one set of the plants was imposed with salt stress of 8 dS m^{-1} whereas the other set was allowed to grow under normal conditions till maturity. Three pots of each genotype were placed inside a circular tub (0.5m height and 1m diameter) for the imposition of salt stress. Each tank was provided with saline water of 8 dS m^{-1}, which was sufficient to saturate the soil of the perforated pots. Application of N:P:K (100:50:50 kg ha^{-1}) was done as per the recommended fertilizer dose.

3.3.1. Evaluation of morphological parameters at the reproductive stage

3.3.1.1. Plant height (cm)

The height of the plant was taken at the time of harvesting with the help of the scale for each genotype × treatment combination in three replications and expressed in centimetres (cm).

3.3.1.2. Number of panicles per plant

Numbers of panicles developed were counted in three replications and for each genotype × treatment combination at the time of harvesting.

3.3.1.3. Panicle length (cm)

Whole matured panicles were plucked from three replications of each genotype × treatment combination at the time of harvesting. The length was measured with the help of a scale and expressed in centimetres (cm).

3.3.1.4. Days to 50% flowering

After the imposition of salt stress at the time of booting, the flowering pattern was observed for each genotype × treatment combination. The days required to attain 50% of flowering were recorded in three replications and the mean value was taken into account.

3.3.1.5. Spikelet sterility percentage (STE), Spikelet degeneration percentage (DEG) (Saha *et al*., 1998)

Spikelet sterility (STE) for each genotype × treatment combination was calculated by applying the following formula

$$\text{Spikelet sterility percentage (\%)} = \frac{\text{Numbers of unfilled grains}}{\text{Total numbers of spikelet}} \times 100$$

Rudimentary and vestigial branches of rachis were considered as degenerated spikelets (DEG) and the degeneration percentage was expressed as

$$\text{Spikelet degeneration percentage (DEG)} = \frac{\text{Numbers of DEG}}{\text{Total numbers of spikelet}} \text{x } 100$$

3.3.1.6. Straw dry weight

Straw dry weight was taken for each genotype × treatment combination in three replications. At the time of harvesting, plants were uprooted with the entire root system and then the shoot portion was separated carefully. The shoot portion was oven dried at 80°C for about a week until the weight became constant. After proper drying, the dry weight was measured with the help of a high-precision weighing machine and the value was expressed in terms of grams (g).

3.3.1.7. Yield and yield-related attributes

In the reproductive stage, after the plant attained full maturity, the yield was recorded from three individual replications and the mean value of three replications was estimated and expressed in terms of g/plant. The salt tolerance and susceptibility

indices of each genotype were calculated by using the following formulas (Bouslama and Schapaugh 1984)

1. Yield stability index (YSI) = Ys/Yp

2. Stress susceptibility index (SSI) = (1 − Ysi/Ypi)/SI;

SI = 1 − Ys/Yp (Fischer and Maurer 1978)

Where, Ysi = PY or yield attributing traits under stress, Ypi = PY or yield attributing traits under non-stress,

Ys and Yp are average yield/yield traits under stress and normal conditions, respectively.

3.4. Estimation of physiological attributes at both early seedling and reproductive stages

All the biochemical attributes were analyzed from the 2nd fully expanded leaf from the top at the seedling stage and the flag leaf for the reproductive stage from each genotype × treatment conditions was taken after 2 weeks of stress imposition in three replications.

3.4.1. Total chlorophyll content (Arnon, 1949)

The total chlorophyll content was estimated by the method given by Arnon (1949) from each genotype × treatment combination after the imposition of stress. At the early seedling stage, the leaf samples were collected from the 2nd leaf from the top, while in the reproductive stage, it was collected from the flag leaf. Fresh leaves of about 25 mg were collected and cut into small pieces. The pieces were taken in test tubes of 10 mL 80% v/v acetone and incubated for 48 hours in dark. After 2 days the absorbance of the extract was taken at 645 and 663 nm in a UV spectrophotometer (UV 2600, Shimadzu, Japan). The total chlorophyll content was calculated as per the formula.

Total chlorophyll content = (20.2× OD at 645 nm) + (8.02 × OD at 663 nm) ×V/1000×W

3.4.2. Chlorophyll fluorescence imaging (Pradhan *et al*., 2018)

ChlF imaging was observed from the same leaves used for chlorophyll estimation. Before the estimation of different fluorescence traits like maximum potential quantum efficiency of PSII (F_v/F_m) and quantum yield of non-regulated energy dissipation [Y(NO)], the leaves were incubated in dark for about half an hour. The traits were measured by an imaging fluorimeter (Imaging PAM—MAXI version, Heinz Walz, Effeltrich, Germany). The images were captured and analysed by using

Imaging Win v2.46i software supplied with the equipment for each genotype × treatment combination, for control and stressed leaves. In each leaf, the measurement was taken from three different points by making a uniform circular area (area of interest AOI) as described by Pradhan *et al.* 2018.

3.4.3. Estimation of tissue Sodium (Na^+), Potassium (K^+) concentration and selective transport of K^+ over Na^+

Assessment of tissue Na^+ and K^+ concentration at vegetative stage whole seedlings were taken out of the hydroponics solution after the SES score of IR29 reached the value 9. The fresh leaves (2^{nd} leaf from the top), stem and roots were separated carefully and dried in an oven at 60°C for a week. Similarly at the reproductive stage, after 30 days of salinity treatment, the plants were uprooted and the root was washed thoroughly with running tap water until totally clean. The developing panicle, flag leaf, middle leaves, stem and roots were carefully separated and dried in an oven at 60°C until all the samples attend a constant dry weight. For the estimation of tissue ion content (both Na^+ and K^+), extraction of 50 mg of the dried sample of each type from three replications was powdered and macerated in 50 mL of 0.1N HCl and kept for 48 hours. The extracts were then filtered by using Whatman #40 filter paper. The Na^+ and K^+ content in the extract measured by using a Flame Photometer (JENWAY PFP7 Photometer of Cole-Parmer scientific experts, India). The selective transport of K^+ over Na^+ was calculated by applying the following equation given by Guo *et al.* 2012: selective transport (K^+) = (K^+/Na^+ in plant part 'X')/(K^+/Na^+ in plant part 'Y'). Higher selective transport (K^+) values indicate a stronger capacity of transport of K^+ over Na^+ from plant tissue 'X' to plant tissue 'Y'.

3.4.4. Leaf gas exchange measurement

Different leaf gas exchange-related measurements like rate of photosynthesis, stomatal conductivity, and transpiration rate for each genotype × treatment combination at both the stages from fully expanded 2^{nd} leaf from the top at both seedling and reproductive stage by using an infra-red gas analyzer (IRGA, Licor 6400 XT, USA). The desired leaf was cleaned thoroughly with the help of clean tissue paper. Then the leaf was inserted into the leaf block, where its temperature was maintained at 25^oC. Two different concentrations of CO_2 were used for taking up reading from different

chambers. A 400 ppm concentration of reference CO_2 was used for FC-a (CO_2) and CC-a (CO_2), whereas, 550 ppm concentration was used for the CE-e (CO_2) condition. The flow rate of the leaf block was 500 ml min $^{-1}$ and the light intensity was maintained at 1200 µmol (photon) m^{-2} s^{-1}.This condition was maintained throughout the whole period while taking observations. The leaf samples were placed inside the chamber for at least 1 to 2 minutes. After achieving stability and the reading showed 2/3 or 3/3 the data was recorded. From the above observations, three different measurements were calculated –photosynthesis (denoted as 'A' and expressed as µmol CO_2 m^{-2} s^{-1}), transpiration rate (denoted as 'E' and expressed as mmol m^{-2} s^{-1}), and the stomatal conductance (denoted as 'g' and expressed as mol H_2O m^{-2} s^{-1}).

3.4.5. Relative water content (Barrs and Weatherly, 1962)

For measurement of relative water content, fresh leaves from each genotype × treatment combination were collected in three replications at both early seedling and reproductive stages. Then it was cut into pieces with a scissor and the fresh weight (FW) was taken immediately. After taking the fresh weight, the same pieces of leaves were placed inside petri plates filled with water and kept overnight. After 24 hours the turgid weight (TW) was recorded. Then the same piece of leaves was dried at 80^{o}C for 72 hours and the dry weight (DW) was taken. After taking the FW, TW, and DW, finally, the relative water content was calculated by applying the formula

$$\text{RWC\%} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

3.4.6. Leaf water potential (Chen *et al*., 2012)

Leaves from each genotype × treatment combinations were collected for measuring the leaf water potential. The leaves were cut into small discs (0.5 cm diameter) using scissors. The small leaf discs were placed on the disc chamber of the psychrometric water potential system (PS9PRO water potential system, Wescor, United States). The data was taken from the screen of the system after waiting for about 5 minutes. The water potential was expressed in terms of mega pascals (Mpa).

3.5. Estimation of different biochemical attributes at both early seedling and reproductive stages

3.5.1. Lipid peroxidation estimation (Heath and Packer, 1968)

MDA (Malonaldehyde) is the secondary end product of fatty acid oxidation and is considered one of the most important indices of lipid peroxidation. For the estimation fresh leaf sample of 500 mg fresh leaf samples were collected from each genotype × treatment combination. The leaf tissue was macerated and homogenized in 10 mL of 0.1% TCA. After homogenization, the extract was centrifuged at 15000 rpm for 15 minutes (HERMLE Z 32 HK centrifuge). The supernatant was further used for the malonaldehyde assay. 1 mL of aliquot was added with 4 mL of 0.5% of TBA in 20% TCA. Then the mixture was heated in the water bath for about 30 minutes at 95°C until the pink colour developed. The solution was cooled in the bath until it reaches room temperature. Again centrifuge it for 10 minutes at 10,000 rpm and the supernatant was collected. The observation was taken at 532 nm and 600 nm OD. The calculation was done by using the following formula

MDA equivalent (nmol. ml^{-1}) = $[(A_{532}-A_{600})/155000]^{10-6}$

3.5.2. Estimation of hydrogen peroxide (H_2O_2) content (Choudhury and Choudhuri 1985)

H_2O_2 content was measured from the fresh leaf tissues for each genotype × treatment combination by following the method described in Choudhury and Choudhuri, 1985. At first, about 200 mg of fresh leaves were macerated with 10 mL of chilled acetone and filtered the extract with filter paper. The extract was then added with 5 mL of ammonium solution and 4 mL of titanium reagent and centrifuged at 1000 rpm at 4°C for about 10 minutes and the supernatant was separated and used further. 10 mL of 2M H_2SO_4 was added and vortex for about 5 minutes and centrifuged for about 10 minutes. The supernatant was then used for measuring the H_2O_2 concentration at 415 nm OD.

3.5.3. Proline content estimation (Bates *et al*., 1973)

About 500 mg of fresh leaf samples from each genotype × treatment combination, was taken and crushed with 10 mL of 3% sulfosalicylic acid. Then the extract was filtered with Whatman #1 filter paper. 2 mL of the above filtrate was then

mixed with 2 mL of Acid ninhydrin solution and 2 mL of glacial acetic acid (Acid ninhydrin reagent was prepared separately by mixing the ninhydrin in a known amount of glacial acetic acid and 6M orthophosphoric acid). This solution mixture was boiled at 100°C for about an hour in the water bath. After boiling it was cooled to room temperature in an ice bath. Later 4 mL of toluene was added to the reaction mixture and thoroughly vortexed. The absorbance was taken at 520 nm of the upper toluene layer. The quantification was done by using the proline standard curve and expressed as mg g^{-1} FW of proline.

3.5.4. Glycine betaine estimation (Grieve and Grattan, 1983)

Glycine betaine is one of the quaternary ammonium osmolytes and it was estimated from the dried tissues. Leaf samples were collected from each genotype × treatment combination and dried at 80°C in a hot air oven for a week. After drying 0.5 g of leaf sample was crushed in 10 mL of deionized water and was kept for 48 hours at room temperature. The crushed solution was filtered with Whatman #1 filter paper and the filtrate was mixed in a ratio of 1:1 with 1N H_2SO_4 keeping it in an ice bath for one hour. After cooling 0.5 mL aliquot was mixed with 0.2 mL of cold potassium triiodide solution and stored in the freezer for about 12 hours. The next day the aliquot was centrifuged at 10,000 rpm for 10 minutes at 40°C temperature. After centrifugation small pallets were visible beneath the test tubes. The supernatant was discarded and dissolved by adding 9 mL of dichloroethane and stored at 4^oC for 2.5 hours and the observations were taken at 365nm.

3.5.5. Antioxidant enzyme assay

The common enzymatic extract (for SOD, CAX, and POX) was prepared by homogenizing the desired tissues in phosphate buffer (pH 7.5). A of 100 mL of 0.1 mM phosphate buffer contains a mixture of solution A and solution B. Solution A was prepared by dissolving 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of double distilled water and solution B was prepared by dissolving 8.71 g of potassium hydrogen phosphate (K_2HPO_4) and the volume was adjusted to 500 mL. Solution A (16 mL) and solution B (84 mL) was mixed and the pH was maintained at 7.5 by adding 1N HCl or 1N NaOH. The final extraction buffer was prepared by adding 0.5 mM EDTA. Extraction was carried out with 500 mg of fresh samples of desired plant part and macerated in mortar and pestle by using liquid nitrogen and uniformly homogenized

with 100 mL of phosphate buffer and the extract was filtered with the help of cheese cloth. After filtration, the extract was centrifuged at 11000 rpm at 4°C for about 10-15 minutes. The aliquot of the solution was further used as the enzyme in the reaction mixture of the different enzymatic assays.

3.5.5.1. Superoxide dismutase assay (Choudhury and Choudhuri, 1985)

The final volume of the enzyme reaction mixture (3 mL) contains 100 mM of phosphate buffer (1.5 mL of pH 7.8), 2.25 mM NBT (0.1 mL), 3.0 mM EDTA (0.1 mL), 2 mM riboflavin (0.1 mL), 1.5 M Na_2CO_3 (sodium bicarbonate) solution (1 mL), 2 mM of methionine (0.2 mL) and enzyme extract of 0.1 mL. The mixture was incubated at room temperature in the presence of a 15W fluorescent lamp in an aluminium foil-lined box for about 10 to 15 minutes until the reduction in NBT produced the desired colour. One positive and one negative control were taken as blank. The positive control contained riboflavin and 0.9 mL of distilled water without enzyme. The negative control contained 0.1 mL enzyme and 0.9 mL of distilled but no riboflavin and was incubated in dark. Before taking the absorbance the blank was set by measuring the absorbance, of the positive control against the negative one. The final absorbance was taken at 560nm.

3.5.5.2. Catalase enzyme assay (Chance and Maehly, 1955)

Extraction of catalase was done in the same above-mentioned procedure. The final volume of the enzyme reaction mixture (3 mL) contained 1.5 mL 100 mM phosphate buffer (100 ml of pH 7 by mixing 39 ml of solution A and 61 ml of solution B), 0.5 mL of H_2O_2, 0.9 mL of distilled water and 0.1 mL of enzyme extract. The absorbance was measured by a spectrophotometer at an OD of 240 nm. One unit of activity was equivalent to 1 mM H_2O_2 degraded per minute and expressed unit per mg of protein.

3.5.5.3. Peroxidase enzyme assay (Chance and Maehly, 1955)

The extraction protocol was the same as it was for other anti-oxidant enzymes. The assay mixture of guaiacol peroxidase contained 1 mL of phosphate buffer (15 mL of solution A and 85 mL of solution B, pH 6.1), 12 mM H_2O_2 (0.5 mL), 96 mM of guaiacol (0.5 mL), 0.1 mL of enzyme extract and rest 0.9 mL distilled water. The increment in absorbance was taken at 420 nm and the enzymatic activity was expressed in a unit of enzyme per mg of protein.

3.5.6. Estimation of protein content (Bradford 1976)

Protein estimation was done from the same extracts as the enzymes by using the Bradford reagent. For preparing 5X Bradford reagent 50 mg Coomassie Blue G250 was first dissolved in 50 mL of methanol to which 100 mL of 85% H_3PO_4 was added. 500 mL distilled water was added to the above solution and mixed thoroughly. Then it was filtered through Whatman #1 filter paper to remove the precipitates. At last 350 mL of water was added and stored at 4^oC for further use. In this experiment, 1X Bradford reagent was prepared by diluting the 5X solution 5 times. 5 mL of 1X Bradford reagent was taken in a test tube and 0.1 mL of enzyme extract was added to it. The solution was vortexed thoroughly and incubated for 30 minutes in dark for uniform colour development. Final absorbance was taken at OD 595 nm and expressed in terms of mg g^{-1} FW.

3.6. RNA isolation and extraction and expression analysis of m-RNA transcripts

3.6.1. RNA isolation and extraction

RNA isolation was done by using Qiagen RNeasy plant mini kit. For extraction 100 mg of plant samples were collected from desired plant part. The fresh tissues were macerated by using liquid nitrogen and then transferred into the micro-centrifuge tube without thawing the tissue. RNA lysis buffer (RLT) (450µL) was added to the powdered tissue. β-mercapto ethanol (10 µL) per one ml was added to the RLT buffer at the time of maceration. It was then incubated for a short 1-3 minutes at 56°C to disrupt the tissue. After incubation, the lysate was transferred to a 'Qia shredder spin column' and placed in a 2 mL collection tube, and centrifuged for 2 minutes at 5000 rpm. The supernatant was carefully transferred to a new micro-centrifuge tube without disturbing the cell debris pellets in the collection tube. The above supernatant was used in subsequent steps. A 0.5 mL of molecular grade ethanol (96- 100%) was added to clear the lysate and mixed immediately by using a pipette. The precipitates were visible after the addition of ethanol. The sample was transferred including the precipitates to another RNeasy spin column and placed over a 2 mL collection tube. The lip of the tube was closed gently and centrifuged for 15 seconds at 8000 rpm and the flow through was discarded. DNase was added to each RNeasy spin column and left for 20-30 minutes. RN_1(350 µL) buffer was added to the RNeasy spin column and the lid was gently closed and centrifuged at 10000 rpm for 15 seconds. The wall of the spin column was washed off and the flow through was discarded. In the next step, 10 µL of DNase I

stock solution and 70 µL of RDD buffer were added and mixed gently by inverting the tube and centrifuged briefly to collect the residual liquid from the sides of the test tubes. This step was repeated 3 to 4 times and the wall was washed with RPE wash 1 solution. Thereafter, the solution was transferred to another new 2 mL collection tube and the old one was discarded. Again it was centrifuged for another minute at 10000 rpm. The RNA spin column was transferred to a 1.5mL tube and 30µL of RNase-free water was added and centrifuged again at 10000 rpm to elute RNA. The isolated RNA was stored at -80°C for further use.

3.6.2. RNA quantification and cDNA synthesis

The quality and quantity of RNA were checked in ND 1000, Thermo, USA, and was diluted further to quantify the concentration of RNA required for DNA preparation. Before the synthesis of cDNA, the template RNA was thawed over ice. gDNAwipeout buffer, Quantiscript reverse transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase free water and template were added to prepare mastermix. A mastermix contains 2 µL gDNAwipeout buffer (7x), 12µL template RNA, and 2 µL RNase free water, which made the total reaction volume of 14µL. The reaction mixture was then incubated at 42^{o}C for 5 minutes and paused for a while (maximum upto 10 minutes) using a programmable PCR machine. PCR tubes containing the reaction mixture were immediately transferred to the ice bath. Reverse transcriptase mix was prepared by adding 1µL Quantitranscript reverse transcriptase, 4 µL Quantitranscript RT buffer, 1µL of RT primer mix, and the total volume was made upto 20 µL for each tube. It was again incubated for 30 minutes at 42°C. The reaction was stopped by incubating at 95°C for 3 minutes and then it was cooled in the ice bath and directly used for expression analysis in real-time qPCR.

3.6.3. Gene expression analysis

Gene expression profiles of the some key genes and pumps related to Na^+, K^+, and H^+ transport in the cell, were analyzed in the root and leaf tissues of four selected genotypes (FL478, AC41585, Rashpanjor, and IR29) at both seedling and reproductive stages. RNA was extracted by using the RNeasy plant mini kit (Qiagen) following the manufacturer's protocol. For total RNA estimation, the samples were collected from the root and leaf (second leaf from the top) tissues in three biological replications. To eliminate the genomic DNA contamination the samples were subjected to DNase I treatment (Qiagen kit) before quantification in Nanodrop (ND 1000, Thermo, USA). A

1µg of total RNA was taken from each biological replicate by using the Quantitech Reverse Transcription Kit (Qiagen) for the synthesis of first cDNA strand. The primer details of the selected genes are listed in Table 2. A20µL reaction mix of the Quantifast SYBR Green PCR kit (Qiagen) was used in quantitative real-time PCR. The reaction mix for expression analysis was prepared by adding the cDNA template of 2µL, 10µL SYBR green, 6µL of distilled water, and 1µL each of forward and reverse primer. The Quant Studio 5 Real-time PCR (Applied Biosystems, Thermo, USA) instrument was used for the expression analysis and the relative expression of treated samples (in terms of respective control samples) was expressed following the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 2. Details of the primers used for real-time q-PCR study for expression analysis of studied genes with their MSU ID/Gene Bank Accession Number

Gene Name	MSU ID/ Gene Bank Acc. No.	Primer Name	Sequences	Amplicon size (bp)
OsSOS1	LOC_Os12g44360.1	*OsSOS1*_F	AGATCGCGCTTACTCTTGCTGTC	66
		*OsSOS1*_R	AGACCTCCAGTGCATCTTGTGC	
OsSOS2	LOC_Os06g40370.1	*OsSOS2*_F	ACTTAGCACTTTGGCCCAGAAAG	130
		*OsSOS2*_R	ACCACATGACCAAACATCTGCTG	
OsSOS3	LOC_Os05g45810.1	*OsSOS3*_F	GAACATGTCACTTCCCTATTTGC	76
		*OsSOS3*_R	GTCATGGGCTTCTGAATGCAT	
OsNHX1	LOC_Os07g47100.1	*OsNHX1*_F	TGACCGTGAGGTTGCCCTTATG	82
		*OsNHX1*_R	GAGAATGCCGCTCAAATCTAGCA	
OsAKT1	LOC_Os01g45990.1	*OsAKT1*_F	GGAGCTGATCCAAATGCCAGAG	142
		*OsAKT1*_R	TGCAAGCGTATAAGCCCGTGTC	
OsHAK1	LOC_Os04g32920	*OsHAK1_F*	AAGAAGGGTTGGGTGTCTCTCG	94
		OsHAK1_R	CGGCCCTGATGTTGAAATGGC	
OsHAK5	LOC_Os01g70490.1	*OsHAK5*_F	TCGCATCTATCCAGAACACGTTG	270
		*OsHAK5*_R	TATGCATTGCCGATCTTGTCTGTA	
OsHKT2;4	LOC_Os06g48800	*OsHKT2.4_F*	AACATCATCTTTGAGGTGATAAG	121
		OsHKT2.4_R	AAGTTGTACGCCTTCTCATGGC	
OsHKT1;1	LOC_Os06g48810.1	*OsHKT1;1*_F	GGCGTTTCTGGCATCAACTGTC	73
		*OsHKT1;1*_R	ATTCCAGTCGACAGCACCGAAC	
OsHKT1;5	LOC_Os01g20160.1	*OsHKT1;5*_F	GTCGTGCTCTACGTGGTGATG	82
		*OsHKT1;5*_R	CTCCCGTTTGCTGGTGTTTGTC	
OsAHA1	LOC_Os03g48310.1	*OsAHA1*_F	ACAGAACCTGGCTTGAGTGTG	133
		*OsAHA1*_R	GGGCAAGCAGCATAAACCCAAA	
OsAHA7	LOC_Os04g56160.1	*OsAHA7*_F	GGAGATCAAGAATGAGGCCG	49
		*OsAHA7*_R	CTCCTCGATCGGTATGTTCTC	
OsV-PPase	LOC_Os06g08080.1	*OsV-PPase*_F	ATGGCTCTCTTCGGAAGGGTTG	141
		*OsV-PPase*_R	GTCACCGACATTGTCAGCAATCA	
OsV-ATPase	LOC_Os11g06890.1	*OsV-ATPase*_F	ACTACCTCTTTGACGGCTACGC	118
		*OsV-ATPase*_R	GCTTTGGTTGCTGTGCATTTGCC	
Os18SrRNA	AK059783	*Os18SrRNA*_F	ACATAGAAGGAGAAGAATGCACCCG	65
		*Os18SrRNA*_R	ACACTTCACCGGACCATTCAA	

3.7. Statistical analysis

The experiment was conducted in Completely Randomized Design (CRD) and the data were subjected to one-way factorial ANOVA. A post hoc analysis for pair-wise comparison of treatment × genotype combination was performed by Tukey's range test using SPSS (version 20.0) software. The charts were prepared using a licensed version of Graphpad Prism 9.5.

RESULTS

In this present study, initially ten (FL478, IR29, AC41585, AC39416A, Sadri, Rashpanjor, CSR27, Binadhan 8, Luna Suvarna and Sabita) rice genotypes of differential response towards salt stress were evaluated under12 dS m^{-1} NaCl stress imposed to 14 days old seedlings in the hydroponic system at the early seedling stage during *rabi* 2020 and 2021. Again in *Kharif* 2020 and 2021, same panel of genotypes were exposed to 8 dS m^{-1} NaCl stress at the booting stage in the net house of ICAR-NRRI, Cuttack. Both experiments were conducted in factorial Completely Randomized Design (FCRD) and the genotypes were evaluated on the basis of various agro-morphological, physiological, biochemical and molecular traits. The data were statistically analyzed and represented through various tables and graphs under different sub-headings in this chapter.

4.1. Agro-morphological Parameters

4.1.1. Early seedling stage

4.1.1.1. Evaluation of visual salt injury based on SES Score under salt stress at the early seedling stage:

The results of the present study showed that, salinity has a very detrimental effect on rice plants' health at the early seedling stage. All 10 genotypes of the panel were shown to have detrimental responses towards salt stress just after 3 days of imposition of stress. Based on visual salt injury the plants were assigned with an appropriate SES score, after 7 days of imposition of salinity. From the SES score three genotypes, FL478, AC41585, and AC39416A having an SES score of '3' were considered to be tolerant to salt stress at the early seedling stage. Four genotypes (CSR27, Rashpanjor, Binadhan 8, and Luna Suvarna) were considered moderately tolerant with a score of '5'. While two genotypes, Sadri and Sabita were seen to have stunted growth along with some severe symptoms of salt stress and were considered moderately susceptible with a score of '7'. Whereas almost all plants of IR29 were dead and found to be highly susceptible with a score of '9' at the early seedling stage under 12 dS m^{-1} salt stress at early seedling stage (Table 3).

Table 3. Evaluation of rice genotypes based on SES score under salt stress at the early seedling stage in rice

SES Score	Damage symptoms	Tolerant/Susceptible	Response of genotypes
1	Normal growth and no symptoms of leaf rolling	Highly tolerant	None
3	Few leaves showed leaf rolling on tips	Tolerant	FL478,AC41585, AC39416A
5	Retardation of growth was associated with leaf rolling	Moderately tolerant	Rashpanjor, CSR27, Binadhan 8 and Luna Suvarna
7	Growth completely stopped. Most leaves were rolled. Some plants are dying	Susceptible	Sadri and Sabita
9	Majority plants died	Highly susceptible	IR29

4.1.1.2. Effect of salt stress on shoot and root length (cm) under salt stress at the early seedling stage in rice:

Significant reduction in both shoot and root length was observed under salt stress (Table 4). Shoot length was significantly decreased in all the genotypes and highest in susceptible genotype IR29 (28.49%) as compared to its control. In moderately tolerant genotypes like Rashpanjor, CSR27 and Luna Suvarna about 20% reduction in shoot length was observed. While least reduction was recorded in FL478 (8.15%) followed by AC41585 (12.92%) and AC39416A (15.45%). Similarly, more than 30% reduction was observed in the root length of IR29 and Sabita, whereas, a little less, yet significant reduction was observed in Rashpanjor (18.65%) and CSR27 (22.90%), Luna Suvarna (21.24%) and Binadhan 8 (24.62%). The lowest reduction was observed in FL478 (8.48%) followed by AC41585 (12.63%) and AC39416A (15.91%).

Table 4. Effect of salt stress on shoot and root length (cm) under salt stress at the early seedling stage in rice

Genotype	Shoot length(cm)		Root length(cm)	
	Control	Stress	Control	Stress
FL478	21.46	12.23 (-8.15%)	8.41	7.70 (-8.48%)
IR29	21.74	15.37 (-28.49%)	8.34	5.60 (-32.88%)
AC41585	29.50	16.58 (-12.92%)	10.61	9.27 (-12.63%)
Sadri	27.81	17.74 (-20.52%)	10.64	7.40 (-30.47%)
AC39416A	29.89	16.78 (-15.45%)	11.69	9.83 (-15.91%)
Rashpanjor	31.22	17.78 (-19.65%)	11.91	9.70 (-18.56%)
CSR27	22.75	13.87 (-20.04%)	8.65	6.67 (-22.90%)
Binadhan 8	24.79	15.23 (-22.32%)	8.62	6.50 (-24.62%)
Luna Suvarna	28.31	16.65 (-21.27%)	9.23	7.27 (-21.24%)
Sabita	27.37	17.52 (-26.22%)	10.76	7.37 (-31.52%)

	G	S	GxS	G	S	GxS
SE(M)±	0.660	0.295	0.934	0.324	0.145	0.459
LSD (*p*<0.05)	1.887	0.844	2.669	0.927	0.415	1.311

4.1.1.3. Effect of salt stress on total biomass (g plant^{-1}), relative water content (RWC in %) and leaf water potential (LWP in MPa) in rice:

Lesser reduction in dry biomass under stress is a positive sign of growth. In this experiment total dry weight of plants in control conditions was higher as compared to plants under salt stress at the early seedling stage (Table 5). There was a significant reduction in total biomass observed in all the genotypes after 7 days of imposition of salt stress. The least reduction of biomass was observed in FL478 (17.67%) followed by AC41585 (22.22%) and AC39416A (26.47%). In moderately tolerant genotypes like Rashpanjor, CSR27, and Binadhan8 ~30% of reduction in total plant biomass was observed. The highest decline of more than 50% in biomass was recorded in susceptible

genotype IR29. Under salinity stressed plants experienced severe osmotic stress and hence maintaining the cellular osmotic potential and water status are two major criteria for determining tolerance. In this present study plant water status was measured through RWC and LWP. Significant difference was observed in both the parameters between stressed plants in comparison with control plants (Table 5). A drastic reduction of almost about 50% in RWC was observed in IR29 followed by Sabita (23.51%) and Sadri (21.05%). Whereas, the decrement was least in the case of FL478 (7.35%) followed by AC41585 (9.85%) and AC39416A (15.43%) as the compared to control.

Table 5. Effect of salt stress on total biomass (g plant^{-1}), Relative water content (RWC in %) and Leaf Water Potential (LWP in MPa) at the early seedling stage in rice

Genotype	Dry weight (g plant^{-1})		Relative Water Content (RWC in %)		Leaf Water Potential (LWP in MPa)	
	Control	Stress	Control	Stress	Control	Stress
FL478	0.297	0.245 (17.67%)	93.33	86.47 (7.35%)	-1.69	-2.49
IR29	0.209	0.095 (54.50%)	91.63	45.88 (-49.93%)	-1.49	-5.66
AC41585	0.341	0.265 (22.22%)	90.10	81.22 (-9.85%)	-1.69	-2.45
Sadri	0.309	0.180 (49.79%)	89.08	70.33 (-21.05%)	-1.65	-4.49
AC39416A	0.376	0.276 (26.47%)	92.63	78.34 (-15.43%)	-1.50	-2.66
Rashpanjor	0.284	0.195 (31.31%)	93.29	77.69 (-16.72%)	-1.39	-2.25
CSR27	0.345	0.235 (31.91%)	91.62	77.91 (-14.96%)	-1.34	-3.14
Binadhan 8	0.247	0.168 (32.18%)	91.62	80.84 (-11.76%)	-1.57	-3.57
Luna Suvarna	0.323	0.208 (35.56%)	94.11	75.64 (-19.63%)	-1.20	-3.49
Sabita	0.352	0.207 (41.26%)	93.09	71.21 (-23.51%)	-1.66	-4.79
	G	**S**	**GXS**	**G**	**S**	**GXS**	**G**	**S**	**GXS**
SE(M)±	0.003	0.001	0.004	0.986	0.441	1.394	0.59	1.40	0.81
LSD (p<0.05)	0.008	0.004	0.011	2.818	1.260	3.985	1.87	0.79	2.59

Similarly the highest retention in LWP was recorded in tolerant genotypes AC41585 (-2.45 MPa) was at par with FL478 (-2.49 MPa) and Rashpanjor (-2.25 MPa) and followed by AC39416A (-2.66 MPa). Less but significant decrement in LWP was observed in moderately tolerant genotypes like CSR27 (-3.14 MPa), Binadhan 8 (3.57 MPa) and Luna Suvarna (-3.49 MPa). Maximum drop in water potential was recorded in IR29 (-5.66 MPa) followed by Sabita (-4.79 MPa).

4.1.2. Reproductive stage

4.1.2.1. Effect of salt stress on plant height (cm), no. of panicles per plant, Panicle length (cm), and Days to 50% flowering in rice

Salinity has a significant impact on plant height (cm), no. of panicles per plant, panicle length (cm) and days to 50% flowering in this present study in all the genotypes as compared to the control plants (Table 6). The highest reduction in plant height was observed in Sabita (30.88%) followed by IR29 (26.38%). Whereas, about an 18-20% decline in plant height was recorded in moderately tolerant genotypes CSR27, Binadhan 8 and Luna Suvarna. However, in tolerant genotypes like AC39416A and FL478, less than 15% drop in plant height was observed. But in Rashpanjor (seedling stage moderately tolerant), the decrement was very low (14.82%) and was at par with AC41585 (14.25%). The number and size of panicle are two major quantitative traits closely associated with the yield of the plant. In our study we found a significant reduction in both number of panicles and panicle length in response to salinity when the salt stress was imposed at the reproductive stage (Table 6). The least decrement in both the traits was recorded in Rashpanjor (of about 9-10%), which was at par with AC41585 (seedling stage tolerant). However, FL478 (seedling stage tolerant) was unable to maintain so and about 37.04% and 26.67% decline in both panicle numbers and length was recorded respectively at the reproductive stage. In moderately tolerant genotypes like CSR27, Binadhan 8 and Luna Suvarna 30-35% reduction in panicle vigour was evident under stress as compared to their control. The highest and more than50% reduction in number of panicles were recorded in Sabita followed by IR29 under stress. Salinity significantly delayed the flowering initiation and attaining 50% flowering in stress-imposed plants as compared to control plant in susceptible genotypes (Table 6). In IR29 flowering was delayed for more than a month as compared to normally grown plants. In moderately tolerant genotypes like CSR27 (~17 days), Binadhan 8 (~12 days) and Luna Suvarna (~11 days) significant delay was observed in attaining 50% flowering, However the least delay was observed in Rashpanjor (~7 days) followed by AC41585 (~9 days) and AC39416A (~10 days).

Table 6. Effect of salt stress on Plant height (cm), No. of panicles per plant, Panicle length (cm) and Days to 50% flowering at the reproductive stage in rice

Genotype	Plant height (cm)		No. of panicles per plant		Panicle length (cm)		Days to 50% flowering	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	83.33	70.33 (-15.60%)	9.00	7.00 (-37.04%)	25.00	18.33 (-26.67%)	75.00	88.00 (+17.33)
IR29	84.67	62.33 (-26.38%)	8.67	4.00 (-53.85%)	24.33	13.67 (-43.84%)	70.33	93.00 (+32.33)
AC41585	133.33	114.33 (-14.25%)	9.33	8.33 (-10.71%)	34.00	29.67 (-12.75%)	102.33	112.00 (+9.45)
Sadri	187.67	137.00 (-27.00%)	10.00	6.00 (-53.33%)	35.67	22.67 (-36.45%)	82.67	96.33 (+16.53)
AC39416A	153.67	126.00 (-18.00%)	11.00	9.33 (-15.15%)	29.00	24.67 (-14.94%)	97.33	107.33 (+10.27)
Rashpanjor	177.67	151.33 (-14.82%)	11.00	10.00 (-9.09%)	32.33	29.33 (-9.28%)	127.67	137.00 (+7.31)
CSR27	127.33	103.67 (-18.59%)	9.00	6.33 (-29.63%)	24.00	16.00 (-33.33%)	83.33	98.00 (+17.60)
Binadhan8	101.67	80.00 (-21.31%)	9.33	6.67 (-28.57%)	28.33	18.33 (-35.29%)	78.00	88.00 (+12.82)
Luna Suvarna	163.00	130.67 (-19.84%)	9.00	6.33 (-29.63%)	26.67	17.00 (-36.25%)	112.33	125.00 (+11.28)
Sabita	167.33	115.67 (-30.88%)	10.00	5.33 (-56.67%)	31.67	18.67 (-41.05%)	123.00	147.33 (+19.78)

	G	S	GXS	G	S	GxS	G	S	GxS	G	S	GxS
SE(M)±	1.364	0.610	1.929	0.354	0.158	0.500	0.681	0.305	0.963	0.968	0.433	1.368
LSD (*p*<0.05)	3.899	1.744	5.514	1.011	0.452	1.429	1.947	0.871	2.753	2.765	1.327	3.911

4.1.2.2. Effect of salt stress on plant dry weight (g plant^{-1}), Spikelet sterility percentage (STE%) Spikelet degeneration percentage (%DEG) in rice

A significant decrement in dry weight was observed under salt stress due to less accumulation of photosynthates (Table 7). The highest reduction in dry weight was recorded in IR29 (36.65%) as compared to its control plants followed by Sabita (36.43%) and Sadri (30.26%). The overall dry matter accumulation was hampered upto 30% in moderately tolerant genotypes like CSR27, Binadhan 8, Luna Suvarna and FL478 in the reproductive stage, while the least decline of about 8% was recorded in AC41585, followed by Rashpanjor (11.03%), and at par with AC39416A (11.05%). Salinity highly affects the floral biology of plants. Production of degenerated and sterile spikelets is one of the most critical symptoms of susceptibility under salinity due to lack of the carbohydrate mobilisation into the growing panicles (Table 7). In our study about 50% of the spikelets were found sterile in susceptible genotypes like IR29, Sabita and Sadri. In some moderately tolerant genotypes like CSR27, Binadhan 8 and ~25-30% spikelets were found to be sterile and degenerated under prolonged period of salt stress. Surprisingly, more than 35% spikelet sterility observed in FL478 the seedling stage tolerant genotype. . While in tolerant genotypes like AC41585, Rashpanjor, and AC39416A spikelet sterility percentage was less yet significant as compared to other genotypes. In the moderately tolerant group (CSR27, Binadhan 8 and Luna Suvarna) ~30-40% of spikelets were degenerated and sterile under stress.

Table 7. Effect of salt stress on plant dry weight (g plant^{-1}), Spikelet sterility percentage (STE%) Spikelet degeneration percentage (%DEG) at the reproductive stage in rice

Genotype	Dry weight (g plant^{-1})			Spikelet Sterility percent (STE%)			Spikelet degeneration percentage (%DEG)		
	Control	Stress		Control	Stress		Control	Stress	
FL478	13.80	10.68 (-26.12%)		7.09	38.05		7.27	36.13	
IR29	18.83	8.74 (-36.65%)		7.61	46.43		6.64	43.53	
AC41585	19.03	17.19 (-8.70%)		8.56	27.75		7.46	24.00	
Sadri	18.95	13.27 (-30.26%)		9.58	49.17		8.07	45.28	
AC39416A	19.50	16.85 (-11.05%)		9.29	27.40		8.67	24.28	
Rashpanjor	16.06	17.35 (-11.03%)		8.74	25.93		7.15	24.11	
CSR27	15.68	12.17 (-24.25%)		9.39	27.01		6.87	29.65	
Binadhan 8	18.26	11.60 (-26.01%)		8.60	30.44		6.13	31.50	
Luna Suvarna	17.37	13.10 (-28.26%)		7.55	40.10		5.36	40.00	
Sabita	13.80	11.04 (-36.43%)		7.77	49.27		5.53	46.68	
	G	S	GXS	G	S	GXS	G	S	GXS
SE(M)±	0.373	0.167	0.527	1.328	0.594	1.878	1.198	0.536	1.694
LSD (p<0.05)	1.066	0.477	1.507	3.795	1.697	5.367	3.423	1.531	4.841

4.1.2.3. Effect of salt stress on Yield (g plant^{-1}), Yield Stability Index (YSI) and Stress Susceptibility Index (SSI) at the reproductive stage in rice

Grain yield per plant was drastically affected in salinity due to ion toxicity and hindrance in photo assimilation in panicles during the grain filling stage. In the present study a significant decline in yield was recorded in all the genotypes and the data was presented in Table 8 in this section. The highest yield reduction was observed in IR29 (64.17%) as compared to the rest of the genotypes and more than 50% reduction was evident in Sadri and Sabita. While about a 20-30% decline in yield was recorded in CSR27, Binadhan 8 and Luna Suvarana. However, the least decline in yield was observed with AC41585 which was at par with Rashpanjor. But about a 40% reduction

in yield was observed in FL478 (seedling stage tolerant) as compared to its control. Based on the yield obtained, the highest Yield Stability Index and Stress Susceptibility Index were calculated to analyze the response of each genotype towards salinity. According to the data presented in Table no 8, the genotype with the YSI was considered to have a tolerance response toward salinity. AC41585 (0.89), Rashpanjor (0.87), and AC39416A (0.85) were having the highest YSI followed by Binadhan 8 (0.76) and CSR27 (0.71). Whereas, the lowest

YSI was recorded in IR29 (0.36) and followed by Sabita (0.44) and Sadri (0.45). In reciprocation the highest SSI indicated the maximum susceptibility towards salinity and was highest for IR29 (1.78) amongst all the ten studied genotypes followed by Sabita (1.56) and Sadri (1.53). In moderately tolerant genotypes like CSR27 and Binadhan 8, SSI was 0.80 and 0.95 respectively. Whereas, for the genotypes like AC41585 (0.31), Rashpanjor (0.36) and AC39416A (0.41), SSI was comparatively lower suggestingtheir tolerance response towards salinity in the reproductive stage. But the the seedling stage tolerant genotype FL478 with YSI of 0.62 and SSI 1.06 was showing moderate response to salt stress at this stage.

Table 8. Effect of salt stress on the yield (g plant^{-1}), YSI (Yield Stability Index) and SSI (Stress Susceptibility Index) at the reproductive stage in rice

Genotype	Yield (g plant^{-1})			YSI (Yield Stability Index)	SSI (Stress Susceptibility Index)
	Control	Stress			
FL478	10.15	6.2 (-38.76%)		0.62	1.06
IR29	13.39	4.80(-64.17%)		0.36	1.78
AC41585	12.55	11.08(-11.71%)		0.89	0.31
Sadri	15.24	6.84(-55.13%)		0.45	1.53
AC39416A	11.97	10.18(-14.94%)		0.85	0.41
Rashpanjor	11.91	10.35(-13.13%)		0.87	0.36
CSR27	13.05	9.29(-28.80%)		0.71	0.80
Binadhan 8	14.15	10.22(-27.77%)		0.76	0.66
Luna Suvarna	12.46	8.21(-34.16%)		0.66	0.95
Sabita	15.64	6.83(-56.35%)		0.44	1.56
	G	S	GXS	GXS	GXS
SE(M)±	0.260	0.116	0.367	3.556	9.877
LSD (p<0.05)	0.743	0.332	1.050	0.105	0.139

4.2. Effect of salt stress on tissue ion concentration (Na^+ and K^+), Na^+/K^+ ratio and selective transport (ST) at both early seedling and reproductive stage in rice

Increment in tissue Na^+ concentration was significant in the root, stem and leaves of all the genotypes at both early seedling and reproductive stages under salinity. At the early seedling stage (Table 9). The highest root Na^+ concentration was observed in FL478 (319.10 mg kg^{-1}, DW), which was at par with AC41585 (304.99 mg kg^{-1}, DW) and AC39416A (307.19 mg kg^{-1}, DW) (Table 9). While in moderately tolerant genotypes like Rashpanjor (243.67 mg kg^{-1}, DW), CSR27 (269.64 mg kg^{-1}, DW) and Binadhan 8 (250.4 mg kg^{-1}, DW) comparatively less Na^+ was accumulated in the root tissue. In genotypes like Sabita (191.57 mg kg^{-1}, DW) and IR29 (217.99 mg kg^{-1}, DW) the root Na^+ concentration was comparatively less than in other genotypes. In stem portions also significant increment in Na^+ concentration was noticed in all the genotypes as compared to control plants under stress. However, the lowest Na^+ accumulation was noticed in the stem region of AC41585 (211.58 mg kg^{-1}, DW) which was at par with AC39416A (214.02 mg kg^{-1}, DW), CSR27 (219.98 mg kg^{-1}, DW) and Binadhan 8 (216.40 mg kg^{-1}, DW). The highest stem Na^+ concentration was observed in Rashpanjor (274.89 mg kg^{-1}, DW). But in the leaf tissues a different pattern of Na^+ accumulation was observed as compared to root tissues. Least Na^+ was accumulated in the photosynthtically active tissues of FL478 (130.89 mg kg^{-1}, DW) followed by AC41585 (141.22 mg kg^{-1}, DW) and AC39416A (145.07 mg kg^{-1}, DW). A moderate amount of Na^+ was deposited in the leaves of CSR27 (172.81 mg kg^{-1}, DW), which was at par with Binadhan 8 (180.33 mg kg^{-1}, DW) and Luna Suvarna (181.76 mg kg^{-1}, DW). However, maximum Na^+ concentration was found in the leaf tissues of IR29 (343.09 mg kg^{-1}, DW) followed by Sabita (306.99 mg kg^{-1}, DW) in the early seedling stage.

In the reproductive stage also, tissue Na^+ concentration was increased significantly in all the genotypes under saline conditions (Table 10). Like the early seedling stage, in the reproductive stage highest Na^+ accumulation was observed in the root tissues of AC41585 (257.33 mg kg^{-1}, DW), which was at par with AC39416A (248.33 mg kg^{-1}, DW) and FL478 (236 mg kg^{-1}, DW). Comparatively lower Na^+ concentration was recorded in root tissues of IR29 (163.33 mg kg^{-1}, DW), which was at

par with Sabita (168.67 mg kg^{-1}, DW). However, in photosynthetically active tissues like middle leaf Na^+ concentration was least in AC41584 (100.67 mg kg^{-1}, DW), which was at par with AC39416A (106.33 mg kg^{-1}, DW). In flag leaves also tissue Na^+ concentration was lowest for AC39416A (50.67 mg kg^{-1}, DW) and AC41585 (51.33 mg kg^{-1}, DW) and was at par with FL478 (63.33 mg kg^{-1}, DW) and Rashpanjor (64.67 mg kg^{-1}, DW). However, the highest Na^+ concentration was recorded in IR29 (92.67 mg kg^{-1}, DW) and Sabita (92 mg kg^{-1}, DW). Similarly, in developing panicles least accumulation of Na^+ was observed in AC39416A (28.67 mg kg^{-1}, DW) followed by AC39416A (32.00 mg kg^{-1}, DW) and Rashpanjor (32.67 mg kg^{-1}, DW). A moderate amount of Na^+ accumulation was evident in CSR27 (42.67 mg kg^{-1}, DW), Binadhan 8 (48.00 mg kg^{-1}, DW) and Luna Suvarna (48.67 mg kg^{-1}, DW). The highest Na^+ concentration in panicles was recorded for IR29 (65.33 mg kg^{-1}, DW) followed by Sabita (60.37 mg kg^{-1}, DW) (Table 10).

Tissue K^+ concentration varied significantly in all the genotypes under salinity. In the early seedling stage, drastic reductions in K^+ concentration in root, stem, and leaf tissues were observed in stressed plants as compared to the control (Table 11). Least K^+ concentration was observed in root tissues of susceptible genotypes like IR29 of 154.06 mg kg^{-1}, DW and followed by Sabita (155.09 mg kg^{-1}, DW). Some moderately tolerant genotypes like Luna Suvarna (157.53 mg kg^{-1}, DW) Binadhan 8 (158.57 mg kg^{-1}, DW) and CSR27 (158.56 mg kg^{-1}, DW).Whereas maximum K^+ retention was observed in the roots of FL478 (180.59 mg kg^{-1}, DW) and was at par with AC41585 (178.37 mg kg^{-1}, DW), AC39416A (168.50 mg kg^{-1}, DW) and Rashpanjor (162.14 mg kg^{-1}, DW).A similar kind of trend was observed in both stem and leaf tissues. The highest leaf K^+ acquisition was observed in AC41585 (348.69 mg kg^{-1}, DW) followed by FL478 (341.09 mg kg^{-1}, DW), AC39416A (320.46 mg kg^{-1}, DW), and Rashpanjor (317.07 mg kg^{-1}, DW). While it was lowest in IR29 (171.13 mg kg^{-1}, DW) followed by Luna Suvarna (185.60 mg kg^{-1}, DW) and Sabita (222.26 mg kg^{-1}, DW).

Table 9. Effect of salt stress on tissue Na^+ ion concentration (mg kg^{-1}, DW) at the early seedling stage in rice

Genotype	Root		Stem		Leaf	
	Control	Stress	Control	Stress	Control	Stress
FL478	103.89^{a}	319.10^{f}	80.71^{a}	226.60^{b}	67.26^{a}	130.89^{b}
IR29	109.53^{a}	217.99^{bc}	81.11^{a}	225.39^{b}	67.59^{a}	343.09^{h}
AC41585	105.23^{a}	304.99^{ef}	82.34^{a}	211.58^{b}	68.62^{a}	141.22^{bc}
Sadri	106.12^{a}	243.80^{cd}	73.99	248.96^{bc}	61.61^{a}	293.76^{g}
AC39416A	102.49^{a}	307.19^{ef}	79.73^{a}	214.02^{b}	66.44^{a}	145.07^{bcd}
Rashpanjor	108.40^{a}	243.67^{cd}	74.78^{a}	274.89^{c}	62.31^{a}	229.08^{f}
CSR27	103.06^{a}	269.64^{de}	70.94^{a}	219.98^{b}	59.12^{a}	172.81^{de}
Binadhan 8	118.16^{a}	250.4^{cd}	75.32^{a}	216.40^{b}	62.77^{a}	180.33^{e}
Luna Suvarna	108.73^{a}	224.55^{bc}	75.47^{a}	218.11^{b}	62.89^{a}	181.76^{e}
Sabita	104.10^{a}	191.57^{b}	81.42^{a}	207.37^{b}	67.85^{a}	306.99^{gh}

	G	S	GXS	G	S	GXS	G	S	GXS
SE(M)±	5.603	2.506	7.924	5.636	2.520	7.197	5.024	2.274	7.105
LSD (*p*<0.05)	16.015	7.162	22.648	16.112	7.205	22.785	14.360	6.421	20.308

Table 10. Effect of salt stress on tissue Na^+ ion concentration (mg kg^{-1}, DW) at the reproductive stage in rice

Genotype	Root		Stem		Middle leaf		Flag leaf		Panicle	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	37.33^{a}	236.00^{cde}	34.00^{a}	173.33^{bc}	16.0^{a}	107.67^{bcd}	12.3^{a}	63.33^{bcd}	8.67^{a}	38.00^{bcd}
IR29	36.00^{a}	163.33^{b}	34.00^{a}	143.67^{b}	21.3^{a}	147.67^{e}	10.7^{a}	92.67^{f}	10.00^{a}	65.33^{g}
AC41585	38.00^{a}	257.33^{f}	38.00^{a}	163.00^{bc}	24.0^{a}	100.67^{b}	15.3^{a}	51.33^{bc}	8.00^{a}	28.67^{b}
Sadri	39.33^{a}	196.67^{bc}	35.33^{a}	149.33^{bc}	20.7^{a}	141.00^{cde}	16.0^{a}	82.00^{ef}	12.67^{a}	54.67^{efg}
AC39416A	39.33^{a}	248.33^{ef}	40.00^{a}	160.67^{bc}	20.0^{a}	106.33^{bc}	14.0^{a}	50.67^{b}	8.00^{a}	32.00^{b}
Rashpanjor	37.33^{a}	229.00^{cde}	29.33^{a}	189.33^{c}	19.3^{a}	122.67^{bcd}	10.0^{a}	64.67^{cd}	9.33^{a}	32.67^{bc}
CSR27	39.33^{a}	210.33^{cd}	32.00^{a}	154.33^{bc}	19.3^{a}	118.67^{bcd}	14.7^{a}	71.33^{de}	12.00^{a}	42.67^{bcde}
Binadhan 8	38.67^{a}	210.00^{cd}	36.00^{a}	168.67^{bc}	20.7^{a}	127.33^{bcd}	13.3^{a}	76.00^{de}	11.33^{a}	48.00^{def}
Luna Suvarna	40.00^{a}	213.67^{cd}	34.00^{a}	162.67^{bc}	22.0^{a}	116.33^{bcd}	11.3^{a}	79.33^{ef}	10.67^{a}	48.67^{def}
Sabita	36.00^{a}	168.67^{b}	30.00^{a}	159.00^{bc}	20.7^{a}	145.00^{de}	12.7^{a}	92.00^{f}	10.67^{a}	60.67^{fg}

	G	S	GXS	G	S	GxS	G	S	GxS	G	S	GxS	G	S	GxS
SE(M)±	5.248	2.347	7.421	5.292	2.367	7.484	4.939	2.207	6.978	1.807	0.808	2.555	2.028	0.907	2.867
LSD (p<0.05)	14.99	6.708	21.21	15.12	6.764	21.39	14.10	6.307	19.94	5.164	2.309	7.303	5.795	2.592	8.196

Similarly, in the reproductive stage also K^+ concentration in all the tissues decreased drastically under salinity (Table 12). Significantly least K^+ retention was observed in the root (22.67 mg kg^{-1}, DW) stem (24 mg kg^{-1}, DW), flag leaf (33.33 mg kg^{-1}, DW) and panicle (36.67 mg kg^{-1}, DW) of IR29, which was at par with Sabita. While, moderately tolerant genotypes like CSR27 and Binadhan 8 were able to retain 31.67 mg kg^{-1}, DW and 30.33 mg kg^{-1}, DW in the root respectively. Least K^+ retention was observed in the root tissues of IR29 (22.67 mg kg^{-1}, DW) followed by Sabita (24.00 mg kg^{-1}, DW).Similar pattern of K^+ acquisition was followed for all the tissues under stress conditions. At the reproductive stage, the flag leaf contributes maximum carbohydrate fixation and translocation. So the maintenance of cellular homeostasis in both the flag leaf and developing panicles is essential for securing yield under prolonged salinity. In both flag leaf and panicles the highest K^+ concentration was in AC41585 with 64.67 mg kg^{-1}, DW and 66.00 mg kg^{-1}, DW respectively, and was at par with both Rashpanjor (66.67 mg kg^{-1}, DW and 63.33 mg kg^{-1}, DW) and AC39416A (64.00 mg kg^{-1}, DW and 63.33 mg kg^{-1}, DW). But in FL478, K^+ concentration in both flag leaf (52.00 mg kg^{-1}, DW) and panicles (54.00 mg kg^{-1}, DW) was dropped significantly as compared to other tolerant genotypes. While the least retention was observed for all the tissue like stem (24.00 mg kg^{-1}, DW), middle leaf (28.00 mg kg^{-1}, DW), flag leaf (33.33 mg kg^{-1}, DW) and panicles (36.67 mg kg^{-1}, DW) of IR29 and was at par with Sabita.

Table 11. Effect of salt stress on tissue K^+ ion concentration (mg kg^{-1}, DW) at the early seedling stage in rice

Genotype	Root		Stem		Leaf	
	Control	Stress	Control	Stress	Control	Stress
FL478	233.25^{a}	180.59^{b}	279.90^{abc}	253.85^{abcd}	361.19^{a}	341.09^{abc}
IR29	229.51^{a}	154.06^{c}	275.42^{abc}	196.02^{f}	349.75^{abc}	171.13^{g}
AC41585	231.68^{a}	178.37^{b}	278.02^{abc}	236.25^{def}	352.95^{ab}	348.69^{abc}
Sadri	236.59^{a}	174.66^{b}	283.91^{ab}	209.35^{ef}	351.57^{abc}	289.80^{d}
AC39416A	228.27^{a}	168.50^{bc}	273.93^{abc}	241.62^{bcde}	358.01^{a}	320.46^{bcd}
Rashpanjor	228.84^{a}	162.14^{bc}	285.55^{a}	256.93^{abcd}	351.56^{abc}	317.07^{abc}
CSR27	228.52^{a}	158.56^{c}	274.23^{abc}	241.19^{bcde}	346.37^{abc}	219.68^{ef}
Binadhan 8	230.58^{a}	158.57^{c}	276.69^{abc}	237.86^{cde}	344.28^{abc}	225.03^{e}
Luna Suvarna	231.45^{a}	157.53^{c}	277.74^{abc}	236.30^{def}	356.40^{a}	185.60^{fg}
Sabita	228.05^{a}	155.09^{c}	273.66^{abc}	199.17^{ef}	367.27^{a}	222.26^{e}

	G	S	GXS	G	S	GXS	G	S	GXS
SE(M)±	5.048	2.257	7.139	5.657	2.527	7.994	4.669	2.088	6.603
LSD (p<0.05)	14.429	6.452	20.405	16.157	7.225	22.849	13.345	5.968	18.872

Table 12. Effect of salt stress on tissue K^+ ion concentration (mg kg^{-1}, DW) at the reproductive stage in rice

Genotype	Root		Stem		Middle leaf		Flag leaf		Panicle	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	53.33 a	31.00 d	64.67 abc	37.00 e	69.33 ab	46.67 def	68.58 a	52.00 bc	72.00 ab	54.00 de
IR29	44.67 b	22.67 e	66.67 ab	24.00 e	72.67 ab	28.00 f	65.92 a	33.33 d	70.67 ab	36.67 f
AC41585	52.67 a	33.33 c	60.00 abcd	45.33 bcde	66.00 abc	54.00 bcd	67.48 a	64.67 ab	71.33 ab	66.00 abcd
Sadri	52.00 a	27.00 de	60.00 abcd	27.00 e	68.67 ab	37.33 ef	66.92 a	40.00 cd	70.00 ab	43.33 ef
AC39416A	48.00 ab	31.33 cd	65.33 abc	42.33 de	66.67 abc	53.00 bcd	65.81 a	64.00 ab	73.33 a	63.33 abcd
Rashpanjor	54.67 a	33.33 c	66.00 ab	44.00 cde	68.00 abc	54.00 bcd	67.17 a	66.67 a	70.00 ab	63.33 abcd
CSR27	48.00 ab	31.67 cd	68.00 a	32.67 e	70.67 ab	44.67 ef	66.43 a	57.33 ab	69.33 ab	58.00 cd
Binadhan8	50.00 a	30.33 d	62.67 abcd	32.00 e	67.33 abc	47.33 cde	64.17 ab	56.67 ab	71.33 ab	62.00 abcd
Luna Suvarna	49.33 ab	30.67 d	64.00 abc	35.00 e	68.00 abc	45.00 ef	64.23 ab	57.33 ab	70.00 ab	59.33 bcd
Sabita	47.33 ab	24.00 e	70.00 a	26.00 e	75.33 a	30.00 f	66.33 a	37.33 d	68.67 abc	35.33 f
	G	**S**	**GXS**	**G**	**S**	**GxS**	**G**	**S**	**GxS**	**G**	**S**	**GxS**	**G**	**S**	**GxS**
SE(M)±	2.477	1.108	3.503	2.824	1.263	3.994	2.747	1.229	3.885	1.673	0.748	2.365	1.844	0.825	2.608
LSD (*p*<0.05)	7.080	3.166	10.02	8.073	3.610	11.48	7.852	3.512	11.110	4.780	2.138	6.761	5.270	2.357	7.453

A significant increment in Na^+/K^+ ratio was also evident under salt stress in both the stages (Table 13). In the seedling stage, the highest Na^+/K^+ ratio was recorded in the root of AC39416A (1.840) followed by FL478 (1.772) and AC41585 (1.726). But in root tissues lowest Na^+/K^+ ratio was observed for Sabita (1.294) followed by IR29 (1.419) However as compared to root less Na^+/K^+ ratio was maintained in leaves of tolerant genotype. In FL478 Na^+/K^+ ratio was lowest (0.383), followed by AC41585 (0.406) and AC39416A (0.453). But in susceptible genotypes leaf Na^+/K^+ ratio was significantly higher than that of tolerant and moderately tolerant genotypes. The highest Na^+/K^+ ratio in the leaf tissues was observed in IR29 (2.010). Similarly at the reproductive stage, comparatively less Na^+/K^+ ratio was maintained in the developing panicles and flag leaf of tolerant genotypes. The least Na^+/K^+ ratio was observed in the panicles of AC41585 (0.442) and was at par with AC39416A (0.513) and Rashpanjor (0.511). Likewise, the Na^+/K^+ ratio in the flag leaves was also lowest in AC39416A (0.790) followed by AC41585 (0.797) and Rashpanjor (0.975). But the ratio was high in both the panicles and flag leaves of the susceptible genotypes like IR29 (1.809 and 2.863, respectively) followed by Sabita (1.715 and 2.505, respectively) and Sadri (1.270 and 2.060, respectively). However, in middle leaf and stem tissues, a relatively higher level of Na^+/K^+ ratio was observed under salinity than that of developing panicles. But some genotypes with greater ion exclusion capacity were able to restrict the Na^+ in the root zone and hence able to maintain a lower Na^+/K^+ ratio in the above-ground portions mostly the photosynthetic tissues. FL478 (8.193) was observed to have the highest Na^+/K^+ ratio in the roots and was at par with AC39416A (8.182) and AC41585 (8.112). But on the contrary, the Na^+/K^+ ratio in the flag leaf (1.238) and panicles (0.704) of FL478 was moderate and was at par with CSR27 (1.225 and 0.735) and Binadhan 8 (1.395 and 0.775).

Preferential transport of K^+ over Na^+ from the root to upper portions of the plant was called selective transport (ST) and is considered to be one of the major criteria of tolerance. Here in this study differential response in selectivity for K^+ over Na^+ was observed and represented in Table no. 14 In the seedling stage under salinity highest ST was observed for FL478 (4.66) followed by AC41585 (4.27) and AC39416A (4.10). In moderately tolerant genotypes like Rashapnajor, CSR27 and Binadhan 8, ST was 2.12, 2.18 and 1.99 respectively. While it was lowest for IR29 (0.71) followed by Sabita (0.91). Similarly in reproductive stage also Selective transport was measured from root to flag leaf under salt stress. According to the data was presented in Table 14 the highest ST at reproductive stage was observed in AC41585 (4.32) followed by AC39416A (4.10), FL478 (3.36), and Rashpanjor (3.18). Whereas, ST was lowest for IR29 (1.40) followed by Sabita (1.76) and Sadri (1.97).

Table 13. Tissue Na^+/K^+ ratio and Selective Transport (ST) at the early seedling stage in rice

Genotype	Root		Stem		Leaf		ST (Root to Leaf)
	Control	Stress	Control	Stress	Control	Stress	
FL478	0.445^{a}	1.772^{c}	0.288^{a}	0.896^{c}	0.186^{a}	0.383^{ab}	4.66^{a}
IR29	0.478^{a}	1.419^{b}	0.295^{a}	1.155^{d}	0.193^{a}	2.010^{e}	0.71^{d}
AC41585	0.454^{a}	1.726^{c}	0.296^{a}	0.898^{bc}	0.194^{a}	0.406^{ab}	4.27^{a}
Sadri	0.448^{a}	1.408^{bc}	0.261^{a}	1.198^{d}	0.175^{a}	1.040^{c}	1.38^{bcd}
AC39416A	0.449^{a}	1.840^{c}	0.291^{a}	0.885^{b}	0.186^{a}	0.453^{ab}	4.10^{a}
Rashpanjor	0.479^{a}	1.511^{b}	0.262^{a}	1.070^{c}	0.177^{a}	0.726^{b}	2.12^{b}
CSR27	0.451^{a}	1.718^{b}	0.259^{a}	0.919^{c}	0.171^{a}	0.791^{b}	2.18^{b}
Binadhan 8	0.513^{a}	1.586^{b}	0.273^{a}	0.922^{cd}	0.182^{a}	0.807^{bc}	1.99^{bc}
Luna Suvarna	0.470^{a}	1.437^{bc}	0.272^{a}	0.931^{c}	0.177^{a}	0.987^{c}	1.46^{bcd}
Sabita	0.457^{a}	1.254^{c}	0.298^{a}	1.048^{cd}	0.185^{a}	1.386^{cd}	0.91^{cd}

	G	S	GXS	G	S	GxS	G	S	GxS	GxS
SE(M)±	0.061	0.027	0.086	0.038	0.017	0.053	0.031	0.013	0.043	0.217
LSD (*p*<0.05)	0.174	0.078	0.246	0.109	0.048	0.154	0.089	0.040	0.125	0.642

Table 14. Tissue Na^+/K^+ ratio and Selective Transport (ST) at the reproductive stage in rice

Genotype	Root		Stem		Middle leaf		Flag leaf		Panicle		ST (Root to Flag leaf)
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	
FL478	0.644^{a}	8.193^{c}	0.53	4.88	0.26	2.39	0.180^{a}	1.238^{b}	0.122^{a}	0.704^{c}	3.36^{ab}
IR29	0.807^{a}	7.230^{bc}	0.51	6.06	0.29	5.31	0.161^{a}	2.863^{e}	0.140^{a}	1.809^{e}	1.40^{d}
AC41585	0.723^{a}	8.112^{c}	0.64	3.89	0.36	1.86	0.228^{a}	0.797^{ab}	0.112^{a}	0.442^{ab}	4.32^{a}
Sadri	0.758^{a}	7.428^{bc}	0.60	5.94	0.30	3.84	0.238^{a}	2.060^{cd}	0.180^{a}	1.27^{d}	1.97^{bcd}
AC39416A	0.821^{a}	8.182^{c}	0.61	3.81	0.30	2.03	0.213^{a}	0.790^{ab}	0.111^{a}	0.513^{ab}	4.10^{a}
Rashpanjor	0.685^{a}	7.052^{bc}	0.45	4.46	0.28	2.30	0.149^{a}	0.975^{b}	0.137^{a}	0.521^{ab}	3.18^{b}
CSR27	0.822^{a}	7.141^{c}	0.47	4.96	0.28	2.75	0.220^{a}	1.255^{b}	0.175^{a}	0.735^{c}	2.76^{bc}
Binadhan 8	0.779^{a}	7.218^{bc}	0.58	5.51	0.31	2.82	0.207^{a}	1.359^{b}	0.161^{a}	0.775^{cd}	2.92^{bc}
Luna Suvarna	0.813^{a}	7.200^{bc}	0.53	4.79	0.32	2.62	0.177^{a}	1.404^{bc}	0.152^{a}	0.819^{cd}	2.76^{bc}
Sabita	0.797^{a}	7.974^{b}	0.43	6.58	0.27	4.87	0.190^{a}	2.505^{de}	0.155^{a}	1.715^{de}	1.76^{cd}
	G	S	GXS	G	S	GxS	G	S	GxS	G	S	GxS	G	S	GxS	GxS
SE(M)±	0.636	0.285	0.900	0.451	0.202	0.637	0.191	0.085	0.269	0.091	0.040	0.122	0.049	0.022	0.069	0.588
LSD (*p*<0.05)	1.818	0.813	2.572	1.228	0.567	1.822	0.545	0.244	0.770	0.206	0.116	0.368	0.141	0.063	0.199	0.831

4.3. Effect of salt stress on chlorophyll content and leaf gas exchange parameters at both the early seedling and reproductive stages in rice

Salinity severely disintegrates the structure and function of chlorophyll. Significant reduction in chlorophyll concentration was observed in all the genotypes as compared to control under salt stress both the early seedling and reproductive stages (Tables 15 and 16). Irrespective of genotypic variation in chlorophyll concentration highest reduction was observed in IR29 (55.32%) followed by Sabita (49.73%) in the early seedling stage. In moderately tolerant genotypes like CSR27, Binadhan 8 and Luna Suvarna ~20-30% reduction in chlorophyll concentration was observed. While in FL478 (12.45%) the decrement was least among all the genotypes and followed by AC39416A (15.15%), AC41585 (18.13%) and Rashpanjor (20.18%). Similarly in the reproductive stage, a significant reduction in total chlorophyll concentration was evident under a prolonged stress period (Table 14). However, the decline was minimum for AC41585 (9.10%) followed by Rashpanjor (14.51%) and AC39416A (18.12%). But in susceptible genotypes like IR29 and Sabita the drop in chlorophyll concentration was ~50%.

Different gas exchange traits like net photosynthesis, stomatal conductance, and transpiration rate were significantly dropped under salt stress in both stages (Table 16). In the seedling stage, genotypes like FL478, AC41585 and AC39416A were able to maintain net photosynthesis and stomatal conductance under 12 dS m^{-1} of salt stress. The least decline in P_N was recorded in FL478 (21.32%) and was at par with AC41585, (23.14%), While the highest reduction in net photosynthesis (74.08%) and stomatal conductance (89.67%) was noticed in IR29 followed by Sabita. More than 30% drop in net photosynthesis and ~40% drop in stomatal conductance were observed in moderately tolerant genotypes like CSR27, Binadhan 8 and Luna Suvarna, Whereas Rashpanjor (moderately tolerant) was able to maintain P_N (23.39%) and g_s (31.48%) even under salt stress at the early seedling stage. Similar results were observed in transpiration also, the highest reduction in transpiration rate was observed in IR29 (85.02%) followed by Sabita (81.71%) and Sadri (80.64%), while it was lowest in

Table 15. Effect of salt stress on chlorophyll concentration and leaf gas exchange parameters at the early seedling stage in rice in rice

Genotype	Chlorophyll concentration (mg g^{-1} FW)		Net photosynthesis (P_N) (µmol CO_2 m^{-2} S^{-1})		Stomatal conductance (g_s) (mmol m^{-2} s^{-1})		Transpiration rate (E) (mmol H_2O m^{-2} s^{-1})	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	2.54	2.23 (12.45%)	21.70	20.22 (21.32%)	0.807	0.539 (33.26%)	6.79	2.46 (-63.82%)
IR29	2.38	1.06 (55.32%)	18.84	1.09 (-74.08%)	0.404	0.081 (79.85%)	6.79	1.02 (-85.02%)
AC41585	2.81	2.30 (18.13%)	18.12	14.45 (23.14%)	0.700	0.450 (35.75%)	5.27	2.24 (-57.58%)
Sadri	2.62	1.45 (44.85%)	18.32	8.17 (-54.97%)	0.610	0.312 (48.86%)	6.63	1.28 (-80.64%)
AC39416A	2.61	2.20 (15.85%)	19.41	13.84 (25.03%)	0.691	0.480 (30.56%)	6.02	1.48 (-75.39%)
Rashpanjor	2.59	2.07 (20.18%)	16.79	14.78 (23.39%)	0.526	0.360 (31.48%)	6.13	1.47 (-75.93%)
CSR27	2.78	2.21 (20.72%)	17.31	11.74 (32.98%)	0.788	0.479 (39.17%)	6.38	1.55 (-75.76%)
Binadhan 8	2.12	1.49 (29.98%)	18.98	10.84 (41.56%)	0.649	0.434 (22.06%)	6.93	1.00 (-85.55%)
Luna Suvarna	2.64	1.61 (38.97%)	20.54	12.17 (44.61%)	0.789	0.427 (-45.0%)	6.42	1.17 (-81.80%)
Sabita	2.48	1.25 (49.73%)	20.27	5.17 (-68.40%)	0.739	0.324 (56.12%)	6.06	1.11 (-81.71%)
	G	**S**	**GXS**	**G**	**S**	**GxS**	**G**	**S**	**GxS**	**G**	**S**	**GxS**
SE(M)±	0.075	0.033	0.106	0.887	0.397	1.254	0.037	0.017	0.052	0.325	0.145	0.460
LSD (p<0.05)	0.214	0.096	0.302	2.535	1.133	3.584	0.106	0.047	0.150	0.929	0.416	1.314

Table 16. Effect of salt stress on chlorophyll concentration and leaf gas exchange parameters at the reproductive stage in rice

Genotype	Chlorophyll concentration (mg g^{-1} FW)		Net photosynthesis (P_N) (µmol CO_2 m^{-2} S^{-1})		Stomatal conductance(g_s) (mmol m^{-2} s^{-1})		Transpiration rate (E) (mmol H_2O m^{-2} s^{-1})	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	4.51	3.16 (-30.06%)	19.13	10.59 (-44.63%)	1.32	0.26 (-80.34%)	10.38	4.08 (-60.68%)
IR29	4.30	2.29 (-48.71%)	18.02	8.18 (-54.63%)	1.10	0.11 (-89.67%)	9.33	2.49 (-73.70%)
AC41585	3.96	3.60 (-9.10%)	20.90	15.51 (-25.76%)	0.91	0.70 (-23.76%)	10.16	6.45 (-36.74%)
Sadri	4.80	3.14 (-34.56%)	17.68	10.74 (-39.26%)	1.00	0.27 (-73.43%)	9.17	3.04 (-66.88%)
AC39416A	4.36	3.57 (-18.12%)	20.11	15.47 (-23.06%)	0.97	0.64 (-34.18%)	9.64	5.41 (-43.85%)
Rashpanjor	4.11	3.52 (-14.51%)	18.07	14.53 (-19.57%)	0.91	0.62 (-32.05%)	9.31	5.51 (-40.89%)
CSR27	4.11	3.19 (-22.37%)	20.03	13.96 (-30.33%)	1.14	0.28 (-75.56%)	10.41	5.26 (-49.47%)
Binadhan 8	3.51	2.53 (-27.37%)	19.57	11.28 (-42.35%)	1.04	0.24 (-76.40%)	9.41	5.23 (-44.48%)
Luna Suvarna	4.17	2.90 (-30.46%)	18.71	10.46 (-44.11%)	1.11	0.16 (-85.39%)	10.97	3.66 (-66.66%)
Sabita	4.45	2.39 (-46.41%)	20.89	8.75 (-58.10%)	1.06	0.08 (-92.61%)	9.67	2.04 (-78.88%)

	G	S	GXS	G	S	GxS	G	S	GxS	G	S	GxS
SE(M)±	0.193	0.086	0.272	0.679	0.304	0.960	0.063	0.028	0.090	0.466	0.208	0.659
LSD (p<0.05)	0.550	0.246	0.778	1.941	0.868	2.748	0.181	0.081	0.256	1.133	0.595	1.882

AC41585 (57.58%) followed by FL478 (63.82%). When these traits were analyzed in the reproductive stage, a significant decline was observed for all the parameters in stressed plants as compared to control plants (Table 17). The total chlorophyll concentration was reduced to half in IR29 followed by Sabita (46.41%) and Sadri (34.56%). Whereas, maximum pigment retention was recorded in AC41585 (9.10%) followed by Rashpanjor (14.51%) and AC39416A (18.12%). Similarly, the net photosynthetic rate, stomatal conductance and transpiration rate was highly compromised in some susceptible genotypes like IR29 and Sabita, While, the least decrement in the rate of the net photosynthesis rate (~20%) was observed in Rashpanjor and followed by AC39416A (~24%) and AC41585 (~25%). Similarly, least drop in both stomatal conductance (~30%) and transpiration rate recorded (~40%) was evident in Rashpanjor amongst all the genotypes in the reproductive stage.

4.5. Effect of salinity on chlorophyll-a fluorescence traits in both early seedling and reproductive stages in rice

In this study maximum potential of quantum efficiency (F_v/F_m) and quantum yield of non-regulated energy dissipation [Y(NO)] trait of PSII was estimated under salt stress (Figures 4 and 5). The drastic drop in F_v/F_m value (0.790 to 0.128) observed in the susceptible genotype (IR29) in quantum yield efficiency of PSII was highly compromised under salt stress. There was less yet significant decline in the maximum potential of quantum efficiency of PSII was recorded in Sadri (0.805 to 0.506), CSR27 (0.785 to 636), Binadhan 8 (0.800 to 0.699) and Luna Suvarna (0.786 to 0.679). However, there was no significant reduction in F_v/F_m was observed for FL478 (0.803 to 0.713) and AC41585 (0.789 to 0.737). On the contrary the quantum yield of non-regulated energy dissipation [Y(NO)] value was higher under stress condition in all the genotypes as compared to the control (Figure 4 B and D). The highest increment was observed in IR29 (0.210 to 0.872) followed by Sadri (0.195 to 0.494). Least increase in [Y(NO)] was observed in AC41585 (0.211 to 0.253) followed by FL478 (0.197 to 0.287).

On the otherhand in the reproductive stage, there was no significant difference observed for the maximum potential of quantum efficiency (F_v/F_m) of PSII, except for susceptible genotypes like IR29 (0.798 to 0.593), Sabita (0.752 to 0.601) and Sadri (0.794 to 0.682) (Figure 5 A and C). Similarly, the values of quantum yield of non-regulated energy dissipation [Y(NO)] trait of PSII was significantly higher for IR29 (0.201 to 0.407) and Sabita (0.197 to 0.395), while in rest of the genotypes there was no significant difference found under prolong stress condition.

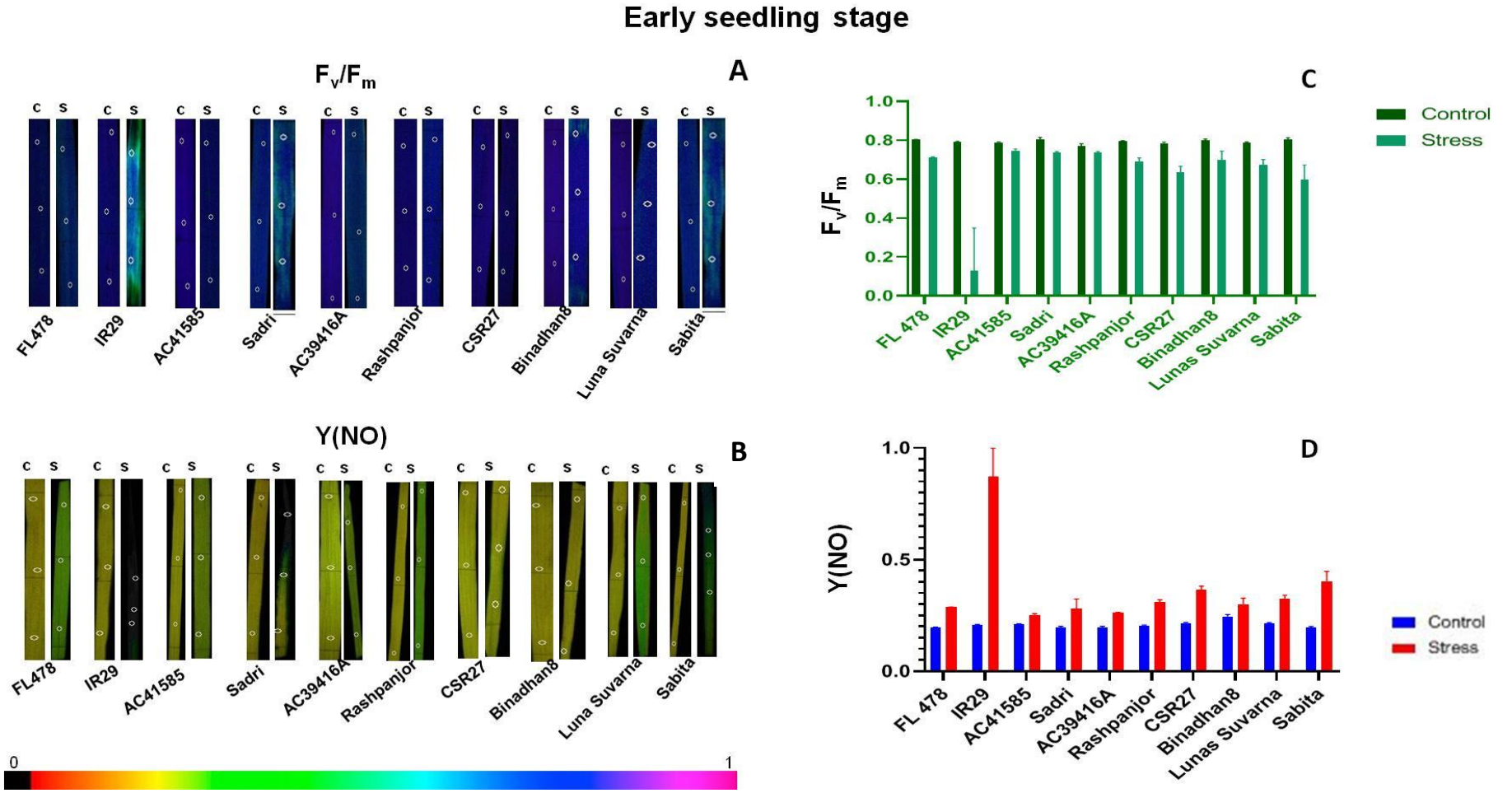


Figure 4. Chlorophyll-a fluorescence images of F_V/F_m (A) and [Y(NO)] (B). The histogram represents value of F_V/F_m (C) and [Y(NO)] (D).

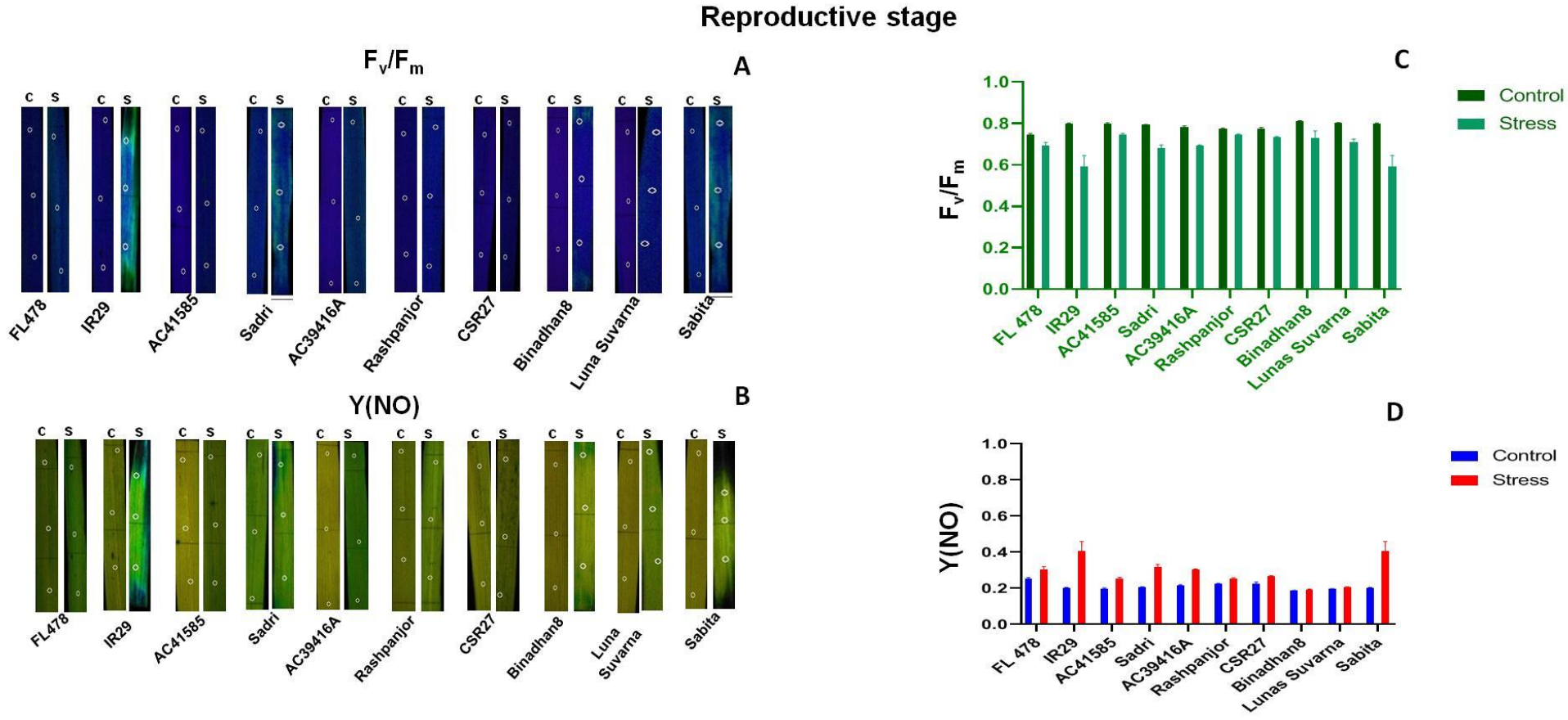


Figure 5. Chlorophyll-a fluorescence images of F_V/F_m (A) and [Y(NO)] (B). The histogram represents value of F_V/F_m (C) and [Y(NO)](D).

4.6. Ranking of the genotypes on the basis of different morpho-physiological traits at early seedling and reproductive stage in rice

Based on the findings the genotypes were ranked in response to salt tolerance at both the early seedling and reproductive stages. We rank the genotypes at the early seedling stage as FL478 > AC41585 > AC39416A > Rashpanjor > CSR27 > Binadhan 8 > Luna Suvarna > Sadri > Sabita > IR29. Likewise, at the reproductive stage, we rank these genotypes as Rashpanjor > AC41585 > AC39416A > CSR27 > Binadhan 8 > Luna Suvarna > FL478 > Sadri > Sabita > IR29 based on several morpho-physiological, yield, and yield-related attributes. It became clear from our evaluation that the tolerance behaviour of the rice genotypes varied depending on the stage and duration of the imposed stress. Overall we found four different categories of rice genotypes i.e., (i) Genotype showing tolerance at both the stages (ii) Genotype highly tolerant at the seedling stage but moderately tolerant at the reproductive stage, (iii) Genotype moderately tolerant to the seedling stage but highly tolerant at the reproductive stage and (iv) Genotype susceptible to both the stages. We selected four genotypes (AC41585, FL478, Rashpanjor, and IR29) from the initial panel to conduct additional research in order to determine the mechanistic differences and the most important factors affecting salt tolerance at these two critical periods.

4.7. Tissue tolerance assay

4.7.1. Chlorophyll-a fluorescence traits and tissue tolerance score in tissue tolerance assay

In this experiment, a sharp decline of F_v/F_m value and a sharp increase of [Y(NO)] fluorescence value was observed in FL478 at the end of 7^{th} day period of stress (Figure 6). In the case of the other three studied genotypes reduction of F_v/F_m or increment of [Y(NO)] values was slower than FL478. Just after 3 days, a sharp decline in F_v/F_m value started in FL478 (0.798 to 0.647). On the other hand a gradual decline was observed in IR29 (0.802 to 0.781), AC41585 (0.791 to 0.703) and Rashpanjor (0.811 to 0.751) after 3 days. In case of IR29, the F_v/F_m value was maximum (0.63) as compared to

the rest three genotypes, at the end of the 7th day of period of stress. This was followed by Rashpanjor (0.52) and AC41585 (0.50). Whereas, the value maximum quantum yield efficiency (F_v/F_m) was almost zero after the 7th day in FL478. Similar to this, based on the [Y(NO)] fluorescence values, FL478 showed the highest increase of [Y(NO)] values just after 3rd day of imbibitions in salt solution and the heat loss in terms of non-regulated energy dissipation was almost '1' at the end of the stress. In reverse, rest three genotypes maintained much lower [Y(NO)] values 0.364 for IR29, 0.47 for Rashpanjor and 0.49 for AC41585 on the 7th day.

Along with this, the chlorophyll breakdown process was highest in the genotype FL478 (Figure 7) from the very beginning. Just after 3rd day of imposition of salt stress about 50% of the chlorophyll concentration was depleted from the initial level. At the end of the 7th day of stress, chlorophyll content was 0.10 mg g^{-1}, FW for FL478. However, chlorophyll retention was maximum in IR29 (0.65 mg g^{-1}, FW) followed by Rashpanjor (0.19 mg g^{-1}, FW) at the end of the 7th day. Although, the imbibitions of sodium in the leaves were nearly similar for all the studied genotypes but chlorophyll breakdown process was different in the case of studied rice genotypes (Figure 7 A-D). Here, IR29 plants showed the least reduction of chlorophyll and total chlorophyll concentration was (0.65 mg g^{-1}, FW) for IR29. This was followed by Rashpanjor (0.50 mg g^{-1}, FW) and AC41585 (0.25 mg g^{-1}, FW). Rest three genotypes were able to maintain the most important photosynthetic pigment chlorophyll to some extent, therefore, able to maintain a higher tissue tolerance score than FL478. Tissue tolerance was calculated based on the LC_{50} values, and a sensitive rice genotype IR29 showed the maximum tissue tolerance score of 433.95 mg Kg^{-1}, DW. This was followed by Rashpanjor (419.45 mg Kg^{-1}, DW), AC41585 (369.75 mg Kg^{-1}, DW) and FL478 (304.56 mg Kg^{-1}, DW).

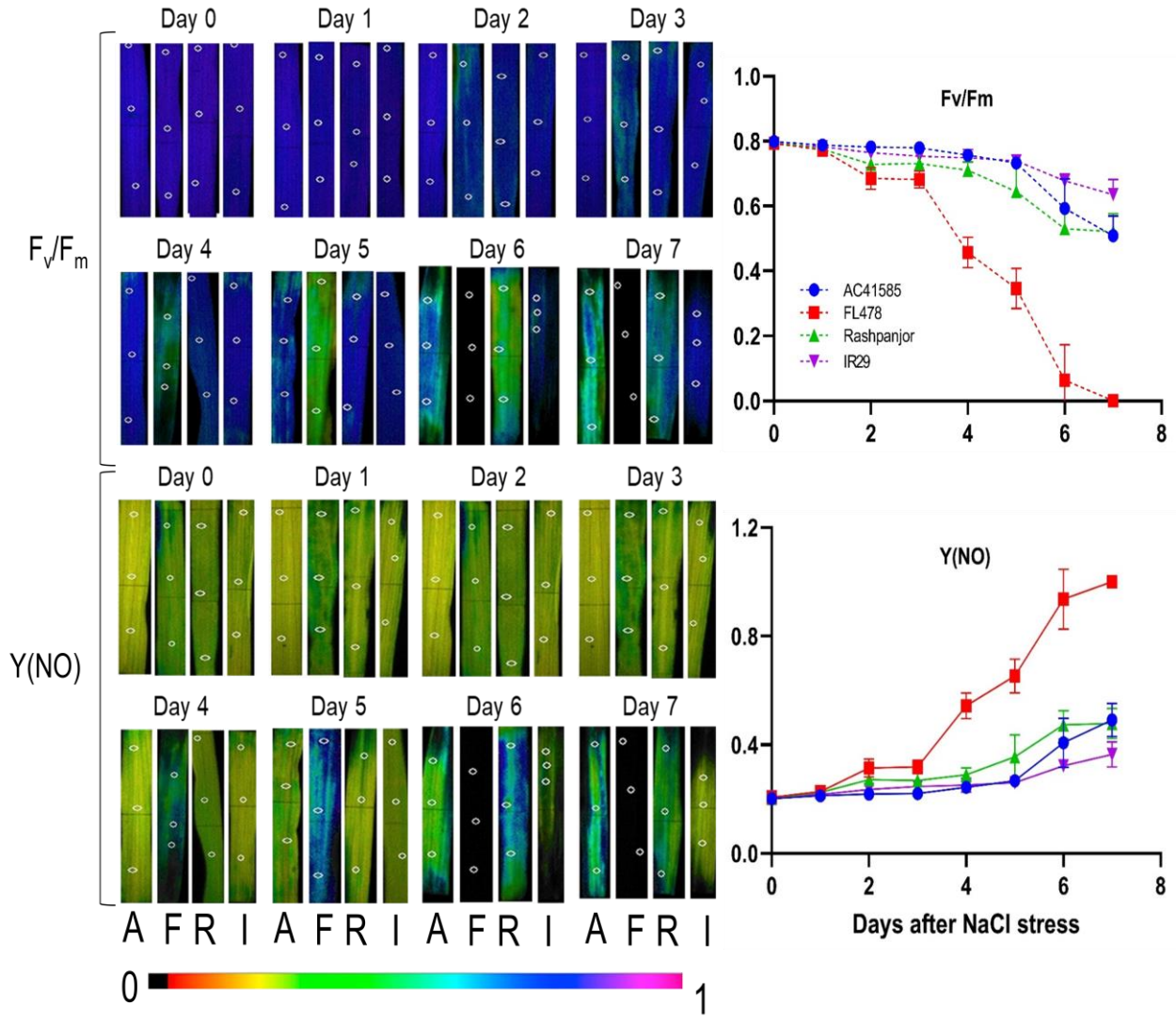


Figure 6. The fluorescence images (A and C) and line diagrams (B and D) of maximum potential quantum efficiency of Photosystem II (Fv/Fm) and quantum yield of non-regulated energy dissipation [Y(NO)] of excised leaf clips of four genotypes subjected to 12 dS m^{-1} salt stress under in vitro conditions, where A, F, R and I represent AC41585, FL478, Rashpanjor and IR29 respectively. Selected images are typical representation of three biological replicates and

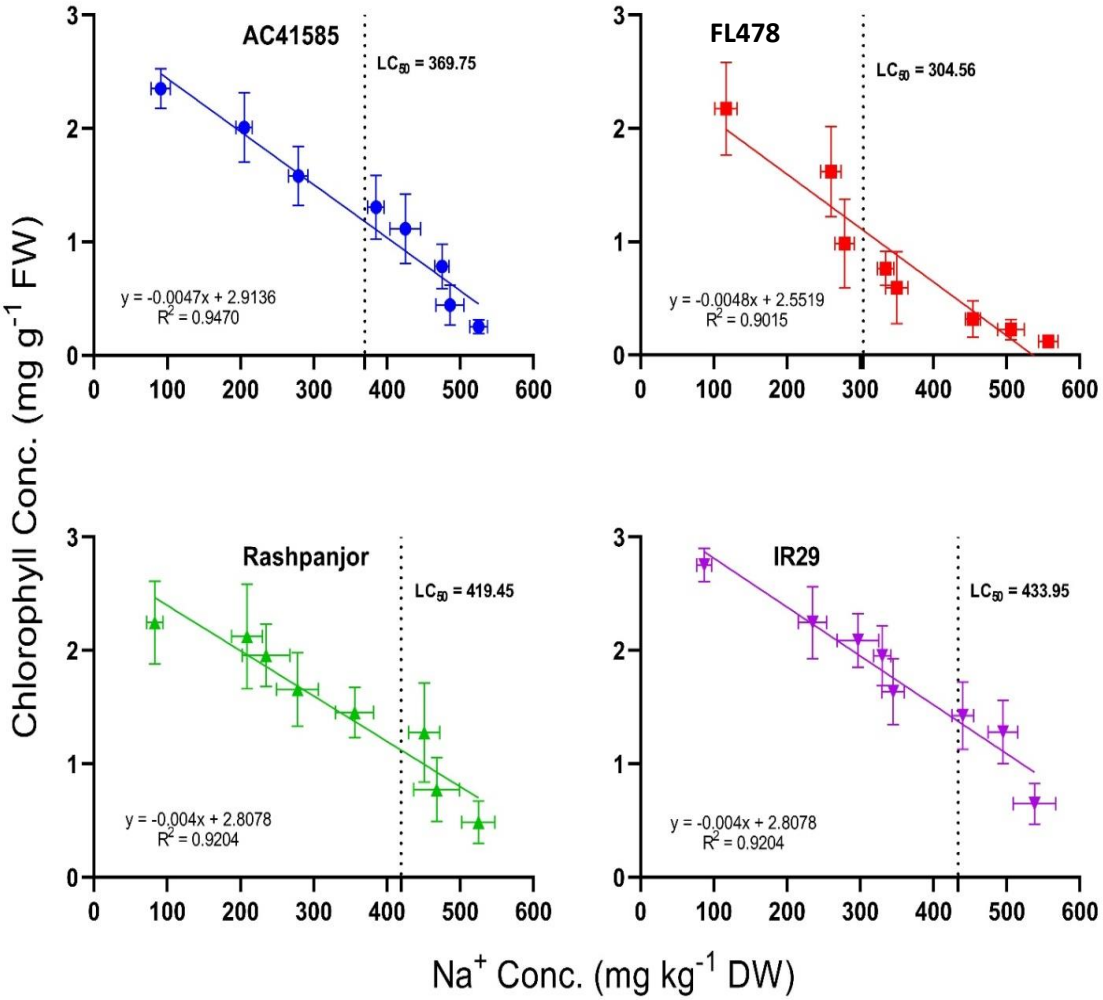


Figure 7. LC_{50} score (A-D) of four genotypes (AC41585, FL478, Rashpanjor, and IR29)

Tissue tolerance score based on LC_{50} value was calculated from the graphs and represented as a dashed vertical line in each plot. Here, 'y′ axis is showing chlorophyll concentration (mean ± SE of mean, where n = 3) and 'x′ axis is showing tissue Na^+ concentration (mean ± SE of mean, where n = 3).

4.8. Effect of salt stress on ROS production

Two major components of oxidative stress damage viz., H_2O_2 and malondialdehyde (MDA) concentration was estimated from the leaves and panicle tissues at both stages of salt stress (Figures 8 and 9). A significant increase in leaf H_2O_2 concentration was observed under salt stress at both seedling and reproductive stages (Figure 8). But the damage was not significant in the panicles of AC41585 and Rashpanjor. A differential response was prominent in all the genotypes at both the stages. The increase was relatively less in AC41585, FL478, and Rashpanjor at the seedling stage although it was still significant ($P < 0.05$). In comparison to the control plants, there was a 40% induction of H_2O_2 burst in AC41585, FL478 and Rashpanjor, however the rise in IR29 was extremely significant ($P < 0.0001$) and > 5.5-fold (Figure 8A). At the reproductive stage, the increase in leaf H_2O_2 concentration under salt stress was not significant in AC41585, while it was significant in Rashpanjor ($P < 0.05$). But in FL478 (3.8-fold) and IR29 (6.5-fold), the induction was highly significant ($P < 0.001$) at reproductive stage (Figure 8B). Similarly in panicles rise in leaf H_2O_2 concentration was not significant for both AC41585 and Rashpanjor $P < 0.05$, while in FL478 the level of H_2O_2 concentration was increased ~ 4 folds and more than 6 fold for IR29 at $P < 0.001$. Apart from measuring the reactive oxygen species, the level of lipid peroxidation was also measured in both leaves and panicles and expressed in terms of MDA (Malondialdehyde) concentration in both stages under salinity (Figure 9). In early seedling stage, no significant changes in MDA concentration was observed in either AC41585 or FL478, but the induction was highly significant ($P < 0.001$) in Rashpanjor (4-fold) and IR29 (6.5-fold) as compared to control. However highly significant increase in leaf MDA concentration was observed in flag leaf tissues of all the genotypes at reproductive stage (Figure 9B). However the results differed when it comes to panicles. The least increase in MDA concentration was observed in AC41585 (2-fold), followed by Rashpanjor and FL478, while the increase was highest in IR29 (8.5-fold) as compared to the control conditions. But in developing panicles of both AC41585 and Rashpanjor, there was no significant increase in MDA concentration at $P < 0.05$ but the induction was significant ($P < 0.05$) for FL478 in the reproductive stage. The highest induction in MDA concentration was observed in both the leaf and panicle of IR29 under both stages.

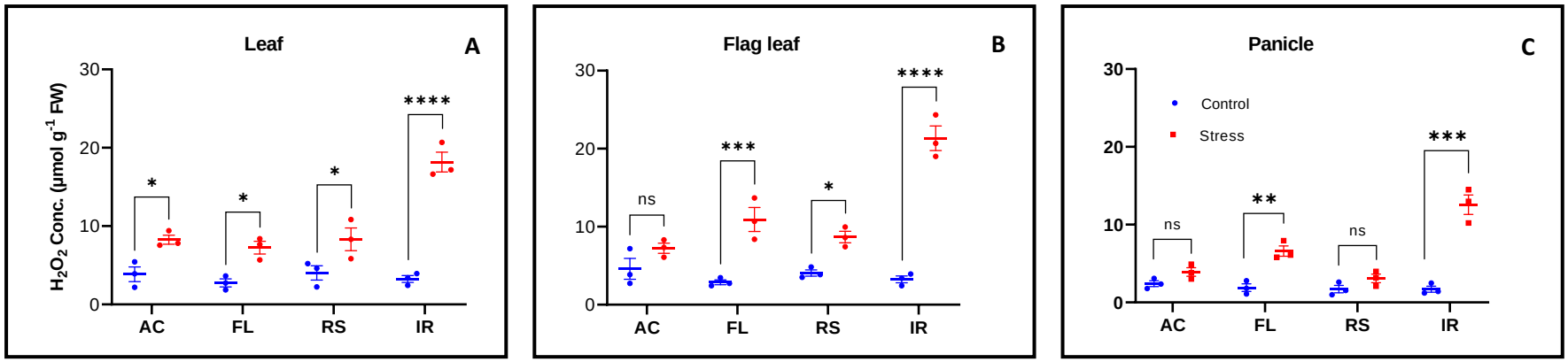


Figure 8. H_2O_2 concentration in (A) leaf tissues at early seedling stage, (B) flag leaf and (C) panicle at reproductive stage under salt stress

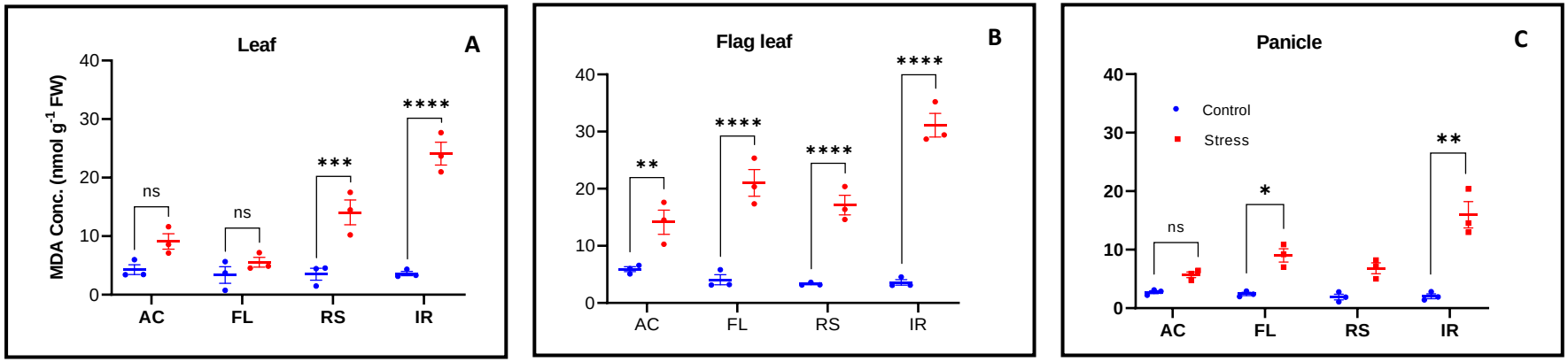


Figure 9. MDA concentration in (A) leaf tissues at early seedling stage, (B) flag leaf and (C) panicle at reproductive stage under salt stress

The histograms represents the mean ± SE of three independent biological replicates and the statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test Values marked with '* *' '***'and '****' with lines are significantly different ($P < 0.05$), ($P < 0.01$), ($P < 0.001$) *and* ($P < 0.0001$). AC, FL, RS and IR represent AC41585, FL478, Rashpanjor and IR29 respectively.

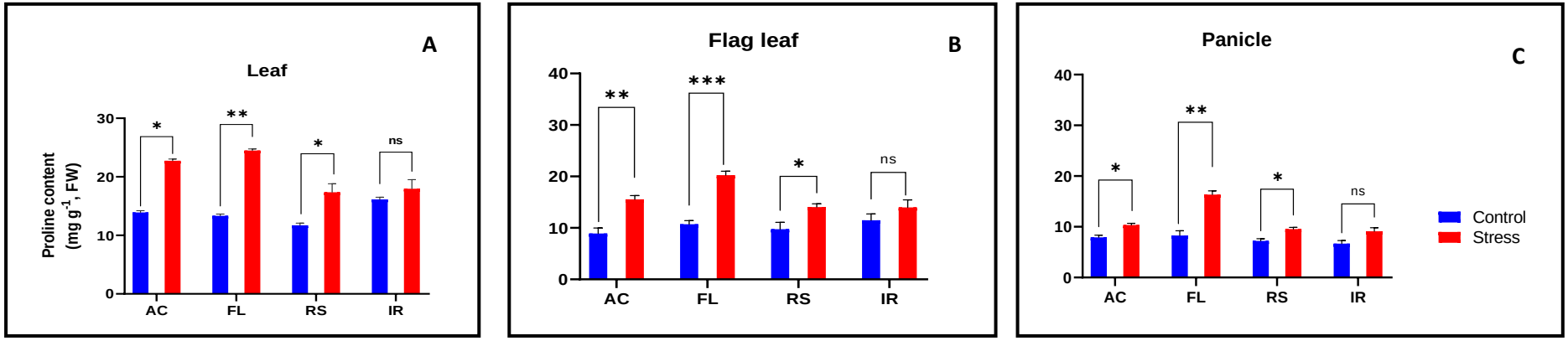


Figure 10. Proline concentration in (A) leaf tissues at the early seedling stage, (B) flag leaf and (C) panicle at the reproductive stage salt stress

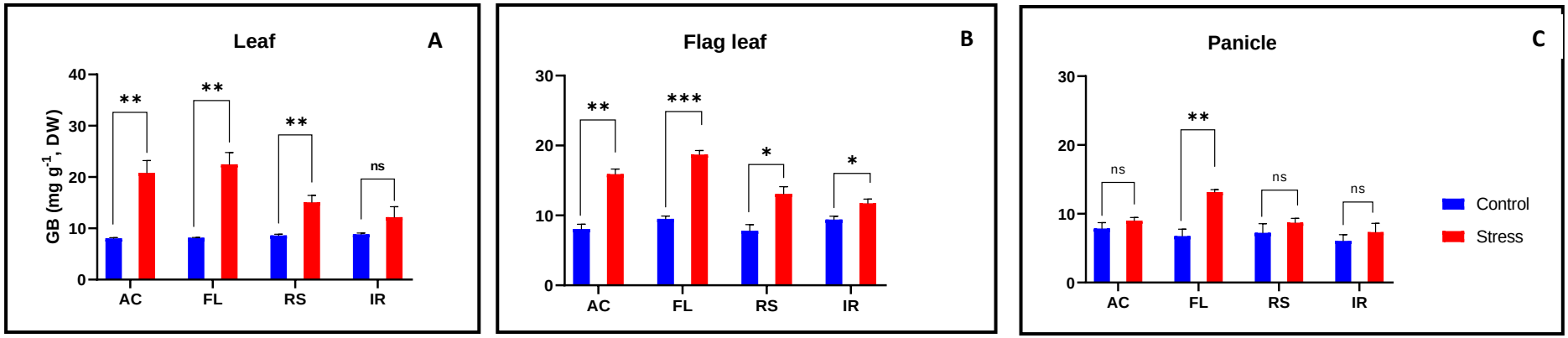


Figure 11. Glycine betaine concentration in (A) leaf tissues at early seedling stage, (B) flag leaf and (C) panicle at reproductive stage under salt stress

The histograms represents the mean ± SE of three independent biological replicates and the statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test Values marked with '* *' '***'and '****' with lines are significantly different (P < *0.05),* (P < *0.01),* (P < *0.001) and* (P < *0.0001).* AC, FL, RS and IR represent AC41585, FL478, Rashpanjor and IR29 respectively.

4.9. Effect of salt stress on compatible osmolytes concentration

Accumulation of compatible osmolytes is very essential for balancing cellular homeostasis. A significant increase in different osmolytes (glycine betaine and proline) was observed in stressed induced plants as compared to its control (Figures 10 and 11). The highest proline concentration was observed in FL478 (22.48 mg g^{-1}, FW) followed by AC41585 in the leaf tissues at the early seedling stage. But no significant increment was noticed in IR29, while the increment was significant for Rashpanjor ($P < 0.05$) in early seedling stage. Like the seedling stage, in the reproductive stage also the increase in proline content in both leaves and panicles of FL478 was highly significant ($P < 0.001$), while in AC41585 and Raspanjor ~ 1.5 fold increase was recorded in leaves and panicles. However the accumulation of proline was not significant in both panicles and leaves of IR29 in reproductive stress. Similarly, the glycine betaine accumulation was also significant under stress in both stages except for IR29 (Figure 11). In early seedling stage, the accumulation was highest in FL478 (24.48 mg g^{-1}, FW) followed by AC41585 (22.47 mg g^{-1}, FW) and Rashpanjor (15.08 mg g^{-1}, FW). While in the reproductive stage, the increment in leaf glycine betaine concentration was significant only in FL478. The highest glycine betaine concentration was observed in leaves (18.71 mg g^{-1}, FW) and panicles (13.42 mg g^{-1}, FW) of FL478. In leaf tissues of both Rashpanjor and AC41585 the increase in glycine betaine concentration was significant ($P < 0.05$) and nearly about 1.5 folds as compared to its respective control plants, while in panicles the increment was not significant.

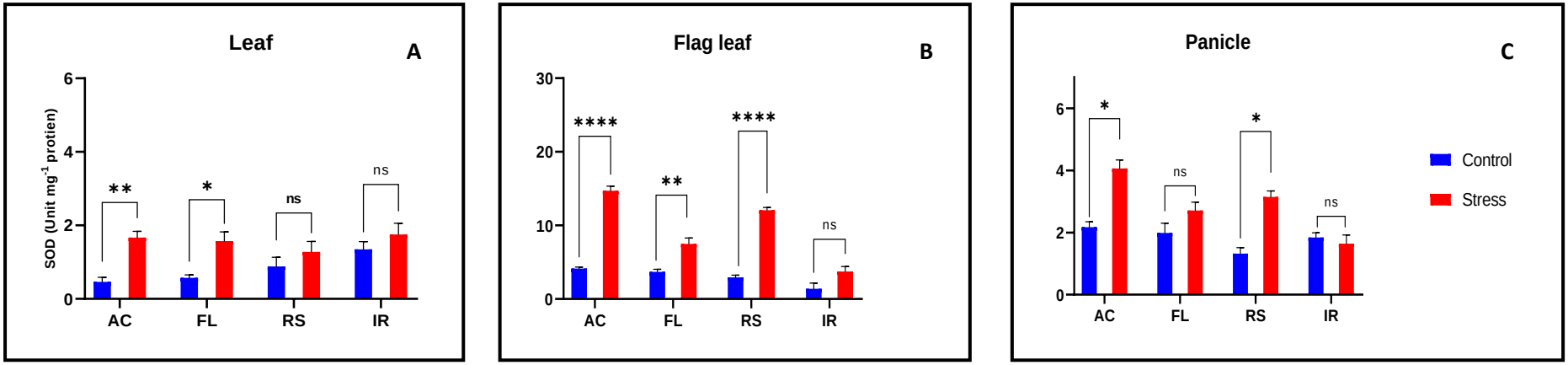


Figure 12. Super oxide dismutase activity in (A) leaf tissues at early seedling stage, (B) flag leaf and (C) panicle at reproductive stage

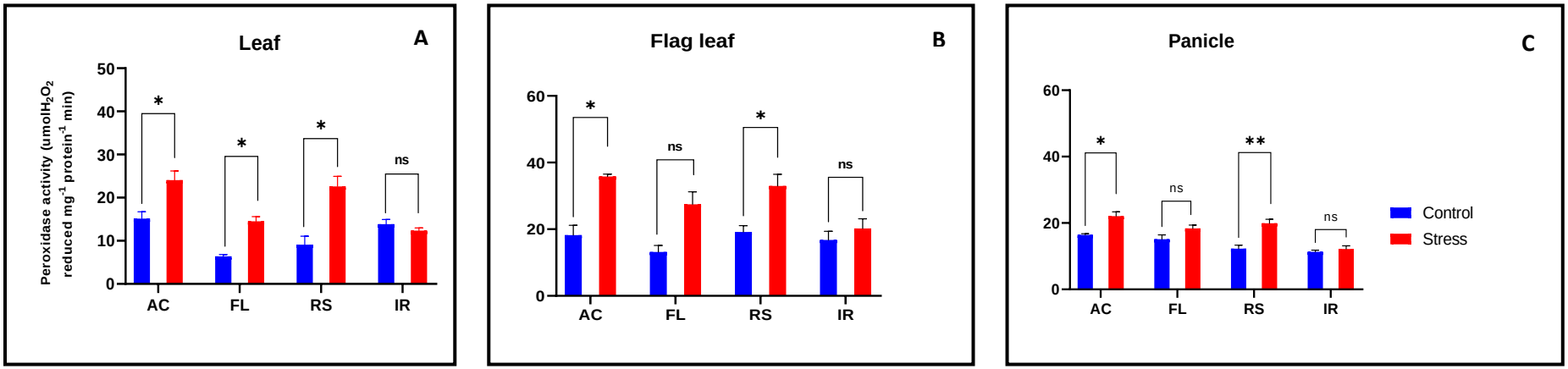


Figure 13. Peroxidase activity in (A) leaf tissues at early seedling stage, (B) flag leaf and (C) panicle at reproductive stage

The histograms represents the mean ± SE of three independent biological replicates and the statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test Values marked with '* *' '***'and '****' with lines are significantly different (P < *0.05),* (P < *0.01),* (P < *0.001) and* (P < *0.0001).* AC, FL, RS and IR represent AC41585, FL478, Rashpanjor and IR29 respectively.

4.10. Effect of salt stress on antioxidant enzymes activity

Significant changes in the activities of different antioxidant enzymes were recorded in both the early seedling (only leaves) and reproductive (both leaf and panicles) stages and the data was presented in Figures 12, 13 and 14. In the seedling stage more than 2-fold increase in SOD activity was recorded in the leaf tissues of AC41585 and FL478. While in both Rashpanjor and IR29, the increment was not significant ($P < 0.05$) at the early seedling stage. Unlike the seedling stage, in the reproductive stage the increment in SOD activity was highly significant ($P < 0.001$) in leaf tissues of AC41585 and Rashpanjor. Less yet significant ($P < 0.01$) induction was observed in the leaves of FL478 as well. But there was no significant activity recorded in leaves or panicles of IR29. However, significant activity was observed in panicles of AC41585 and Rashpanjor but not in FL478 ($P < 0.05$). Peroxidase (POX) activity was significantly increased ($P < 0.05$) in the leaf tissues of all the genotypes except IR29. Whereas, in the reproductive stage ~2-fold increase was observed in both leaves and panicles of AC41585 and Rashpanjor. But no significant ($P < 0.05$) activity was observed in FL478 and IR29 in both leaf and panicle tissues. Similarly, catalase (CAT) activity was highly induced in the leaves of AC41585 and Rashpanjor ($P < 0.01$) and > 4-fold increment was observed in FL478 at the early seedling stage. The activity was not significant for IR29 in this stage under stress. While in the reproductive stage, significantly ($P < 0.05$) high activity of CAT was recorded in both leaves and panicles of AC41585 and Rashpanjor. On the other hand the activity recorded in leaves and panicles of both FL478 and IR29 was not significant in the reproductive stage.

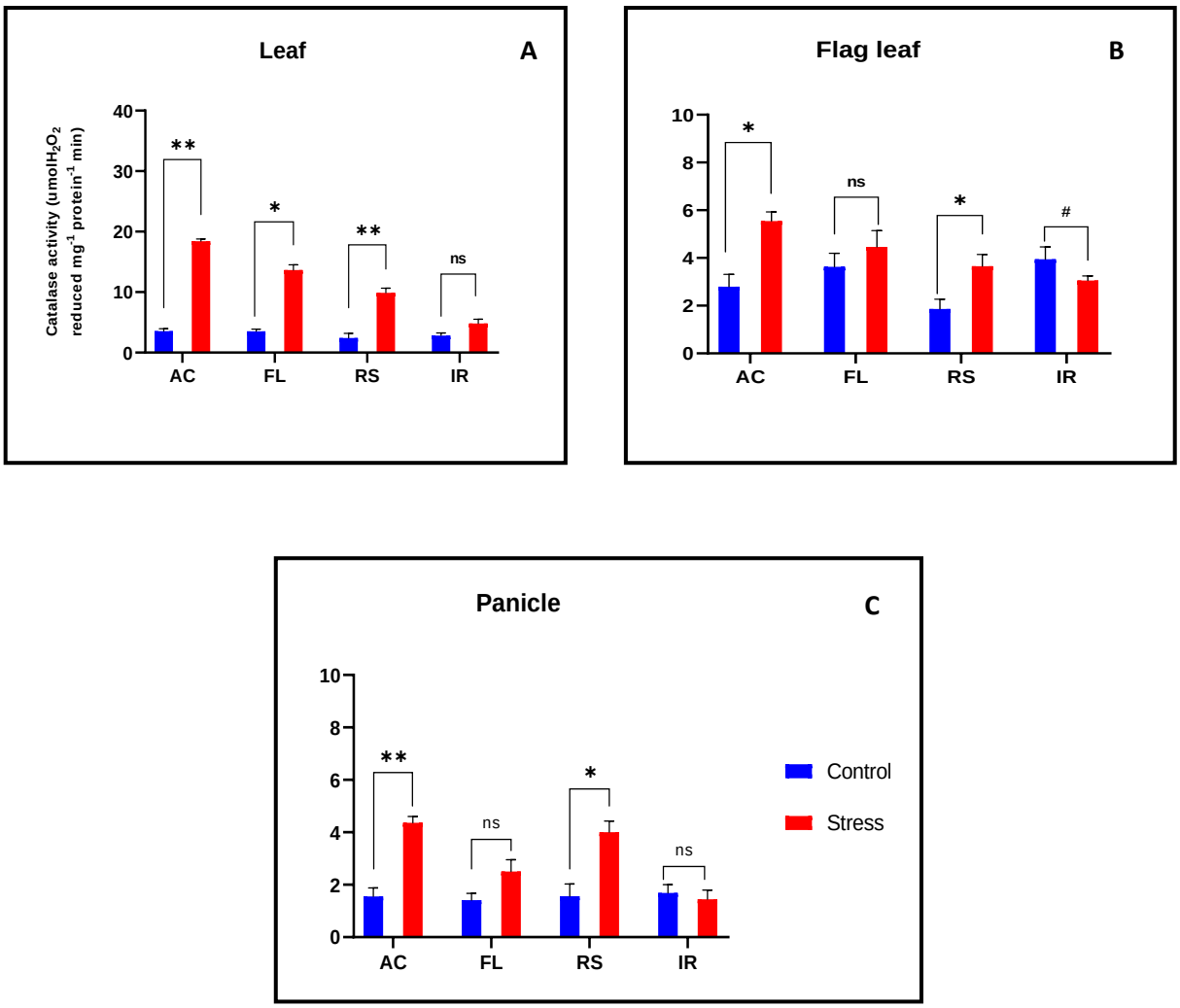


Figure 14. Catalase activity in (A) leaf tissues at early seedling stage, (B) flag leaf and (C) panicle at reproductive stage under salt

The histograms represents the mean ± SE of three independent biological replicates and the statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test Values marked with '* *' '***'and '****' with lines are significantly different (P < *0.05),* (P < *0.01),* (P < *0.001) and* (P < *0.0001).* AC, FL, RS and IR represent AC41585, FL478, Rashpanjor and IR29 respectively.

4.11. Expression analysis of different Na^+ and K^+ specific transporters/ ion channels/ pumps

4.11.1. Changes in the expressions profiles of SOS family and *OsNHX1*

In the present study, significant changes in the expression of SOS family and *OsNHX1* were analyzed in both root and leaf tissues (Figures 15, 16 and 17). In the seedling stage, the activity of *OsSOS1* was mostly upregulated in both the tissues and in all the genotypes except for IR29 (Figure 15A). The upregulation in *OsSOS1* was highest in roots of FL478 (~3.26 -fold) followed by AC41585 (2.45 -fold) and Rashpanjor (1.38 -fold). About 2 -fold of upregulation was recorded in the leaf tissues of FL478. *OsSOS1* expression was much higher in the roots of AC41585 and FL478 during the reproductive stage compared to its leaves. The expressions of transcript was highly induced in both root and leaf tissues of Rashpanjor, and in the leaves of AC41585 (1.57 -fold) and FL478 (1.13-fold) during reproductive stage. The expression pattern of *OsSOS2 (OsCIPK24)* and *OsSOS3 (CBL4)* was almost similar as *OsSOS1* in all the genotypes (Figures 15B and C). In FL478, ~2-fold upregulation of *OsSOS2 and OsSOS3* was observed in the root at the seedling stage. While in root tissues of both Rashpanjor and AC41585, there was an induction of more than 2-fold for *OsSOS2* transcript while no significant change was observed in the susceptible genotype, IR29 in the reproductive stage. But surprisingly the expression of *OsNHX1* (Figure 15D) was extremely distinct from the *SOS* transcripts since it showed significant upregulation in the leaf tissues of IR29. ~2-fold of increment in both roots and leaves was observed in AC41585 and Rashpanjor at the seedling stage but at the reproductive stage highest induction of *OsNHX1* was observed in the leaf tissue of Rashpanjor (1.87 -fold) followed by AC41585 (1.80 -fold),while for FL478, it remained unchanged or downregulated at both stages.

4.11.2. Changes in the expression of High-affinity Potassium Transporters (HKTs) and K^+ specific transporters/ channels

The upregulation in the expressions of class I HKT family transporters was remarkable, specifically for *OsHKT1.1* in all the genotypes, except IR29 under salt stress at both stages (Figures 16 A, B, and C). It was highly upregulated in the leaves of AC41585 (2.2 -fold) followed by FL478 (1.8 -fold) and Rashpanjor (1.5 -fold) than the root tissues of AC41585 (1.9-fold), FL478 (1.1 -fold) and Rashpanjor (0.9 -fold) at the

seedling stage. Similar to that in the reproductive stage, leaves of Rashpanjor (2.1-fold) and AC41585 (1.9-fold) showed significantly higher expression than roots. *OsHKT1.5* (*SKC1*), another important member of the class I HKT family, was significantly upregulated during early seedling stage in the leaves (2.3-fold) and roots (3.7 -fold) of FL478 followed by in the roots of AC41585 (3.1-fold) (Figure 16A). Unlike the seedling stage, in reproductive stage in FL478, the upregulation of the *OsHKT1.5* transporter was very less in leaf tissues. Whereas, In the case of AC41585 there was less yet significant up regulation was observed in the roots (2.4 -fold) and leaves (1.4 -fold) followed by in the leaves of Rashpanjor (2.1-fold). In case of the Class II HKT family, *OsHKT2.4* was highly upregulated in the leaf tissue of AC41585 (3.8 -fold) and in the root of FL478 (2.8 -fold) in seedling stage (Figure 16C). On the contrary in the reproductive stage there was slight up regulation was observed in all the genotypes but in IR29 it was mostly downregulated in the leaves.

Similar to Na^+-specific transporters and HKTs, same kind of upregulation was observed in K^+-specific transporters in both the stages (Figures 16 D, E and F). During salt stress, the expression pattern of two important K^+ transporters, *OsHAK1* and *OsHAK5* was greatly increased. The induction of *OsHAK1* was very high in the roots than the leaves. The upregulation was highest in the roots of Rashpanjor (2.2 -fold) followed by AC41585 (1.6 -fold) in both seedling and reproductive stages. On the other hand, *OsHAK5* expression was more prevalent in the leaves than the roots during both stages (Figure 16E). It was highest in leaves of FL478 (3.5 -fold) followed by AC41585 (2.9 -fold) and Rashpanjor (2.1 -fold) in seedling stage. While nearly 2 -fold of upregulation in expression was noted in the roots and leaves of AC41585 followed by Rashpanjor at reproductive stage. But it was found to be downregulated in the roots of IR29. Another inward rectifying K^+ channel *OsAKT1*, whose expression was mostly induced in the root tissues at both the stages under stress conditions than control (Figure 16F). Maximum induction was observed in the roots of FL478 (2 -fold) followed by AC41585 (1.8 -fold) in the seedling stage. However, it was highest in the root of Rashpanjor (3.0 -fold) followed by AC41585 (2.3 -fold) under prolonged period of stress. In FL478 and IR29 it was mostly unaffected in the reproductive stage.

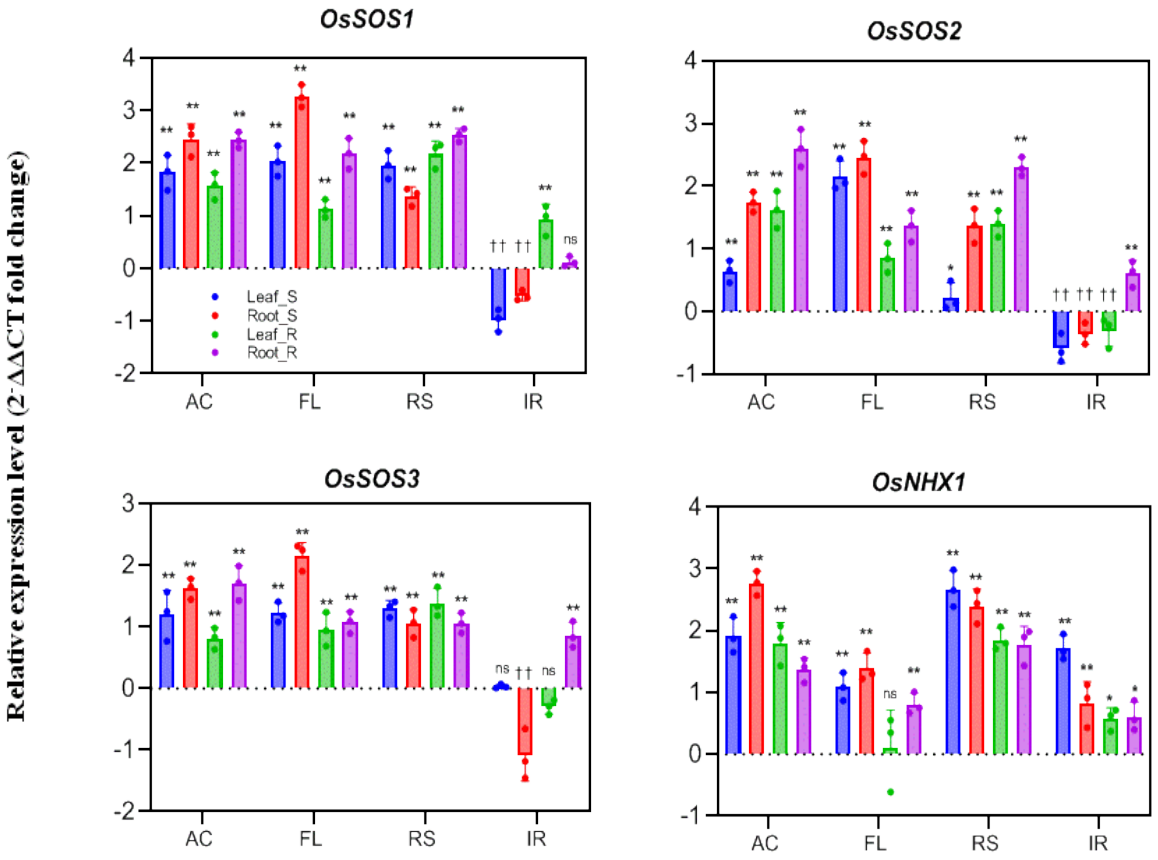


Figure 15. Expression analysis of (A) *OsSOS1*, (B) *OsSOS2*, (C) *OsSOS3* and (D) *OsNHX1* in leaf and root tissues at both the stages

The histogram represents changes in gene expression profiles. Gene expression values are depicted as fold change ($2^{-\Delta\Delta CT}$) compared to the control presented in Log_2 scale and values presented are the mean ± SE of mean (n = 3).The statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test. '**' and '*'denotes significant upregulation and, '††'and '†' denotes significant down-regulation of gene expressions as compared to control at $P < 0.01$ *and* $P < 0.05$, respectively.

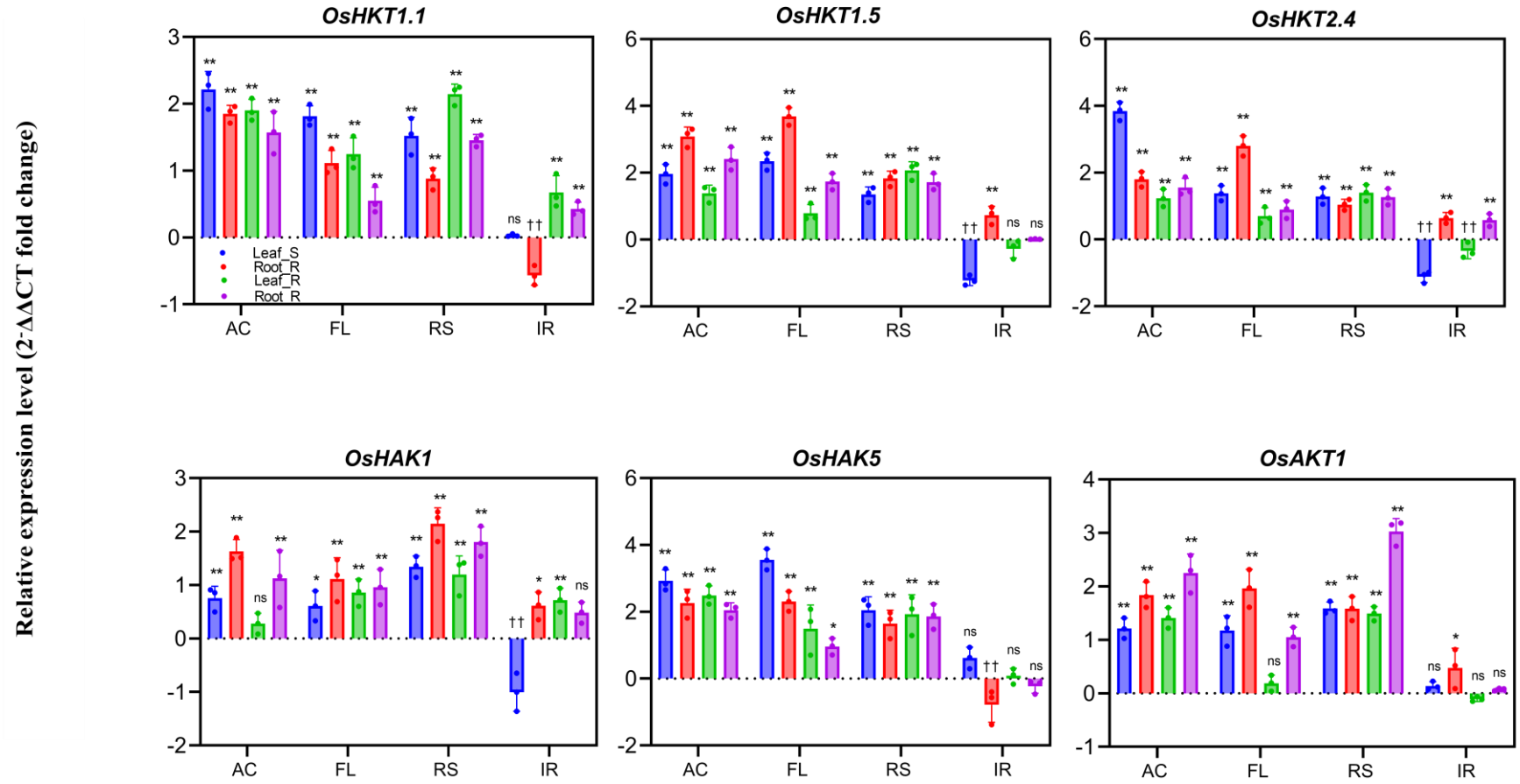


Figure 16. Expression analysis of (A) *OsHKT1.1*, (B) *OsHKT1.5*, (C) *OsHKT2.4* , (D) *OsHAK1* (E) *OsHAK5* and (F) *OsAKT1* in leaf and root tissues at both the stages

The histogram represents changes in gene expression profiles. Gene expression values are depicted as fold change ($2^{-\Delta\Delta CT}$) compared to the control presented in Log_2 scale and values presented are the mean ± SE of mean (n = 3).The statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test. '**' and '*'denotes significant upregulation and, '††'and '†' denotes significant down-regulation of gene expressions as compared to control at $P < 0.01$ *and* $P < 0.05$, respectively.

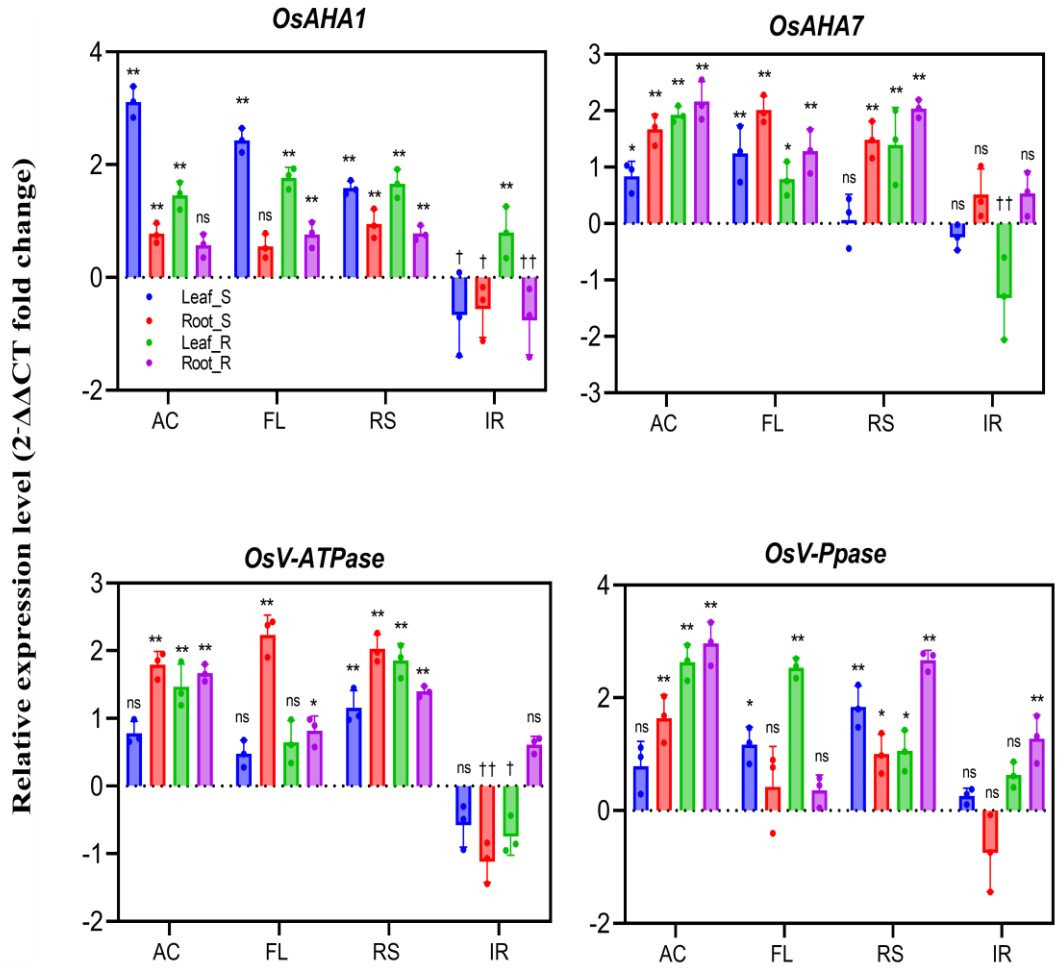


Figure 17. Expression analysis of (A) *OsAHA1*, (B) *OsAHA7*, (C) *OsV-ATPase* and (D) *OsV-PPase* in leaf and root tissues at both the stages

The histogram represents changes in gene expression profiles. Gene expression values are depicted as fold change ($2^{-\Delta\Delta CT}$) compared to the control presented in Log_2 scale and values presented are the mean ± SE of mean (n = 3).The statistical significance was tested using one-way ANOVA, followed by Tukey's multiple comparison test. '**' and '*'denotes significant upregulation and, '††'and '†' denotes significant down-regulation of gene expressions as compared to control at $P < 0.01$ *and* $P < 0.05$, respectively.

4.11.3. Changes in the expression of different proton pumps

All genotypes, with the exception of IR29, has shown dramatical induction in the expression of two plasma membrane H^+ pumps (*OsAHA1* and *OsAHA7*) and two vacuolar H^+ pumps (*OsV-ATPase* and *OsV-PPase*) in response to salt stress (Figure 17). The expression of *OsAHA1* was highly upregulated in the leaf tissues than the root of AC41585 (3.1 -fold) followed by FL478 (2.4 -fold) and Rashpanjor (1.6-fold) in the seedling stage. But induction was highest in the leaves of FL478 in the reproductive stage (Figure 17A). Considerably higher expression of *OsAHA7* was observed in the root portion than in the leaves (Figure 17B) under salt stress. The roots of FL478 had the maximum induction at the seedling stage (2.0 fold), but AC41585 and Rashpanjor had the highest induction at the reproductive stage (2.2 fold) (2.0 –fold) respectively. The activity of both the PM-proton pumps was downregulated in IR29 under stress in both the stages. Similarly both *OsV-ATPase* and *OsV-PPase* showed a higher degree of upregulation in the roots and leaves of Rashpanjor at both seedling and reproductive stages (Figures 17 C and D). While in AC41585 the expression of *OsV-PPase* was significantly up regulated in roots as well as in leaves in the reproductive stage.

DISCUSSION

Salinity has a negative impact on the growth and development of plants. In rice particularly, both the early seedling and reproductive stages are very sensitive to salt stress (Ahmadizadeh *et al*., 2016). Interestingly, in rice the response to salinity at both these stages is significantly different and may be independent from each other (Singh *et al*., 2008; Ferdose *et al*., 2009). This means the genotypes with excellent salt tolerance capability in the seedling stage may not have the same level of tolerance at the reproductive stage. Therefore, the overall objective of this present study is to find out the genotypes having differences in stage-specific salt tolerance ability and the underlying mechanistic differences for tolerance to salinity at both seedling and reproductive stages.

5.1. Response to salinity might differ in rice genotypes at both the stages

5.1.1. Early seedling stage

Some previous studies showed that the substantial tolerance to salinity at an early stage can be determined by estimating the relative growth and biomass accumulation. The productivity varies considerably with the reduction in biomass and vigour (Ali *et al*., 2014). For that in this present study, the preliminary assessment at the early seedling stage was done based on visual salt injury scoring and reduction in root and shoot length of 10 genotypes at the seedling stage (Tables 3 and 4). According to the VSI scoring under 12 dS m^{-1} of salt stress, FL478 was the most tolerant genotype followed by AC41585 and AC39416A, whereas, the tolerance response to salinity was moderate in the case of Rashpanjor, CSR27, Binadhan 8, and Luna Suvarna. On the other hand, Sadri, Sabita, and IR29 came under the susceptible category. Apart from the VSI score, the least reduction in both root and shoot and seedling dry weights were observed in FL478, the most tolerant genotype after one week of imposition of saline stress. Besides maintaining the dry weight and vigour, maintenance of the leaf water status is considered one of the major criteria of tolerance in the seedling stage. According to Suriya-arunroj *et al*. (2004) relative water content (RWC) is strongly positively correlated with the salt tolerance index at the early stages of the growth. In our study, both FL478 (86.67%) and AC41585 (81.22%) were able to maintain high water status even under salt stress. However, in case of susceptible genotypes like IR29 both the RWC and leaf water

potential dropped drastically due to poor osmotic regulation (Table 5). Many studies have reported that the maximum decrement in leaf water potential and relative water content was drastically decreased in sensitive genotypes (Hossain *et* al., 2015; Nounjan 2018).

5.1.2. Reproductive stage

Similarly, in the reproductive stage, salinityhas severely affected the growth parameters, especially the yield and yield-related attributes (Negrao *et al*., 2017). Plant height, number of tillers, total biomass (Zeng and Shanon, 2001), and flowering time were greatly affected by salinity stress. In this study, plant height was decreased in all the genotypes under stress probably due to excess accumulation of toxic Na^+ in the plant tissues which ceases the plant growth. Salinity mostly affects the viability of the pollen grains which leads to poor grain filling and induced spikelet sterility (Chattopadhyay *et al*., 2018). The increased spikelet sterility in IR29, Sabita, and Sadri might be due to poor ionic homeostasis, high accumulation of Na^+ in the flag leaf or in floral parts that leads to a reduction in grain filling (Table7) as also observed by Chakraborty *et al.* (2019). Other than that, competition between the spikelets of the panicle for limited carbohydrate reserve renders the seed setting under salt stress (Zeng and Shannon, 2001). Moreover, grain filling, panicle formation, and panicle length are also greatly affected under salinity. Poor rate of photosynthesis and limited carbohydrate translocation from the vegetative sink to the source (panicle) in IR29 and Sabita might be the reason for decreased panicle growth (Table 6) under stress in the present study. Similar restriction in carbohydrate translocation in response to salt stress was reported by Abdullah *et al*. (2001). Apart from grain filling, salinity also hampers the rice phenology. Studies by Gerona *et al*. (2019) reported that rice genotypes under salt stress at the reproductive stage showed a delay in the maturity period. However, in case of tolerant genotypes (Rashpanjor, AC41585, and AC39416A), we observed a minimum difference in attaining 50% flowering between the controlled and treated plants (Table 6).

Yield is severely affected in susceptible genotypes in this present study. The results of previous studies showed a strong correlation between the yield and YSI (Yield Stability Index) and SSI (Stress Susceptibility Index) in crops like wheat and barley (Jamshidi and Javanmard, 2016). The least SSI value indicates the genotypes with the highest tolerance level under salinity and vice versa. Accordingly, the highest YSI

resembles the least differences in yield between non-stress and stress conditions. In our case, the result of YSI was positively correlated with yield and supported the fact with the previous study of Chakraborty *et al*. (2019), that with highest YSI and least SSI have a great correlation with salt tolerance. We also found in the reproductive stage, tolerant genotypes like AC41585, Rashpanjor, and AC39416A were least affected by the ill effects of salinity with comparatively lower SSI (Stress Susceptibility Index) and were able to maintain the yield (Table 8). However surprisingly, FL478 showed moderate levels of tolerance under prolonged salt stress with a significant drop in grain yield (Table 8). The observed differential response indicated that the salt stress-induced yield reduction depends on many factors including the duration, intensity, and stage of salt stress (Shereen *et al*.,2005). We also noticed a significant reduction in YSI and a simultaneous increase in SSI values in susceptible genotypes which might be due to the disruption in photosynthesis and carbohydrate supply under high salt accumulation (Khatun *et al*., 1995; Moradi *et al*., 2003).

5.2. Differential tissue ion concentration and ionic homeostasis in rice genotypes in response to salt stress at both stages

Previous studies showed that tissue-specific accumulation of Na^+ and K^+ ions and ionic discrimination under salinity play a major role in imparting salt tolerance. Rapid Na^+ buildup causes ion toxicity and nutrient imbalance and reduces plant growth and development after 14 days of salt stress (Mousa *et al*., 2013). It was also reported that a low Na^+/K^+ ratio is one of the key criteria to identify salt tolerance (Piers *et al*., 2015). Maintenance of ionic balance and restricting the movement of excess Na^+ in actively growing plant tissues are very crucial to sustain under salt stress (Tester and Davenport, 2003). These findings are in line with the findings from our study. The genotypes used in present study had shown significant variation in Na^+ accumulation, K^+ retention, and in maintaining a definite Na^+/K^+ ratio in metabolically active plant parts. High Na^+concentration and Na^+/K^+ ratio in the leaf tissue of IR29 and Sabita indicated its poor ionic regulation capability under stress in the early stage (Table 11). Whereas, high Na^+ concentrationin the roots of FL478, AC41585, and AC39416A signified their ability to restrict the upward flow of Na^+ from the very beginning. Findings of some studies revealed the existence of strong correlation between salt exclusion and salinity tolerance (Munns *et al*., 2006 and Platten *et al*., 2013). This is what exactly supports the fact that

salt-tolerant genotype like FL478 and AC41585 excludes excess Na^+ through roots or restricts the upward movement of Na^+ to the leaves, The failure of this discrimination process ingenotypes like IR29 and Sabita perhaps led to its susceptibility under 12 dS m^{-1} salt stress at the seedling stage. Meanwhile some moderately tolerant genotypes like Rashpanjor, CSR27, and Binadhan 8 were able to maintain ample amounts of K^+ for managing the cellular equilibrium under a heavy load of Na^+. The presence of better selectivity and retention capacity for K^+ under a high load of Na^+ is another crucial factor of tolerance under salt stress (Munns and Tester 2008). Further from our selective transport (ST), it was quite evident that tolerant genotypes like FL478 (4.66), AC41585 (4.27), and AC39416A (4.10) were having high ST values and higher ionic discrimination ability than others (Tables 13). Whereas, another moderately tolerant (at the seedling stage) genotype like Rashpanjor had less preferential selectivity than the tolerant ones but was able to maintain a much lesser Na^+/K^+ ratio in the leaf tissue and might have the ability to sequester the excess Na^+ in the older and less photosynthetically active tissues, which is another effective mechanism to maintain the cellular homeostasis (Reddy *et al*., 2017). While incapability of both ion exclusion and K^+ retention leads to higher Na^+/K^+ ratio were some of the reasons for susceptibility of genotypes like IR29, Sabita, and Sadri towards salt stress in the seedling stage. However in the reproductive stage, the response to salinity varied across the genotypes, but the moderately tolerant genotype Rashpanjor (at the seedling stage), performed much better when the stress was imposed at the reproductive stage and for a prolonged period. From the findings of different previous studies, it was quite clear that ionic discrimination and selective coordinated transport of Na^+ and K^+ are equally important for achieving salinity tolerance at the reproductive stage (Chakraborty *et al*., 2019). The recent study indicted that the susceptible genotypes (IR29, Sabita, and Sadri) were incapable of maintaining a proper ionic balance under prolonged salt stress. However, some moderately tolerant genotypes like CSR27, Binadhan8, and Luna Suvarna were able to sustain under stress by following moderate levels of ion exclusion with moderate selectivity for K^+. Whereas, FL478, AC41585, and AC39416A (seedling stage tolerant genotypes) could able to maintain a comparatively higher rate of selective transport and able to restrict its upward movement of Na^+ from the root to mesophyll tissues, perhaps owing to superior xylem unloading in the reproductive stage as well. Now the question arises, irrespective of highion exclusion, why FL478 could not able to compensate for the yield loss? On the other hand, Rashpanjor (seedling stage moderately tolerant) might have adopted the Na^+

exclusion strategy only when the Na^+ buildup is more under prolonged stress and thus probably conserved greater energy and resources for grain filling. It was also seen that with an increase in salt stress, many glycophytes store an ample amount of Na^+ in the plant tissues and maintain a low Na^+ concentration in the cytosol to survive the salt stress (Hossain,2014). Under high external Na^+ concentration, sequestration of excess Na^+ in the vacuole and keeping desirable K^+ concentration is one of the desirable traits for salt tolerance (Sabhala and Cuin, 2008).

5.3. Differential response in maintaining pigment stability and photosynthetic efficiency under salinity at both stages

5.3.1. Cholorophyll concentration and Chlorophyll-a fluorescence

The reduction in total chlorophyll content under salinity was reported in several plants. In mustard (Mittal *et al*., 2012), rice (Mishra *et al*., 1997), and sugarcane (Cha-um *et al*., 2012) the reduction in the chlorophyll content was due to the deposition of a noxious amount of Na^+ in the leaf mesophyll tissues. In our study, we also observed a significant reduction in total chlorophyll content both at the seedling (12 dS m^{-1}) and reproductive stage (8 dS m^{-1}). Although there was high genotypic variation, the degradation in chlorophyll content was maximum in susceptible genotypes (IR29, Sabita, and Sadri) under stress at both the stages (Tables 15 and 16), while the reduction was lowest in FL478 in the seedling stage and in AC41585 atthe reproductive stage. Disruption in quantum yield of PSII (F_v/F_m) and energy loss in heat dissipation Y(NO) is one of the important assessments to estimate the pigment stability under various abiotic stresses in rice (Sarkar and Panda, 2009). In our study, at the seedling stage, there was a significant decrease in the value of F_v/F_m and a simultaneous increment in the Y(NO) value in susceptible genotypes (IR29, Sabita, and Sadri) might be due to damage in photochemical apparatus and disruption in energy distribution to PSII (Gilmore *et al*., 1996; Oukarroum *et al*., 2007) in young tender leaves (Figure 4). However, we also found a negligible impact of salt stress on the quantum yield of tolerant genotypes like Rashpanjor, AC41585, and AC39416A but the efficiency of F_v/F_mdropped significantly in susceptible genotypes (IR29 and Sabita) at the reproductive stage (Figure 5). Some previous reports suggested that in some rice cultivars, F_v/F_m was almost not affected by salt stress, whereas qN increased in sensitive cultivars with increasing salt stress (Dionisio-Sese and Tobita, 2000).In this present study, we also found a similar kind of

response at the reproductive stage. Chakraborty *et al*., (2019), also reported similar kind of findings, where the F_v/F_m value in flag leaves remained unchanged under stress in the reproductive stage. Moradi and Ismail (2007), also reported salt stress at the reproductive stage doesn't significantly alter the quantum yield rather it affects the non-photochemical quenching in tolerant genotypes at the flowering stage in response to salinity.

5.3.2.Leaf gas exchange traits

Salinity has greatly diminished the photosynthetic efficiency of the plant. It was noticed that in salt-sensitive genotypes, the net photosynthetic rate, PSII photochemical efficiency, stomatal conductance, and intercellular CO_2 concentration were significantly reduced. It might be due to the direct effect of stomatal restriction (closure of guard cell) which is indirectly induced by the reduction in intercellular CO_2 partial pressure (Yeo *et al*.,1985; Dionisio-Sese and Tobita, 2000). In the seedling stage, a substantial reduction in net photosynthetic rate along with stomatal conductance and transpiration was evident in all the genotypes under stress. A minimum decline in net photosynthesis was observed in the tolerant genotypes, FL478 (21.32%) followed by AC41585 (23.14%) (Table 15).Apart from net photosynthesis least decline in stomatal conductance (g_s) (36.26%) and transpiration rate (63.82%) was observed in FL478. But all the gas exchange traits were highly declined in the case of IR29 (susceptible) suggesting the damage done to the photosynthetic apparatus due to the higher accumulation of salt which leads to a decline in stomatal conductance and water status of the plant (James *et al*., 2001)

In the reproductive stage as well, the reduction in all gas exchange traits was observed as it was in the seedling stage. Rashpanjor was observed to have the least reduction of about 20% followed by AC41585 (25%) in the net photosynthetic rate of the flag leaf (Table 16). Maintaining high stability of the photosystem and efficiency of the photosynthetic reaction center (high value of F_v/F_m) might be the reason behind higher photosynthesis of tolerant genotypes even under salt stress in the present study, similar to the observations of Sui *et al*. (2015). However, this scenario was a little bit different for FL478. About a 45% decline in photosynthetic efficiency was observed in the genotype which was highly tolerant at the seedling stage as compared to its non-treated plants. Other than the net photosynthesis rate (pN), stomatal conductance (g_s) and transpiration rate (E) was also significantly hampered under salt stress. In this experiment highest inhibition in stomatal conductance was observed in IR29 in both the seedling and

reproductive stages respectively (Table 16). Some studies showed a decline in stomatal conductance and transpiration had a negative influence on net CO_2 fixation. The possible explanation might be a higher accumulation of noxious ions in the mesophyll cells restricted the internal CO_2 concentration and eventual reduction in stomatal conduction (Maxwell and Johnson, 2000). Other than that an apparent decline in leaf water potential slowed down the transpiration rate from root to shoot and ABA-induced stomatal closure (Zheng *et al*., 2001). Additionally, the effect of ion accumulation on enzymatic factors of photosynthesis cannot be completely ruled out. The activity of the Rubisco enzyme was also highly dropped in sensitive bean plants under salinity. Excess Na^+ accumulation in the photosynthetic tissues not only changed the membrane permeability but also caused swelling and disorganization of grana under salinity (Flowers, 1985).

5.4. Differential ROS signalling and osmoregulation in rice genotypes under salt stress at both stages

5.4.1. ROS load and activity of anti-oxidant enzymes

Previous studies have reported that salinity has caused severe oxidative damage to plants by producing reactive oxygen species (ROS) like H_2O_2, OH^- and O_2^-. In rice hyperaccumulation of NaCl induced H_2O_2 and disturbed the membrane stability and hampered the growth and development (Vaidyanathan *et al*., 2003).Generally a low amount of reactive oxygen species (ROS) was produced during the process of photosynthesis. But during salinity due to a reduction in stomatal conductance and limited CO_2 availability, the cellular redox state is affected and that lead to an excessive production of ROS (Allakhverdiev *et al*., 2002). Hyperaccumulation of ROS in the cell caused severe oxidative damage and lipid peroxidation of the cellular membrane (Hossain and Dietz, 2016). A previous study by Solis *et al.* (2021) reported that the sensitive genotypes highly suffered due to excessive accumulation of H_2O_2 in the leaf. On the contrary, the genotype with greater Na^+ exclusion capacity and minimal tonoplast leakage was able to check the oxidative damage. In this study, increased levels of H_2O_2 in the leaves of susceptible genotype IR29, showed the clear-cut impact of oxidative stress (Figure 8). Another indicator of severe oxidative damage is hyperaccumulation of malondialdehyde (MDA), which is produced during the process of lipid peroxidation due to excessive ROS load (Jain *et al*., 2001). On the contrary genotypes like FL478 and AC41585 were experiencing a lesser degree of oxidative damage, perhaps due to the

presence of a robust enzymatic antioxidant defense system (Figure 9). Some of the previous studies revealed significant increments in SOD, CAT, and POX activities that appeared to be correlated with the reduced level of MDA under salt stress (Turkana and Demiral, 2009). In this experiment, for instance, it was witnessed that, the genotypes (AC41585 and FL478) pose a significantly higher levelof SOD activity as compared to the sensitive IR29. It might be due to the ability to detoxify the excess ROS load generated as a result of superfluous transport of Na^+ in the upper portion (Figure 12). This result was similar tothe findings of Sarkar *et al.* (2013) where they observed high SOD activity in cv. Pokkali, under salt stress as compared to the sensitive genotype. However, in the reproductive stage significant increment in SOD, CAT, and POX activity in both flag leaf and panicles of Rashapanjor and AC41585 indicates abetter ROS scavenging network even under prolonged salt stress as compared to FL478 (seedling stage tolerant). At the seedling stage, induction in CAT activity in the leaf tissues of FL478, AC41585, and Rashpanjor implies the detoxification of ROS (especially H_2O_2) but reduced activity in IR29, possibly promoting the accumulation of H_2O_2 and leads to severe lipid peroxidation under stress (Figure 14). Similar kind of findings was found in the study of Abu-Muriefah 2015. As CAT, POX was also involved in the detoxification of H_2O_2 in the chloroplast (Dionisio and Toiba, 1998). The increased activity of POX in FL478 (tolerant) and Rashpanjor (moderately tolerant) enhanced the capacity to scavenge ROS vis-a-vis under higher salt tolerance at the seedling stage (Figure 13). However,in the reproductive stage, POX activity was not significant in FL478 and IR29 in both flag leaf and panicle, which might lead to a higher degree of oxidative damage.

From the above study, it can be fairly said that no doubt there were substantial differences present in salt tolerance mechanisms for different genotypes. Some of the genotypes (like FL478 and AC41585) had a higher degree of ionic and osmotic regulation for salt tolerance at the seedling stage. But in some other genotypes (Rashpanjor), with comparatively less ionic discrimination can maintain a fair osmotic equilibrium in the reproductive stage. But in both of these scenarios, the ROS detoxification cascade played a very important role in maintaining vital physiological functions in plants. Hence the genotypes with a strong and well-regulated ROS scavenging system lead to greater protection and could effectively reduce the ill effects of salinity

5.4.2. Compatible osmolyte accumulation

Preliminarily due to the presence of excess solutes in the rhizosphere, plants face osmotic stress immediately after exposure to salt stress. A high concentration of Na^+ ion makes the plants difficult to draw water at the root zone. Hence maintaining optimum water content is considered one of the essential traits of tolerance under saline stress (Uddin *et al*., 2016). Consistent with the literature, our study confirmed that genotypes (FL478, AC41585) with higher RWC were able to cope with the salinity much more efficiently than the susceptible (IR29) in both seedling and reproductive stages. Many osmoprotectants like proline, trehalose, and glycine betaine were found to maintain a favourable osmotic potential between cells and the surrounding (Sivakumar *et al*., 2001). In this present study, the significant increment in proline content in the leaf tissues of tolerant genotypes might confer better protection against salt stress. Some of the previous studies revealed the presence of a higher amount of glycine betaine in the leaf might protect the photosynthetic apparatus (fluidity of thylakoid membrane) under drought and salt stress conditions (Wang *et al*., 2010). A significant level of osmotic protection was evident in the case of FL478 and AC41585 under salt stress in both stages (Figures 10 and 11). However, Rashpanjor, was able to maintain a decent water status with minimal accumulation of osmoprotectants at the reproductive stage. Irrespective of having a comparatively lower level of osmolyte content some of the genotypes were able to maintain a quite decent level of tolerance in salinity because of their ability to use some of the excess Na^+ as cheap compatible osmolytes and to minimize the ROS production (Solis *et al*., 2021). A previous report by Yu and Assmann *et al*. (2016) suggested that sometimes Na^+ in the guard cell could mimic the nature of K^+ for maintaining stomatal turgidity to improve the stomatal conductance and *p*N even under salt stress, which reduces the risk of oxidative damage. Our study justified the fact that genotypes with a good degree of osmoticprotection (FL478) were able to maintain the cellular osmotic balance for some time but it was quite an energy-consuming process. On the other hand some genotypes (Rashpanjor) with minimal osmolyte production and using some of the available Na^+ as inorganic compatible solutes to maintain their cellular homeostasis were very effective when it comes to long-duration stress. Meanwhile a delicate balance of both of the processes was quite effective for some of the genotypes (AC41585). But failing to follow any of the mechanisms leads to absolute susceptibility in the genotypes like IR29.

5.5. Tissue tolerance trait might help to improve salinity tolerance under prolong period of salt stress

Apart from ion exclusion, tissue tolerance is another specialized mechanism rendering salinity tolerance in many wild accessions and landraces of rice (Prusty *et al*., 2018; Chakraborty *et al*., 2020) in seedling stage. Tissue tolerance is the ability to compartmentalize excess of Na^+ in the vacuoles and maintaining comparatively low cytosolic Na^+ load and maintaining the cellular stability (Munns *et al*., 2016). From the results of leaf clip based tissue tolerance assay, it was confirmed that both IR29 and Rashpanjor possessed considerably higher tissue-tolerance ability with high LC_{50} value of 433.95 and 419.45 respectively (Figure 7). Hence both of the genotypes were able to maintain the integrity of chlorophyll pigment system under high leaf Na^+ load. While in FL478 more than 50% of initial chlorophyll was destroyed even under low Na^+ concentration. Our result was lined up with findings of Chakraborty *et al*. (2020), where existence of superior tissue tolerance trait might help Kamini (landrace) to spend less energy and maintain the NaCl load by effective vacuolar sequestration to overcome the salt stress effectively. It was also reported that, most of the halophytes and non-halophyte (Barley) adopted tissue tolerance mechanism to efficiently conserve energy in salt stress (Munns and Tester, 2008). In our study both Rashpanjor and AC41585 might have a greater balance of both ion-exclusion and tissue tolerance under prolonged salt stress and hence able to sustain the salinity for longer period.

5.6. Differential regulation of some key Na^+/K^+ transporter in response to salt stress at both stages

5.6.1. SOS family transporters

In our study, we found the expression of SOS family genes (*OsSOS1*,*OsSOS2*, and *OsSOS3)*wassignificantly upregulated in the root and leaf tissues in all the genotypes. The SOS (*S*alt *O*verly *S*ensitive) family genes, particularly *OsSOS1*, a Na^+/H^+ antiporter play a key role in actively pumping out Na^+ to the apoplast or rhizospheric region during uptake and help to maintain a low Na^+/K^+ ratio in the leaf (Martinez-Atinenza *et al*., 2007; Chakraborty *et al*., 2012). In the seedling stage, *OsSOS1* activity was very high in both root and leaf and at the reproductive stage in root tissues of FL478, which indicates active exclusion of Na^+ out of the cell under salt stress (Figure 15A). The

less tissue Na^+concentration in leaves of FL478 clearly showed apositive correlation. Similarly in AC41585, significant upregulation in the expression of SOS family genes was observed in both leaf and root tissues at both stages, which supported the strong ionic exclusion mechanism operating in this genotype. However, in the case of Rashpanjor, the expression was much higher in both root and leaves in the reproductive stage than in the seedling stage. It indicated that the genotypes might adopt Na^+ exclusion under prolonged salt stress when it was most necessary. In IR29, there was no induction in SOS family gene expression noticed even under high Na^+ load in both stages.

5.6.2. HKT family transporters

Besides SOS family members, we observed a clear-cut difference in the expression pattern of HKT family transcripts. High-affinity potassium transporters (HKT family) are one of the major groups of transporters reported to play a crucial role in salinity tolerance. Many previous studies have shown, class I HKT family members have high specificity for the Na^+ ions and promote the Na^+ exclusion at xylem parenchyma (Munn and Tester, 2008; Hamamoto *et al*.,2015). According to Wang *et al*. (2015), *OsHKT 1.1*, a member of the HKT I family, mainly functions in the leaf blades and helps limiting the Na^+ accumulation in the leaves. Another member of the HKT family, *OsHKT1.5* present in xylem parenchyma plays a major role in imparting salinity stress by preventing the transport of Na^+ to the upper portions in some tolerant genotypes like Nonabokra and Koshihikari (Ren *et al*., 2005). Here in our study, we found higher expression of *OsHKT 1.1* in the leaves and *OsHKT1.5* in the roots of AC41585 and FL478 in both stages (Figure 16 A and B). This perfectly explained how these two genotypes were able to maintain lower tissue Na^+/K^+ ratio in their leaves and able to maintain a high selective transport under salt stress. A similar kind of result was reported for FL478 in the seedling stage (Chakraborty *et al*., 2020) and AC41585 in the reproductive stage (Chakraborty *et al*., 2019). However, there was high Na^+ content in the roots of FL478 at the reproductive stage due to the high xylem unloading and greater activity of *OsHKT1.5* which leads to the maximum accumulation of Na^+ in the roots and prevents upward movement of Na^+. In case of Rashpanjor, *OsHKT1.5* transcripts were much more upregulated in the reproductive stage than the seedling stage. This indicates that Rashpanjor might not be an ideal excluder at the seedling stage but employs Na^+

exclusion under prolonged salinity when the Na^+ load crosses a particular threshold level while in IR29 it was mostly downregulated. Unlike other HKTs, the *OsHKT2.4* transporter is highly selective to K^+ and particularly has a low permeability to Na^+ (Sassi *et al*., 2012). Some previous reports suggested that it was actively expressed in the plasma membrane and could be involved in both nutritional K^+ uptake and its long-distance transport (Lan *et al*., 2010; Horie *et al*., 2011). In our study, *OsHKT2.4* was highly upregulated in the leaves of AC41585, FL478, andRashpanjor in both stages and might be responsible for high K^+ retention in the leaves under salt stress (Figure 16 C).

5.6.3. HAK and AKT family transporters

Other than Na^+ exclusion, maintenance of a desirable amount of K^+ under salinity in leaf mesophyll cells is another crucial trait for tolerance in barley (Chen *et al*., 2007), cotton (Wang *et al*., 2016) and Brassica (Chakraborty *et al*., 2016). High affinity K^+ uptake from the soil is mostly mediated through the KT/HAK/KUP family of transporters under salt stress (Nieves-Cordones *et al*., 2010). *OsAKT1* is a shaker-type K^+ channel, predominantly active in rice roots (Golldack *et al*., 2003; Li *et al*., 2014). In the present study, high induction in *OsAKT1* transcript was observed in the roots of FL478 and AC41585 in the seedling stage, and in Rashpanjor and AC41585 in the reproductive stage which might have rendered to higher uptake in K^+ under stress in these genotypes. However, in high salinity and low availability of K^+, the uptake is mostly stimulated by the HAK transporter family (Su *et al*., 2002).The HAK family members *HAK1* and *HAK5*, are upregulated mostly in K^+-deprived conditions and induced high uptake of K^+ (Nieves-Cordones *et al*., 2010). Previous studies revealed, *OsHAK5* improves the K^+ acquisition in leaves (Yang *et al*., 2014), while *OsHAK1* was responsible for significant root and shoot K^+ content (Chen *et al*., 2015). Here we found high expression of *OsHAK1* in the root of Rashpanjor and AC41585 in the seedling as well as reproductive stages, which was responsible for an acceptable amount of K^+ uptake under stress (Figure 16D). However, the high induction of *OsHAK5* in the leaf tissue of all three genotypes except IR29 indicated greater K^+ acquisition plays a crucial role in salt tolerance (Figure 16E).

5.6.4. *OsNHX1*, plasma membrane and vacuolar H^+-pumps

Besides Na^+ exclusion, compartmentalization of excess Na^+ into vacuoles, older leaves, or leaf sheaths is one of the most energy-efficient mechanisms adopted to impart salt toleranceby some of the tolerant genotypes (Apse and Blumwald, 2002; Jiang *et al*., 2010). Under high external Na^+ concentration, sequestration of surplus Na^+ inside the vacuole and possession of a desirable K^+ concentration is another desirable trait for salt tolerance (Shabala and Cuin, 2008). Some wild relatives of rice including halophytic member such as *O. coarctata*, were reported to have a high degree of tissue tolerance which might play a dominant role in salt tolerance in these species (Prusty *et al*., 2018; Mondal *et al*., 2018). Previous studies revealed higher expression of *OsNHX1* preferred to compartmentalize the excess Na^+ in the vacuole to regulate high apoplastic Na^+ flow and conferring salt tolerance (Yamaguchi *et al*., 2001; Agarwal *et al*., 2013). The expression of *OsNHX1* was highly induced in the root tissues of Rashpanjor and AC41585 and in leaf tissues of all three genotypes except FL478, which suggests the higher vacuolar sequestration in the root and the leaf of Rashpanjor and AC41585 (Figure 15D). In the case of IR29 (susceptible), vacuolar compartmentalization was more prominent in the leaf tissues. Significant downregulation of *OsNHX1* in the leaves of FL478 is an indication of the poor sequestering ability of the genotype. The stimulation of *NHX* activity was seen to be highly coordinated with the increment activity of vacuolar proton pumps, which provides the H^+ driving force across the tonoplast (Gaxiola *et al*., 2001). In many halophytes, *V-ATPase* was found to be of the the major contributors to salt tolerance (Wang *et al*., 2001). Significant upregulation of two vacuolar proton pumps (*V-ATPase* and *V-PPase*) in the roots and leaves of Rashpanjor and in the leaves of AC41585 found to be associated with the higher expression of *OsNHX1* and signifies active compartmentalization of Na^+ in root as well as in leaf tissues. Whereas, in the seedling stage the slight upregulation in *V-ATPase* was positively correlated to the activity of PM- H^+ transporters in the root which might responsible for the active accumulation of Na^+ (Figure 17 C and D). Salinity causes membrane depolarisation and reduction in membrane potential. The phenomenon of Na^+ exclusion either at the time of uptake or xylem loading and K^+ acquisition are all very energy consuming processes and it is energized by the proton gradient (H^+) across the plasma membrane and tonoplast (Chakraborty *et al*., 2018). The activation energy comes from the hydrolysis of ATP for the establishment of the electrochemical gradient, by the

PM-ATPase pumps. Out of the eleven variants of AHAs, *AHA1* and *AHA7* were two major plasma membrane-bound H^+- ATPase pumps reported to be upregulated in the tolerant genotypes. Here in our study also, the transcripts of *OsAHA1* and *OsAHA7* were highly induced in the leaves and root of AC41585 and FL478 in both seedling and reproductive stages (Figure 17A and B). The significant upregulation suggested the requirement of active pumping of proton against the concentration gradient across the plasma membrane to meet up the energy expenses of Na^+ exclusion either due to the activity of *SOS* or *HKT* group of the transporter (Chen *et al*., 2010; Fuglsang *et al.*,2007; Chakraborty *et al*., 2020).

Hence the study reveals that the overall phenomenon of ionic exclusion is highly energy-demanding. Out of four genotypes, FL478 was most tolerant at the seedling stage, but despite having a robust ion exclusion and ionic discrimination mechanism, it was unable to maintain the yield under reproductive stage salinity stress. This implies the high energy demand of ionic exclusion under prolonged stress conditions might be the possible reason for poor yield of FL478. on the contrary, Rashpanjor with a moderate level of ion exclusion ability was able to withstand the prolonged salt stress in the reproductive stage. This indicates that both Rashpanjor and AC41585 might have an additional contribution of other traits *viz*., tissue tolerance to aid the ion exclusion strategy which made them fitter to withstand the prolonged salt stress in the reproductive stage.

SUMMARY AND CONCLUSION

The experiment on "Differential salt tolerance mechanism in rice (*Oryza sativa* L.) at early seedling and reproductive stages" was carried out in both *kharif* and *rabi* seasons of 2020 and 2021 in the net house of ICAR-NRRI, Cuttack. Ten rice genotypes having differential salt tolerance responses were evaluated based on different morpho-physiological and biochemical attributes at both early seeding and reproductive stages. From the initial panel, four genotypes having differential stage-specific salinity responses at both these stages were selected and critically evaluated with detailed biochemical and molecular traits to understand the difference in salt tolerance mechanism at these two stages. The findings were presented in chapter IV and discussed elaborately in chapter V. This chapter summarises the most important findings and draws important conclusions from the present study.

Based on the results of assessment for different morpho-physiological (SES Score, root and shoot length, plant dry weight, relative water content, leaf water potential, and leaf gas exchange parameters) and biochemical (total chlorophyll content, chlorophyll-a fluorescence traits, and tissue Na^+ and K^+ concentration) traits at the early seedling stage, we found FL478 was the most tolerant genotype followed by AC41585 and AC3946A. Rashpanjor, CSR27, Binadhan 8, and Luna Suvarna were found to be moderately tolerant, while IR29, Sabita, and Sadri were grouped as susceptible based on their response to salinity at the early seedling stage. In the reproductive stage, again the same panel of genotypes was evaluated based on different morpho-physiological (Plant height, numbers of panicles per plant, panicle length, and leaf gas exchange parameters), biochemical (total chlorophyll concentration, chlorophyll-a fluorescence traits, and tissue Na^+ and K^+ concentration), yield and yield attributing traits (Yield Stability Index, Stress Susceptibility Index, Spikelet Sterility Percentage, Spikelet Degeneration Percentage and days to 50% flowering). In response to salinity stress at the reproductive stage, we found Rashpanjor and AC41585 to be the most tolerant genotype followed by AC39416A.While FL478, the most salt-tolerant genotype at the seedling stage was found to be moderately tolerant under prolonged periods of salt stress at the reproductive stage along with CSR27, Binadhan 8, and Luna Suvarna. Like the seedling stage, IR29, Sabita, and Sadri were found susceptible at the reproductive stage as well. Based on the preliminary findings, it was evident that the

tolerance response of the studied rice genotypes varied significantly according to the growth stages and stress duration. From this study, we found four different categories of rice genotypes i.e., (i) tolerant at both seedling and reproductive stage (e.g. AC41585), (ii) highly tolerant at the seedling stage but moderately tolerant at the reproductive stage (e.g. FL478), (iii) moderately tolerant at the seedling stage but highly tolerant at the reproductive stage (e.g. Rashpanjor) and (iv) susceptible to salt stress at both the stages (e.g. IR29). To find out the mechanistic differences and the most critical factors rendering salt tolerance at these two critical stages, four genotypes were chosen (AC41585, FL478, Rashpanjor, and IR29) to carryout further studies.

At both stages, the tissue ion (Na^+ and K^+) accumulation pattern was critically studied to comprehend the exact mechanism(s) adopted for imparting salinity tolerance. Less Na^+/K^+ ratio in the leaf tissues and greater selective transport (ST) of K^+ over Na^+ from root to upper plant parts in both FL478 and AC41585 suggested a strong Na^+ exclusion and preferential restriction in the upward transport of Na^+ from roots to leaves was followed in these genotypes at the early seedling stage. As a matter of fact, in this stage, FL478 was found to be the most tolerant genotype with respect to Na^+ exclusion and maintenance of the least Na^+/K^+ ratio in photosynthetically active tissues. On the other hand, at the reproductive stage, both FL478 and AC41585 were still able to maintain a good selective transport for K^+ and relatively lower leaf Na^+/K^+ ratio by restricting most of the superfluous Na^+ in the root zone. This indicates strong ionic exclusion mechanism operating in both AC41585 and FL478 to limit the upstream transport of Na^+ and critical maintenance of K^+ to encounter salt stress. Besides, a high upregulation in expression of both *OsSOS* and *OsHKT* family transporters in both root and leaf tissues of FL478 and AC41585 suggests the reliance of these two genotypes on ion exclusion strategy for salt tolerance at both stages. But in case of Rashpanjor, greater upregulation of these SOS and HKT family genes were more pronounced at the reproductive stage, which indicates this genotype adopts Na^+ exclusion more under prolonged stress when the tissue Na^+ load crosses a particular threshold level. On the other hand, IR29 was unable to follow ion exclusion strategy even under a heavy load of Na^+, which is why it was found susceptible to salinity at both stages.

Despite having a superior ion exclusion at both stages, FL478 could not able to sustain the prolonged salt stress at the reproductive stage, whereas AC41585 and Rashpanjor could. To find out the precise reason, we studied the differences in the

tissue tolerance trait of these genotypes. For this, a separate excised leaf clip based assay was carried out where the condition of restricted movement of Na^+ to the mesophyll tissue was completely ruled out. The results of the experiment confirmed that both Rashpanjor and IR29 could able to retain high chlorophyll integrity in the leaf mesophyll cells even under a high Na^+ load (LC_{50} score). However, AC41585 was able to maintain a fair LC_{50} score even when the ion exclusion trait was completely eliminated. On the contrary, the least LC_{50} score of FL478 indicated a poor tissue tolerance ability of this genotype, which might made this genotype relatively vulnerable under prolonged salt stress imposed at the reproductive stage. Comparatively higher upregulation in *OsNHX1* transcripts in Rashpanjor and AC41585 and almost no induction in the leaves of FL478 at the reproductive stage has pointed out that contribution of tissue tolerance trait was fairly low in FL478. This suggested that, despite having an excellent ion exclusion capacity, FL478 could not sustain well under prolonged salt stress at the reproductive stage due to limited contribution of tissue tolerance. But a superior ion exclusion and better tissue tolerance trait might helped AC41585 to withstand the salinity stress at both stages.

To reaffirm the above finding, detailed studies on the accumulation pattern of compatible osmolytes, generation of reactive oxygen species (ROS), and activities of antioxidant enzymes in four selected genotypes were done at both stages. We found sensitive genotype (IR29) suffered a lot due to due to excessive accumulation of H_2O_2 in the leaf tissues and the highest lipid peroxidation at both stages. In the seedling stage, the ROS damage was comparatively less in FL478, AC41585, and Rashpanjor. But in contrast, a very high H_2O_2 concentration was observed in the flag leaf and panicles of FL478 at the reproductive stage. We found much higher activities of stress-induced antioxidant enzymes like SOD, CAT, and POX in the tolerant genotypes under salt stress. The higher activities of SOD, CAT, and POX might effectively detoxified the ill effect of ROS in AC41585 and FL478 in seedlings and AC41585 and Rashpanjor in the reproductive stage.

Taken together, from the entire study, we conclude thatthe response of different rice genotypes to salinity stress varies significantly at the seedling and reproductive stages. We observed that there are two basic strategies – (i) on exclusion and (ii) tissue tolerance, adopted by the studied genotypes to overcome the ill effects of salt stress. The contribution of these two traits toward salt tolerance showed genotypic variations.

Superiority in Na^+ exclusion and K^+ retention to maintain a lower Na^+ load is found to be the principal criterion for rendering salt tolerance irrespective of the stages in cultivated rice. But to tolerate the prolonged salt stress at the reproductive stage, suitable complementation of tissue tolerance is also found necessary. To summarize the whole study, the following inferences can be drawn which may help in developing reproductive stage salt-tolerant rice cultivars in the future,

i) Ion exclusion alone could be sufficient to impart salt tolerance at the seedling stage, but it might not be enough to provide an acceptable level of tolerance at the reproductive stage (eg. FL478).

ii) A fine balance between ion exclusion and tissue tolerance is crucial for the reproductive stage salt tolerance in rice (eg. AC41585 and Rashpanjor).

iii) Even with high tissue tolerance ability, strong ion exclusion is necessary to impart a high level of salt tolerance at the seedling stage in cultivated rice (eg. Rashpanjor).

iv) Only tissue tolerance without sufficient supplementation of ion exclusion may not result in either seedling or reproductive stage salt tolerance in the studied rice genotypes (eg. IR29).

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