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Potential determinants of salinity tolerance in rice (*Oryza sativa* L.) and modulation of tolerance by exogenous ascorbic acid application

Md. Musfiqur Rahman¹, Israt Jahan¹, Md. Mahmud Al Noor²,
Mst. Fatema Tuzzohora¹, Abdullah Al Mamun Sohag³, Sharif-Ar-Raffi¹,
Mirza Moffazzal Islam², David J. Burritt⁴, Mohammad Anwar Hossain^{1*}

¹Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, ²Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Bangladesh Agricultural University Campus, Mymensingh, Bangladesh, ³Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, ⁴Department of Botany, The University of Otago, Dunedin 9016, New Zealand

ABSTRACT

Rice is a relatively salt-sensitive crop with the reproductive and seedling stages being the most sensitive. Two separate experiments were conducted to isolate potential determinants of salinity tolerance and to investigate the possibility of modulating salt tolerance by exogenous ascorbic acid (AsA) application. Rice plants were imposed to salinity (EC= 10.0 dS m⁻¹) both at the seedling and reproductive phases of growth. Salinity at the seedling stage resulted a sharp decline in shoot and root growth related traits including leaf chlorophyll content, while hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) levels increased. Plants experienced with salinity at the reproductive phases of growth showed a significant reduction in yield attributing traits while the tissue levels of H₂O₂ increased. Exogenous AsA application reversed the negative impact of salt stress, modulating the root and shoots growth and yield related traits and lowering H₂O₂ and MDA levels. FL-478 was identified as the most tolerant genotype at the seedling stage, with Binadhan-10 being the most tolerant at the reproductive stage. Grain yield panicle⁻¹ significantly and positively corrected with number of filled grains panicle⁻¹, panicle length, plant height, and spikelet fertility, and negatively correlated with H₂O₂ levels. Stress tolerance indices clearly separated the tolerant and susceptible genotypes. A principal component analysis revealed that the first two components explained 87% of the total variation among the genotypes. Breeding efforts could therefore to undertake for developing salinity tolerance by manipulating endogenous AsA content in rice.

KEYWORDS: Rice, Ascorbic Acid, Salt Stress, Oxidative Stress, Seedling Stage, Reproductive stage

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***Corresponding Author:**
Mohammad Anwar Hossain
Email: anwargpb@bau.edu.bd

INTRODUCTION

Saline soils are one of the most severe constraints to crop productivity worldwide and thus are a major concern for global food security [1]. It has been projected that more than 20% of the world's arable land and 50% of irrigated areas, which include around 30% of rice growing areas, already suffer from salinity problems [2]. To feed an increasing global population, it will be essential to utilize these saline soils either by using reclamation to reduce salinity or by growing salt tolerant crop plant [3]. Conventional plant breeding to increase crop yields in saline environments is often slow, due to our poor understanding of the molecular and genetic mechanisms of salt stress tolerance as well as lack of suitable phenotyping and genotyping techniques [4].

Hence, there is a pressing need to improve our understanding of the complex mechanisms associated with salt tolerance, as well as to develop appropriate phenotyping and genotyping techniques to be used for the development of modern crop varieties that are more resilient to salt stress. An understanding of salinity tolerance mechanisms at the various phases of plant development and identification of potential traits associated with salt stress determinants by using genotypes with variable degrees of salt stress tolerance will enable us to develop robust salt tolerant crop varieties or management techniques for sustainable crop production [5].

Rice (*Oryza sativa* L.) is an essential crop that provides food for more than half of the world population. It is the staple

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food of most people in Bangladesh and it is the world's second most important grain. In Bangladesh, rice occupies about 70% of the total cropped area, about 13.9 million hectares. Of the 2.85 million hectares of coastal arable land about 1.056 million hectares are affected by various salinity levels and crop production in those areas is very limited [6]. Importantly, it has also been predicted that increases in soil salinity may lead to a decline in rice yield by 15.6% by 2050 [7]. So, there is a critical need for developing rice varieties that can withstand high levels of salt stress and maintain satisfactory yields under both saline and non-saline conditions.

Rice is relatively tolerant to stress during seed germination, active tillering, and at maturity but is very sensitive at the early seedling stage and at the reproductive stage [2, 8]. Very poor correlation exists between tolerance at the reproductive and seedling stages, suggesting that these two sensitive stages are independent of each other and are controlled by dissimilar sets of genes [9]. Despite much research having been conducted for the salt tolerance at the seedling stage very little attention has been paid to the reproductive stage, although the reproductive stage is most decisive as it finally governs grain yield [10]. This is mainly because of a lack of reliable reproductive-stage-specific phenotyping protocols [2].

Salinity imposes ionic and osmotic stresses on plants [11] and increased levels of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$) and hydroxyl radical, and this ultimately leads to oxidative stress [12-14]. Consequently, metabolic dysfunction and damage to cellular structures, inhibition of photosynthesis contribute to growth perturbances, reduced fertility, and premature senescence in plants [15]. The most injurious effect of salinity, at the reproductive stage, is on panicle initiation, pollen viability, spikelet formation, pollen germination and fertilization, and significant effects have been observed on panicle weight, panicle length, primary branches panicle⁻¹, number of filled grains panicle⁻¹, number of unfilled grains panicle⁻¹, total grain number panicle⁻¹, total grain weight panicle⁻¹, 1000-seed weight and total grains plant⁻¹ [16-18]. Though pollen and spikelet fertility and pollen germination are the most important determinates of rice yields under salt stress [19,20], there are no comprehensive reports concerning the relationship between ROS metabolism and pollen or spikelet fertility in response to salinity, although high Na^+ concentrations are known to be associated with pollen sterility [21]. Importantly, drought induced spikelet sterility was reported to be associated with an abrupt increase in ROS and/or inefficient antioxidant defenses [22]. Enhanced antioxidant defense is one of the mechanisms plants use to adapt to adverse environments, including salt stress [13-14, 23]. Changes in the amount and the activities of antioxidant enzymes in response to salinity were found to differ between salt sensitive and tolerant cultivars of various crop plants [23,24]. While several studies have been conducted to decipher the mechanisms of salt tolerance at the seedling stage, little emphasis has been given to the biochemical mechanisms of salinity tolerance during panicle initiation and flowering. Therefore, assessment of popular salt tolerant and high yielding rice varieties, tolerant breeding lines and sensitive varieties, for spikelet fertility in

relation to ROS metabolism could open up new possibilities to breed rice varieties for higher salt tolerance.

Ascorbic acid (AsA) is a highly water-soluble antioxidant molecules and plays a vital role in plant defense, including ROS detoxification through the ascorbate-glutathione pathway and through cellular signalling that triggers adaptive responses [25]. Optimum levels of glutathione and AsA were found to improve the overall productivity of plants through the modulation of osmoregulation, water use efficiency, photosynthetic performance and plant water and nutrient utilization efficiency [25]. Under stressful conditions higher rates of AsA degradation have been observed and without increased rates biosynthesis this can cause an imbalance in cell redox homeostasis [26-27]. Exogenous application of AsA was found to improve salt tolerance in various crop plants through protection of lipids and proteins from oxidative damage, particularly at seedling stage [28-30]. Transgenic plants over-expressing AsA biosynthetic pathway genes have been shown to possess higher abiotic stress tolerance, including salinity tolerance, by improving ROS and methylglyoxal (MG) detoxification [31-33]; however, many aspects of the role of exogenous AsA in modulating salt stress tolerance particularly at reproductive phase is remain unknown. In rice, research related to spikelet fertility/sterility in relation to salt-induced ROS accumulation and AsA metabolism is unknown. Considering the above, the present research was conducted to identify potential morphological and biochemical determinants and stress tolerance indices in rice at the seedling and reproductive stages, using contrasting genotypes having difference in salinity tolerance, using an appropriate phenotyping protocol. The potential of exogenous AsA-mediated salt stress tolerance was also investigated as an addition to future breeding strategies.

MATERIALS AND METHODS

Plant Materials

Seven diverse rice genotypes including one salt tolerant advanced breeding line (FL-478), two salt sensitive varieties (Binadhan-6, BRRI dhan28), and four moderately salt tolerant varieties (Binadhan-8, Binadhan-10, BRRI dhan67, BRRI dhan78) were used as plant materials.

Seedlings Growth Conditions and Salt Stress Treatments

Two separate experiments were conducted with four replications and four treatments (viz., control, 2mM AsA, salinity (EC = 10.0 dSm⁻¹) and salinity +2mM AsA) under a completely randomized design (CRD). In experiment I, rice seedlings were cultured in professional peter solution under hydroponic condition. After 13 days of seedling growth, two groups of seedlings of each genotype were pre-treated with 2mM AsA in peter solution for 24 h. After 14 days of seedling growth, AsA pre-treated and untreated seedlings were then imposed to salinity (10 dS m⁻¹ NaCl solution) in hydroponic peter solution for 96 hours. The other group of AsA pre-treated seedlings was also grown under control condition.

In experiment II, rice plants were grown in perforated pots filled with field soil and at the end of the rice growth stage 4 (young panicle about to emerge from flag leaf) two groups of rice plants of each genotype were pruned, leaving the penultimate leaf and flag leaf, and then subjected to 10 dS m⁻¹ salt stress in hydroponic tank and allowed to grow up to the ripening stage [2]. One group of salt-stressed rice plants and one group of control rice plants were sprayed with a 2mM AsA solution containing 0.001% Tween-20, using a hand sprayer, after 2 days and 4 days of salinity treatment. The same amount of distilled water was sprayed onto control plants.

Data on Morphological Traits Recorded at the Seedling and Reproductive Stage

In experiment I, data on shoot length, root length, fresh root weight, and fresh shoot weight were calculated from ten seedlings per genotype for each replication. Data on dry root weight and dry shoot weight were estimated after oven-drying of the samples at 60° C for 3 days.

In experiment II, data on yield and yield attributing traits (days to maturity, panicle length, plant height, number of unfilled grains panicle⁻¹, number of filled grains panicle⁻¹, spikelet fertility (%), 100-seed weight, grain yield panicle⁻¹) were recorded from ten plants replication⁻¹ for each genotype, after harvesting. Data on H₂O₂ was measured from flag leaf tissues after 8 days of salt stress.

Determination of Chlorophyll Content

Chlorophyll content was determined from leaf tissues (0.5 g) of the seedlings by soaking in 80% acetone as described by Sohag et al. [34]. The absorbance of the acetone extracts was measured at 645 and 663 using a UV-VIS spectrophotometer (Shimadzu, UV-1201, Japan). The total chlorophyll content was expressed as mg g⁻¹FW.

Determination of Hydrogen Peroxide (H₂O₂)

Hydrogen peroxide from leaf tissues at the seedling stage and from flag leaf tissues at the reproductive stage was measured following the method of Velikova et al. [35] and the H₂O₂ content was calculated by utilizing 0.28 μM⁻¹cm⁻¹ extinction coefficient [36].

Determination of Malondialdehyde (MDA)

MDA was measured from leaf tissues (at seedling stage) following the standard method as described by Heath and packer [37] and the MDA content was determined utilizing 155 mM⁻¹cm⁻¹ extinction coefficient and expressed as nmol g⁻¹ FW [38].

Stress Tolerance Indices

Different indices of stress tolerance were calculated based on the data on grain yield panicle⁻¹ using following equations:

SSI = $(1 - (Y_s/Y_p)) / (1 - (\bar{Y}_s/\bar{Y}_p))$ according to Fisher and Maurer [39]

TOL = $Y_p - Y_s$ according to Rosielle and Hamblin [40]

STI = $(Y_p \times Y_s) / (\bar{Y}_p)^2$ according to Fernandez [41]

YSI = Y_s/Y_p according to Bouslama and Schapaugh [42]

Where, in all above equations, Y_s and Y_p are stress and normal yield panicle⁻¹ of a given genotype, respectively. \bar{Y}_s and \bar{Y}_p are average yield of all genotypes under stress and normal conditions, respectively.

Statistical Analysis

Data analysis was carried out using the Minitab 17 statistical software package (Minitab Inc. State College, Pennsylvania) or R, version 3.3.2. A one-way analysis of variance was carried following CRD design with two factors in mixed model, in which replicates were random and factors were fixed. Significant difference in treatment means was tested at $P < 0.05$ level using Tukey's multiple comparison test. Principal component analysis (PCA) and phenotypic correlation co-efficient was done using Minitab 17 statistical software.

RESULTS

Effect of AsA, Salt Stress and Salt+2mM AsA Treatments on Rice Genotypes at the Seedling Stage

The results of analysis of variance for all the characters (*viz.*, root length, shoot length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, total chlorophyll, H₂O₂, MDA) showed highly significant ($P \leq 0.001$) variations due to genotypes as well as treatments (Supp. Table 1). Root length, shoot length, fresh shoot weight, dry root weight, dry shoot weight, total chlorophyll, H₂O₂, MDA were found to have significant ($P \leq 0.001$) G × T interactions, whereas shoot fresh weight showed no significant G × T interaction (Supp. Table 1).

Root Length

The greatest root length under control conditions was found for BRRI dhan28 (12.23 cm), whereas the lowest was found for Binadhan-8 (7.32 cm) (Table 1). Root length showed a significant decrease under salt stress in comparison with control for all the genotypes studied. The greatest reduction was observed in salt susceptible Binadhan-6 (15.51%), whereas the least reduction was found for Binadhan-10 (0.19%) (Table 1). In response to exogenous AsA pre-treatment, the salt susceptible variety Binadhan-6 showed the greatest increase in root length (14.70%), followed by BRRI dhan28, BRRI dhan67, BRRI dhan78, Binadhan-10, Binadhan-8 and FL-478 (10.53, 5.96, 5.55, 2.82, 1.08 and 0.42%, respectively), in comparison with the seedlings imposed to salinity without AsA pretreatment. Importantly, exogenous AsA pre-treated non-stressed seedlings have showed no significant increase in root length in comparison with control (Table 1).

Table 1: Effects of exogenous AsA on different morphological and biochemical traits of seven rice genotypes grown under salt stress condition at the seedling stage. Data are presented with mean values of four independent replicates. Different letters in the same column indicate significant differences between the treatments at $p < 0.05$ level using the Tukey's multiple comparison tests. Control (Peter solution), AsA (2mM AsA in Peter solution), Salt (10.0 dSm⁻¹ in Peter solution), Salt+AsA (10.0 dSm⁻¹+ 2mM AsA in Peter solution)

Genotype × Treatment	Root length (cm)	Shoot Length (cm)	Root fresh weight (mg)	Shoot fresh weight (mg)	Root dry weight (mg)	Shoot dry weight (mg)	Total chlorophyll (mg g ⁻¹ FW)	H ₂ O ₂ (nmol g ⁻¹ FW)	MDA (nmol g ⁻¹ FW)	
FL-478	Control	10.15ef	31.74a	166.67a	460.00a	42.00a	72.33a	1.87c-e	35.41e-g	28.55kl
	AsA	10.17d-f	32.19a	160.00ab	450.00a	37.67b	70.33ab	1.95bc	37.14d-f	24.63mn
	Salt	9.45fg	27.82bc	130.00c-g	303.33d	25.00cd	62.00cd	1.58h-j	41.55bc	34.51hi
	Salt + AsA	9.49f	28.84b	154.00a-c	326.67cd	27.33c	65.67bc	1.79d-f	39.23cd	30.54jk
Binadhan-6	Control	10.31de	26.08c-f	110.00f-j	316.67cd	22.99de	49.68fg	1.88cd	33.60g-i	33.74hi
	AsA	10.46c-e	26.38c-e	113.33e-i	296.67d	22.67de	45.67g-i	1.94bc	29.19k-n	29.50k
	Salt	8.71g	20.80k	83.33j-l	143.33j	17.00h-m	34.33kl	1.33lm	45.09a	58.24ab
	Salt + AsA	9.99ef	22.35h-k	100.00h-k	176.67h-j	20.00e-h	41.67ij	1.61hi	38.50c-e	53.56c
Binadhan-8	Control	7.32h	23.20g-j	77.00kl	220.00e-g	12.00o-r	32.67kl	1.95bc	25.61o-q	29.25k
	AsA	7.09hi	23.36g-j	82.67j-l	230.00ef	12.33n-r	31.67kl	2.18a	26.39n-q	26.15lm
	Salt	6.46i	22.11i-k	70.00l	170.00h-j	9.33r	29.33l	1.89cd	31.48i-l	48.93d
	Salt + AsA	6.53i	23.20g-j	83.00j-l	190.00f-h	10.67qr	35.33k	1.96bc	30.21j-m	38.49f
Binadhan-10	Control	10.64c-e	29.33b	123.33d-h	376.67b	17.33g-l	58.00de	2.16a	27.69m-o	32.63ij
	AsA	10.69c-e	28.84b	133.33b-f	386.67b	18.00f-l	61.67cd	2.17a	28.46l-o	30.25jk
	Salt	10.63c-e	27.58bc	101.00h-k	293.33d	16.00i-n	49.67fg	1.99b	31.60h-l	45.87e
	Salt + AsA	10.93b-d	27.88bc	120.67d-h	333.33cd	18.33f-k	57.00de	1.86c-e	29.74j-m	37.50fg
BRR1 dhan28	Control	12.23a	26.52cd	120.00d-h	303.33d	21.67d-f	56.00de	1.48jk	27.61m-o	33.48hi
	AsA	12.17a	26.42c-e	113.33e-i	300.00d	21.00e-g	57.00de	1.66gh	27.23m-p	28.71kl
	Salt	10.54c-e	22.74g-k	87.00i-k	140.00j	12.33n-r	36.67jk	1.03n	43.11ab	60.25a
	Salt + AsA	11.65ab	24.15f-i	103.33g-k	186.67g-i	15.67j-o	51.33f	1.27m	32.37g-k	57.25b
BRR1 dhan67	Control	11.13bc	23.38g-j	110.00f-j	246.67e	14.67k-p	43.00hi	1.66gh	24.11pq	23.17n
	AsA	11.64ab	23.27g-j	100.00h-k	230.00ef	14.33l-q	44.67g-i	1.65h	23.60g-i	25.39mn
	Salt	10.60ef	20.67k	76.67kl	146.67ij	11.67p-r	35.33k	1.41kl	34.80f-h	43.37e
	Salt + AsA	10.66c-e	21.79jk	100.00h-k	176.67h-j	13.33m-q	42.00ij	1.52ij	30.31j-k	35.33gh
BRR1 dhan78	Control	12.09a	24.49d-h	156.67a-c	350.00bc	19.67e-i	51.33f	1.78e-f	35.11fg	23.06n
	AsA	12.05a	24.24e-i	140.00a-e	356.67bc	19.33e-j	52.67ef	1.84d-f	32.56g-j	23.67mn
	Salt	10.64c-e	23.09g-j	123.33d-h	210.00e-h	16.33h-m	42.00ij	1.64h	40.31b-d	35.98f-h
	Salt + AsA	11.23bc	24.77d-g	143.33a-d	233.33e	18.33f-k	48.00f-h	1.75fg	37.93d-f	29.16k

Shoot Length

On average, shoot length was the highest in FL-478 (31.74cm) and lowest in Binadhan-8 (23.20 cm), under control conditions (Table 1). Exposure of rice seedlings to salinity caused a significant reduction in shoot length, with the greatest reduction observed for Binadhan-6 (20.24%) followed by BRR1 dhan28, FL-478, BRR1 dhan67, Binadhan-10, BRR1 dhan78 and Binadhan-8 (14.26, 12.35, 11.59, 5.97, 5.72 and 4.70%, respectively), compared to controls (Table 1). AsA pre-treatment was found to increase shoot length, the greatest increase (7.45%) was noted for Binadhan-6 followed by BRR1 dhan78, BRR1 dhan28, BRR1 dhan67, Binadhan-8, FL-478 and Binadhan-10 (7.28, 6.20, 5.42, 4.93, 3.67 and 1.09%, respectively) in comparison with the seedlings imposed to salinity without AsA pre-treatment (Table 1).

Fresh Root Weight

Under control conditions, the greatest fresh root weight was found in FL-478 (166.67 mg) and lowest in Binadhan-6 (77.00 mg) (Table 1). A decrease in fresh root weight was observed in response to salinity; the greatest reduction was found for BRR1 dhan67 (30.30%) and least reduction was found for Binadhan-8 (9.09%), compared to their controls. AsA pre-treatment was found to increase the root fresh weight, the

greatest increase (30.43%) was found for BRR1 dhan67, followed by Binadhan-6, Binadhan-10, BRR1 dhan28, Binadhan-8, BRR1 dhan78 and FL-478 (20.00, 19.48, 18.77, 18.57, 16.22 and 15.58%, respectively), in comparison with the seedlings imposed to salinity without AsA pre-treatment (Table 1).

Fresh Shoot Weight

The greatest fresh shoot weight was found for FL-478 (460.00 mg) and the lowest for Binadhan-8 (220.00 mg) (Table 1) under control conditions. In response to salinity, shoot fresh weight showed a significant decrease in all of the genotypes, with the greatest reduction for Binadhan-6 (54.74%) and the least reduction for Binadhan-10 (22.13%), as compared to controls. AsA pre-treatment was found to reduce the decrease of fresh root weight under the condition of salinity. The highest decrease was found in BRR1 dhan28 (33.34%) followed by Binadhan-6, BRR1 dhan67, Binadhan-10, Binadhan-8, BRR1 dhan78 and FL-478 (23.26, 20.45, 13.64, 11.76, 11.11 and 7.69%, respectively) as compared to the seedlings treated with salt stress only (Table 1).

Dry Root Weight

The maximum dry root weight was observed in FL-478 (42 mg) and minimum in Binadhan-8 (12 mg) under control treatments

(Table 1). In response to salt stress dry root weight was found to decrease, the greatest reduction was recorded for BRR1 dhan28 (43.10%) and the least for Binadhan-10 (7.67%). Exogenous AsA application showed to mitigate the negative impacts of salt stress and increase root dry weight. The greatest increase (27.09%) was for BRR1 dhan28 followed by Binadhan-6, Binadhan-10, Bindhan-8, BRR1 dhan67, BRR1 dhan78, and FL-478 (17.65, 14.56, 14.36, 14.22, 12.25 and 9.32%, respectively), in comparison with the seedlings imposed to salinity without pretreatment (Table 1).

Dry Shoot Weight

The highest shoot dry weight was recorded for FL-478 (72.33 mg) and the lowest for Binadhan-8 (32.67 mg) under control treatments (Table 1). Salt stress lead to a significant decrease in shoot dry weight for all the genotypes studied. The greatest reduction was observed in salt susceptible BRR1 dhan28 (34.51%) whereas the least reduction was found for Binadhan-8 (10.22%) compare to their control. Pre-treated AsA salt stressed seedlings showed an increase in shoot dry weight, the greatest increase (39.97%) was found in BRR1 dhan28 followed by Binadhan-6, Binadhan-8, BRR1 dhan67, Binadhan-10, BRR1 dhan78, and FL-478 (21.38, 20.45, 18.88, 14.76, 14.29 and 5.92%, respectively), in comparison with the seedlings imposed to salinity without pretreatment (Table 1).

Total Chlorophyll

The highest total chlorophyll content was found for Binadhan-10 (2.16 mg g⁻¹ FW) and the lowest in BRR1 dhan28 (1.48 mg g⁻¹ FW) (Table 1) under control condition. Due to salt stress, the greatest decrease was found for BRR1 dhan28 (30.41%) and the least for Binadhan-8 (3.08%) (Table 1). AsA pre-treated seedlings had higher total chlorophyll contents in comparison with the seedlings imposed to salinity without pretreatment. The greatest increase (23.30%) was recorded for BRR1 dhan28 followed by Binadhan-6, FL-478, BRR1 dhan67, BRR1 dhan78, Binadhan-10, and Binadhan-8 (21.05, 13.29, 7.80, 6.71, 6.53 and 3.7%, respectively) (Table 1).

Hydrogen Peroxide

The highest level of H₂O₂ was found in FL-478 (35.41 nmol g⁻¹ FW), with the lowest in BRR1 dhan67 (24.11 mg g⁻¹ FW) under control conditions (Table 1). Under salinity the greatest increase in H₂O₂ was recorded in susceptible BRR1 dhan28 (56.14%) and the lowest in Binadhan-10 (14.12%) as compared to controls. Exogenous AsA pre-treatment was found to improve salt stress tolerance by lowering H₂O₂ levels. The greatest reduction (24.91%) in H₂O₂ was found for BRR1 dhan28, followed by Binadhan-6, BRR1 dhan67, BRR1 dhan78, Binadhan-10, FL-478 and Binadhan-8 (14.61, 12.90, 5.90, 5.89, 5.58 and 4.03%, respectively) as compared to plants treated with salt stress only (Table 1).

Malondialdehyde (MDA)

The highest level of MDA was found in Binadhan-6 (33.74 nmol g⁻¹ FW) and the lowest in BRR1 dhan78

(23.06 nmol g⁻¹ FW) under control conditions (Table 1). A sharp increase in MDA contents in response to salinity was observed for all of the genotypes, with the highest level found in BRR1 dhan28 (79.95%) and the lowest in FL-478 (20.88%) as compared to controls. Application of exogenous AsA under salt conditions resulted in a reduction in the MDA content with the greatest reduction was found for Binadhan-8 (21.33%) followed by BRR1 dhan78, BRR1 dhan67, Binadhan-10, FL-478, Binadhan-6 and BRR1 dhan28 (19.12, 18.54, 18.25, 11.50, 8.04 and 4.98%, respectively) in comparison with the seedlings imposed to salinity without pretreatment (Table 1).

Effect of AsA, Salt Stress and salt+2mM AsA Treatments on Rice Genotypes at the Reproductive Stage

The result of the analysis of variance for all the characters showed highly significant ($P \leq 0.001$) variation due to genotypes and treatments (Supp. Table 2). Days to maturity, numbers of filled grains panicle⁻¹, number of unfilled grains panicle⁻¹, spikelet fertility, 100-seed weight, grain yield panicle⁻¹, H₂O₂ also showed significant ($P \leq 0.001$) G × T interactions, whereas plant height and panicle length showed no significant G × T interaction (Supp. Table 2).

Days to Maturity

The maximum number of days to maturity under control conditions was found for BRR1 dhan78 (164), whereas the least was found for BRR1 dhan67 (148) (Table 2). A significant decrease in days to maturity was observed under salinity stress in all the genotypes studied. The greatest reduction was observed in salt susceptible Binadhan-6 (11.40%), whereas the least reduction was found for BRR1 dhan78 (8.10%) (Table 2). Exogenous AsA showed no significant influence on days to maturity (2.27, 1.47, 1.39, 0.97, 0.96, 0.74 and 0.65% by BRR1 dhan28, FL-478, Binadhan-6, Binadhan-8, Binadhan-10, BRR1 dhan67, and BRR1 dhan78, respectively) in comparison with the seedlings imposed to salinity without pretreatment. Exogenous AsA applied non-stressed showed no significant increases in days to maturity as compared to control (Table 2).

Plant Height

On average, plant height was the highest in Binadhan-6 (84.15 cm) and lowest in FL-478 (55.39 cm) under control conditions (Table 2). Exposure to salinity stress caused a decrease in plant height with the greatest decrease observed for BRR1 dhan28 (5.82%) followed by FL-478, Binadhan-6, BRR1 dhan67, BRR1 dhan78, Binadhan-8 and Binadhan-10 (4.89, 4.58%, 4.35, 4.13, 2.56 and 2.49 %, respectively) compared to control. Little increase in plant height was noted in response to AsA, application however the greatest increase was found for FL-478 (3.83%) followed by BRR1 dhan28, Binadhan-6, BRR1 dhan67, BRR1 dhan78, Binadhan-8 and Binadhan-10 (3.73, 3.41, 2.89, 2.70, 1.44 and 0.81% respectively) in relation to the seedlings imposed to without AsA pretreatment (Table 2).

Table 2: Effects of exogenous AsA on different morphological and biochemical traits of seven rice genotypes grown under salt stress condition at the reproductive stage. Data are presented with mean values of four independent replicates. Different letters in the same column indicate significant differences between the treatments at $p < 0.05$ level using the Tukey's multiple comparison tests. Control (water), AsA (2mM AsA as foliar spray), Salt (10.0 dSm⁻¹ in water); Salt+AsA (10.0 dSm⁻¹+ 2mM AsA)

Genotype × Treatment	Days to maturity	Plant height (cm)	Panicle length (cm)	Number of filled grains panicle ⁻¹	Number of unfilled grains panicle ⁻¹	Spikelet fertility (%)	100-seed weight (g)	Grain yield panicle ⁻¹ (g)	H ₂ O ₂ (nmol g ⁻¹ FW)	
FL-478	Control	150.00e-g	55.39m	19.76h-j	61.28g	27.08h-j	69.35b	2.56a	1.61e	33.76h-j
	AsA	150.00e-g	55.83m	19.77g-j	62.74g	27.61h-j	69.50b	2.50a	1.66e	33.70h-j
	Salt	136.00kl	52.68m	18.61j	13.24l	75.63ab	14.99j	1.58hi	0.21l	43.32a
	Salt + AsA	138.00j-l	54.70m	19.23ij	19.76l	69.42a-c	22.19i	1.92f	0.35l	39.33b-d
Binadhan-6	Control	161.00b	84.15a	23.13bc	109.37a	21.29ij	83.72a	2.26b-d	2.50a	31.74ij
	AsA	162.00ab	84.00ab	23.24b	106.31ab	20.92ij	83.55a	2.29bc	2.48a	31.72ij
	Salt	144.00i	80.30c-e	21.82b-e	50.29h-j	80.16a	38.61gh	1.23l	0.77h-j	42.66ab
	Salt + AsA	146.00hi	83.04a-c	22.53b-d	60.66gh	70.87a-c	46.18d-f	1.40jk	0.85f-h	34.54f-j
Binadhan-8	Control	150.67ef	67.50ij	22.50b-d	90.14c-f	15.92j	84.98a	2.57a	2.20b	35.71e-h
	AsA	151.67de	68.10i	22.42b-e	94.78c-e	16.81j	84.94a	2.57a	2.13bc	35.65e-h
	Salt	137.67j-l	65.77i-k	21.73b-f	50.33h-j	55.28de	47.65de	1.63gh	0.77g-i	39.45b-d
	Salt + AsA	139.00jk	66.72ij	22.35b-e	53.51g-j	53.58ef	49.99d	1.69gh	0.81f-i	38.91c-e
Binadhan-10	Control	154.00cd	77.59e-g	22.88b-d	85.45ef	17.88j	82.68a	2.37b	1.94d	34.62f-j
	AsA	154.00cd	77.53e-g	22.50b-d	83.04f	17.53j	82.54a	2.37b	1.95d	34.70f-i
	Salt	138.00j-l	75.66f-h	22.14b-e	59.26gh	40.82fg	59.21c	1.65gh	0.93fg	36.42d-h
	Salt + AsA	139.33j	76.27fg	22.72b-d	62.14g	38.09gh	62.05c	1.75g	0.94f	33.18h-j
BRR1 dhan28	Control	149.00e-h	64.58jk	22.08b-e	91.51c-f	14.84j	86.10a	2.27b-d	2.02cd	31.28j
	AsA	150.00e-g	64.21jk	21.42c-h	89.32d-f	16.87j	84.14a	2.29bc	2.03cd	31.29j
	Salt	132.00m	60.82l	20.03f-j	38.88k	67.10b-d	36.65h	1.34kl	0.51k	41.56a-c
	Salt + AsA	135.00lm	63.09kl	21.50c-g	45.98jk	59.98c-e	44.30d-g	1.50ij	0.61jk	35.45f-h
BRR1 dhan67	Control	148.00f-h	76.09f-h	21.93b-e	98.78b-d	15.58j	86.39a	2.18c-e	2.08b-d	33.85h-j
	AsA	147.00g-i	76.06f-h	21.67b-f	97.35b-d	16.26j	85.68a	2.16de	2.04cd	33.82h-j
	Salt	136.00kl	72.78h	20.74e-i	45.50jk	68.54a-c	39.91f-h	1.34kl	0.66i-k	41.32a-c
	Salt + AsA	137.00j-l	74.88gh	21.38d-h	48.27i-k	65.75b-e	42.32e-h	1.51ij	0.70h-j	35.25f-h
BRR1 dhan78	Control	164.00ab	82.00a-d	26.23a	96.02b-d	31.82g-i	75.19b	2.07e	1.99cd	34.18g-j
	AsA	165.00a	82.28a-c	26.26a	99.75a-c	33.68g-i	74.79b	2.12e	2.01cd	34.19g-j
	Salt	154.00cd	78.61d-f	25.10a	54.81g-j	73.64ab	42.65e-h	1.41jk	0.81f-i	37.85d-f
	Salt + AsA	155.00c	80.73b-e	25.73a	56.85g-i	71.75a-c	44.23d-g	1.51ij	0.85f-h	37.35d-g

Panicle Length

The highest panicle length was found for BRR1 dhan78 (26.23) and least for FL-478 (19.76) under control conditions (Table 2). Salt stress resulted in a decrease in panicle length for all genotypes with the greatest reduction for BRR1 dhan28 (9.28%) and least reduction for Binadhan-10 (3.23%) compared to control conditions. Exogenous AsA application under salt stress was found to increase panicle length, with the greatest increase for BRR1 dhan28 (6.86%) followed by FL-478, Binadhan-6, BRR1 dhan67, Binadhan-8, Binadhan-10 and BRR1 dhan78 (3.33, 3.25, 3.09, 2.85, 2.62 and 2.51%, respectively) as compared to the plants treated with salt treatment only (Table 2). AsA treated non-stressed genotypes showed no significant differences in panicle length in comparison with controls.

Number of Filled Grains Panicle⁻¹

The highest number of filled grains panicle⁻¹ was found for Binadhan-6 (109.37) under control conditions and the lowest was for FL-478 (62.74) (Table 2). Imposition of salinity resulted in a significant decrease in the number of filled grains panicle⁻¹ among all of the genotypes with the greatest reduction found for FL-478 (78.39%) followed by BRR1 dhan28, Binadhan-6, BRR1 dhan67, Binadhan-8, BRR1 dhan78 and Binadhan-10 (57.51, 54.02, 53.94, 44.16, 42.92 and 30.65%, respectively) as compared to controls.

Application of exogenous AsA under salt stress was increased the number of filled grains panicle⁻¹ as compared to the plants treated with salt stress only. The greatest increase was found for FL-478 (49.24%) followed by Binadhan-6, BRR1 dhan28, Binadhan-8, BRR1 dhan67, Binadhan-10 and BRR1 dhan78 (20.62, 18.26, 6.32, 6.09, 4.86 and 3.72%, respectively). AsA treated non-stressed genotypes showed no significant differences in the number of filled grains panicle⁻¹ in comparison with controls (Table 2).

Number of Unfilled Grains Panicle⁻¹

The maximum number of unfilled grains panicle⁻¹ was observed for BRR1 dhan78 (31.82) and minimum for BRR1 dhan28 (14.84) under control treatments (Table 2). The number of unfilled grains panicle⁻¹ showed a significant decrease in response to salinity, with the greatest recorded for BRR1 dhan28 (352.14%) followed by BRR1 dhan67, Binadhan-6, Binadhan-8, FL-478, BRR1 dhan78 and Binadhan-10 (339.92, 276.51, 247.24, 179.28, 131.42 and 128.30%, respectively). Exogenous AsA application was found to mitigate the negative impacts of salt stress by reducing the number of unfilled grains panicle⁻¹. The greatest reduction was found for Binadhan-6 (11.58%) followed by BRR1 dhan28, FL-478, Binadhan-10, BRR1 dhan67, Binadhan-8 and BRR1 dhan78 (10.61, 8.21, 6.69, 4.07, 3.07 and 2.57%, respectively) as compared to plants treated with salt stress only (Table 2).

Spikelet Fertility (%)

Imposition of salinity showed a substantial decrease in the percentage spikelet fertility for all of the genotypes compared to controls. The highest percentage of spikelet fertility was found for BRR1 dhan67 (86.39%) under control condition whereas the lowest for FL-478 (69.35%) (Table 2). Due to salt stress, the greatest reduction in percentage spikelet fertility was found for FL-478 (78.38%) and the least for Binadhan-10 (28.39%). The salt + 2mM AsA treatment was found to increase the percentage fertility as compared to the plants treated with salt stress only. The greatest increase was recorded for FL-478 (48.03%) followed by BRR1 dhan28, Binadhan-6, BRR1 dhan67, Binadhan-8, Binadhan-10 and BRR1 dhan78 (20.87, 19.60, 6.04, 4.91, 4.80 and 3.0%, respectively) (Table 2).

100-seed Weight

The highest 100-seed weight was recorded for Binadhan-8 (2.57g) and the lowest in BRR1 dhan78 (2.07g) under control treatments (Table 2). Salt stress resulted in a meaningful decrease in 100-seed weight for all of the genotypes studied in comparison with controls. The greatest reduction was observed in salt susceptible Binadhan-6 (45.58%) followed by BRR1 dhan28, BRR1 dhan67, FL-478, Binadhan-8, BRR1 dhan78 and Binadhan-10 (40.97, 38.53, 38.28, 36.58, 31.88 and 30.38%, respectively). AsA treated salt-stressed plants showed an increase in 100-seed weight in comparison with plants subjected to salt stress without AsA application. The greatest increase was found for FL-478 (21.52%) followed by Binadhan-6, BRR1 dhan67, BRR1 dhan28, BRR1 dhan78, Binadhan-10 and Binadhan-8 (13.82, 12.69, 11.94, 7.09, 6.06 and 3.68%, respectively) (Table 2).

Grain Yield Panicle⁻¹

Grain yield panicle⁻¹ was a maximum for Binadhan-6 (2.50 g) and minimum for FL-478 (1.61 g) under control conditions (Table 2). Grain yield panicle⁻¹ showed a significant decrease in response to salinity in all of the genotypes with the greatest decrease for FL-478 (86.96%), followed by BRR1 dhan28, Binadhan-6, BRR1 dhan67, Binadhan-8, BRR1 dhan78 and Binadhan-10 (74.75, 69.20, 68.27, 65.00, 59.30 and 52.06%, respectively) related to control. Application of exogenous AsA under the condition of salinity stress was found to mitigate the negative impact of salt stress, with an increased grain yield panicle⁻¹ in relation with the seedlings imposed to salt stress only. The greatest increase was found for FL-478 (66.67%) followed by BRR1 dhan28, Binadhan-6, BRR1 dhan67, Binadhan-8, BRR1 dhan78, and Binadhan-10 (19.61, 10.39, 6.06, 5.19, 4.94 and 1.08%, respectively) (Table 2).

Hydrogen Peroxide

The highest H₂O₂ level was found in Binadhan-8 (35.71 nmol g⁻¹ FW) whereas lowest was in BRR1 dhan28 (31.28 nmol g⁻¹ FW) under control conditions (Table 2). In response to salt stress, H₂O₂ levels showed a significant increase in comparison with controls. The highest levels were found in susceptible Binadhan-6 (34.40%) followed by BRR1 dhan28, FL-478, BRR1 dhan67, BRR1 dhan78, Binadhan-8 and Binadhan-10 (32.86, 28.32, 22.07, 10.74, 10.47

and 5.20%, respectively). Application of AsA under salt stress conditions reversed the negative effects of salt stress by decreasing the H₂O₂ levels in comparison with the plants subjected to salinity without AsA. The greatest reduction in H₂O₂ levels was found for Binadhan-6 (19.03%), followed by BRR1 dhan28, BRR1 dhan67, FL-478, Binadhan-10, Binadhan-8 and BRR1 dhan78 (14.70, 14.69, 9.21, 8.90, 1.37 and 1.32% respectively) (Table 2).

Estimation of Correlation Co-efficient Among Nine Characters of Rice Genotypes Under Control and Salt Stress Conditions at the Reproductive Stage

Days to maturity showed a significant positive correlation with plant height and panicle length under both control and salt stress conditions, but showed significant positive correlation with number of filled and unfilled grains panicle⁻¹ and negative correlation with 100-seed weight and H₂O₂ content under control conditions. Days to maturity showed a significant positive correlation with grain yield panicle⁻¹ under stress conditions (Table 3). Plant height showed a significant positive correlation with panicle length and number of filled grains panicle⁻¹ and grain yield panicle⁻¹ under both control and salt stress conditions, but a significant negative correlation with 100-seed weight under control conditions. Plant height showed a significant positive correlation with grain yield panicle⁻¹ and negative correlation with H₂O₂ (Table 3). Panicle length showed a significant positive correlation with number of filled grains panicle⁻¹ under both control and salt stress conditions, but a significant negative correlation with 100-seed weight under control conditions. Under salt stress conditions, panicle length showed a significant positive correlation with spikelet fertility and grain yield panicle⁻¹, but showed a significant negative correlation with 100-seed weight and H₂O₂ content. Number of filled grains panicle⁻¹ showed a significant positive correlation with spikelet fertility and grain yield panicle⁻¹ under both control and stress conditions, but showed significant negative correlation with 100-seed weight under control conditions. Under salt stress conditions number of filled grains panicle⁻¹ showed a significant negative correlation with number unfilled grains panicle⁻¹ and H₂O₂ content. Number of unfilled grains panicle⁻¹ showed a significant negative correlation with spikelet fertility under both control and stress conditions, but showed significant negative correlation with grain yield plant⁻¹ and H₂O₂ under stress condition. Spikelet fertility showed a significant positive correlation with grain yield panicle⁻¹ and negative correlation with H₂O₂ under salt stress conditions. 100-seed weight and grain yield panicle⁻¹ showed a significant negative correlation with H₂O₂ under salt stress conditions (Table 3).

Principal Components (PCs) for Nine Morphological and Biochemical Traits in Seven Rice Genotypes from PCA

The first two principal components PC1 and PC2 explained 66.5% and 20.5% of total variation, respectively (Table 4). Because PC1 collectively explained more than half (66.5%) of the variation and contributed more to the separation of genotypes into different categories, they were used to classify the 84 groups of 7 rice genotypes into four major groups including highly salt sensitive, moderately salt sensitive, moderately salt tolerant and highly

Table 3: Phenotypic correlation co-efficient among nine characters of rice genotypes grown under control and salt stress condition at the reproductive stage

Traits		Days to maturity	Plant height	Panicle length	Number of filled grains panicle ⁻¹	Number of unfilled grains panicle ⁻¹	Spikelet fertility	100-seed weight	Grain yield panicle ⁻¹
Plant height	C	0.708***							
	S	0.651**							
Panicle length	C	0.792***	0.753***						
	S	0.850***	0.786***						
Number of filled grains panicle ⁻¹	C	0.438*	0.796***	0.597**					
	S	0.431	0.847***	0.771***					
Number of unfilled grains panicle ⁻¹	C	0.630**	0.111	0.386	-0.246				
	S	0.262	-0.322	-0.192	-0.618**				
Spikelet fertility	C	0.258	0.324	0.061	0.671**	-0.872***			
	S	0.061	0.636**	0.501*	0.879***	-0.911***			
100-seed weight	C	-0.501*	-0.686**	-0.652**	-0.641**	-0.202	-0.180		
	S	-0.188	-0.360	-0.053	-0.062	0.317	0.179		
Grain yield panicle ⁻¹	C	0.364	0.663**	0.398	0.862***	-0.277	0.620	-0.282	
	S	0.455*	0.864***	0.779***	0.973***	-0.609**	0.870***	0.015	
H ₂ O ₂	C	-0.076*	-0.056	0.022	-0.314	0.062	-0.160	0.319	-0.256
	S	-0.351	-0.453*	-0.666***	-0.673***	0.563**	-0.696***	-0.462*	-0.695***

***, ** and * indicates significant at 0.1%, 1% and 5% level of probability respectively. Here, 'C' indicates control condition and 'S' indicates salinity stress condition.

Table 4: Principal components (PCs) for nine morphological and biochemical traits in seven rice genotypes from principal component analysis (PCA)

Variable	PC1	PC2
Days to maturity	0.332	0.243
Plant height	0.188	0.594
Panicle length	0.206	0.581
Number of filled grains panicle ⁻¹	0.396	0.063
Number of unfilled grains panicle ⁻¹	-0.358	0.251
Spikelet fertility (%)	0.390	-0.143
100-seed weight	0.321	-0.383
Grain yield panicle ⁻¹	0.396	-0.095
H ₂ O ₂	-0.339	0.090
% variation explained	66.5%	20.5%

salt tolerant. From the biplot, it was found that the PC1 scores of FL-478 under the salt stress treatment completely separated from those of Binadhan-6 under control and AsA treatments. The variation between FL-478 under control conditions and Binadhan-6 under control and AsA treatments were due to a higher negative coefficient of the traits: number of unfilled grains panicle⁻¹ (UFG/P) and H₂O₂ compared to the positive coefficients of the traits of number of filled grains panicle⁻¹, grain yield panicle⁻¹ and spikelet fertility (Figure 1). Similarly, PC2 scores of BRRI dhan78 under Salt+ 2mM AsA treatment completely separated from those of FL-478 control and AsA treatments, due to higher positive coefficients of the traits of plant height, panicle length, number of unfilled grains panicle⁻¹, H₂O₂, days to maturity compared to the negative coefficients of the traits of 100-seed weight.

Estimation of Stress Tolerance Indices Based on Grain Yield Panicle⁻¹ Obtained from Control and Salt Stress Conditions

The highest SSI was recorded for the genotype FL-478 (1.26) followed by BRRI dhan28 (1.09), Binadhan-6 (1.01), BRRI dhan67 (0.99), Binadhan-8 (0.95), BRRI dhan78 (0.86), Binadhan-10 (0.76). The highest value of TOL was recorded in Binadhan-6

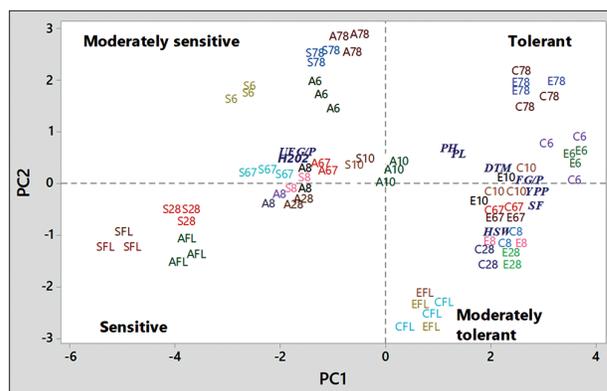


Figure 1: Biplot from the principal component analysis of the morphological and biochemical traits of seven rice genotypes under control, 2mMAsA, Salt, Salt+2mM AsA treatments. Here, DTM= Days to maturity, PH= Plant height, PL= Panicle length, FG/P= Number of filled grains panicle-1, UFG/P= Number of unfilled grains panicle-1, SF= Spikelet fertility (%), HSW= 100-seed weight, YPP= Grain yield panicle-1, H₂O₂= Hydrogen peroxide. 'C', 'E', 'S', 'A' before number indicates treatment under control, AsA, salt, salt+2mM AsA and FL, 6, 8, 10, 28, 67, 78 indicates genotypes FL-478, Binadhan-6, Binadhan-8, Binadhan-10, BRRI dhan28, BRRI dhan67, BRRI dhan78 respectively

(1.73) followed by BRRI dhan28 (1.51), Binadhan-8(1.44), BRRI dhan67 (1.42), FL-478 (1.4), BRRI dhan78 (1.18), Binadhan-10 (1.01) (Table 5). Maximum STI was obtained from the genotype Binadhan-10 (0.43) followed by Binadhan-8 (0.40), BRRI dhan78 (0.38), Binadhan-6 (0.36), BRRI dhan67 (0.33), BRRI dhan28 (0.25), and FL-478 (0.08). The highest YSI was obtained for the genotype Binadhan-10 (.48) followed by BRRI dhan78 (0.41), Binadhan-8 (0.35), BRRI dhan67 (0.32), Binadhan-6 (0.30), BRRI dhan28 (0.25), and FL-478 (0.13) (Table 5).

Ranking based on Morphological and Biochemical Traits of Rice Genotypes at the Seedling and Reproductive Stages

Considering all the traits at the seedling stage under control conditions, FL-478 was in first position followed by

Table 5: Stress tolerance indices in rice genotypes, estimated from grain yield panicle⁻¹ obtained from control and salt stress conditions

Genotype	SSI	TOL	STI	YSI
FL-478	1.26	1.4	0.08	0.13
Binadhan-6	1.01	1.73	0.36	0.30
Binadhan-8	0.95	1.44	0.40	0.35
Binadhan-10	0.76	1.01	0.43	0.48
BRR1 dhan28	1.09	1.51	0.25	0.25
BRR1 dhan67	0.99	1.42	0.33	0.32
BRR1 dhan78	0.86	1.18	0.38	0.41

Here, SSI: Stress susceptibility index; TOL: Tolerance index; STI: Stress tolerance index; YSI: Yield stability index.

Binadhan-10, BRR1 dhan78, BRR1 dhan28, Binadhan-6, BRR1 dhan67, Binadhan-8. The genotype FL-478 secured the first position followed by Binadhan-10, BRR1 dhan78, BRR1 dhan67, Binadhan-8, BRR1 dhan28, Binadhan-6 under the condition of salt stress (Supp. table 3). At the reproductive stage, Binadhan-10 was in the first position followed by Binadhan-8, Binadhan-6, BRR1 dhan67, BRR1 dhan78, BRR1 dhan28, FL-478 under control conditions, whereas under salt stress condition, Binadhan-10 was in the first position followed by BRR1 dhan78, Binadhan-8, BRR1 dhan67, Binadhan-6, FL-478, BRR1 dhan28 (Supp. Table 3).

DISCUSSION

Rice is currently registered as the most salt-sensitive cereal crop with a threshold of 3dSm⁻¹ for most cultivated varieties [43]. Salinity caused a substantial reduction in plant development and growth as compared to respective control plants during all growth stages and it prevented plants from fully expressing their full genetic potential [44]. Salt stress triggers a reduction in intra-cellular water potential and water availability and so roots fail to absorb sufficient water and nutrients for adequate plant growth [45]. The current study showed that the imposition of salt stress significantly reduced the shoot growth and root characteristics and as well as leaf chlorophyll content (Table 1). The reduction of shoot and root growth and chlorophyll content was greatest in the sensitive cultivars compared to tolerant genotypes. Salinity reduced growth of plants obviously due to the negative consequence of salt stress that restricts cell division [46] and arrests plant growth at least in part due to the initiation of oxidative stress [47]. Additionally, the reduction in development and growth of salt-stressed seedlings could be due to the negative effects of the high osmotic potential of the nutrient solution that lowered uptake of water and nutrients [48]. The reduction in morphological parameters and chlorophyll content due to salinity was also found by other researchers [36, 49-52]. The findings of the present experiment also show that the salt treatment led to increased H₂O₂ and MDA contents in all of the genotypes tested, but that the accumulations of H₂O₂ and MDA were lower in salt-tolerant genotypes compared to salt-sensitive genotypes (Table 1). The lower accumulation of MDA and H₂O₂ in the salt-tolerant genotypes implies greater protection against oxidative damage by better regulating mechanism to ROS formation to perform their signaling function [53, 54] and therefore, these genotypes displayed more salinity tolerance [23, 55,56]. In

contrast, the higher accumulation of H₂O₂ and MDA contents in salt-sensitive genotypes was probably due to higher rates of ROS production as well as inactivation of antioxidant enzymes [57], leading to oxidative stress and membrane lipid damage [58,59]. Generation of oxidative stress in response to short term salt stress in rice was also reported by others [13, 60]. Importantly, an exogenous AsA pre-treatment resulted in greater root and shoot length as well as chlorophyll content for plants of all the genotypes tested, compared to plants treated with the salt stress only (Figure 2). Similar results were also indicated by other researchers [49, 61-64]. Exogenous AsA led to a reduction in ROS and MDA content, however the greatest reduction was noted in the sensitive genotypes in comparison with tolerant genotypes that could mean that the tolerant genotypes synthesize more AsA in comparison with sensitive genotype or produces lower ROS levels. Therefore, the higher growth rates of AsA pre-treated seedlings might due to efficient ROS detoxification and /or lower ROS synthesis and accumulation, and also better ion homeostasis, particularly the maintenance of low Na⁺/K⁺ ions through the mechanisms like salt exclusion, ion partitioning and compartmentation of Na⁺ into shoots [61] and the proper signaling function of AsA and ROS for up-regulation of stress responsive genes [65-67] (Figure 3). Significant variations in days to maturity due to treatments within a particular genotype were found because of direct salt inclusion from root zone to panicle. Salt stress forced the plants to mature earlier, but tolerant genotypes showed a similar number of days to maturity when grown under control or salt stress conditions. The yield contributing traits like number of filled grains panicle⁻¹, panicle length, plant height, spikelet fertility %, 100-seed weight, and grain panicle⁻¹ and yield were significantly reduced by the imposition of salt stress whereas the number of unfilled grains increased (Table 2). The reduction of yield attributing traits and yield due to salinity were also mentioned by other researchers [18, 59, 68,69]. Importantly, application of exogenous AsA was found to improve yield attributing traits and yields under salinity stress condition. The increase in yield and yield attributing traits due to AsA under salt stress were also found by other researchers [28]. Importantly the level of H₂O₂ increased significantly however the highest increase noted in the genotype those were salt susceptible. An increase in the MDA content in flag leaves at the reproductive stage in response salt stress was also reported by Moradi and Ismail [70] and similar results were found by the others [49, 61]. Application of exogenous AsA was also found to lower H₂O₂ and MDA level under salt stress in other studies [67, 71,72].

The phenotypic correction (Table 3) study among the yield attributing and yield traits reflects a significant positive correlation of grain yield panicle⁻¹ with the other morphological traits studied, whereas yield panicle⁻¹ showed significant negative correlation with the biochemical traits measured (ROS and MDA). A similar positive correlation with yield was also reported by others [73-76]. The increase panicle length, filled grains and spikelet fertility ensures increased grain numbers, which contributes to increased grain yield. Furthermore, positive correlation of 100-seed weight with yield indicates the importance of individual grain weight for increased yields. However, the number of unfilled grains panicle⁻¹ showed a

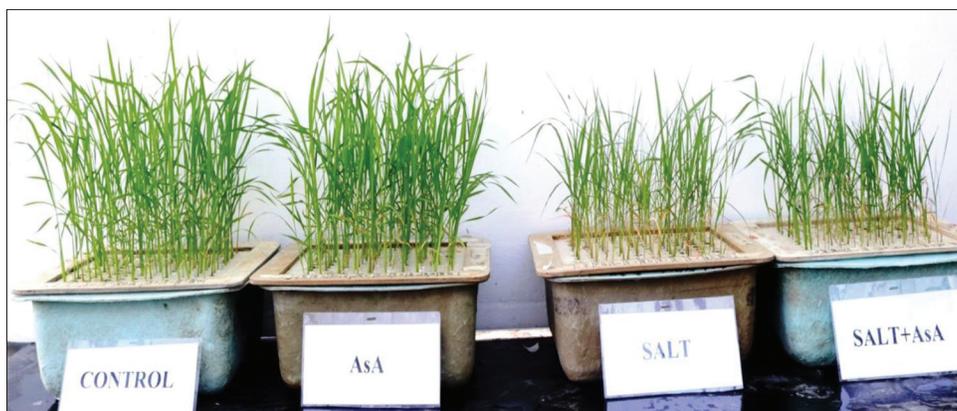


Figure 2: Phenological appearance of control, AsA-pretreated control, salt and AsA-pretreated salt-stressed seedlings after 96 h of salt stress (10 dSm^{-1}) treatment

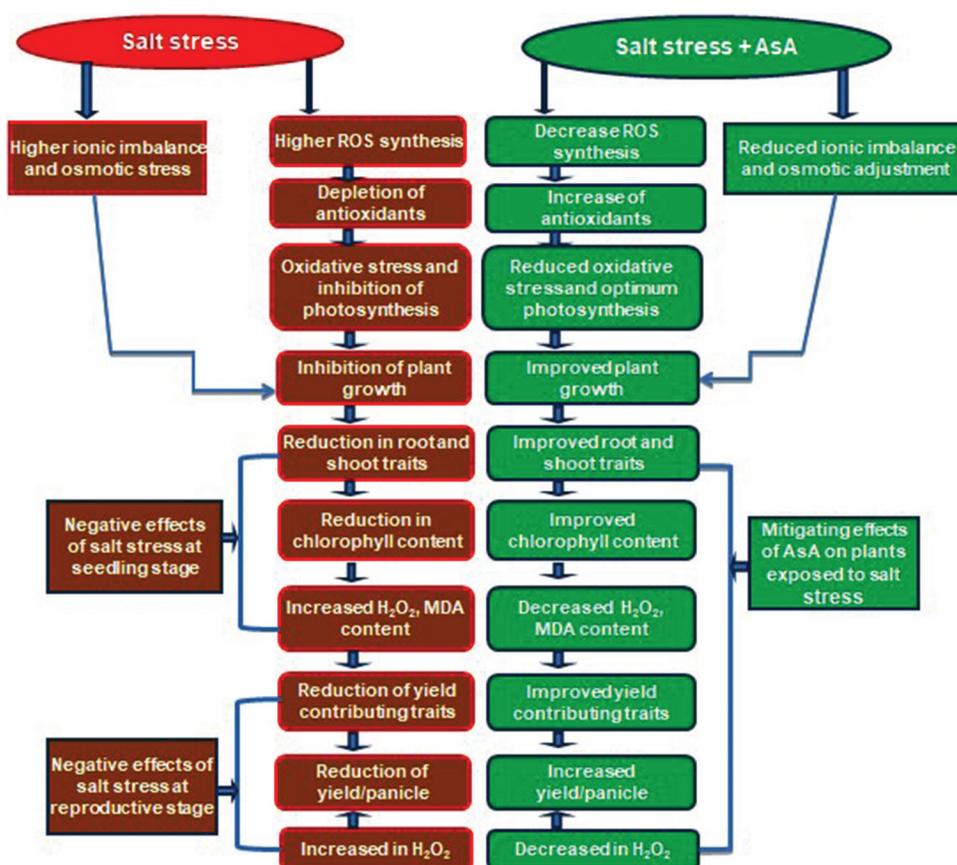


Figure 3: A schematic diagram showing the possible effects of AsA on the mitigation of salt stress in rice at the seedling and reproductive stages. Common negative effects of salt stress on plants are ionic imbalance and osmotic stress, which can trigger the accumulation of toxic compounds, e.g. reactive oxygen species (ROS), and depletion of antioxidants, e.g. AsA. These negatively affect plant growth and development, and consequently yield-contributing parameters and final yields. Application of exogenous AsA to plants under salt stress resulted in the maintenance of non-toxic levels of ROS, all of which contributed to the alleviation of salt-induced damage, leading to enhanced growth and development, and resulted higher yields

significant positive correlation with H_2O_2 and MDA. This result indicates that ROS and MDA levels might be the most important biochemical determinants of grain fertility. Increased level of ROS and MDA might be responsible for pollen and spikelet sterility which finally conferred the higher of number of unfilled grains panicle⁻¹. A similar negative correlation with

ROS and rice grain yield were reported by Selote and Khanna-Chopra [22].

PCA provide an explanation and indication of the decisive component traits contributing to salt tolerance for the genotypes and treatments under study [77]. In this research

experiment, PCA analysis disclosed that PC1 is negatively correlated with number of unfilled grains panicle⁻¹ and H₂O₂ (Table 4). This is in contrast to grain yield panicle⁻¹, spikelet fertility, number of filled grains panicle⁻¹, days to maturity, and 100-seed weight, which are positively correlated with PC1. Therefore grain yield panicle⁻¹, spikelet fertility, number of filled grains panicle⁻¹, days to maturity, and 100-seed weight traits are positively associated with salinity tolerance in the present study. Under these hypothetical conditions, the H₂O₂ level was negatively correlated with spikelet fertility and 100-seed weight. The results suggest that plants with higher H₂O₂ level contents have lower spikelet fertility and 100-seed weights under saline conditions. Genotypes showing the highest values for the positively correlated traits for PC1 and PC2, were considered as highly saline tolerant genotypes and taking the place in the upper-right corner of the biplot. Genotypes with moderate values for PC1 and PC2, located in the lower right and upper left corner of the graph, were considered as moderately salt tolerant and moderately salt sensitive, respectively (Figure 1). On the contrary, genotypes displaying the low values of positively correlated traits fall in the lower left portion of the biplot and were categorized as salt sensitive. Similar four major groups also categorized by Kakar et al. [78] using 74 rice genotypes. Under salt stress + AsA condition, only Binadhan-10, a salt tolerant rice genotype, is in the upper-right corner of the biplot, which has both positive effects for PC1 and PC2 and this genotype is therefore classified as highly saline tolerant genotype.

Stress tolerance indices such as SSI, TOL, STI and YSI values estimated from grain yield panicle⁻¹ were found to be effective in separating the susceptible and tolerant genotypes (Table 5). Among the stress tolerance indicators, Krishnamurthy et al. [79] suggested that higher values of TOL and SSI represent relatively more sensitivity to stress, thus a reduced value of TOL and SSI for a given genotype indicates the higher stability of the genotype in stress and no stress environments. Selection based on these two criteria favours genotypes with high yields under stress conditions. On the other hand, higher values of STI and YSI represent relatively a more tolerant genotype under salt stress than genotypes with lower values [80] and lower grain yield stability in stress conditions [81]. From the stress tolerance indices, FL-478 was the most susceptible genotype whereas Binadhan-10 was the most tolerant at reproductive stage of the seven rice genotypes tested. These selection indices are therefore effective for separating salt susceptible and tolerant genotypes. Additionally, individual ranking of the genotypes considering all of the traits at two different phases of plant growth indicates that tolerance at the reproductive and seedling stages are not correlated. Similar results were also reported by others [68, 82].

In conclusion, our studies clearly demonstrated that salinity at the seedlings stage and/or reproductive stages significantly impacted plant growth and development, and reduce yields and yield attributing traits. A clear genotypic difference in salt tolerance was observed with respect to developmental stage. Exogenous AsA application at the seedling and/or reproductive stages improved salinity tolerance in rice through the positive modulation of growth, yield and yield attributing traits (summarized in figure 3). The findings of our current study

are useful for rice breeding programs and further research on molecular aspects of AsA-mediated salinity tolerance in rice is warranted, with the aim to enhance endogenous AsA levels using a genetic engineering approach. However, field trials with various concentrations of AsA and salinity levels will be needed to provide more definite information for management of salt stress in rice, as well as for the genetic manipulation of rice plants to enhance productivity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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