Philippine Journal of Science
154 No. 1: 159-167, February 2025
ISSN 0031 - 7683
Date Received: 21 Aug 2024

Mitigation of Morpho-biochemical Attributes of Saline Stressed Rice (*Oryza sativa* L.) Seedling with 50 and 100-µM Abscisic Acid (ABA) Treatments

Ikram-ul Haq[1]*, Nazia Parveen Gill[2], Faheeda Soomro[3], Zainab Soomro[1], Mehwish Baloch[1], Chanda Bakhtawar Baloch[1], Sanam Nawaz[1], Asra Mahar[1], and Javeria Nizamani[1]

[1]Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro 76080 Pakistan
[2]Department of Statistics, University of Sindh, Jamshoro 76080 Pakistan
[3]Department of Linguistics and Human Sciences, Begum Nusrat Bhutto Women University, Sukkur 65200 Pakistan

Phytohormones act as signal transmitter molecules to manage abiotic stresses and regulate plant morphogenesis. Abscisic acid (ABA) responds under toxic salt stress as a signal passed at danger in the regulation of plant metabolism. The seed germination rate has been considered as the key to final crop yields. In this study alleviation effect of ABA (50 and 100 µM) on germination than subsequent growth of Basmati-370 cultivar of rice (*Oryza sativa* L.) seedlings stressed under 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) for 2 wk were assessed. In the 1st week, both ABA and salts were sprayed to moisturize the filter paper on which seeds were sown, whereas in the 2nd week, only ABA was foliarly sprayed. ABA decreases in gibberellic acid (GA_3) concentrations and α-amylases activities of control (without salts) and salt-stressed seeds, which results in a significant decrease in seed germination (%) rate. Salinity significantly decreases seedling growth, relative water contents, pigments, K^+, proteins, and total sugars, whereas reducing sugars, malondialdehyde (MDA), proline contents, hydrogen peroxide (H_2O_2), Na^+ and Cl^- increased ($p \leq 0.05$). These above observations were measured as reversed due to the ameliorated effects of ABA supplementation. In conclusion, an increase in ABA levels leads to a decrease in GA_3 contents at the seed germination stage, which contributes toward a lower seed germination rate, whereas after germination exogenous ABA applications enhance salt tolerance by promoting nutrient uptake and balancing endogenous hormones to accelerate seedlings growth effectively.

Keywords: gibberellic acid, ionic contents, organic solutes, salinity, seed germination

INTRODUCTION

Plant growth as well as its yields have been decreasing continuously mainly due to salinity as among the environmental stresses (van Zelm *et al*. 2020). It is represented as the major hurdle in the expansion of agricultural areas to increase yields of crops (Reddy *et al*. 2017). Increasing soil salinity has distributed nearly 71 % of the Earth's area (Li *et al*. 2018). Mainly the retention of NaCl with or Na_2SO_4 (neutral) and $NaHCO_3$ with or Na_2CO_3 (alkaline) salts causes soil salinization. This process continues, and simultaneously, salinization

*Corresponding author: rao.ikram@yahoo.com

is increasing all over the world's arid and semi-arid areas (Yang *et al*. 2007). Both cases are adding pH stress with specific osmotic and ionic toxicity on plant developmental processes (Wang *et al*. 2020) and also induce negative effects on the quality and quantity of final crop yields.

Plant vegetative and reproductive growths decrease with high salinity or pH. A few methods have been brought up to increase salinity tolerance. Like as application of Ca as $CaSO_4.2H_2O$ (Suhayda *et al*. 1997), K as K_2SO_4 salts (Kaya *et al*. 2002), and Zn as $ZnSO_4$ (Al-Zahrani *et al*. 2021) showed significant amelioration effects on growth and fruit developments. Meanwhile, other methods like arbuscular mycorrhizal biofertilizers improve soil quality, as well as expedite the organic matter decomposition helping to improve plant health (Thirkell *et al*. 2017). Up to now, few research-based reports have been cited for decreasing salt effects on crops with different supplementations of crop growth enhancement in the soil. The physiological responses under saline stresses, as well as the improvement of salinity tolerance, are still unclear.

Plants are sessile organisms, and their growth depends on nearby available soil nutrients and growth regulators, whereas phytohormones are active molecular agents of plant signal cascade to induce specific responses under abiotic stressed conditions (Quamruzzaman *et al*. 2021). Among flowering plants, abscisic acid (ABA) has been found a major stress hormone that directs specific molecular mechanisms to tolerate the applied abiotic environmental stresses (Sun *et al.* 2020). It triggers alterations in gene expression with the closure of leaf stomata and rapid changes in intra-membranous ionic fluxes (Wege *et al*. 2014). The ABA levels are increased when plants grow under abiotic stressed conditions within a few minutes and may be retained for several hours, depending on the stress type and its severity (Sofy *et al*. 2022). According to Wang *et al*. (2020), up-regulation of ABA signaling was observed in newly aseptically regenerated plantlets during acclimatization. Pretreatment of either seeds or foliar sprays of ABA alleviates the NaCl inhibitory effects on normal plant vegetative, as well as yield growth along with translocation rates of assimilates (Li *et al*. 2020).

Rice (*Oryza sativa* L.) is one of the main staple food crops, and it has been facing various yields reducing saline stresses. Its salt tolerance improvement is necessary (Reddy *et al*. 2017). Like some reports about physiological mechanisms against NaCl stress and methods that may be used to enable salt tolerance in different crops (Li *et al*. 2020). Either by applications of necessary inorganic elements in soils or foliar spray of various hormones (Quamruzzaman *et al*. 2021). The salinity shows differential behaviors against plant's antioxidation and causes concomitant oxidative damage of lipids, as well as chlorophyll contents of plants (Taïbi *et al*. 2016). Meanwhile, very few reports are available to assess the effect of hormones or other necessary inorganic elements on seed germination, biosynthesis of gibberellic acid (GA_3), α-amylases activities, and latterly chlorophyll contents and lipid peroxidation during initial seedling-growth of rice crop. As the seed germination stage is a key to final plant yields, this work was planned to explore the mitigative performance of ABA in seed germination, seedling growth rate, and the biochemical contents of rice grown under saline toxic conditions.

MATERIALS AND METHODS

Under aseptic conditions, an experiment was performed to assess the effectiveness of ABA and saline conditions on rice seed germination. For this experiment, the following steps were taken.

Plant Seeds Collections and Its Germination

The taxonomically authenticated seeds of salt-sensitive Basmati-370 of indica rice (*Oryza sativa* L.) cultivar were collected from Ayyub Agriculture Research Institute in Faisalabad, Pakistan. An aseptic study in glass petri dishes (50 x 12 mm) was conducted for seed germination and its seedling growth evaluation. Healthy and uniform-sized seeds were selected and washed in running tap water and sterilized for surface-growing microbes disinfection with 5% bleach (5% NaOCl) solution, followed by washing twice in sterile distilled H_2O (Wani *et al.* 2010). Ten (10) seeds were sown on the bed of four layered Whatman # 1 filter paper. They were moisturized with different solutions (Table 1) with a volume of 10 mL and incubated for 12 h (overnight) in the dark for 2 wk at 25 ± 1 °C with light (27 μmol $m^{-2}s^{-1}$ intensity) and dark conditions for 16 and 8 h, respectively.

Treatment Applications

Seeds arranged in Petri plates were moisturized with two levels of salt stresses as [a] 0-mM salts (control, dH_2O, pH 6.45 ± 1) and [b] 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) for 2 wk (Table 1). ABA solution (1st dissolved in absolute ethanol) in dH_2O was then applied in two levels – 50 and 100 μM ABA. During the 1st week, the seeds were moisturized with ABA, whereas in the 2nd week, they were sprayed foliarly on seedlings and salts applied thoroughly to seeds or filter-paper moisturization. Seeds under control treatment were moisturized with an equivalent amount of dH_2O with no salt in the absence of ABA. Four replicates per treatment were maintained. All chemicals and hormones used in this experiment were purchased from Sigma-Aldrich (Taufkirchen, Germany) company.

Seedlings Growth, Its Biomass, and Pigments

At the end of 2nd week, all seedlings were harvested and washed first with tap-H_2O and then dH_2O. Seed germination (SG) was recorded as germinated if the seedling has ≥ 0.5 cm and ≥ 1 cm shoot and root lengths, respectively. The number of germinated seeds was counted, and the SG rate was calculated as in Equation 1, with the seedling vigor index (SVI) as per Maisuria and Patel (2009) with the formula given in Equation 2:

SG (%) = (Germinated seeds / Total seeds sown) x 100 (l)
SVI = (Shoot length + Root length) x SG (%) (2)

Four seedlings from each treatment were immediately preserved at –20 °C for the next usage for different biochemical analyses. The remaining seedlings were subjected to plant growth and biomass measurements. The seedling's fresh weight (FW) of the plumule or shoot (g) and the radical or root (g) and length (cm) were measured. The same seedlings-stuff was dried in an electric oven by incubation at 72 °C for 3 d, and then their dry weight (DW) was recorded (g). In 0.50 g of fresh shoots, the chlorophyll content and total carotenoids were analyzed (Wellburn *et al*. 1994), whereas the chlorophyll stability index (CSI) was calculated with the formula as in Equation 3 (Mohan *et al*. 2000):

CSI: Chl ab contents in treated seedlings/Chl ab in control seedlings (3)

Determination of Mineral Contents

Exact amounts of 50 mg dried tissues (shoot and root) were boiled in 10 ml ddH_2O at 100 ºC for 1 h. Its filtrate was subjected to analysis of different inorganic and organic contents, whereas the dry sample (50 mg) was roasted in 10-mL sulfosalicylic acid (3 %, w/v), then its filtrate was used for proline content determination. For minerals (Na^+ and K^+) determination, the above filtrate sample was used for their analysis [Flame-Photometer (Model 410, UK)] by following its manual (Barnes *et al*. 1945). The chlorides (Cl^-) were measured in the same filtrate as the chlorometer (Chapman and Pratt 1961).

Determination of Organic Solutes

The total proteins, sugars, reducing sugars, and proline contents were analyzed by following the methods of Lowery (1951), Dubois *et al*. (1956), Miller (1959), and Bates *et al*. (1973), respectively. Hydrogen peroxide (H_2O_2) was determined with H_2O_2 and titanium tetrachloride complex formation and lipid peroxidation by production of thiobarbituric acid for analyzing free malondialdehyde (MDA) content formation (Brennan and Frenkel 1977; Lutts *et al*. 1996). For ascorbic acid, fresh leaf tissues (0.20 g) were ground and then stuff homogenized in 2-mL TCA [trichloroacetic acid, 10 % (w/v)], and its supernatant was subjected to reduction with dehydroascorbate, dithiothreitol, and $FeCl_3$ than its OD525 recorded (Law *et al*. 1983). Total phenolics and ABA were determined by Ti *et al*. (2014) and Simura *et al*. (2018), whereas GA_3 was by Lin and Stafford (1987). For α-amylase activities measurements, its crude extract was prepared (Sharma *et al*. 2017) by grinding in 50-mL dH_2O, and the mixture was squeezed after 10 min through nylon cloth. By following 3,5-dinitrosalicylic acid as per Miller's (1959) method, its OD540 was recorded, and the maltose standard curve was drawn for calculation of α-amylases activities.

Statistical Data Analysis

Data collected from each treatment of seedlings with four replicates was analyzed with a one-way analysis of variance for significance. Mean values and their standard errors were also recorded at different salt stresses with or without ABA applications, which compared with LSD at $p \leq 0.05$ (Sadooghi-Alvandi *et al*. 2012).

RESULTS

In this study, the effects of foliar sprays of ABA on germination and the subsequent seedling growth of seeds of Basmati-370 rice (*Oryza sativa* L.) cultivar under salt stress (Table 1) were assessed. The results of Table 2 showed a significant decrease in the rate of seed germination from control (95.00 ± 2.887%) to saline-stressed (60.00 ±

Table 1. Composition and schedule of treatment applications.

#s	Treatments	Composition	Treatments applications
01.	T_0	ddH_2O (control)	a. Seed germination stage (1s day of 1st week)
02.	T_1	50-µM ABA	
03.	T_2	100-µM ABA	b. Foliar spray on seedlings (7th day of 1st week)
04.	T_3	150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1)	
05.	T_4	150-mM salts + 50-µM ABA	
06.	T_5	150-mM salts + 100-µM ABA	

[ddH_2O] deionized distilled water; [ABA] abscisic acid; [NaCl] sodium chloride; [Na_2SO_4] sodium sulfate

Table 2. Effects of abscisic acid (ABA) on seed germination and its related attributes of indica rice (*Oryza sativa* L.) cv., Basmati-370 germinated under 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) stresses, as shown in Table 1.

#s.	Treatments	GS (%)	GA_3 (ηg g^{-1} FW)	ABA (ηg g^{-1}.FW)	GA_3/ABA	α-amy (U • S^{-1}, 96 h)	SVI (%)
01.	T_0	95.00 ± 2.887^{a}	0.528 ± 0.007^{a}	13.24 ± 0.018^{f}	0.040 ± 0.001^{a}	20.55 ± 0.282^{a}	1086 ± 37.45^{a}
02.	T_1	85.00 ± 2.887^{b}	0.506 ± 0.007^{ab}	15.33 ± 0.020^{e}	0.033 ± 0.000^{b}	16.52 ± 0.146^{b}	982.8 ± 31.81^{b}
03.	T_2	60.00 ± 4.082^{c}	0.493 ± 0.015^{b}	16.65 ± 0.016^{d}	0.030 ± 0.001^{c}	15.33 ± 0.098^{c}	708.1 ± 48.01^{c}
04.	T_3	60.00 ± 4.082^{c}	0.353 ± 0.012^{c}	23.68 ± 0.005^{c}	0.015 ± 0.001^{d}	14.40 ± 0.149^{d}	337.2 ± 22.10^{d}
05.	T_4	55.00 ± 2.887^{cd}	0.332 ± 0.005^{cd}	27.71 ± 0.006^{b}	0.012 ± 0.000^{e}	13.95 ± 0.069^{d}	350.8 ± 17.25^{d}
06.	T_5	47.50 ± 2.500^{d}	0.312 ± 0.009^{d}	33.63 ± 0.219^{a}	0.009 ± 0.000^{f}	11.58 ± 0.221^{e}	367.1 ± 21.34^{d}
	F-significance	32.10***	97.9***	7852***	585***	293***	115***
	CVC	9.748	0.0296	0.2682	1.546E	0.5232	93.655
	Mean ± SE	67.08 ± 3.734	0.421 ± 0.019	21.71 ± 1.523	21.71 ± 1.523	15.39 ± 0.578	0.914 ± 0.108

[GS] germination of seed; [Amy] amylases; [U • S^{-1}] unit per seed after 96 h of imbibition; [SVI] seedling vigor index; [SE] standard error; [GA_3] gibberellic acid; [ABA] abscisic acid; [CVC] critical value for comparisons. The presented values are means ± SE; the mean values followed with dissimilar letters, which represent differences among the treated groups by LSD (least significant difference) test, whereas *, **, and *** were used for significant and highly significant *p*-values respectively affected with treatments at $p \leq 0.05$

Table 3. Effects of abscisic acid (ABA) on growth attributes of indica rice (*Oryza sativa* L.) cv., Basmati-370 seedlings under 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) stresses as shown in Table 1.

#s	Treatments	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	SRWC (%)	RRWC (%)
01.	T_0	4.743 ± 0.034^{b}	6.693 ± 0.040^{b}	0.800 ± 0.004^{c}	0.332 ± 0.004^{c}	0.345 ± 0.003^{c}	0.181 ± 0.003^{c}	56.86 ± 0.542^{a}	45.35 ± 1.509^{bc}
02.	T_1	4.840 ± 0.030^{b}	6.725 ± 0.031^{b}	0.843 ± 0.003^{b}	0.355 ± 0.003^{b}	0.369 ± 0.004^{b}	0.198 ± 0.004^{b}	56.26 ± 0.293^{a}	44.21 ± 1.499^{bc}
03.	T_2	4.955 ± 0.033^{a}	6.848 ± 0.027^{a}	0.886 ± 0.003^{a}	0.376 ± 0.005^{a}	0.387 ± 0.004^{a}	0.217 ± 0.002^{a}	56.34 ± 0.310^{a}	42.31 ± 1.247^{c}
04.	T_3	2.658 ± 0.030^{e}	2.965 ± 0.050^{e}	0.382 ± 0.004^{f}	0.163 ± 0.002^{e}	0.221 ± 0.003^{e}	0.087 ± 0.003^{de}	41.98 ± 1.121^{c}	46.72 ± 1.626^{bc}
05.	T_4	3.135 ± 0.029^{d}	3.248 ± 0.037^{d}	0.479 ± 0.003^{e}	0.180 ± 0.003^{d}	0.250 ± 0.003^{d}	0.079 ± 0.003^{e}	47.80 ± 0.355^{b}	55.94 ± 2.263^{a}
06.	T_5	4.105 ± 0.030^{c}	3.615 ± 0.034^{c}	0.520 ± 0.005^{d}	0.187 ± 0.005^{d}	0.230 ± 0.004^{c}	0.093 ± 0.004^{d}	55.71 ± 0.935^{a}	50.10 ± 3.266^{ab}
	F-significance	1050.0***	2665.0***	3220.0***	675.00***	499.00***	402.00***	83.800***	5.920***
	CVC	0.089	0.1104	0.011	0.0113	9.942	9.302	2.005	6.006
	Mean ± SE	4.075 ± 0.185	5.015 ± 0.365	0.651 ± 0.041	0.265 ± 0.019	0.453 ± 0.092	0.142 ± 0.012	35.26 ± 9.279	47.44 ± 1.187

[SL] shoot length; [RL] root length; [SFW] shoot fresh weight; [RFW] root fresh weight; [SDW] shoot dry weight; [RDW] root dry weight; [SRWC] shoot relative water contents; [RRWC] root relative water contents; [SE] standard error; [CVC] critical value for comparisons. The presented values are means ± SE; the mean values followed with dissimilar letters, which represent differences among the treated groups by LSD (least significant difference) test, whereas *, **, and *** were used for significant and highly significant *p*-values respectively affected with treatments at $p \leq 0.05$.

4.082%) seeds with or without provision of either ABA at 50-µM ABA (85.00 ± 2.887% at control and 55.00 ± 2.887% at salt-stressed) or 100-µM ABA (60.00 ± 4.082% at control and 47.50 ± 2.500% at salt-stressed) levels. ABA performed opposite activities when sprayed at the initial stages of seed germination to later seedling growth stages. Seeds after the 96th hour of ABA moisturization caused a decrease in GA_3 contents and α-amylases activities, whereas an increase in endogenous ABA contents ($p \leq 0.05$) was observed in control and salt-stressed seedling's culture.

Regarding the seedling vitality, as noted in Table 2, overall salinity and ABA applications reduced SVI significantly, especially when seeds germinated under salinity stresses with ABA supplementation. Meanwhile, after the germination of seeds, their subsequent seedling growth depended on the growth status of initial plumules and radicals. As the results in Table 3 showed, the seedling growth rate of rice and its biomass was reduced or slowed down due to the inhibitory effects of salt ($p \leq 0.05$). Differential reductions in the biomass of seedlings (shoots and roots) were noted at the end of 2nd week of seed germination cultures under salt-stressed conditions. However, seedlings with ABA applications (T_1 and T_2) showed an increase in their FW and DW from control (T_0), as well as from saline-stressed (T_3) ones significantly, but values remained lower in cultures with salts and ABA supplied (T_4 and T_5). Both ABA concentrations either low

Table 4. Effects of abscisic acid (ABA) on pigmentation and carotenoids of indica rice (*Oryza sativa* L.) cv., Basmati-370 seedlings under 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) stresses, as shown in Table 1.

#s	Treatments	Chl a (mg g^{-1}.FW)	Chl b (mg g^{-1}.FW)	Chl ab (mg g^{-1}.FW)	Chl a/Chl b	Carot. (mg g^{-1}.FW)	Chl ab/ Carot.
01.	T_0	3.643 ± 0.015^a	1.657 ± 0.015^b	5.300 ± 0.010^b	2.199 ± 0.027^c	1.320 ± 0.006^a	4.016 ± 0.026^b
02.	T_1	3.697 ± 0.006^a	1.735 ± 0.027^a	5.432 ± 0.025^a	2.132 ± 0.034^c	1.305 ± 0.009^a	4.163 ± 0.030^a
03.	T_2	3.319 ± 0.004^b	1.534 ± 0.009^c	4.853 ± 0.011^c	2.165 ± 0.011^c	1.272 ± 0.004^b	3.816 ± 0.005^c
04.	T_3	2.353 ± 0.008^d	0.988 ± 0.008^f	3.340 ± 0.004^f	2.383 ± 0.027^b	1.004 ± 0.007^e	3.329 ± 0.026^e
05.	T_4	2.888 ± 0.071^c	1.115 ± 0.005^e	4.001 ± 0.074^e	2.590 ± 0.060^a	1.102 ± 0.005^d	3.631 ± 0.066^d
06.	T_5	2.911 ± 0.034^c	1.218 ± 0.007^d	4.128 ± 0.035^d	2.391 ± 0.030^b	1.138 ± 0.005^c	3.628 ± 0.031^d
	F-significance	242***	502***	536***	25.6***	394***	71.4***
	CVC	0.0984	0.041	0.1053	0.1036	0.0192	0.1055
	Mean ± SE	3.135 ± 0.099	1.374 ± 0.059	4.509 ± 0.157	2.310 ± 0.036	1.190 ± 0.025	3.764 ± 0.059

[Chl] chlorophyll; [Carot] carotenoids; [MDA] malondialdehyde; [SVI] seedling vigor index; [CVC] critical values of comparisons; [SE] standard error. The presented values are means ± SE; the mean values followed with dissimilar letters, which represent difference among the treated groups by LSD (least significant difference) test, whereas *, **, and *** were used for significant and highly significant *p*-values respectively affected with treatments at $p \leq 0.05$.

Table 5. Effects of abscisic acid (ABA) on mineral ionic contents (mM g^{-1} DW) of indica rice (*Oryza sativa* L.) cv., Basmati-370 seedlings under 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) stresses, as shown in Table 1.

#s	Treatments	Na^+ (mM g^{-1})	K^+ (mM g^{-1})	Cl^- (mM g^{-1})	Na^+/K^+	Na^+/Cl^-	K^+/Cl^-	STI	SI
01.	T_0	0.578 ± 0.003^d	0.893 ± 0.005^d	0.887 ± 0.005^d	0.645 ± 0.006^d	0.651 ± 0.006^c	1.007 ± 0.003^d	100.0 ± 0.000^b	0.000 ± 0.000^b
02.	T_1	0.476 ± 0.007^e	0.983 ± 0.004^c	0.723 ± 0.004^e	0.484 ± 0.005^e	0.659 ± 0.013^c	1.361 ± 0.012^b	106.9 ± 1.811^b	6.931 ± 1.811^b
03.	T_2	0.413 ± 0.004^f	1.222 ± 0.004^a	0.625 ± 0.004^f	0.338 ± 0.004^f	0.660 ± 0.006^c	1.957 ± 0.018^a	112.2 ± 1.789^a	12.22 ± 1.789^a
04.	T_3	1.871 ± 0.007^a	0.545 ± 0.008^f	1.876 ± 0.024^a	3.438 ± 0.043^a	0.998 ± 0.012^a	0.290 ± 0.003^f	64.16 ± 1.361^b	-35.8 ± 1.361^b
05.	T_4	1.266 ± 0.010^b	0.876 ± 0.004^e	1.422 ± 0.004^b	1.444 ± 0.005^b	0.891 ± 0.009^b	0.616 ± 0.004^e	72.40 ± 0.632^b	-27.6 ± 0.632^b
06.	T_5	0.882 ± 0.007^c	1.118 ± 0.004^b	0.983 ± 0.006^c	0.789 ± 0.005^c	0.898 ± 0.012^b	1.138 ± 0.011^c	66.69 ± 1.380^b	-33.3 ± 1.380^b
	F-significance	7476***	2040***	1934***	4037***	239***	3251***	11.5***	11.5***
	CVC	0.0194	0.0155	0.0321	0.0545	0.0296	0.0303	106.46	106.47
	Mean ± SE	0.914 ± 0.108	0.940 ± 0.045	1.086 ± 0.091	1.190 ± 0.222	0.793 ± 0.029	1.062 ± 0.111	87.07 ± 4.154	–12.9 ± 4.153

[Na^+] sodium ion; [K^+] potassium ion; [Cl^-] chloride ion; [mM] millimole; [STI] salinity tolerance index; [SI] sensitivity index; [CVC] critical values of comparisons; [SE] standard error. The presented values are means ± SE; the mean values followed with dissimilar letters, which represent differences among the treated groups by LSD (least significant difference) test, whereas *, **, and *** were used for significant and highly significant *p*-values respectively affected with treatments at $p \leq 0.05$.

(50 µmol) or high (100 µmol) have increased seedling growth and biomass significantly.

Salinity caused a decreasing trend in photosynthetic pigments of seedlings, as shown in Table 4. The chlorophyll contents – including chlorophyll (Chl) a and b – and total carotenoid contents in salt-stressed (150-mM NaCl) seedlings *vs.* control ($p \leq 0.05$) were decreased. Total chlorophyll (Chl ab) content also decreased significantly, especially in seedlings that were not treated with ABA. Applications of ABA increased the ratios of pigments (Chl a/ Chl b and Chl ab/ carotenoids) significantly higher in salt-stressed than shoots of seedlings in control (T_0). Increases in photosynthetic pigments and total carotenoids in seedling leaves of salt-stressed, as well as in controls with ABA applications (Table 4).

Under salt-stressed conditions, Na^+ and Cl^- contents were increased significantly, whereas K^+ contents decreased ($p \leq 0.05$) from control seedlings (Table 5). However, concentrations of these mineral ionic contents were observed to be reversed ($p \leq 0.05$) with applications on applications of both ABA levels. Shoots either of glycophytic or halophytic tolerate at certain levels of toxic Na^+ concentrations, as Na^+ was observed to be the main soil poisoner. Usually, plants under salt-stressed conditions prefer to maintain low Na^+ concentrations and low ratios of Na^+/K^+ and Na^+/Cl^-, whereas high K^+ and K^+/Cl^- ratios in their cytoplasm. Table 5 results showed maximum increases in salt tolerance index (STI) in control seedlings with application of ABA and also increased ($p \leq 0.05$) among the salt-stressed seedlings.

Table 6. Effects of abscisic acid (ABA) on physio-biochemical contents of indica rice (*Oryza sativa* L.) cv., Basmati-370 seedlings under 150-mM salts (NaCl: Na_2SO_4, 9:1, pH 6.45 ± 1) stresses, as shown in Table 1.

#s	Treatments	Proteins (μM g^{-1})	Sugars (mg g^{-1})	R. Sugars (mg g^{-1})	H_2O_2 (μM g^{-1})	Proline (μM g^{-1})	Phenolics (mg g^{-1})	MDA (ηM g^{-1})
01.	T_0	28.24 ± 0.024^{c}	3.320 ± 0.009^{b}	0.313 ± 0.007^{d}	108.0 ± 5.479^{d}	8.439 ± 0.020^{f}	0.862 ± 0.018^{d}	9.541 ± 0.161^{d}
02.	T_1	29.55 ± 0.068^{b}	3.352 ± 0.004^{ab}	0.326 ± 0.011^{d}	94.11 ± 1.232^{de}	10.03 ± 0.027^{e}	0.871 ± 0.012^{d}	9.316 ± 0.191^{d}
03.	T_2	30.11 ± 0.008^{a}	3.371 ± 0.011^{a}	0.345 ± 0.017^{d}	82.23 ± 0.235^{e}	13.54 ± 0.016^{d}	0.894 ± 0.007^{d}	9.101 ± 0.122^{d}
04.	T_3	19.73 ± 0.033^{f}	2.092 ± 0.016^{e}	0.512 ± 0.008^{c}	238.1 ± 6.246^{a}	22.40 ± 0.010^{c}	0.999 ± 0.011^{c}	18.24 ± 0.226^{a}
05.	T_4	21.56 ± 0.027^{e}	2.405 ± 0.010^{d}	0.706 ± 0.010^{b}	183.6 ± 3.172^{b}	27.76 ± 0.022^{b}	1.263 ± 0.007^{b}	14.50 ± 0.159^{b}
06.	T_5	24.17 ± 0.064^{d}	2.667 ± 0.012^{c}	0.843 ± 0.013^{a}	140.1 ± 9.727^{c}	30.19 ± 0.015^{a}	1.313 ± 0.010^{a}	11.57 ± 0.115^{c}
	F-significance	10272***	2633***	369***	123***	241341***	325***	481***
	CVC	0.1282	0.0322	0.0346	16.059	0.0564	0.0335	0.4962
	Mean ± SE	25.56 ± 0.833	2.868 ± 0.106	0.508 ± 0.043	141.0 ± 11.58	18.73 ± 1.775	1.034 ± 0.039	12.04 ± 0.700

[R. sugars] reducing sugars; [H_2O_2] hydrogen peroxide; [MDA] malondialdehyde; [μM] micromoles; [ηM] nanomoles; [CVC] critical values of comparisons; [SE] standard error. The presented values are means ± SE; The mean values followed with dissimilar letters, which represent differences among the treated groups by LSD (least significant difference) test, whereas *, **, and *** were used for significant and highly significant *p*-values respectively affected with treatments at $p \leq 0.05$.

Meanwhile, the sensitivity index (SI) presented in -ve values in salt-stressed seedlings with or without ABA applications. It means that the Basmati-370 cultivar of rice is salt-sensitive, which may yield best with applications of ABA or any other regulators whenever sowed in saline.

Reduction in seedling growth occurs due to the accumulation of toxic ions under salinity stresses to reduce the assimilation rate by photosynthetic pigments, whereas it was reversed with ABA application significantly (Tables 3–5). Morphophysiological expressions depend on the intra-cellular accumulations of organic solutes, as per the results shown in Table 6. The total protein and sugar contents were measured as low, whereas reducing sugars, proline, H_2O_2, and MDA were observed higher in salt-stressed seedlings. Applications of ABA increased sugars and proteins in salt-stressed seedlings; similarly, reducing sugars and proline contents also increased significantly, but H_2O_2 and MDA were observed as decreased ($p \leq 0.05$).

DISCUSSION

Fast seed germination has remained the major key to final crop yield, which depends on seed-imbibition conditions and biosynthesis of GA_3 with active α-amylases. Seeds of the Basmati-370 cultivar of rice (*Oryza sativa* L.) germinated under salt stress showed a decrease in seed germination rate, whereas ABA performed opposite metabolic activities when sprayed at the seed germination stage. The ABA moisturized seeds showed a decrease in GA_3 contents and α-amylases activities, whereas there was an increase in ABA contents ($p \leq 0.05$) in both control and salt-stressed conditions. It means that the primary action of salt stress is the retardation of water uptake due to osmotic inhibition during the imbibition period, which is crucial for seed-germination (Ali and Elozeiri 2017), whereas both ABA and salinity led to a decrease in GA_3, which further decreases α-amylases activities, as per Liu *et al*. (2018) and Wang *et al*. (2018). The above-observed facts have shown directly proportional relationships for seed germination at the 96th hour after sowing, whereas works in opposite directions after seed germination for the growth of seedlings.

Regarding the seedling vitality, overall salinity and ABA applications reduce SVI significantly, just as the findings reported in wheat (Karimi *et al*. 2021) and soya bean (Jasim *et al*. 2016; El Kholy *et al*. 2021) for decreases in FW, DW, and RWC (relative waters contents) by salt stresses, as well as significant increase in wheat (Li *et al*. 2020) and jojoba (Karimi *et al*. 2021) with applications of ABA. Plant hormones play very important signal transduction roles for plant development regulation to improve environmental stress tolerance. ABA application enhances shoot growth with the induction of specific and effective morpho-physiological changes in the roots of plants stressed with salts (Li *et al*. 2020).

Salinity decreases in the synthesis of pigments, whereas applications of ABA increase pigment (Chl a/ Chl b; Chl ab/ carotenoids) ratios significantly higher in salt-stressed than shoots of seedlings in control (T_0). ABA is also an endogenous phytohormone that has many key effects in controlled morphogenesis, and it promotes senescence (Chen *et al*. 2020), although its exogenous applications induce the same effects (Li *et al*. 2020). Various researchers have reported that ABA deals with stress tolerance in growing seedlings in abiotic stressed

cultures (Sofy *et al.* 2022), including delayed chlorophyll degradation (Muchate *et al*. 2016; Ahmed *et al*. 2021). Plant pigments decrease under salt-stressed conditions could be due to changes in ratios of lipid and protein or degradation of chlorophyll by chlorophyllase or ribulose bisphosphate carboxylase activities due to high ionic toxic contents accumulations (Karimi *et al.* 2021). It might be caused by a disruption in the absorption of Fe and Mg due to Na richness, which is involved in major central chlorophyll structurization (Zhao *et al*. 2019). Their deficiency is eliminated by ABA application, as has been observed in the stability of chlorophyll and carotenoid concentrations under saline stress by Karimi *et al*. (2021).

In plants, Na^+ and Cl^- increase by salinity but are reversed with ABA applications ($p \leq 0.05$) (Chen *et al.* 2020; Hao *et al*. 2021; Ouertani *et al*. 2021). Results (Table 5) show maximum STI in control seedlings with the application of ABA, as it also increased ($p \leq 0.05$) among the salt-stressed seedlings. The SI had negative values in salt-stressed seedlings with or without ABA applications, which means that the Basmati-370 cultivar of rice is salt-sensitive. The same STI and SI were applied to assess quantitative salt tolerance in salinity stressed and control (without salinity) conditions by Munawar *et al*. (2021), which means that higher STI and or SI indicates the highest performances by cultivar with the highest tolerance against stress (Kordrostami and Rabiei 2019).

Salinity causes many complications from ionic toxicity to genotoxicity, which results in r*eactive oxygen species* generation, as shown by van Zelm *et al*. (2020). For instance, a 2–4-fold increase in H_2O_2 production under NaCl (200 mM) stresses was observed compared to the control seedlings. The H_2O_2 contents were produced first in roots, followed by mature leaves and then young leaves (Rehman *et al.* 2019), which coupled with lipid peroxidation or biosynthesis of MDA for instances reported in barley by Zeeshan *et al*. (2020) and Ouertani *et al*. (2021), as well as in maize by Abdelgawad *et al*. (2016) and Taïbi *et al*. (2016). However, ABA applications drastically reduce detrimental salinity effects with regulations of concentrations of other hormones (Sofy *et al*. 2022). It reflects that those specific concentrations of ABA applications on seedlings grown under salinity stress lead to adjustments in the physiological aspects of plants with upregulation of phytohormones (Li *et al*. 2020). This study suggests that equilibrium among biosynthesis of hormones must be established by applications of ABA or other auxin to regulate the salinity tolerance in plants with accumulation of organic protective contents, including proline and antioxidants.

CONCLUSION

The toxic salinity effects on rice are alleviated by ABA applications effectively through the amelioration of Na^+ toxicity in rice seedlings. Both osmotic stress of salts and ABA applications showed directly proportional relationships in the reduction of GA_3 – including α-amylases, which significantly led to a decrease in seed germination rates. Under salt stresses, a decrease in chlorophyll and carotenoid contents was observed with an increase in Na^+ and Cl^- toxicity, which has been alleviated with ABA applications. It demonstrates that ABA supplementation alleviates toxic ionic stressed injuries in seedlings, which are represented by H_2O_2 and MDA biosynthesis. For seed germination, both salinity and ABA remained inhibitors, whereas at the seedling growth stage, ABA was observed as a growth ameliorator against ionic toxicity. This work could be useful in the future to enhance seed germination under saline-stressed conditions with applications of ABA or any other neighbor phytohormone. In the end, seed germination and seedling attributes could be considered as principal keys to final plant yields of salt-sensitive cultivars like Basmati-370 whenever sowed in saline soils.

ACKNOWLEDGMENTS

We are thankful to the host institute for the cooperation for proper access to available material facilities at the research laboratory in the form of chemicals and apparatus for the completion of this proposed study.

STATEMENT ON CONFLICT OF INTEREST

No conflict in this study is shown by the authors.

REFERENCES

ABDELGAWAD H, ZINTA G, HEGAB MM, PANDEY R, ASARD H, ABUELSOUD W. 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front Plant Sci 7*:* 276.

AHMED KBM, SINGH S, SADIQ Y, KHAN MMA, UD-DIN M, NAEEM M, AFTAB T. 2021. Photosynthetic and cellular responses in plants under saline conditions. Frontiers in Plant-Soil Interaction. p. 293–365.

ALI AS, ELOZEIRI AA. 2017. Metabolic processes during seed germination. Adv Seed Biol 2017: 141–166.

AL-ZAHRANI H, ALHARBY H, HAKEEM K, REHMAN R. 2021. Exogenous application of zinc to mitigate the salt stress in *Vigna radiata* (L.) Wilczek-Evaluation of physiological and biochemical processes. Plants 10(5): 1005.

BARNES R, RICHARDSON D, BERRY J, HOOD R. 1945. Flame photometry a rapid analytical procedure. Indust Engr Chem Analyt Ed 17(10): 605–611.

BATES L, WALDREN R, TEARE I. 1973. Rapid determination of free proline for water-stress studies. Plant Soil Sci 39(1): 205–207.

BRENNAN T, FRENKEL C. 1977. Involvement of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol 59(3): 411–416.

CHAPMAN H, PRATT P. 1961. Method of analysis for soils, plants, and waters. University of California Riverside, Berkeley, CA, USA.

CHEN K, LI G, BRESSAN R, SONG C, ZHU J, ZHAO Y. 2020. Abscisic acid dynamics, signaling, and functions in plants. J Integ Plant Biol 62(1): 25–54.

DUBOIS M, GILLES K, HAMILTON J, REBERS P, SMITH F. 1956. Colorimetric method for determination of sugars and related substances. Analyt Chem 28(3): 350–356.

EL KHOLY RI, SAYED AI, EL-SHAER HF, HANAFY MS. 2021. Impact of sea salt stress on growth and some physiological attributes of two soybean (*Glycine max* L.) cultivars. Al-Azhar J Agric Res 46(2): 88–100.

HAO S, WANG Y, YAN Y, LIU Y, WANG J, CHEN S. 2021. A review on plant responses to salt stress and their mechanisms of salt resistance. Horticulturae 7(6): 132.

JASIM AH, AL TIMMEN WA, ABID AS. 2016. Effect of salt stress on plant growth and free endogenous hormones of primed radish (*Raphanus sativus* L.) seeds with salicylic acid. Int J Chem Tech Res 9(6): 339–346.

KARIMI R, EBRAHIMI M, AMERIAN M. 2021. Abscisic acid mitigates NaCl toxicity in grapevine by influencing phytochemical compounds and mineral nutrients in leaves. Sci Horticult 288: 110336.

KAYA C, HIGGS D, SALTALI K, GEZEREL O. 2002. Response of strawberry grown at high salinity and alkalinity to supplementary potassium. J Plant Nutr 25(7): 1415–1427.

KORDROSTAMI M, RABIEI B. 2019. Salinity stress tolerance in plants: physiological, molecular, and biotechnological approaches. Plant Abiot Stress Tol 2019: 101–127.

LAW M, CHARLES S, HALLIWELL B. 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts: the effect of hydrogen peroxide and of paraquat. Biochem J 210(3): 899–903.

LI P, QIAN H, WU J. 2018. Conjunctive use of groundwater and surface water to reduce soil salinization in the Yinchuan Plain, north-west China. Int J Water Resour Develop 34(3): 337–353.

LI X, LI S, WANG J, LIN J. 2020. Exogenous abscisic acid alleviates harmful effect of salt and alkali stresses on wheat seedlings. Int J Environ Res Publ Health 17(11): 3770.

LIN J, STAFFORD A. 1987. Comparison of the endogenous gibberellins in the shoots and roots of vernalized and non-vernalized Chinese Spring wheat seedlings. Phytochem 26(9): 2485–2488.

LIU L, XIA W, LI H, ZENG H, WEI B, HAN S, YIN C. 2018. Salinity inhibits rice seed germination by reducing α-amylase activity *via* decreased bioactive gibberellin content. Front Plant Sci 9: 275.

LOWERY O. 1951. Protein measurement with the Folin-Phenol reagent. J Biol Chem 193: 256–261.

LUTTS S, KINET J, BOUHARMONT J. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Annal Bot 78(3): 389–398.

MAISURIA K, PATEL S. 2009. Seed germinability, root and shoot length, and vigour index of soybean as influenced by rhizosphere fungi. Karnataka J Agric Sci 22: 1120–1122.

MILLER G. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analyt Chem 31(3): 426–428.

MOHAN M, NARAYANAN S, IBRAHIM S. 2000. Chlorophyll stability index (CSI): its impact on salt tolerance in rice. Int Rice Res Notes 25(2): 38–39.

MUCHATE NS, NIKALJE GC, RAJURKAR NS, SUPRASANNA P, NIKAM TD. 2016. Plant salt stress: adaptive responses, tolerance mechanism and bioengineering for salt tolerance. The Bot Rev 82: 371–406.

MUNAWAR W, HAMEED A KHAN MKR. 2021. Differential morphophysiological and biochemical responses of cotton genotypes under various salinity stress levels during early growth stage. Front Plant Sci 12: 622309.

OUERTANI NR, ABID G, KARMOUS C, BEN CHIKHA M, BOUDAYA O, MAHMOUDI H, MEJRI S, JANSEN RK, GHORBEL A. 2021. Evaluating the contribution of osmotic and oxidative stress components on barley growth under salt stress. AoB Plant 13(4): plab034.

QUAMRUZZAMAN M, MANIK S, SHABALA S, ZHOU M. 2021. Improving performance of salt-grown crops by exogenous application of plant growth regulators. Biomolecules 11(6): 788.

REDDY I, KIM B, YOON I, KIM K, KWON T. 2017. Salt tolerance in rice: focus on mechanisms and approaches. Rice Sci 24(3): 123–144.

REHMAN S, ABBAS G, SHAHID M, SAQIB M, FAROOQ A, HUSSAIN M, FAROOQ A. 2019. Effect of salinity on cadmium tolerance, ionic homeostasis, and oxidative stress responses in *Conocarpus* exposed to cadmium stress: Implications for phytoremediation. Ecotoxicol Environ Safety 171: 146–153.

SADOOGHI-ALVANDI SM, JAFARI AA, MARDANI-FARD HA. 2012. One-way ANOVA with unequal variances. Comm Statistics–Theory Method 41(22): 4200–4221.

SHARMA V, MISRA S, SRIVASTAVA A. 2017. Developing a green and sustainable process for enhanced PHB production by *Azohydromonas australica*. Biocatal Agric Biotechnol 10: 122–129.

SIMURA J, ANTONIADI I, SIROKA J, TARKOWSKA D, STRNAD M, LJUNG K, NOVAK O. 2018. Plant hormonomics: multiple phytohormone profiling by targeted metabolomics. Plant Physiol 177(2): 476–489.

SOFY M, MOHAMED H, DAWOOD M, ABU-ELSAOUD A, SOLIMAN M. 2022. Integrated usage of *Trichoderma harzianum* and biochar to ameliorate salt stress on spinach plants. Arch Agron Soil Sci 68(14): 2005–2026.

SUHAYDA C, YIN L, REDMANN R, LI J. 1997. Gypsum amendment improves native grass establishment on saline-alkali soils in northeast China. Soil Use Manag 13(1): 43–47.

SUN Y, PRI-TAL O, MICHAELI D, MOSQUNA A. 2020. Evolution of abscisic acid signaling module and its perception. Front Plant Sci 11: 934.

TAÏBI K, TAÏBI F, ABDERRAHIM LA, ENNAJAH A, BELKHODJA M, MULET JM. 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation, and antioxidant defence systems in *Phaseolus vulgaris* L. South Afr J Bot 105: 306–312.

THIRKELL T, CHARTERS M, ELLIOTT A, SAIT S, FIELD K. 2017. Are mycorrhizal fungi our sustainable saviours? Considerations for achieving food security. J Ecol 105(4): 921–929.

TI H, LI Q, ZHANG R, ZHANG M, DENG Y, WEI Z, ZHANG Y. 2014. Free and bound phenolic profiles and antioxidant activity of milled fractions of different indica rice varieties cultivated in southern China. Food Chem 159: 166–174.

VAN ZELM E, ZHANG Y, TESTERINK C. 2020. Salt tolerance mechanisms of plants. Ann Rev Plant Biol 71(1): 403–433.

WANG W, LIANG J, WANG G, SUN M, PENG F, XIAO Y. 2020. Overexpression of PpSnRK1α in tomato enhanced salt tolerance by regulating ABA signaling pathway and reactive oxygen metabolism. BMC Plant Biol 20(1): 1–15.

WANG Y, CUI Y, HU G, WANG X, CHEN H, SHI Q, ZHANG Y. 2018. Reduced bioactive gibberellin content in rice seeds under low temperature leads to decreased sugar consumption and low seed germination rates. Plant Physiol Biochem 133: 1–10.

WANI SH, LONE AA, Da SILVA T. GOSAL SS. 2010. Effects of NaCl stress on callus induction and plant regeneration from mature seeds of rice (*Oryza sativa* L.). Asia Aust J Plant Sci Biotechnol 4(1): 57-61.

WEGE S, DE ANGELI A, DROILLARD M, KRONIEWICZ L, MERLOT S, CORNU D, THOMINE S. 2014. Phosphorylation of the vacuolar anion exchanger AtCLCa is required for the stomatal response to abscisic acid. Sci Signal 7(333): ra65-ra65.

WELLBURN A. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144(3): 307-313.

YANG C, CHONG J, LI C, KIM C, SHI D, WANG D. 2007. Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. Plant Soil 294(1): 263-276.

ZEESHAN M, LU M, SEHAR S, HOLFORD P, WU F. 2020. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. Agronomy 10(1): 127.

ZHAO H, LIANG H, CHU Y, SUN C, WEI N, YANG M, ZHENG C. 2019. Effects of salt stress on chlorophyll fluorescence and the antioxidant system in *Ginkgo biloba* L. seedlings. HortSci 54(12): 2125–2133.