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Alleviating effects of salicylic acid spray on stage-based growth and antioxidative defense system in two drought-stressed rice (*Oryza sativa* L.) cultivars

ASMA[1,2], Iqbal HUSSAIN[2], Muhammad Yasin ASHRAF[3], Muhammad Hamzah SALEEM[4], Muhammad Arslan ASHRAF[2], Baber ALI[5,*], Aisha SHEREEN[1], Ghulam FARID[6], Muhammad ALI[1], Muhammad Ubaidullah SHIRAZI[1], Ammara SALEEM[7], Yasser S. MOSTAFA[8], Mohamed HASHEM[8,9], Ghulam YASIN[10]

[1]Nuclear Institute for Agriculture (NIA), Tandojam, Hyderabad, Pakistan
[2]Department of Botany, Government College University (GCU), Faisalabad, Pakistan
[3]Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan
[4]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China
[5]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[6]Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad
[7]Institute of Botany, University of the Punjab, Lahore, Pakistan
[8]King Khalid University, College of Science, Department of Biology, Abha, Saudi Arabia
[9]Department of Botany and Microbiology, Assiut University, Faculty of Science, Assiut, Egypt
[10]Department of Botany, BahauddinZakariya University, Multan, Pakistan

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Abstract: Approximately 33% of the arable land around the world is vulnerable to drought, which is a very serious issue affecting the yield and productivity of cereal crops. Two contrasting rice (*Oryza sativa* L.) genotypes, HTT-138 (drought tolerant) and (HTT-39 drought-sensitive), under various levels of water treatments, i.e. control flooded (CF) as recommended for rice (control), control saturated (CS) 100% field capacity (FC), 80% FC, and 60% FC without or with foliar spray (100 mg L^{-1}) of salicylic acid (SA) evaluated to enhance the yield for food security. The results showed that leaf gas exchange parameters, water use efficiency and water related parameters reduced under all levels of water deficient conditions. Drought stress increased oxidative stress (superoxide anions and hydrogen peroxide) and decreased after foliar spray of SA due to enhancement in antioxidant activity (catalase, ascorbate peroxidase, peroxidase, superoxide dismutase). Ascorbic acid, total soluble protein, total soluble sugar, total phenolics, proline, anthocyanin, salicylic acid, and amylase activity were reduced under drought stress and increased after foliar spray of SA. HTT-138 showed more tolerance to the drought stress than HTT-39 under the same levels of water deficient conditions. Although drought-stress was ameliorated by the foliar spray of SA which not only increased plant growth, dry weight, and metabolism or metabolic activities but also increased the nutritional status of the plant by decreasing the concentration of reactive oxygen species (ROS) in the membranous bounded organelles. In conclusion, the foliar spray of SA is useful to enhance plant growth and yield in cereal crops especially those grown in abiotic stress environments.

Key words: Rice, salicylic acid, gas exchange characteristics, oxidative stress, antioxidant capacity, water deficient conditions

1. Introduction

Rice (*Oryza sativa*. L) is cultivated in most significant areas of the world as an important cereal crop after maize and wheat. Demand for rice has increased with the increase in human population (Wu et al., 2019), 50% will be required by the year 2025 (Rizwan et al., 2016), and its supply is adversely affected by nature's wrath in the form of various abiotic (Ali et al., 2022a–2022d; Ma et al., 2022a–2022c) and biotic stress factors (Al-Zaban et al., 2022; Metayi et al., 2022; Solanki et al., 2022; Wahab et al., 2022). Abiotic stress is the principal cause of crop failure worldwide, reducing the average yield for most major crops by more than 50%, this stress threatens the sustainability of the agricultural industry (Wei et al., 2015; Ahlem et al., 2021; Ijaz et al., 2021; Adnan et al., 2022; Ahmad et al., 2022; Bibi et al., 2022; Nawaz et al., 2022; Naz et al., 2022; Saeed et al., 2022; Saini et al., 2022; Hussain et al., 2023).

Rice is severely affected by drought stress due to its water-loving nature, which reduces yield by 15%–50% depending on the intensity and period of stress, moderate drought stress can drastically reduce grain yield. Drought-resistant varieties, higher yields, and genetic control of drought-tolerant traits will be a better option for water-limiting environments to improve stable characteristics

* Correspondence: baberali@bs.qau.edu.pk

reduction (Hussain et al., 2016). This requires a good knowledge of the physiological traits under drought contributing to drought tolerance (Dola et al., 2022; Farooq et al., 2022; Wahab et al., 2022; Yaseen et al., 2020; Yasmin et al., 2022). Additionally, efforts to increase yield for drought also pay attention to improve secondary traits such as root architecture, leaf water potential, panicle water potential, osmotic adjustment, and relative water content (RWC) (Khan et al., 2020; Yadav et al., 2020).

Water scarcity caused a variety of biochemical and physiological responses in plants, including stomatal closure, suppression of cell growth, photosynthesis, and activation of respiration. Physiological studies related to sugar, sucrose, alcohols, and amino acids have been reported under drought stress in different plants (Yin et al., 2014; Rizwan et al., 2016; Akram et al., 2018; Yadav et al., 2020). Salicylic acid (SA) derives biochemical pathways to mediate growth and development through signal transductions under stress conditions (Guo et al., 2007). The SA is naturally produced phenolic compound and has a positive role in regulations of physiological and biochemical mechanisms in the plant during stress condition (Kohli et al., 2018). Production of salicylic acid improves plant growth by regulating uptake and transportation of ions, photosynthesis process, respirational activities, stomatal conductance, and anti-ROS activities in the plant (Kong et al., 2021), by increasing the concentration of cytosolic Ca^{2+} and K^{+}. Salicylic acid has the ability to regenerate the antioxidant activities, membrane stability (MS), and osmoprotectants by reducing malondialdehyde (MDA) and to detoxify the negative impacts of ROS (Ali et al., 2022e; Amna et al., 2021; Faryal et al., 2022; Hussein et al., 2012; Khattak et al., 2021; Mehmood et al., 2021; Saleem et al., 2022; Zainab et al., 2021). However, many plants lack the ability to naturally synthesize stress-tolerant metabolites (Afridi et al., 2022; Aziz et al., 2018; Perveen et al., 2021). The objectives of the present study are to find out the alleviating effects of salicylic acid spray on rice genotypes under drought stress to determine yield. The present study also helped in finding out the contributory and indicator traits for tolerance.

2. Materials and methods

The five seedlings of rice genotype HTT-138 (drought-tolerant) and HTT-39 (drought-sensitive) were grown in plastic pots (25 cm diameter × 22 cm length) containing 7 kg soil (which reduced to one plants per pots after the thinning) in the growth chamber of the stress physiology laboratory at the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, 38000, Pakistan; with four different water treatments, i.e. control flooded (CF) as recommended for rice (control), 100% field capacity (FC) is control saturated (CS), 80% FC and 60% FC. Salicylic acid (100 mg L^{-1}) with a final pH adjusted 5.5 with NaOH (1.0 N) solution was adjusted. Salicylic acid (SA) was sprayed at the reproductive stage; twice fullest, twice throughout a week. A total volume of 50 mL of SA was sprayed per plant to ensure full coverage throughout the experiment. A complete randomized design was used to record a factorial arrangement of three replicates in this experiment. Plants were harvested for recording growth and physiological and biochemical changes at the reproductive stage (flowering stage) and yield parameters at the maturity stage.

2.1. Leaf gas exchange characteristics

Instantaneous gas exchange measurements, i.e. net photosynthetic rate (*A*), transpiration rate (E), sub stomatal CO_2 concentration (C_i) and stomatal conductance (gs) of fully expanded penultimate leaf of each plant by using a photosynthesis measuring system CI-340 portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These observations were recorded before and after SA foliar application from 9.00 to 11.00 AM. The water use efficiency (WUE) was measured as the ratio between *A* and *E*.

2.2. Estimation of photosynthetic pigments

According to Arnon (1949), the pigment contents (Chl. a, b) was extracted from fresh leaves of rice. The supernatant was used to measure absorbance at 645,663 on a spectrophotometer; the following formulas were used to calculate the content of the pigment:

Chlorophyll a (mg/100 mL) = 0.999A663 – 0.0989A645

Chlorophyll b (mg/100 mL) = 0.328A663 + 1.77A645

2.3. Leaf water relation estimation

The leaf water potential (ψ_w) of fully expanded leaf was recorded (-MPa) from 10.00 to 11.00 AM using a Scholander type pressure chamber (Pressure Bomb Arimad 2, Germany). Leaf tissues were quickly frozen in a freezer at –30 °C. After 7 days, osmotic potential (ψ_s) was recorded (-MPa) with vapor pressure on an osmometer (Model-Wescor 5500, Logon, USA).

Turgor potential (ψ_p) was the difference between the water (ψ_w) and osmotic potential (ψ_s) values (-MPa) and can be determined by the following formula:

$$\psi_p = \psi_w - \psi_s$$

Relative water contents (RWC) was calculated for each treatment according to (Mahmood et al., 2005) by using the following formula:

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

2.4. Biochemical attributes

2.4.1. Determination of nitrate and nitrite reductase

The nitrite reductase (NiR) activity in leaf samples was determined using (Mahmood et al., 2005). Fresh leaves (0.5 g) were chopped in 4.5 mL of phosphate buffer (0.02

M; pH 5.0) and 0.5 mL (phosphate buffer (0.02 M; pH 5.0) of $NaNO_2$ was added in a test tube (25 mL). These test tubes were incubated in the dark at 30 °C for 30 min. The samples were then boiled in a water bath and then removed and placed on an ice-cooling system at room temperature. In a test tube, 1 mL of reaction mixture with 0.5 mL of sulphanilamide (1%) and 0.5 mL of 0.02% N (diamine dihydrochlorideiamine) was taken. The standard minute later, a color developed, and the made volume up to 10 mL and the absorption of the reaction mixture were measured on a spectrophotometer (540 nm). A standard curve was also developed with $NaNO_2$. The above procedure was repeated with NO_2 substrate and the blank had no $NaNO_2$. NiR activity was calculated in terms of μmol of NO_2 g^{-1} fresh weight per hour.

2.4.2. Estimation of sugar and protein contents

According to Vázquez-Ortiz et al. (1995), total free amino acid (TFA) was measured. Prepared samples were read at 570 nm using a spectrophotometer (Hitatchi, 220, Japan). A standard curve with Leucine was developed and free amino acids were calculated using the formulae given below:

Total amino acids (μg g^{-1} fresh wt) = (Graph reading of sample) X (Dilution factor)

Dilution Factor = (Volume of the sample/weight of the sample)

Classics Lowry et al.'s (1951) method was used to determine the total soluble protein (TSP). The optical density (OD) was read at 620 nm on a spectrophotometer (Hitachi, 220, Japan). The protein concentration was calculated using a standard curve developed by different concentrations calculated using a standard curve of Bovine serum albumin (BSA).Total soluble sugars (TSS) were estimated by Dubois et al.'s (1956) method. The proline concentration was determined using the method of Bates et al. (1973). Its absorbance was noted at 520 nm using the above mentioned model of a spectrophotometer (Hitachihi, 220, Japan). Toluene was used as a blank. The proline concentration was calculated by using a standard curve developed by analar grade proline and calculated on a fresh weight basis.

2.4.3. Total soluble phenolics (Tphe.)

Leaf samples were extracted in 80% acetone to determine total soluble phenolics (Tphe) tannic acid-equivalent soluble phenolics were then determined using a spectrophotometer as described by Julkenen-Titto (1985).

2.4.4. Estimation of enzyme activity

For the estimation of α-amylase activity, a leaf sample (1 g) was ground with 10 mL of phosphate buffer (pH 7.0) and left for 24 h at 4 °C. The enzyme activity was determined from the supernatant by using the dinitrosalicyclic acid (DNS) method (Bernfeld 1955). The activities of peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) were determined spectrophotometrically (Hitatchi, 220, Japan). Leaf extract was prepared by homogenizing leaves in a medium composed of 50 mM phosphate buffer with 7.0 pH and 1 mM dithiothreitol (DTT) as described by (Dixit et al., 2001).

The superoxide dismutase (SOD) activity was measured as the reduction in photochemical nitro blue tetrazolium (NBT) through the method of Giannopolitis and Ries (1977). Readings were taken at 560 nm with a spectrophotometer (Hitachi U-2100, Tokyo, Japan). One unit of SOD activity was defined as the amount of enzyme that inhibited the photochemical reduction of NBT by 50%.

The POD activity was calculated by measuring the peroxidation of H_2O_2 with guaiacol (as an electron donor) using the Chance and Maehly (1955) method. The reaction mixture of POD consists of 50 mM of phosphate buffer (pH 5), 20 mM of guaiacol, 40 mM of H_2O_2, and 0.1 mL of enzyme extract. The increase in the absorbance due to the formation of tetraguaiacol at 470 nm was assayed after every 20 s. One unit of the enzyme was considered the amount of the enzyme that was responsible for the increase in OD value of 0.01 in 1 min. The enzyme activity was determined and expressed as unit min^{-1} g^{-1} fresh weight basis.

Catalase (CAT) activity was measured as the conversion rate of hydrogen peroxide to water and oxygen molecules, following the method described by Chance and Maehly (1955). The decline in absorbance at 240 nm after every 20 s due to consumption of H_2O_2 was the measurement of catalase activity. An absorbance change of 0.01 units min^{-1} was defined as one unit of catalase activity.

Ascorbate peroxidase (APX) activity was measured through the method described by Cakmak et al. (1994). The APX activity was measured by observing the decrease in absorbance of ascorbic acid at 290 nm (extinction coefficient 2.8 mM cm^{-1}) on a spectrophotometer.

2.4.5. Salicylic acid determination

The method of Malamy et al. (1992) was used for the determination of salicylic acid.

2.4.6. Malondialdehyde contents (MDA)

Lipid peroxidation was determined using the thiobarbituric acid (TBA) method (Dhindsa et al., 1981). The absorbance of the supernatant was determined at 532 and 600 nm. MDA content was calculated with the following formula:

MDA (nmol/g. fwt) = [(A532-A600) /155] /1000 * dilution factor

2.4.7. Hydrogen peroxide contents

The method of Alexieva et al. (2001) was used to measure hydrogen peroxide (H_2O_2). The reaction mixture was read at 390 nm with the help of spectrophotometer. Values

are calculated using the standard hydrogen peroxide and hydrogen peroxide using serial dilution with distilled water.

2.4.8. Ascorbic acid contents (AsA)

Ascorbic acid was determined by following Mukherjee and Choudhuri's (1983) method. The absorbance was measured at 530 nm with a UV–Vis spectrophotometer (Hitachi U-2910, Tokyo, Japan). Ascorbic acid content was determined from a standard with a known concentration of ascorbic acid.

2.4.9. Determination of ions and nutrients

The analysis of K^+, Na, Ca^{2+}, and Mg^{2+} was done by the methods described by (Yoshida and Coronel, 1976).

The reading of K^+ was read on a flame photometer (Jenway PFP 7). The standard curve of K was drawn by a graded series of standards (ranging from 5 ppm to 100 ppm). The values of unknown K^+ samples were determined by comparing them with standard curves. The same procedure was followed for the Na ion determination. The Ca^{2+} and Mg^{2+} were determined by an atomic absorption spectrophotometer. The Ca^{+2} and Mg^{+2} were measured at 422.7 and 285.2 nm, respectively.

For Mg^{2+}, the stock solution was prepared with 1 g of magnesium ribbon dissolved in HCl (1+1) HCl by heating and then its volume was increased up to 1000 mL with 1% (v/v) HCl, which gave a concentration of 1000 ppm. Next, 10 mL of this solution was increased to 100 mL to get 100 ppm Mg^{2+}. Final quantities were computed comparing the sample readings with the standard curves.

Phosphorus (P) was determined by a spectrophotometer. The extracted material (2 mL) was dissolved in 2 mL of Barton's reagent, and the total volume was 50 mL. These samples were kept for half an hour before analyzing P. The values of P were calculated by using a standard curve.

Nitrogen (N) was estimated by micro-Kjeldhal's method (Bremner and Keeney, 1965). The N was determined using reagents (3% Boric acid solution, 0.01 N sulfuric acid standard, and a mixed indicator on bromocresol methylene red). Five milliliters of digested material and NaOH (5 mL of 40%) were taken in Kjeldhal's tubes. The following formula was used to estimate N:

$$N\,(\%) = \frac{(V2 - V1) \times N \times 0.014}{W} \times 100,$$

where V_2 = Volume of standard H_2SO_4 required to titrate the sample solution, V1 = Volume of standard H_2SO_4 necessary to titrate the blank solution, N = Normality of H_2SO_4, W = Weight of the sample.

2.5. Growth and yield determinations

Growth and yielding parameters were measured after foliar application of SA at the reproductive stage. A complete randomized design with three replicates was used to measure the plant height, plant fresh matter, plant dry matter, panicle length, grain yield per plant, 1000-grain weight, r plant, 1000-grain weight, and harvest index (HI).

2.6. Statistical analysis

A two-way analysis of variance test (ANOVA) was conducted in order to test the significance of SA application and drought effects on plant biomass and biochemical variables. Tukey's post hoc was used for the multiple means comparison technique. Statistical analysis was performed with SPSS for Windows Software v. 19. Correlation and principal component analysis were constructed among various rice cultivars variables using R Studio software.

3. Results and discussion

Better productivity under water stress conditions needs a good understanding of changes in morphology and physiological traits related to water stress because tolerance could be used to select and create new varieties of crops (Akram et al., 2018; Hussain et al., 2018; Parveen et al., 2019). Plant responses to water stress are significant depending on plant species, stress duration, intensity, and growth stage (Ghafar et al., 2021; Nawaz et al., 2021).

3.1. Plant growth attributes

Drought stress affects plant growth, elongation and expansion (Bashir et al., 2020; Kong et al., 2021; Naz and Perveen, 2021). Salicylic acid (SA) plays a vital role in regulating plant growth and productivity. It also affects the different physiological and biochemical activities of plants (Hayat et al., 2010; Naz et al., 2021). In this study, plant height was significantly affected by drought stress especially in rice due to its water-loving nature. Thus, Audebert et al. (2000) observed a reduction in height under water-deficient condition in rice. Plant height (PH) was significantly ($p < 0.001$) reduced under drought stress (Table 1). The PH of 85.67 cm was noted in HTT-138 and 75.67 cm in HTT-39 cm with a percent reduction of 44% (HTT-138) and 60% (HTT-39) under 60% FC. Less reduction (14% and 16%) was observed under CS, followed by 80% FC (27% and 31% in HTT-138 and HTT-39, respectively). A similar kind of reduction was reported in plant height under drought stress (Bashir et al., 2020; Naveed et al., 2014; Perveen et al., 2019). Plant height has been recovered after foliar spray of SA in both genotypes (HTT-138 and HTT-39). The maximum recovery of 35% was observed in HTT-138 under 60% FC, followed by 20%, 18%, and 9% under 100%, 80%, and CF conditions. However, in HTT-39, the maximum increase has been observed in CF (19%), followed by 17%, 13%, and 10% in CS, 80% and 60% FC after SA (100 ppm) spray (Table 1). Another observed effect of water stress on crop plants is reduced fresh and dry biomass (Khan et al., 2019a; Sarker and Oba, 2018). The plant fresh mass (PFM) showed a significant ($p < 0.001$) difference in genotypes, with foliar

Table 1. Effect of foliar-applied salicylic acid (SA) on growth attributes and chlorophyll pigments in rice (*Oryza sativa* L.) genotypes grown under drought stress.

	PH (cm)	PFM (g)	PDM (g)	Chl.*a* (mg g^{-1} FW)	Chl.*b* (mg g^{-1} FW)	Total Chl. (mg g^{-1} FW)	Chl. a/b
HTT-138							
CF	$128.66^{a} \pm 1.20$	$501.33^{d} \pm 4.17$	$22.57^{b} \pm 0.21$	$2.34^{bc} \pm 0.05$	$1.42^{b} \pm 0.06$	$3.77^{b} \pm 0.09$	$0.62^{kl} \pm 0.008$
CS	$116.33^{cd} \pm 0.88$	$466.67^{e} \pm 7.70$	$20.83^{c} \pm 0.38$	$2.17^{de} \pm 0.08$	$1.16^{d} \pm 0.01$	$3.34^{cd} \pm 0.08$	$0.65^{hi} \pm 0.011$
FC (80%)	$113.00^{de} \pm 1.15$	$420.44^{g} \pm 5.50$	$18.52^{d} \pm 0.27$	$2.17^{de} \pm 0.03$	$1.08^{ef} \pm 0.01$	$3.25^{cd} \pm 0.04$	$0.67^{efg} \pm 0.005$
FC (60%)	$85.67^{h} \pm 2.33$	$387.33^{h} \pm 4.81$	$16.86^{e} \pm 0.24$	$1.89^{g} \pm 0.05$	$0.83^{jk} \pm 0.03$	$2.72^{gh} \pm 0.08$	$0.69^{cd} \pm 0.002$
CF +SA (100 mg/g)	$132.66^{a} \pm 1.76$	$629.33^{a} \pm 3.29$	$26.47^{a} \pm 0.16$	$2.53^{a} \pm 0.08$	$1.58^{a} \pm 0.03$	$4.11^{a} \pm 0.05$	$0.61^{l} \pm 0.013$
CS + SA (100 mg/g)	$120.33^{bc} \pm 0.66$	$550.44^{b} \pm 3.32$	$22.52^{b} \pm 0.16$	$2.43^{ab} \pm 0.05$	$1.39^{b} \pm 0.04$	$3.82^{b} \pm 0.08$	$0.63^{jk} \pm 0.002$
80 % FC+SA (100 mg/g)	$117.00^{cd} \pm 1.53$	$509.56^{cd} \pm 3.86$	$20.48^{c} \pm 0.19$	$2.45^{ab} \pm 0.07$	$1.29^{c} \pm 0.04$	$3.75^{b} \pm 0.11$	$0.65^{ij} \pm 0.004$
60 % FC+SA (100 mg/g)	$89.67^{h} \pm 2.34$	$467.78^{e} \pm 4.05$	$18.39^{d} \pm 0.20$	$1.95^{fg} \pm 0.02$	$0.95^{hi} \pm 0.02$	$2.90^{fg} \pm 0.04$	$0.68^{def} \pm 0.002$
HTT-39							
CF	$118.66^{bc} \pm 1.20$	$470.67^{e} \pm 2.34$	$21.03^{c} \pm 0.12$	$2.07^{ef} \pm 0.08$	$0.96^{gh} \pm 0.02$	$3.04^{ef} \pm 0.10$	$0.68^{de} \pm 0.010$
CS	$106.33^{fg} \pm 0.33$	$430.22^{fg} \pm 1.24$	$19.01^{d} \pm 0.06$	$1.92^{fg} \pm 0.01$	$0.87^{ij} \pm 0.01$	$2.79^{gh} \pm 0.01$	$0.69^{cd} \pm 0.003$
FC (80%)	$103.00^{g} \pm 1.53$	$391.78^{h} \pm 2.71$	$17.09^{e} \pm 0.13$	$1.85^{g} \pm 0.04$	$0.76^{k} \pm 0.03$	$2.61^{h} \pm 0.06$	$0.71^{c} \pm 0.005$
FC (60%)	$75.67^{i} \pm 1.76$	$356.00^{i} \pm 4.02$	$15.30^{f} \pm 0.20$	$1.50^{h} \pm 0.05$	$0.40^{l} \pm 0.01$	$1.90^{i} \pm 0.07$	$0.79^{a} \pm 0.002$
CF +SA (100 mg/g)	$122.66^{b} \pm 0.66$	$558.89^{b} \pm 8.45$	$22.94^{b} \pm 0.42$	$2.25^{cd} \pm 0.05$	$1.15^{de} \pm 0.01$	$3.40^{c} \pm 0.05$	$0.66^{fghi} \pm 0.006$
CS + SA (100 mg/g)	$110.33^{ef} \pm 0.67$	$518.44^{c} \pm 2.26$	$20.92^{c} \pm 0.11$	$2.14^{de} \pm 0.06$	$1.03^{fg} \pm 0.01$	$3.17^{de} \pm 0.06$	$0.67^{efgh} \pm 0.004$
80 % FC+SA (100 mg/g)	$107.00^{fg} \pm 2.00$	$477.11^{e} \pm 1.90$	$18.85^{d} \pm 0.09$	$1.92^{fg} \pm 0.04$	$0.97^{gh} \pm 0.02$	$2.89^{fg} \pm 0.05$	$0.66^{fghi} \pm 0.005$
60 % FC+SA (100 mg/g)	$79.67^{i} \pm 2.19$	$438.00^{f} \pm 3.68$	$16.90^{e} \pm 0.18$	$1.59^{h} \pm 0.02$	$0.48^{l} \pm 0.01$	$2.06^{i} \pm 0.02$	$0.77^{b} \pm 0.006$

Means are followed by standard errors. Means were compared with least significance difference ($LSD_{\alpha \leq 0.05}$) and different letters indicate that means are different at 95% confidence level. Abbreviations: CF = Control flooded; FC = Field capacity; CS = Control saturated (100% FC); SA = Salicylic acid; PH = plant height; PFM = Plant fresh mass; PDM = Plant dry mass; Chl. = Chlorophyll.

spray of SA and with different levels of drought stress PFM decreased the most (22% and 24%) under 60% FC stress and the least under CF condition. A positive correlation was observed between SA spray and plant fresh mass. A maximum increment of 25% and 18% in PFM was observed in HTT-138 under CF and in HTT-39 under 60% FC, respectively. The SA spray increased PFM under all levels of drought stress in both genotypes. The genotype HTT-39 showed an increase of 17% under both CS and 80% along with 15% under the CF conditions. A remarkable increase (21%) has been shown in HTT-138 under 80% FC stress after SA spray (Table 1).

Under drought stress, a high amount of dry mass is considered a supportive trait for the survivability of the plant (Vardharajula et al., 2011). Foliar spray of SA significantly (Table 1) increased the plant dry mass (PDM). The highest increases (17% and 9.4%) were seen after SA spray under CF and 60% FC stress in HTT-138 and HTT-39, respectively. Overall, SA spray increased PDM in both genotypes under all levels of drought stress as well as in normal (Table 1). According to Fariduddin et al. (2003) dry matter aggregation was significantly increased in *Brassica juncea* when sprayed with lower concentrations of salicylic acid. Water deficiency reduces PDM significantly, with the highest decrease (25.2% and 27.2%) observed under 60% FC stress and the lowest (7.6% and 9.6%) observed under CF condition in both genotypes (HTT-138 and HTT-39, respectively). The process of dry matter portioning and temporal biomass distribution is indirectly related to plant production under water stress (Ahmad et al., 2017; Ijaz et al., 2021).

3.2. Chlorophyll pigments (mg/g) and gaseous exchange attributes

Under drought stress, leaf color changes due to membrane and chlorophyll breakage, which leads to leaf senescence (Ahmad et al., 2017; Khan et al., 2019b). The chlorophyll pigments decreased on the application of drought treatments. In this study, chlorophyll a (chlo.a), chlorophyll b (chlo.b) and total chlorophyll (T.chlo.) showed the same pattern of decline on imposing drought stress (Table 1). The interaction of genotypes and drought stress is significant ($p < 0.001$) for chlo.b and chlo a/b but not significant ($p > 0.05$) for chlo.a and Tchlo. (Table1). The highest decline

(58% and 41%) was noted in chlo.b as compared to chlo.a (27% and 19%), and Tchlo. (37% and 27%) under 60% FC in HTT-39 and HTT-138, respectively. Under 80% FC stress chlo.b (21% and 24%) was reduced more than chlo.a (27 and 19%) and Tchlo (3 and 27%) in HTT-39 and HTT-139, respectively. However, the situation improved by SA (100 ppm) spray because chlorophyll pigments recovered after this spray. According to Khodary (2004), SA spray enhanced the growth characteristics, pigment contents, and photosynthetic rate in maize. After SA spray chlo.b restored 16% and 15%, chlo.a recovered 5% and 3% along with Tchlo. 7% and 6% under 60% FC stress in HTT-39 and HTT-138, respectively. The highest restoration (19% and 21%) of chlo.b has been observed, which is more than CF (10% and 15%) in HTT-138 and HTT-39 (respectively) under 80% FC stress. The chlo. a/b ratio increased under drought stress and a maximum (11% and 15%) a/b ratio was found under 60% FC than CF (4.5% and 0.8%) in HTT-138 and HTT-39. The SA spray decreased under 60% FC stress more than other drought treatments.

Decline in photosynthesis is the primary sign of leaf senescence (Javed et al., 2020; Nazar et al., 2020; Saleem et al., 2020). The net photosynthesis (*A*, umol m^{-2} s^{1}) was greatly affected by drought stress because water deficiency directly affects the stomatal performance (Table 2). Similar results were documented by Talbi et al. (2020). The interaction between genotypes and drought ($p < 0.001$), genotypes and SA spray ($p < 0.05$) was found to be significant and nonsignificant for drought and SA spray ($p > 0.05$). In this study, the highest reduction (60% and 44%) has been observed under high level of drought stress (60% FC) in HTT-39 and HTT-138, respectively. Net photosynthesis was reduced less under CS (14% and 16.2%), followed by 27% and 31% in HTT-138 and HTT-39, respectively. Both genotypes behave differently towards SA spray (100 ppm) under drought stress. The maximum increase (35%) in net photosynthesis has been noted in HTT-138 under 60% FC, but HTT-39 showed a high increase (19% and 17%) under CF and CS conditions. Hence, SA spray enhanced the net photosynthesis in both genotypes under all levels of drought stress (including CF) (Table 2).

Under drought stress, reduced stomatal conductance

Table 2. Effect of foliar-applied salicylic acid (SA) on gas exchange attributes in rice (*Oryza sativa* L.) genotypes grown under drought stress.

	A **(μmol CO_2 m^{-2} s^{-1})**	***E*** **(mmol H_2O m^{-2} s^{-1})**	***gs*** **(mmol CO_2 m^{-2} s^{-1})**	***Ci*** **(μmol m^{-2} s^{-1})**	**WUEi (*A/E*) (μmol CO_2/mmol H_2O)**	***Ci/C*** $_a$
HTT-138						
CF	$25.73^{b} \pm 0.74$	$12.76^{a} \pm 0.13$	$0.63^{b} \pm 0.012$	$126.0^{b} \pm 0.58$	$2.02^{efg} \pm 0.08$	$0.252^{b} \pm 0.001$
CS	$22.06^{c} \pm 0.29$	$10.46^{b} \pm 0.20$	$0.55^{cd} \pm 0.004$	$111.3^{d} \pm 0.88$	$2.11^{def} \pm 0.04$	$0.223^{d} \pm 0.002$
FC (80%)	$18.70^{de} \pm 0.46$	$9.93^{bc} \pm 0.06$	$0.54^{d} \pm 0.003$	$109.3^{e} \pm 0.66$	$1.88^{gh} \pm 0.06$	$0.218^{e} \pm 0.001$
FC (60%)	$14.30^{g} \pm 0.58$	$8.33^{f} \pm 0.12$	$0.43^{h} \pm 0.007$	$86.0^{l} \pm 0.58$	$1.72^{hi} \pm 0.09$	$0.172^{l} \pm 0.001$
CF +SA (100 mg/g)	$28.13^{a} \pm 0.18$	$12.43^{a} \pm 0.06$	$0.68^{a} \pm 0.004$	$146.6^{a} \pm 0.88$	$2.26^{cd} \pm 0.01$	$0.293^{a} \pm 0.002$
CS + SA (100 mg/g)	$26.53^{b} \pm 0.60$	$9.73^{cd} \pm 0.12$	$0.63^{b} \pm 0.007$	$127.0^{b} \pm 0.58$	$2.73^{a} \pm 0.09$	$0.254^{b} \pm 0.001$
80 % FC+SA (100 mg/g)	$22.10^{c} \pm 0.55$	$9.20^{de} \pm 0.17$	$0.57^{c} \pm 0.010$	$114.0^{c} \pm 0.58$	$2.40^{bc} \pm 0.09$	$0.228^{c} \pm 0.001$
60 % FC+SA (100 mg/g)	$19.36^{d} \pm 0.46$	$8.86^{ef} \pm 0.03$	$0.47^{g} \pm 0.005$	95.0i ± 0.58	$2.18^{de} \pm 0.05$	$0.190^{j} \pm 0.001$
HTT-39						
CF	$20.76^{c} \pm 0.46$	$8.86^{ef} \pm 0.39$	$0.49^{ef} \pm 0.001$	$100.0^{h} \pm 0.58$	$2.40^{bc} \pm 0.12$	$0.200^{h} \pm 0.001$
CS	$17.40^{ef} \pm 0.40$	$8.50^{f} \pm 0.34$	$0.44^{h} \pm 0.001$	$88.67^{k} \pm 0.33$	$2.05^{defg} \pm 0.04$	$0.177^{k} \pm 0.001$
FC (80%)	$14.13^{g} \pm 0.18$	$6.93^{g} \pm 0.28$	$0.40^{i} \pm 0.003$	$80.66^{m} \pm 0.66$	$2.04^{efg} \pm 0.05$	$0.161^{m} \pm 0.001$
FC (60%)	$8.26^{h} \pm 0.62$	$5.23^{h} \pm 0.29$	$0.29^{k} \pm 0.003$	$58.66^{o} \pm 0.88$	$1.57^{ij} \pm 0.05$	$0.117^{o} \pm 0.002$
CF +SA (100 mg/g)	$25.66^{b} \pm 0.46$	$9.70^{cd} \pm 1.00$	$0.51^{e} \pm 0.004$	$102.6^{g} \pm 0.88$	$2.65^{a} \pm 0.07$	$0.205^{g} \pm 0.002$
CS + SA (100 mg/g)	$20.96^{c} \pm 0.28$	$8.33^{f} \pm 0.16$	$0.48^{fg} \pm 0.006$	$105.6^{f} \pm 0.33$	$2.52^{ab} \pm 0.07$	$0.211^{f} \pm 0.001$
80 % FC+SA (100 mg/g)	$16.30^{f} \pm 0.58$	$8.50^{f} \pm 0.29$	$0.41^{i} \pm 0.010$	$92.0^{j} \pm 0.58$	$1.92^{fgh} \pm 0.11$	$0.184^{j} \pm 0.001$
60 % FC+SA (100 mg/g)	$9.23^{h} \pm 0.20$	$6.70^{g} \pm 0.20$	$0.33^{j} \pm 0.006$	$71.66^{n} \pm 0.66$	$1.38^{j} \pm 0.02$	$0.143^{n} \pm 0.001$

Means are followed by standard errors. Means were compared with least significance difference ($LSD_{\alpha \leq 0.05}$) and different letters indicate that means are different at 95% confidence level. Abbreviations: CF = Control flooded; FC = Field capacity; CS = Control saturated (100% FC); SA = Salicylic acid; *A* = Net photosynthetic rate; *E* = Transpiration rate; *gs* = Stomatal conductance; *Ci* = Substomatal CO_2 concentration; C_a = Carboxylation efficiency (*A/Ci*); WUEi = Intrinsic water use efficiency.

decreases transpiration by closing the stomata. Genotype that reduces transpiration is considered good for drought tolerance (Farooq et al., 2013). The plant transpiration rate (*E,* mmol H_2 Om^{-2} s^{-1}) showed a significant ($p < 0.001$) difference in genotypes (Table 2), with SA spray and drought stress conditions along with a significant difference among these factors ($p < 0.05$). Drought stress directly affects the performance of stomata and reduces the transpiration rate (*E,*mmol H_2 Om^{-2} s^{-1}). The highest reduction in transpiration rate (39% and 34%) was found under 60% FC stress followed, by 80% FC (22% and 20%) stress and CS (18 and 1%) in HTT-138 and HTT-39. After foliar spray of SA (100 ppm), a further reduction in transpiration rate has been observed in HTT-138 under CF (2%), CS (7%), and 80% FC (7%) except 60% FC stress (6.4% increase). Foliar application SA increases the transpiration rate CF (10%), 80% FC (18%), and 60% FC (21%), except CS (–2%).

Drought stress limits gas exchange by stomatal closure (Javed et al., 2020; Nazar et al., 2020; Saleem et al., 2020). The plant stomatal conductance (*gs*) showed a significant ($p < 0.001$) difference in genotypes, with SA spray and drought levels along with a nonsignificant difference among these factors ($p > 0.05$). In this study, *gs* followed the same pattern as Ci/Ca by decreasing more under severe drought stress (60% FC) conditions. Under 60% FC stress, the more reduced (27% and 19%) *gs* have been noted under HTT-138 and HTT-39, respectively. The 80% FC stress caused a reduction of 7% and 10% in HTT-138 and HTT-39, respectively. Both genotypes showed the same reduction (7%) under the CS condition. At the beginning of drought stress, *gs* reduced the photosynthesis (Nikolaeva et al., 2010), but prolonged drought stress may cause tissue dehydration, which leads to metabolic impairment (Mafakheri et al., 2010). The foliar application of SA restored the gs in both genotypes even under drought stress. The SA spray restored (12% and 11%) more gs in HTT-138 than in HTT-39 (3% and 10%) under 80% FC and CS conditions respectively. However, under 60% FC stress, HTT-39 recovered 5% more than HTT-138 (3%). Both genotypes recovered the same (8%) in CF condition after SA spray (Table 2).

Stomatal closure, which reduces CO_2 influx into mesophyll cells (Farooq et al., 2013), reduces the activity and content of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Mohamed et al., 2020), as well as ribulosebisphosphate (RuBP) regeneration. Drought stress affects the stomatal conductance, which results in low intercellular CO_2 conductance (C_i) (Table 2). The *Ci* showed a significant ($p < 0.001$) difference in genotypes and under drought conditions. The minimum *Ci* was found in both genotypes HTT-138 (31%) and HTT-39 (41%) under 60% FC stress. HTT-138 showed 13% while HTT-39 showed 19% reduction in *Ci* under 80% FC. A minimum reduction of 11% was noted under CS conditions in both genotypes. Foliar spray ($p < 0.001$) enhanced the *Ci* in both genotypes. Both genotypes behave differently towards SA spray, HTT-138 showed high increase under CF (16%) followed by CS (14%), 60% FC (10%) and 80% FC (4%) gradually. Genotype HTT-39 showed more increase under 60% FC (18%), CS (16%) and 80% FC (12%) as compared to HTT-138.

The *Ci*/Ca ratio (μmol m^{-2} s^{-1}) showed a significant difference in genotypes ($p < 0.001$) and with foliar applied SA ($p < 0.001$). The intercellular to ambient CO_2 ratio (*Ci*/Ca) was significantly ($p < 0.001$) reduced on imposing drought stress. Its high reduction (31% and 41%) has been seen in severe drought stress (60% FC) stress in HTT-138 and HTT-39, respectively (Table 2).

3.3. Water relations

The water use efficiency (WUE) significantly ($p < 0.001$) decreased under drought stress (Table 2). More reduction (14% and 34%) has been observed under 60% FC stress followed by 80% FC stress (6% and 15%) and CS (4% and 14%) in HTT-138 and HTT-39, respectively. The reduction in WUE was noted more in HTT-39 than in HTT-138 under drought stress. Foliar application of SA significantly ($p < 0.001$) increased (27%) the WUE under high level of drought stress (60% FC) and 80% FC stress more than CF (12%), but the highest increase was noted under CS (29%) in the HTT-138 genotype. On the other hand, SA spray differently affected the HTT-39 and increased WUE only under CF (9%) and CS (18%) but negatively affected it under 80% FC (6%) and 60% FC (15%) stress.

Drought stress reduced water content, diminished leaf osmotic potential, turgor pressure, stomatal activity, decreased cell enlargement and growth in plant (Farooq et al., 2020; Nawaz et al., 2021; Shemi et al., 2021) under high drought stress arrest of photosynthesis, a disorder in metabolism, and finally the death of plant (Hussain et al., 2008; Kosar et al., 2021).

The water potential (Ψ_p) showed significant difference in genotypes ($p < 0.001$) and after foliar spray of SA ($p < 0.001$). Less availability of water has led to lower water content in the leaves and reduced the stomatal size by losing turgor pressure (Ψt) in guard cells which results in stomatal closure (Karimpour, 2019). The data (Table 3) related to Ψ_p showed a significant difference in genotypes ($p < 0.01$) and after foliar spray of SA ($p < 0.001$). The Ψ_t decreased more under drought stress in both genotypes. The highest reduction in Ψ_t was found under 80% FC stress in both genotypes HTT-138 (74%) and HTT-39 (87%) followed by 60% FC stress (66% and 78% in HTT-138 and HTT-39, respectively). The SA spray significantly enhanced the stomatal closure by decreasing more Ψ_t in leaves to save the water content. In HTT-39, the highest

Table 3. Effect of foliar applied salicylic acid (SA) on water relation attributes and nitrate, nitrite reductase activity reductase activity in rice (*Oryza sativa* L.) genotypes grown under drought stress.

	Ψp (-Mpa)	Ψs (-Mpa)	Ψt (-Mpa)	RWC (%)	NRA (μmol NO_3 g^{-1} FW h^{-1})	NiRA (μmol NO_2 g^{-1} FW h^{-1})
HTT-138						
CF	$0.76^{ij} \pm 0.006$	$0.92^{i} \pm 0.009$	$0.158^{a} \pm 0.003$	$83.46^{a} \pm 1.921$	$7.67^{a} \pm 0.076$	$5.18^{b} \pm 0.005$
CS	$0.81^{i} \pm 0.008$	$0.87^{j} \pm 0.014$	$0.061^{d} \pm 0.005$	$76.40^{b} \pm 0.069$	$7.13^{bc} \pm 0.102$	$4.87^{c} \pm 0.058$
FC (80%)	$1.00^{fg} \pm 0.003$	$1.04^{gh} \pm 0.008$	$0.040^{ef} \pm 0.006$	$65.30^{cd} \pm 0.085$	$6.46^{d} \pm 0.042$	$4.50^{d} \pm 0.102$
FC (60%)	$1.36^{b} \pm 0.006$	$1.42^{b} \pm 0.041$	$0.053^{de} \pm 0.005$	$55.10^{e} \pm 1.619$	$5.45^{gh} \pm 0.184$	$3.68^{gh} \pm 0.151$
CF +SA (100 mg/g)	$0.90^{h} \pm 0.006$	$1.00^{h} \pm 0.004$	$0.099^{c} \pm 0.003$	$74.46^{b} \pm 0.867$	$7.70^{a} \pm 0.022$	$5.49^{a} \pm 0.026$
CS + SA (100 mg/g)	$0.97^{g} \pm 0.011$	$1.01^{h} \pm 0.018$	$0.037^{ef} \pm 0.004$	$64.60^{d} \pm 1.763$	$7.29^{b} \pm 0.031$	$5.13^{b} \pm 0.029$
80 % FC+SA (100 mg/g)	$1.22^{c} \pm 0.020$	$1.25^{d} \pm 0.003$	$0.030^{fg} \pm 0.003$	$55.36^{e} \pm 1.185$	$6.87^{c} \pm 0.058$	$4.66^{cd} \pm 0.063$
60 % FC+SA (100 mg/g)	$1.46^{a} \pm 0.033$	$1.50^{a} \pm 0.029$	$0.034^{f} \pm 0.011$	$45.00^{g} \pm 1.734$	$5.82^{ef} \pm 0.120$	$3.88^{fg} \pm 0.051$
HTT-39						
CF	$0.59^{k} \pm 0.005$	$0.72^{l} \pm 0.004$	$0.128^{b} \pm 0.003$	$76.10^{b} \pm 1.322$	$5.91^{ef} \pm 0.210$	$4.03^{f} \pm 0.048$
CS	$0.62^{k} \pm 0.012$	$0.71^{l} \pm 0.006$	$0.084^{c} \pm 0.004$	$64.30^{d} \pm 1.539$	$5.32^{h} \pm 0.082$	$3.48^{hi} \pm 0.167$
FC (80%)	$1.04^{ef} \pm 0.008$	$1.06^{fg} \pm 0.007$	$0.016^{ghi} \pm 0.002$	$56.30^{e} \pm 1.251$	$5.14^{h} \pm 0.042$	$3.54^{h} \pm 0.072$
FC (60%)	$1.12^{d} \pm 0.057$	$1.14^{e} \pm 0.001$	$0.027^{fgh} \pm 0.003$	$47.30^{fg} \pm 0.962$	$4.37^{j} \pm 0.193$	$2.68^{j} \pm 0.022$
CF +SA (100 mg/g)	$0.61^{k} \pm 0.008$	$0.73^{l} \pm 0.006$	$0.118^{b} \pm 0.004$	$68.30^{c} \pm 0.908$	$6.33^{d} \pm 0.063$	$4.66^{cd} \pm 0.074$
CS + SA (100 mg/g)	$0.71^{j} \pm 0.008$	$0.78^{l} \pm 0.017$	$0.066^{d} \pm 0.009$	$58.36^{e} \pm 1.034$	$6.03^{e} \pm 0.045$	$4.64^{d} \pm 0.032$
80 % FC+SA (100 mg/g)	$1.08^{de} \pm 0.003$	$1.09^{ef} \pm 0.001$	$0.011^{hi} \pm 0.001$	$48.80^{f} \pm 0.700$	$5.70^{fg} \pm 0.080$	$4.25^{e} \pm 0.017$
60 % FC+SA (100 mg/g)	$1.33^{b} \pm 0.033$	$1.34^{c} \pm 0.033$	$0.005^{i} \pm 0.001$	$40.86^{h} \pm 0.677$	$4.78^{i} \pm 0.057$	$3.29^{i} \pm 0.022$

Means are followed by standard errors. Means were compared with least significance difference ($LSD_{\alpha \leq 0.05}$) and different letters indicate that means are different at 95% confidence level. Abbreviations: CF = Control flooded; FC = Field capacity; CS = Control saturated (100% FC); SA = Salicylic acid; Ψp = water potential; Ψs = Osmotic potential; Ψt = turgor potential; RWC = Relative water contents; NRA = Nitrate reductase activity; NiRA = Nitrite reductase activity.

decrease (48%) was observed under 80% FC, followed by 60% FC (41%), CS (26%) and CF (8%). In HTT-138, the highest reduction was observed under CF and CS (37%), followed by 60% FC (36%) and 80% FC (15%) after SA foliar spray.

The data related to osmotic potential (Ψ_s) showed a significant ($p < 0.001$) difference in genotypes, with SA spray and under drought conditions. Drought stress reduced the Ψs, and severe drought stress reduced it even more. The high reduction (59% and 53%) in Ψ_s was noted under 60% FC stress, followed by under 80% FC stress (47% and 12%) in HTT-39 and HTT-138, respectively. The foliar spray of SA recovered the Ψ_s in the leaf and maximum (20%) recovery was observed in HTT-138, under 80% FC stress, followed by HTT-39 (14%) under 60% FC stress. Foliar spray recovered the Ψ_s under all levels of drought stress, even under CF conditions (Table 3).

The relative water content (RWC) decreased under drought stress (Table 3). The RWC decreased in plants facing drought stress and was found to have a high reduction under high intensity of stress. In HTT-39 and HTT-138, the maximum reduction (37% and 33%) was found under 60% FC stress, followed by 80% FC stress reduction (26% and 21%). Less reduction (15% and 8%) was found under CS condition in HTT-39 and HTT-138, respectively. The SA spray did not affect the RWC in both genotypes.

3.4. Nitrate reductase activity (NRA, μmol NO_3 g^{-1}Fw h^{-1})

Under drought stress (Table 3), nitrate reductase activity (NRA) is reduced. In this study, more reductions (29% and 26%) have been noted under 60% FC stress. The stress of 80% FC reduced the NRA 15% and 12% in HTT-138 and HTT-39, respectively. Less reduction (7% and 10%) was noted under the CS condition in HTT-138 and HTT-39, respectively. Foliar spray of SA recovered the NRA in both genotypes under drought stress. The high recovery (11%, 9%, 8%, and 6%) was found in HTT-39 followed by HTT-138 (2%, 6%, 6%, and 0.2%) under CS, 80% FC, 60% FC, and CF condition, respectively, after SA spray.

3.5. Nitrite reductase activity (NiRA, μmol NO_3 g^{-1}Fw

h^{-1})

Nitrite reductase activity (NiRA) decreased under drought stress (Table 3). The decline in NiRA activity increased with the increase in stress intensity. The highest decline was (33% and 28%) was noted under 60% FC stress, followed by 80% FC stress (12% and 13%), along with CS (13% and 5%) in HTT-39 and HTT-138, respectively. High increment was observed in HTT-39 after SA spray ($p < 0.001$) under 60% FC (18%), 80% FC (16%), CS (24%), and CF (13%). Less recovery was noted in HTT-138, under 60% FC (5%), 80% FC (3%), CS (5%), and CF (5%), after SA spray.

3.6. Oxidative damage

The production of hydrogen peroxide (H_2O_2) initiation was significantly ($p < 0.001$) increased under stress conditions (Table 4). Thus, H_2O_2 produced more (31% and 18%) under a high level of drought stress (60% FC) in HTT-138 and HTT-39, respectively. Under 80% FC stress, H_2O_2 increased by 27% and 17% in HTT-138 and HTT-39, respectively. Foliar spray of SA ($p < 0.001$) reduced H_2O_2 more in HTT-39 than HTT-138. A maximum reduction (19%) was noted under CF and 12% under both CS and 80% FC in HTT-39. The HTT-138 genotype showed 5%, 7%, and 5.8% reductions in H_2O_2 under CF, CS, and 80% FC stress, but no reduction has been observed under 60% FC stress (Table 4).

The production of reactive oxygen species (ROS) should be prohibited and ROS-induced oxidative damage should be mitigated through lipid peroxidation (MDA), protein and DNA oxidation (Akram et al., 2022). Lipid peroxidation was measured in malondialdehyde (MDA), which increased under stress conditions (Table 4). HTT-39 showed a significant increase in MDA (31%, 14%, 4%) under 60% FC, 80% FC, and CS conditions, compared to HTT-138 under 60% FC (22%), 80% FC (16%), and (3%) CS condition. The foliar spray at the reproductive stage reduced the MDA content under normal and drought stress in both genotypes. The high reduction (32%, 11%, 15%, and 13%) was noted in HTT-39 and less reduction (6%, 9%, 7%, and 5%) was observed in HTT-138 under CF,

Table 4. Effect of foliar applied salicylic acid (SA) on oxidative and biochemical attributes in rice (*Oryza sativa* L.) genotypes grown under drought stress.

	H_2O_2 (μmol g^{-1} FW)	MDA (nmol mL^{-1} g^{-1} FW)	AsA (mg g^{-1} FW)	TSP (mg g^{-1} FW)	FAA (mg g^{-1} FW)	TSS (mg g^{-1} FW)
HTT-138						
CF	$30.46^{i} \pm 0.15$	$2.96^{g} \pm 0.032$	$37.7^{j} \pm 0.69$	$10.25^{b} \pm 0.11$	$4.25^{ij} \pm 0.07$	$3.34^{k} \pm 0.04$
CS	$35.62^{g} \pm 0.05$	$2.86^{h} \pm 0.012$	$45.9^{i} \pm 1.33$	$9.72^{cd} \pm 0.11$	$4.52^{hi} \pm 0.01$	$3.44^{k} \pm 0.12$
FC (80%)	$38.75^{d} \pm 0.17$	$3.44^{d} \pm 0.010$	$70.7^{d} \pm 0.38$	$9.41^{e} \pm 0.04$	$5.39^{g} \pm 0.04$	$4.32^{hi} \pm 0.16$
FC (60%)	$40.10^{c} \pm 0.14$	$3.63^{c} \pm 0.066$	$50.53^{h} \pm 0.79$	$8.24^{h} \pm 0.07$	$7.05^{e} \pm 0.04$	$6.64^{d} \pm 0.12$
CF +SA (100 mg/g)	$28.76^{j} \pm 0.51$	$2.77^{i} \pm 0.027$	$48.26^{hi} \pm 0.55$	$10.46^{a} \pm 0.05$	$5.38^{g} \pm 0.08$	$3.88^{jk} \pm 0.01$
CS + SA (100 mg/g)	$33.08^{h} \pm 0.37$	$2.60^{j} \pm 0.008$	$65.56^{e} \pm 1.65$	$10.24^{b} \pm 0.02$	$6.03^{f} \pm 0.01$	$4.24^{j} \pm 0.07$
80 % FC+SA (100 mg/g)	$36.50^{f} \pm 0.20$	$3.19^{f} \pm 0.018$	$83.26^{b} \pm 0.69$	$9.72^{cd} \pm 0.08$	$7.23^{d} \pm 0.06$	$4.64^{h} \pm 0.06$
60 % FC+SA (100 mg/g)	$39.92^{c} \pm 1.24$	$3.42^{de} \pm 0.063$	$70.06^{d} \pm 0.87$	$8.92^{f} \pm 0.02$	$8.07^{b} \pm 0.04$	$6.34^{e} \pm 0.09$
HTT-39						
CF	$36.64^{ef} \pm 0.08$	$3.37^{de} \pm 0.008$	$31.56^{k} \pm 2.40$	$9.61^{d} \pm 0.01$	$4.13^{j} \pm 0.06$	$3.34^{k} \pm 0.01$
CS	$38.77^{d} \pm 0.20$	$3.52^{cd} \pm 0.063$	$48.13^{hi} \pm 1.42$	$8.68^{g} \pm 0.05$	$4.68^{h} \pm 0.12$	$3.97^{j} \pm 0.02$
FC (80%)	$43.10^{a} \pm 0.16$	$3.87^{b} \pm 0.012$	$69.23^{d} \pm 0.36$	$8.29^{h} \pm 0.04$	$5.48^{g} \pm 0.27$	$5.15^{f} \pm 0.04$
FC (60%)	$43.46^{a} \pm 0.12$	$4.42^{a} \pm 0.012$	$55.43^{g} \pm 0.17$	$6.38^{j} \pm 0.04$	$7.65^{d} \pm 0.06$	$7.68^{b} \pm 0.04$
CF +SA (100 mg/g)	$30.71^{i} \pm 0.11$	$2.54^{j} \pm 0.012$	$58.23^{f} \pm 0.58$	$9.89^{c} \pm 0.04$	$6.15^{f} \pm 0.05$	$4.90^{g} \pm 0.04$
CS + SA (100 mg/g)	$34.51^{h} \pm 0.05$	$3.15^{f} \pm 0.012$	$79.36^{c} \pm 1.63$	$9.42^{e} \pm 0.07$	$7.23^{d} \pm 0.06$	$6.20^{e} \pm 0.03$
80 % FC+SA (100 mg/g)	$38.32^{d} \pm 0.11$	$3.35^{e} \pm 0.018$	$97.63^{a} \pm 0.67$	$8.84^{fg} \pm 0.02$	$7.98^{c} \pm 0.07$	$7.44^{c} \pm 0.05$
60 % FC+SA (100 mg/g)	$41.04^{b} \pm 0.19$	$3.89^{b} \pm 0.017$	$71.56^{d} \pm 0.35$	$6.84^{i} \pm 0.02$	$9.46^{a} \pm 0.05$	$9.31^{a} \pm 0.08$

Means are followed by standard errors. Means were compared with least significance difference ($LSD_{\alpha \leq 0.05}$) and different letters indicate that means are different at 95% confidence level. Abbreviations: CF = Control flooded; FC = Field capacity; CS = Control saturated (100% FC); SA = Salicylic acid; H_2O_2 = Hydrogen peroxide; MDA = Malondialdehyde; AsA = Ascorbic acid; TSP = Total soluble protein; FAA = Free amino acids; TSS = Total soluble sugar.

CS, 80% FC, and 60% FC stress, respectively.

3.7. Total ascorbic acid (AsA), sugar, and phenolics contents

Ascorbic acid (AsA) has been reported as a contributor to drought tolerance in *O. Africana* (Hassan et al., 2021). The AsA increased under drought stress. The AsA showed significant differences in genotypes, SA spray, different levels of drought, and a significant difference among these factors ($p < 0.001$). The genotype HTT-39 showed an increment of 119%, 75%, and 52% under 80% FC, CS, and 60% FC stress respectively, 87%, 34%, and 21% have been shown to have a minimum increase under 80% FC, 60% FC, and CS conditions. Salicylic acid spray enhanced the AsA concentration in both genotypes (Table 4). The increase in AsA concentration was noted in HTT-138 under CS (42%), 60% FC (38%) and in HTT-39 under CF (45%), CS (39%), 80% FC (29%) along with 60% FC (22%).

The total soluble sugar (TSS) increased under drought stress conditions (Table 4). The highest increase in amount (129% and 98%) of TSS was found under severe drought stress (60% FC stress) in HTT-39 and HTT-138, respectively. Under CF, less than 2% and 18% were found in HTT-138 and HTT-39, respectively. After SA spray TSS concentration increased and showed the highest values (31% and 16%) under CF condition followed by CS (35% and 23%) in HTT-39 and HTT-138 respectively. However, in HTT-138 there was a decline (4%) in TSS under 60% FC stress as compared to HTT-39, which showed an increase (17%).

Total phenolic (Tphe) decreased under drought stress and this decrease increased with the increase in stress intensity (Table 5). The maximum decrease (33% and 19%) has been noted under 60% FC, followed by 80% FC (13% and 8%) and CS (9% and 5%) in HTT-39 and HTT-138, respectively. The foliar application of SA (100 ppm) increased the amount of Tphe under all water stress conditions along with normal. The maximum increase (8%) in Tphe was seen in HTT-138 under 60% FC stress and in HTT-39 (7%) under CS condition. A minimum increase (2%) was noted in both genotypes under CF condition. The increment has been observed in other conditions also.

3.8. Protein contents

Table 5. Effect of foliar-applied salicylic acid (SA) on biochemical attributes in rice (*Oryza sativa* L.) genotypes grown under drought stress.

	Total phenolic (**mg g^{-1} TA eq.**)	Proline (**nmol $mL^{-1}g^{-1}$ FW**)	Anthocyanin (**mg g^{-1} FW**)	Salicylic acid (**µg g^{-1} FW**)	α-Amylase activity (**unit g^{-1} protein**)
HTT-138					
CF	$4.55^{b} \pm 0.05$	$201.24^{l} \pm 0.38$	$11.73^{g} \pm 0.13$	$6.99^{c} \pm 0.14$	$5.26^{n} \pm 0.026$
CS	$4.32^{cd} \pm 0.05$	$226.84^{j} \pm 1.19$	$14.88^{d} \pm 0.25$	$7.88^{d} \pm 0.18$	$6.57^{l} \pm 0.032$
FC (80%)	$4.18^{e} \pm 0.02$	$226.23^{h} \pm 1.56$	$16.99^{b} \pm 0.07$	$10.75^{a} \pm 0.05$	$8.35^{i} \pm 0.060$
FC (60%)	$3.66^{h} \pm 0.03$	$354.86^{d} \pm 0.87$	$13.16^{ef} \pm 0.15$	$8.02^{b} \pm 0.11$	$10.66^{f} \pm 0.120$
CF +SA (100 mg/g)	$4.65^{a} \pm 0.02$	$192.06^{m} \pm 0.76$	$14.90^{d} \pm 0.10$	$12.92^{c} \pm 0.11$	$5.81^{m} \pm 0.029$
CS + SA (100 mg/g)	$4.55^{b} \pm 0.01$	$229.66^{j} \pm 1.20$	$17.02^{b} \pm 0.12$	$14.19^{d} \pm 0.13$	$8.11^{j} \pm 0.003$
80 % FC+SA (100 mg/g)	$4.32^{cd} \pm 0.04$	$268.23^{h} \pm 1.65$	$18.59^{a} \pm 0.13$	$17.87^{a} \pm 0.14$	$8.91^{h} \pm 0.016$
60 % FC+SA (100 mg/g)	$3.97^{f} \pm 0.01$	$325.50^{e} \pm 1.76$	$13.63^{e} \pm 0.17$	$13.56^{b} \pm 0.18$	$11.76^{d} \pm 0.081$
HTT-39					
CF	$4.27^{d} \pm 0.01$	$206.96^{k} \pm 0.67$	$7.18^{k} \pm 0.45$	$5.11^{c} \pm 0.32$	$6.91^{k} \pm 0.029$
CS	$3.85^{g} \pm 0.02$	$249.93^{i} \pm 0.89$	$8.74^{j} \pm 0.27$	$6.23^{d} \pm 0.19$	$9.69^{g} \pm 0.044$
FC (80%)	$3.68^{h} \pm 0.02$	$298.00^{f} \pm 0.92$	$13.46^{ef} \pm 0.07$	$8.93^{a} \pm 0.05$	$11.54^{e} \pm 0.008$
FC (60%)	$2.84^{j} \pm 0.02$	$387.90^{b} \pm 1.36$	$9.62^{i} \pm 0.03$	$6.85^{b} \pm 0.02$	$13.33^{c} \pm 0.043$
CF +SA (100 mg/g)	$4.40^{c} \pm 0.02$	$289.86^{g} \pm 0.67$	$10.72^{h} \pm 0.11$	$7.66^{c} \pm 0.08$	$9.12^{h} \pm 0.021$
CS + SA (100 mg/g)	$4.18^{e} \pm 0.03$	$366.33^{c} \pm 4.10$	$13.06^{f} \pm 0.31$	$9.30^{d} \pm 0.22$	$11.91^{d} \pm 0.101$
80 % FC+SA (100 mg/g)	$3.93^{fg} \pm 0.01$	$386.56^{b} \pm 2.83$	$15.86^{c} \pm 0.13$	$11.30^{a} \pm 0.09$	$13.93^{b} \pm 0.202$
60 % FC+SA (100 mg/g)	$3.04^{i} \pm 0.01$	$429.66^{a} \pm 3.28$	$11.44^{g} \pm 0.07$	$9.51^{b} \pm 0.05$	$16.44^{a} \pm 0.033$

Means are followed by standard errors. Means were compared with least significance difference ($LSD_{\alpha \leq 0.05}$) and different letters indicate that means are different at 95% ±confidence level. Abbreviations: Abbreviations: CF = Control flooded; FC = Field capacity; CS = Control saturated (100% FC); SA = Salicylic acid.

The total soluble protein (TSP) decreased under drought stress (Table 4). The data related to TSP showed a significant difference in genotypes ($p < 0.001$) and after foliar spray of SA ($p < 0.001$). In this study, TSP decreased and a high decline has been observed under conditions of high intensity of stress. The highest decline was noted under 60% FC stress in HTT-138 (19%) and HTT-39 (33%). The lowest increment was noted under CF condition in HTT-138 (5%) and HTT-39 (9%). The foliar spray of SA recovered the TSP in both genotypes under normal as well as stressed condition. The highest recovery (8%) of TSP was found under 60% FC in HTT-138 and under CS (7%) in HTT-39.

The concentration of free amino acid (FAA) increased in plants facing stress (Table 4). The highest increments (85% and 65%) were seen in HTT-39 and HTT-138 under 60% FC stress. Under the minimum stress CS condition, less increment (6% and 13%) was observed. Foliar spray of SA enhanced the amount of FAA in genotypes under normal and drought stress. The high increments (32% and 26%; 35% and 33%; 31% and 34%) have been observed under CF, CS, and 80% FC stress in HTT-39 and HTT-138 respectively. The increment under 60% FC stress has been noted to be less (19% and 14%) in HTT-39 and HTT-138, respectively as compared to other water treatments.

The free proline (prol.) increased under stress conditions in plants facing them (Table 5). The highest amounts (87% and 76%) were noted under 60% FC stress, followed by 80% FC (43% and 32%) and CS (20% and 12%) in HTT-39 and HTT-138, respectively. From the results, it is clear that proline increased significantly ($p < 0.001$) with the increase in stress intensity. Proline quantity increased (9%, 22%, 31%, and 28%) in HTT-39 after foliar application of SA (100 ppm) under 60% FC, 80% FC, CS, and CF conditions, respectively. The foliar application of SA decreased ($p < 0.001$) the quantity of free proline (8% and 4%) under 60% FC and CF condition.

3.9. Anthocyanine (umol/g F.wt.)

The concentration of anthocyanine (Anth) was found to be higher in HTT-138 than in the HTT-39 genotype (Table 5). The highest amounts (87% and 44%) of Anth were noted under 80% FC stress in HTT-39 and HTT-138, respectively. However, under 60% FC stress, more Anth was observed in HTT-138 (39%) than in HTT-39 (34%). A lesser increase in amount was noted under CF in HTT-138 (26%) and HTT-39 (21%). Foliar application of SA (100 ppm) increased the amount of Anth. at greater rate in HTT-39 than in HTT-138. The highest increases 33% and 32% of Anth. was noted under CS and CF in HTT-39, respectively, after SA spray. In HTT-138 genotype, high amount (27%) of Anth. was recorded under CF followed by CS (14%), and 80% FC (9%), but under 60% FC, Anth. decreased (16%).

3.10. Salicylic acid (SA)

Under drought stress ($p < 0.001$) salicylic acid (SA) found in high concentration (74% and 53%) in HTT-39 and HTT-138, respectively (Table 5). Under 80% FC stress, FC increments of less than 60% (34% and 14%) have been observed in HTT-39 and HTT-138, respectively. In HTT-138 and HTT-39, less growth (12% and 21%, respectively) was observed under CS. Foliar spray of SA (100 ppm) has increased ($p < 0.001$) the concentration (84%, 79%, 66%, and 69%) under CF, CS, 80% FC and 60% FC in HTT-138, respectively. The HTT-39 showed an increment of 33% under both CF, CS and 20%, 27% under 80%, and 60% FC stress after SA spray (Table 5).

3.11. α-amylase activity (unit/g)

The highest increases, 102% and 92%, have been seen under high levels of drought stress (60% FC) in HTT-138 and HTT-39, respectively. Under 80% FC stress conditions, HTT-39 and HTT-138 increased by 66% and 58%, respectively. Genotype HTT-138 showed a less dramatic increase (24%) under CS as compared to HTT-39 (40%). Foliar spray of SA (100 ppm) increased ($p < 0.001$) the α-amylase activity in both genotypes under all levels of water stress conditions. The foliar spray of SA increased the α-amylase activity more (18%, 17%, and 24%) in HTT-138 than in HTT-39 (10%, 6%, and 10%) under 60% FC, 80% FC and CF, respectively.

3.12. Antioxidant enzymes

A significant increase in antioxidants (CAT, SOD, POD, and glutathione reductase) has been observed in *O. africana* under drought stress (Talbi et al., 2020). Antioxidant activity starts in a plant facing stress. This is the defense mechanism of the plant to face or tolerate stress condition. The highest amount of APX was found under 60% FC (57% and 53%), followed by 80% FC (52 and 46 %) and CS (13% and 18%) in HTT-39 and HTT-138, respectively. After foliar spray of SA (100 ppm), the maximum increase ($p < 0.001$) has been seen under CF (17%) and 60% FC (15%) in HTT 138. However, in HTT-39, a high amount was found under CF (13%) and CS (11%) after SA spray. The foliar spray of SA (100 ppm) increased the amount of APX under all drought stress conditions (Figure 1).

The catalase (CAT) activity significantly increased under stress condition in plants to tolerate the stress (Figure 1). Under 60% FC stress, both genotypes (HTT-138 and HTT-39) had high levels of CAT (80 and 75%). CAT activity was also found to be high in HTT-138 and HTT-39 under 80% FC (17% and 35%) and CS (33% and 15%) conditions. After foliar application of SA (100 ppm), CAT activity increased more in HTT-138 under 60% FC (13%), 80% FC (17%), CS (16%) and CF (26%) as compared to HTT-39 (5%, 6%, 8%, and 7%, respectively).

Peroxidase (POD) activity increased under stress condition in plants (Figure 1 and Table 1). The highest amount of POD was noted in HTT-138 under 60% FC

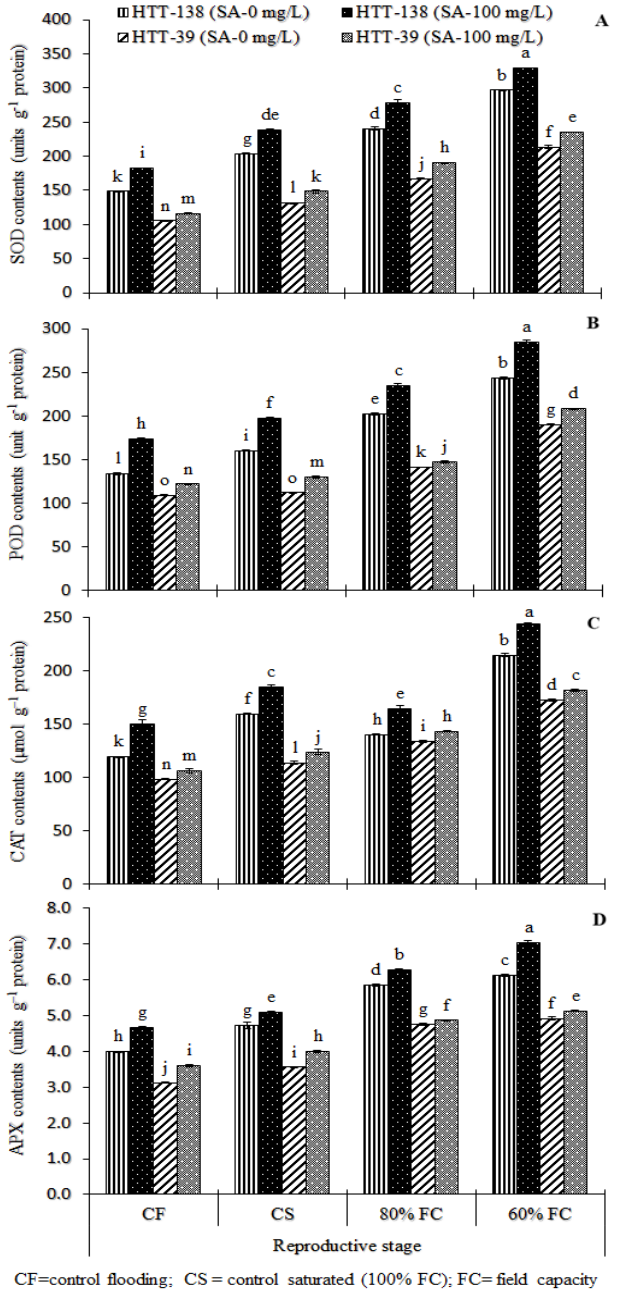

Figure 1. Changes in antioxidants (SOD, POD, CAT, and APX) in two rice genotypes treated with salicylic acid (SA) under drought stress (n = 3 ± SE). Bars expressed with different letters indicate that means are different at 95% confidence level.

(81%), 80% FC (50%), and CS (19%) as compared to HTT-39 (74%, 29%, and 2% under 60% FC, 80% FC, and CS respectively). After SA foliar application, POD increased with same pattern more in HTT-138 as compared to HTT-39. The highest increase (29%) has been shown under CF, followed by CS (22%), 80% FC (16%) and 60% FC (17%) in HTT-138 genotype (Figure 1). In the HTT-39, a high increment was seen under CS (13%) followed by CF (10%), 60% FC (8%), and 80% FC (4%) stress condition.

The superoxide dismutase (SOD) activity increased under stress condition (Figure 1). The SOD The highest amount (102% and 100%) was noted under 60% FC stress in HTT-39 and HTT-138, respectively (Figure 1). The least increment has been seen under CS (24% and 37%) in HTT-39 and HTT-138, respectively. Foliar spray of SA increased SOD amounts under all levels of drought stress as well as normal condition. The highest increase (23% and 12%) was seen in HTT-138 and HTT-39 under CF and 80% FC stress, respectively. It increased under 60% FC (10 and 9%) stress in HTT-138 and HTT-39, respectively. In HTT-138, an increase of 15% and 16% was noted under 80% FC and CS condition, respectively. According to Flexas et al. (2006), plants with high photosynthesis and antioxidant capacity show tolerance and rapid recovery.

3.13. Ion contents

Drought stress reduces plant growth by affecting photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism (Umar et al., 2022), and growth promoters (Haider et al., 2022; Kosar et al., 2021; Mumtaz et al., 2021; Zafar et al., 2021). The concentration of sodium ion (Na) increased under drought stress (Figure 2).

Foliar spray also reduced the Na concentration under other drought stresses (Figure 2). The data related to calcium (Ca) showed a significant difference in genotypes ($p < 0.001$), with foliar applied SA ($p < 0.001$), and with different drought stress along with a significant difference among these factors ($p < 0.01$). The calcium (Ca) concentration increased under all drought stress conditions as well as normal in both genotypes except HTT-39 under 60% FC (–8%). The foliar spray of SA (100 ppm) enhanced the Ca concentration in both genotypes under normal and stressed conditions (Figure 2). The high concentration (17.2%, 17.1%, and 14.9%) was noted under 60% FC, CS, and CF conditions, respectively in HTT-138. Genotype HTT-39 showed the highest increase (12%) in 60% FC stress as compared to 80% FC (1.7%), CS (4%), and CF (8%) conditions.

The data related to magnesium (Mg) showed significant differences in genotypes (Figure 2), with foliar applied SA and with different drought stress along significant difference among these factors. The concentration of magnesium (Mg) increased under drought stress, especially highest increase (68 and 42%) was noted in HTT-39 under 80% FC stress and CS condition (Fig. 2). But HTT-138 showed a decrease (3.5%) in concentration under 60% FC stress as compared to 80% FC and CS which showed an increment of 28% and 11%, respectively. Foliar spray further enhanced the concentration and showed high values under CS (18%) and CF (13%) in HTT-138,

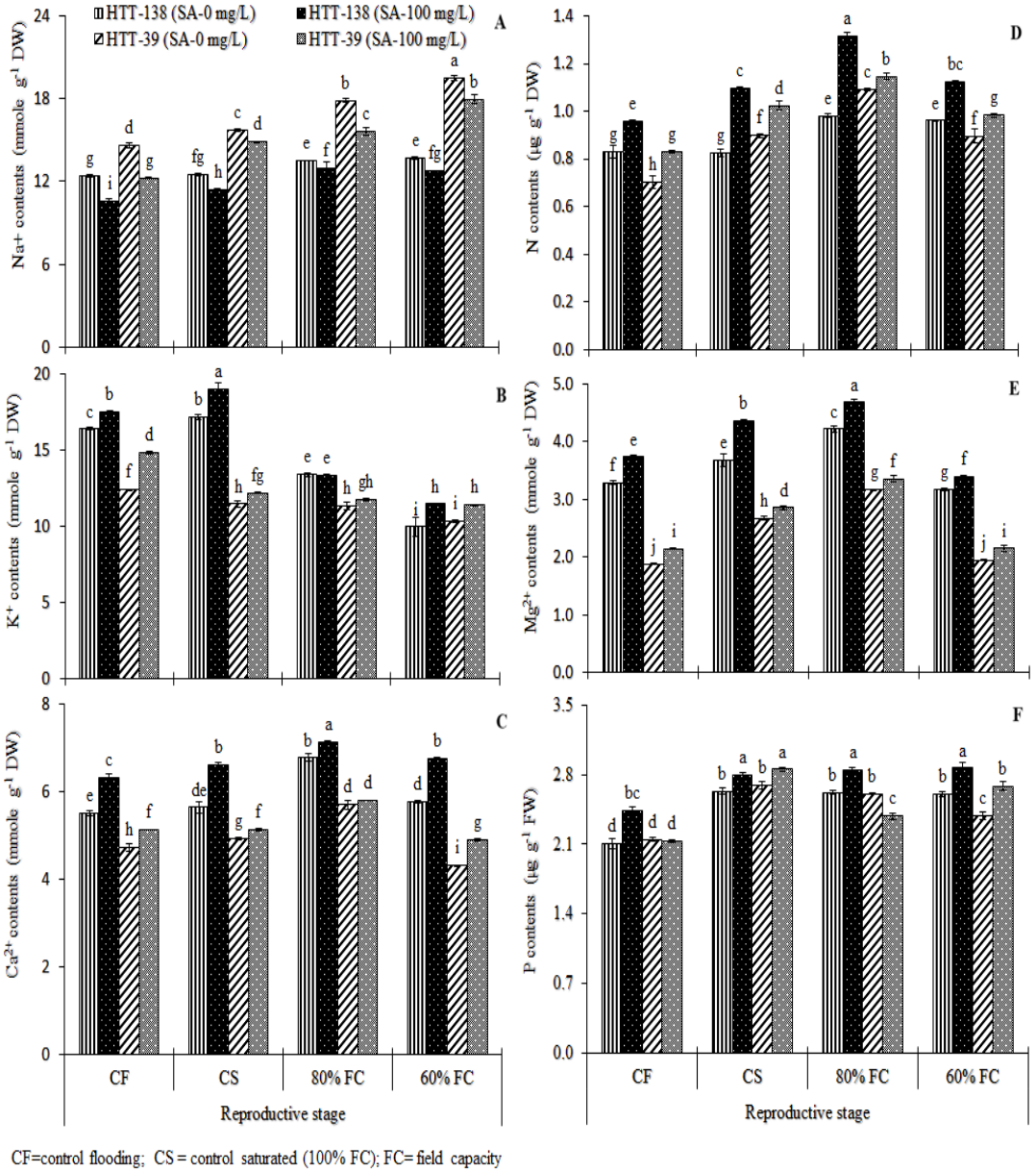

Figure 2. Changes in mineral contents (Na^+, K^+, Ca^{2+}, N, Mg^{2+}, and P) in the root of two rice genotypes with salicylic acid (SA) under drought stress (n = 3 ± SE). Bars expressed with different letters indicate that means are different at a 95% confidence level condition.

although HTT-39 showed high values under CF (12%) and 60% FC stress (9.6%).

Potassium (K) concentration decreased with the increase in water deficiency (Figure 2). In the present study, K ion decreased ($p < 0.001$) under water deficiency and the highest decrease (39 and 16%) was observed under 60% FC stress in HTT-138 and HTT-39 genotypes, respectively. But it enhanced (4%) in HTT-138 under CS as compared to HTT-39 (-7.5%). Foliar spray of SA (100 ppm) improves ($p < 0.001$) the concentration K. The highest values 16 and 15% were noted in HTT-39 (16%) under CF and in HTT-138 (15%) under 60% FC stress. The SA spray enhanced the K concentration in all kinds of drought stress in both genotypes.

The concentration of phosphorus (P) increased under drought stress. The concentration of P increased in the current study under drought stress conditions, with the highest values observed in HTT-138 under CS (25%), 80% FC (24.5%), and 60% FC (23.7%). Genotype HTT-39 showed high increment in CS (25%), 80% FC (21%) stress condition and low under 60% FC stress (11%). The foliar spray of SA (100 ppm) enhanced the concentration ($p <$

0.001) of P in HTT-138 under all levels of water condition, highest value found under CF (16%) condition. In HTT-39, a high increment value (11%) of P was found under 60% FC stress (Figure 2).

Nitrogen concentration increased under drought as showed in Figure 2. In this study, the highest concentration of nitrogen due to drought stress conditions was noted in HTT-39 under 80% FC (55%), CS (28%), and 60% FC (27%). Less increment was noted in HTT-138 under 60% FC (15%), 80% FC (17%) with 1% reduction under CS as compared to HTT-39. Foliar spray of SA increased N concentration (33%, 34%, and 16%) in HTT-138 under CS, 80% FC, and 60% FC, respectively, as compared to HTT-39 (12%, 4.8%, and 8.8%, respectively). Thus, SA spray increased ($p < 0.001$) the concentration of N in HTT-138 than HTT-39 (Figure 2).

3.14. Yield-related attributes

Water scarcity has a global impact on agricultural production and productivity, potentially resulting in significant yield losses (Afridi et al., 2022; Salam et al., 2022; Saleem et al., 2022; Yasmeen et al., 2022). Rice is probably more susceptible to drought due to its water-loving nature as compared to other crops (Filgueiras et al., 2020).

Drought stress conditions decreased the (HI) in genotypes under CS, 80%, and 60% FC stress (Figure 3). The maximum decrease (18%) was observed under high drought stress (60% FC) in HTT-39, followed by HTT-138 (16%). Swain et al. (2010) evaluated eighteen rice genotypes under drought stress and reported reduced panicle number (72%) and grain yield (12%). Foliar spray of SA increases the harvest index (HI), high increment in HI was seen under 60% FC stress in HTT-138 (13.8%) and HTT-39 (10.6%).

Drought stress significantly decreased the number of grains per plant. Behind this reduction, the principal reasons are reduction in net photosynthesis, metabolic limitations, oxidative damage to chloroplasts, stomatal closure, poor grain set and development (Farooq et al., 2020; Hameed et al., 2014). In this study, the highest reduction (16.8% and 15%) was seen under 60% FC in HTT-138 and HTT-39, respectively. A lesser decrease (3.7% and 6.7%) was observed in CS followed by 80% FC (9.2% and 8.5%) in HTT-138 and HTT-39, respectively. Foliar spray of SA (100 ppm) increased the grain number per plant under all levels of drought stress. The high increment (16.8% and 15%) was observed under 60% FC, followed by 80% FC (9.2 and 8.5%) and CS (3.7 and 6.7%) in HTT-138 and HTT-39 respectively. Foliar spray of SA (100 ppm) effectively increased grains under highest level of drought stress (60% FC) in both genotypes. It means SA spray is effective even under intense drought stress.

Thousand grains weight is also affected by drought

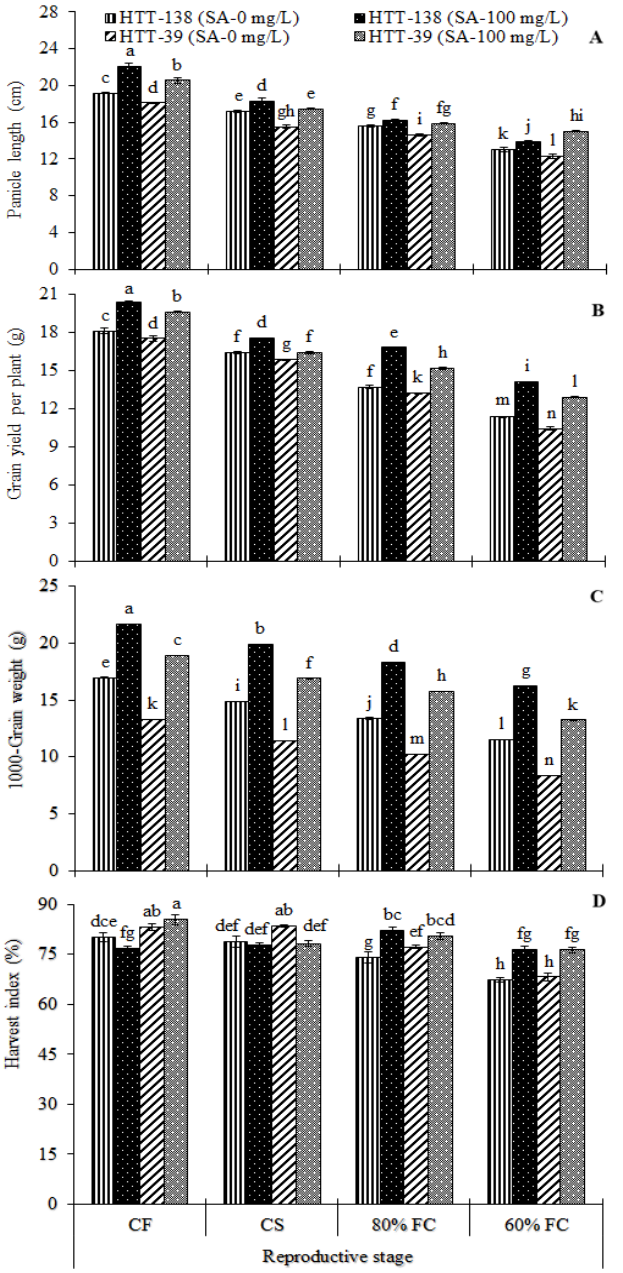

Figure 3. Changes in yield attributes of two rice genotypes treated salicylic acid (SA) under drought stress (n = 3 ± SE). Bars expressed with different letters indicate that means are different at 95% confidence level.

stress significantly and decreased (Figure 3). The highest decrease (37% and 32%) was observed under 60% FC, followed by 80% FC (22.6 and 21%) and CS (13.7 and 12.2%) in HTT-39 and HTT-138, respectively. Foliar spray of SA increased the 1000 grain weight under all level of drought stress even under 60% FC (37.2% and 32.3%) in both genotypes (HTT-39 and HTT-138, respectively). The

foliar spray of SA also effectively increased ($p < 0.001$) the 1000 grain weight under 80% FC in HTT-138 (21.2%) and HTT-39 (22.6%). The foliar spray of SA (100 ppm) increased 1000 grain weight under 60% FC as compared to CS in HTT-138 (12.2%) and HTT-39 (13.7%).

Drought stress reduced the grain yield per plant of rice drastically due to its water-loving nature (Figure 3). A maximum reduction (40.4% and 37.2%, respectively) was observed under 60% FC in HTT-39 and HTT-138. Minimum reduction (9.3%) was noted under CS condition followed by 24% under 80% FC in both genotypes. Swain et al. (2010) also reported reductions in several yielding traits including grain yield under drought stress in rice. Foliar spray of SA enhanced the grain yield not even under CF but also under all levels of drought stress. Genotype HTT-138 showed the highest increase in 60% FC (24%) followed by 80% FC (22%), CS (6.8%), and CF (12.4%) after SA (100 ppm) spray. Foliar spray of SA positively affected the HTT-39 and showed increments of 19%, 13%, 3.1%, and 10.6% under 60% FC, 80% FC, CS, and CF conditions.

Drought stress greatly reduced the panicle length (PL) in both genotypes (Figure 3). Drought stress (60% FC) reduced panicle length by 32% in both genotypes more than 80% FC (19% and 18% in HTT-39 and HTT-138, respectively) and CS (14.3% and 10.2% in HTT-39 and HTT-138%, respectively). Foliar spray of SA (100 ppm) at the reproductive stage increases ($p < 0.001$) the panicle length, which might increase the grain yield. The SA spray increased the panicle length in the sensitive genotype (HTT-39) more than in the tolerant one (HTT-138). The HTT-39 showed an 18% increase in panicle length as compared to HTT-138 (7%) under 60% FC stress. Under CF, the highest increase (15%) was noted in HTT-138 and 11% in HT-39. Under 80% FC and CS stress HTT-138 showed 3.8% and 6.3% as compared to HTT-39 (7.7% and 11.2%, respectively) after SA (100 ppm) spray.

3.15. Correlation

A Pearson correlation analysis was conducted to explore different morphophysiological traits and ion uptake in both cultivars of *O. sativa* (Figure 5). In HTT-138, the sodium accumulation was positively correlated with hydrogen peroxide initiation, malondialdehyde content, and proline content while negatively correlated with relative water content, plant height, nitrate reductase activity, total chlorophyll content, intercellular CO_2, potassium content, calcium content, magnesium content, peroxidase activity, ascorbate peroxidase activity, salicylic acid content, anthocyanin content, superoxidase dismutase activity, catalase activity, nitrogen content, ascorbic acid content, and phosphorus contents. The same pattern was also observed for the HTT-39 cultivar and it was shown that sodium uptake in the plants was positively correlated

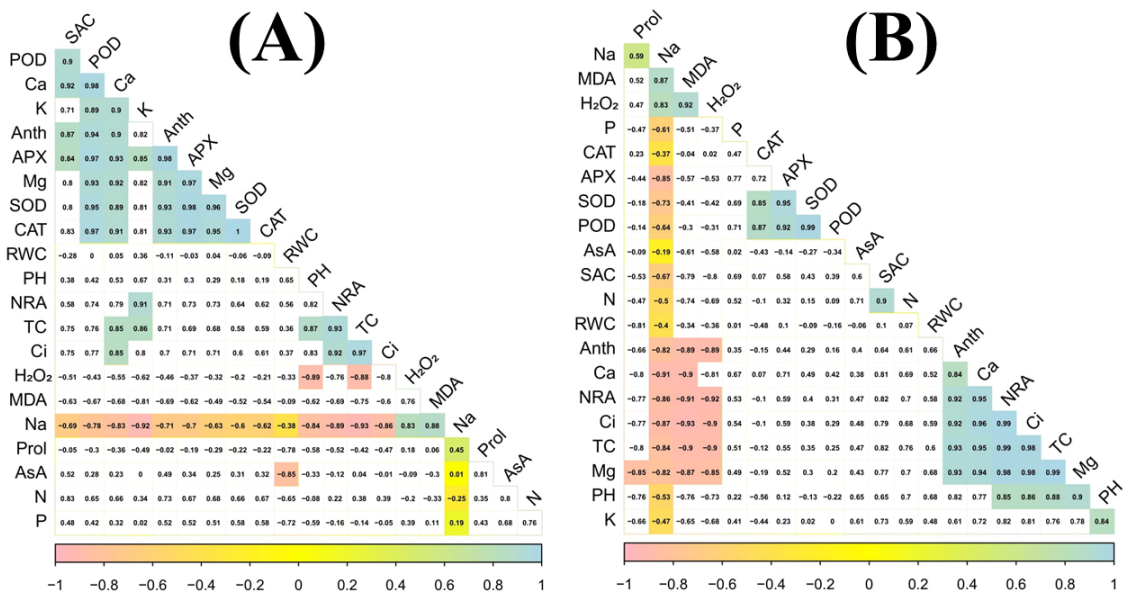

Figure 4. Correlation between different morphological traits, physiological attributes, and ion accumulation in HTT-138 (A) and HTT-39 (B) cultivars grown in drought stressed environments with the exogenous application of SA. The different abbreviations used in this figure are as follows: RWC (relative water content), PH (plant height), NRA (nitrate reductase activity), TC (total chlorophyll content), Ci (intercellular CO_2), K (potassium content), Ca (calcium content), Mg (magnesium content), POD (peroxidase activity), APX (ascorbate peroxidase activity), SAC (salicylic acid content), Anth (anthocyanin content), SOD (superoxidase dismutase activity), CAT (catalase activity), N (nitrogen content), AsA (ascorbic acid content), P (phosphorus content), MDA (malondialdehyde content), H_2O_2 (hydrogen peroxide initiation), Na (sodium content) and Prol. (proline content).

with hydrogen peroxide initiation, malondialdehyde content, and proline contents while negatively correlated with relative water content, plant height, nitrate reductase activity, total chlorophyll content, intercellular CO_2, potassium content, calcium content, magnesium content, peroxidase activity, ascorbate peroxidase activity, salicylic acid content, anthocyanin content, superoxidase dismutase activity, catalase activity, nitrogen content, ascorbic acid content, and phosphorus content. This relationship demonstrates a close connection between growth and ion uptake in both cultivars of *O. sativa*.

3.16. Principal component analysis

A principal component analysis (PCA) was also conducted for both *O. sativa* cultivars to study different morphophysiological traits, ion accumulation, and oxidative damage induced by water stress under the application of SA (Figure 5). In the HTT-138 cultivar, Dim1 (PCA-1) comprised 60.6% and Dim2 (PCA-2) comprised 25%, while in HTT-39 cultivar Dim2 (PCA-2) comprised 61.3% and Dim2 (PCA-2) comprised 21.8% of the whole database. In both cultivars of *O. sativa*, all the variables dispersed successfully in whole database, and it was also noticed that sodium content, hydrogen peroxide initiation, malondialdehyde content, and proline content were most dispersed from all other variables in the database. This gave a clear indication that water stress significantly affected morphophysiological attributes of both cultivars of *O. sativa*. It was also noticed that sodium content, hydrogen peroxide initiation, malondialdehyde content and proline contents were positively correlated in the database while relative water content, plant height, nitrate reductase activity, total chlorophyll content, intercellular CO_2, potassium content, calcium content, magnesium content, peroxidase activity, ascorbate peroxidase activity, salicylic acid content, anthocyanin content, superoxidase dismutase activity, catalase activity, nitrogen content, ascorbic acid content and phosphorus content were negatively correlated with other variables in the database.

4. Conclusion

On the basis of these findings, it can be concluded that the negative impact of water stress can be overcome by the external application of SA. Our results show that a water deficit environment induced severe toxicity in both genotypes of rice (HTT-39 and HTT-138), by increasing the generation of ROS in the form of oxidative stress and also increasing the content of Na in the plant tissues. Hence, the toxic effects of drought stress were eliminated by the application of SA, which also enhanced the antioxidant capacity and decreased the oxidative damage induced by MDA and H_2O_2. Thus, using SA as a primary

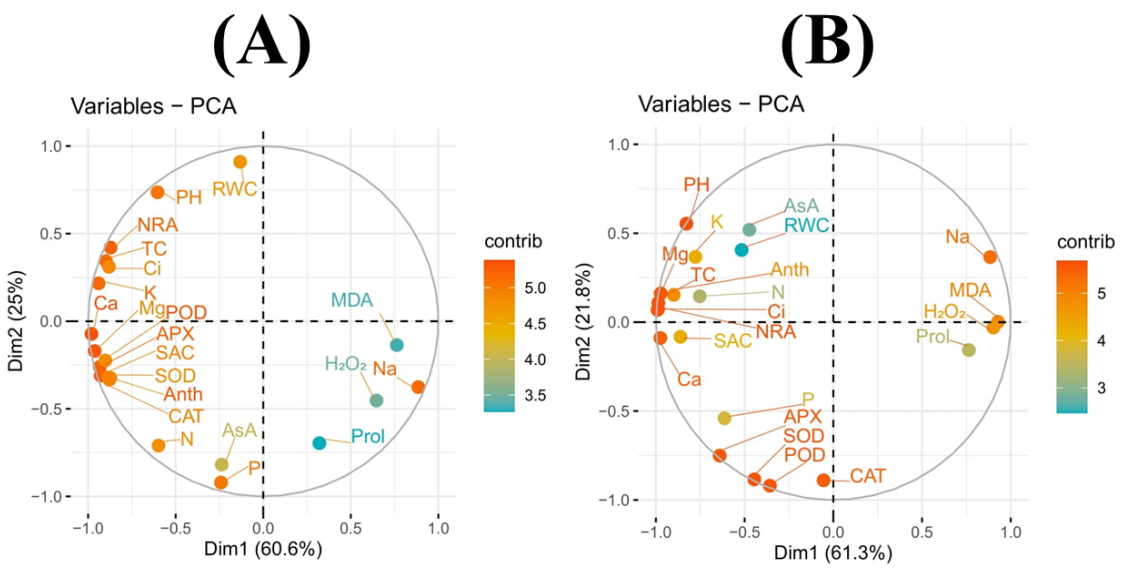

Figure 5. Loading plots of principal component analysis on different morphophysiological traits and ions accumulation in HTT-138 (A) and HTT-39 (B) cultivars grown in drought-stressed environment with the exogenous application of SA. The different abbreviations used in this figure are as follows: RWC (relative water content), PH (plant height), NRA (nitrate reductase activity), TC (total chlorophyll content), Ci (intercellular CO_2), K (potassium content), Ca (calcium content), Mg (magnesium content), POD (peroxidase activity), APX (ascorbate peroxidase activity), SAC (salicylic acid content), Anth (anthocyanin content), SOD (superoxidase dismutase activity), CAT (catalase activity), N (nitrogen content), AsA (ascorbic acid content), P (phosphorus content), MDA (malondialdehyde content), H_2O_2 (hydrogen peroxide initiation), Na (sodium content) and Prol (proline content).

goal to increase crop yield and photosynthetic efficiency in a drought-stressed environment can be recommended. Taken together, these results suggest that cultivation of rice as a forage crop using SA as a foliar application for higher biomass and yield in abiotic stressed environment is possible.

Author contributions

Conceptualization, Asma and Muhammad Yasin Ashraf; Data curation, Asma, Yasser S. Mostafa, Mohammed Hashem, Iqbal Hussain and Muhammad Ali; Formal analysis, Asma and Ghulam Farid; Funding acquisition, Iqbal Hussain, Yasser S. Mostafa, Mohammed Hashem, Aisha shereen and Muhammad Ali; Investigation, Huda Alshaya and Muhammad Ubaidullah Shirazi; Methodology, Asma, Muhammad Yasin Ashraf, ; Resources, Iqbal Hussain and Muhammad Yasin Ashraf; Software, Ghulam Yasin, Shafaqat Ali; Supervision, Muhammad Saleem and Ghulam Farid; Validation, Ghulam Farid; Visualization, Muhammad Saleem and Shafaqat Ali; Writing – original draft, Asma, Review & editing, Muhammad Saleem, Baber Ali, Ghulam Yasin, Basit Latief Jan and Asma. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Not applicable for their financial support to complete this project.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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