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IMPACT OF FOLIAR APPLICATION OF SALICYLIC ACID ON PHYSIOLOGICAL PERFORMANCE OF SORGHUM (*Sorghum bicolor* L.) UNDER SALT STRESS

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ABSTRACT

Although salt stress is a worldwide agricultural and eco-environmental problem and many PGRs have been tried to combat the salinity effects but limited information is available on the impact of salicylic acid in sorghum under salt stress. To assess the impact, seeds of sorghum varieties (HJ 513 and HJ 541) were grown with four different salt levels (0, 5.0, 7.5 and 10.0 dS m^{-1}) and foliar spray of three salicylic acids (0, 25 and 50 ppm) levels with twelve differeLimited water availability hampers the sustainability of crop production. Assessment of the physiological parameters *i.e.* increase in MDA and H_2O_2 content from 0.115 to 0.260 µmole g^{-1} DW and 29.87 to 66.00 nmole g^{-1} DW, respectively and declining trend was observed in total chlorophyll content 3.16 to 2.02 mg g^{-1} DW, SPAD Value 43.61 to 25.19 and photochemical quantum yield (F_v/F_m) 0.736 to 0.651, respectively, in leaf samples at vegetative stage from control to 10 dS m^{-1} of salt stress. Overall yield attributes and yield *i.e.,* panicle length, panicle weight, seed yield plant^{-1}, 100 seed weight and harvest index reduced significantly under salt stress. Furthermore, seed yield plant^{-1} ranged from 13.2 to 9.7 g in HJ 513 and 11.9 to 8.7 g in HJ 541 from control to 10 dS m^{-1} of salt stress. Foliar application of salicylic acid @ 50 ppm significantly influenced the physiological performance of sorghum over control and 25 ppm. Seed yield plant^{-1} increased by 9.8 % after application of 50 ppm SA under salt stress (10 dS m^{-1}) over control. Thus SA may be an alternative to ameliorate the adverse effect of salt stress on sorghum crop.

Keywords: Chlorophyll content, Malondialdehyde, Salicylic acid, Salt stress, Sorghum

The largest grower countries of sorghum are USA, Nigeria, Ethiopia, Mexico and India. India contributes 7.62% of the world's sorghum production with 4.5 million metric tonnes of total production (USDA, 2019). The major constraints *i.e.,* abiotic and biotic stresses that reduce sorghum productivity. Sorghum is moderately salt tolerant and being used as a dual purpose- food and fodder. Salt stress toxicity is a worldwide agricultural and eco-environmental problem. Approximately one-third of the world's land surface is arid and semi-arid, out of which half is affected by salt stress (Liang *et al*., 1996). In India, approximately 6.7 lakh hectare of the cultivated land area are adversely affected by salt stress (Sharma and Singh, 2015). Salt stress in plants retards their physiological performances, yield attributes and finally yield. Salt stress in plants associated with many secondary physiological changes such as reduction of enzyme activity, increase of oxidative stress, membrane dysfunction, reduced photosynthesis and other morpho-anatomical alterations (Tiwari *et al*., 2021). Exposure of plants to salt stress results in increased generation of reactive oxygen species, as by-products, which damage the cellular component. Reactive oxygen species cause chlorophyll degradation and membrane lipid peroxidation, reducing membrane fluidity and selectivity (Taibi *et al*., 2016). Salt stress reduces plant growth and yield due to a reduction in uptake of water and changes the plant metabolic processes (Rady and Mohamed, 2015). Adverse environmental conditions cannot be tolerated by plants, and they are not able to move from one place to another, so for survival, plants do some metabolic changes under stress condition like synthesis of some organic metabolites or some growth regulators. In plants, phenolic compound *i.e.,* Salicylic acid (SA) play a regulatory role in activating biochemical pathways associated with tolerance mechanisms (Stevens *et al.*, 2006).

Salicylic acid is a plant hormone endogenous regulator, which can regulate plant growth and also provides protection against biotic and abiotic stresses such as salt stress (Ma *et al.*, 2017). The effect of salicylic acid on physiological processes varies and depends on species, developmental stage and environmental conditions (Khan *et al.*, 2015). Imposition of SA positively influences a wide variety of plant processes, including stomatal regulation, chlorophyll content, photosynthesis and also seed yield. Combat effect of SA under abiotic stress conditions on growth of crop

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plants may have been due to its role in enhancement of nutrient uptake, maintaining the water status and stomatal regulation (Ma *et al.*, 2017). It has been shown that SA can markedly improve germination under salt stress, and exogenously applied SA can significantly increase plant growth under both saline and non-saline conditions, particularly at the 500 uM L^{-1} (Kovacik *et al*., 2009). Therefore, an experiment was conducted to assess the foliar application of SA could induce salt tolerance in sorghum by taking key parameters of membrane, photosynthetic pigment, yield attributes and yield to elucidate mechanism associated for improved salt tolerance in sorghum.

MATERIALS AND METHODS

Plant material

Seeds of sorghum varieties (HJ 513 and HJ 541) were collected from Forage section, Dept. of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana), India. Experiment was conducted under controlled conditions and plastic pots were filled with 10kg dune sand. Seeds of sorghum varieties were sterilized in 1% sodium hypochlorite (NaOCl) solution for 5 min to avoid contamination. Before sowing, pots were saturated with desired levels of salt *i.e.,* Control (0), 5.0, 7.5 and 10.0 dS m^{-1}. According to Hoagland and Arnon, (1950), nutrient solution was applied to each pot as and when required. Moisture content and salt movement was maintained by applying water through pipe present in the pot. Salicylic acid (0, 25 and 50 ppm) was applied exogenously with the help of manual sprayer 30 days after sowing (DAS). Arrangement of the experiment was completely randomized with three replications. The sampling was done at 40 DAS. The data was analysed statistically for ANOVA using completely randomized design (CRD) by using OPSTAT programme (Sheoran *et. al.,* 1998). Treatments were compared with CD values at 5% level of significance

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content present in leaf tissues. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968). H_2O_2 content was estimated by the method of Sinha (1972). Total chlorophyll content was estimated by method described by Sawhney and Singh (2002) and its content was calculated from the formula:

$$\text{Chl "a"} = \frac{12.3\, A_{663} - 0.86\, A_{645}}{a \times W} \times V$$

$$\text{Chl "b"} = \frac{19.3\, A_{645} - 3.6\, A_{663}}{a \times W} \times V$$

Where, V= Volume of DMSO, a = Path length, W = Weight of tissue taken (mg)

Chlorophyll content was determined by SPAD 502 plus instrument by measuring the absorbance of the leaf in two wavelength regions (Blue 400-500 nm and Red 600-700 nm). Chlorophyll fluorescence was recorded in intact plants using chlorophyll fluorometer (OS-30p, Opti-Science, Inc., Hudson, USA) at mid-day. Photochemical quantum yield was then calculated by F_v/F_m ratio.

Mean length (cm) of the ten panicles, which was measured from the base to the tip of the head of panicle. Panicle weight (g) is determined by taking random five heads of each pot weighed and divided by five. Seed yield per plant (g) was measured by taking the seed weight in grams from each plant. 100 seed weight (g) was calculated by counting of 100-seed and then weighed by electrical balance. Harvest index (%) is represented in terms of percentage. The harvest index for each plot was calculated by dividing the economic (grain) yield by the biological yield (seed + stover yield) of the same pot and multiplied by 100 as given below:

HI = (Economic yield / Biological yield) x 100

RESULTS AND DISCUSSION

Malondialdehyde (MDA)

It is depicted from Fig. 1, that lipid peroxidation in terms of MDA content increased with the increasing levels of salt stress from control to 10 dS m^{-1} in both varieties. MDA content was measured maximum at 10 dS m^{-1} of salt level in both varieties *i.e.,* 0.220 in HJ 513 and 0.300 in HJ 541. Kukreja *et al.* (2006) reported that MDA content increased consistently with increasing levels of salt stress due to accumulation of H_2O_2 content. With regard to malondialdehyde (MDA), significant differences were found between genotypes in salt treatments. According to Sevengor *et al*. (2011), salt stress led to a gradual increase in the levels of MDA in genotypes. MDA accumulation in sensitive genotypes was higher (132.82 and 140.48 %) than tolerant genotypes (Iskenderun-4: 53.03%, and AB- 44: 34.65 %).

However, foliar application of SA decreased the MDA content in both varieties, but percent decrease was on higher side in HJ 513 as compared to HJ 541 *i.e.,* 31.8% and 30.0% respectively at 10 dS m^{-1} of salt level over their respective control. Decreased MDA content after application of salicylic acid was due to the enhancement in the defensive mechanism and resulted in reduced breakdown of membrane lipids which ultimately reduce the synthesis of MDA content. Drought stress increased the MDA (104%) compared to

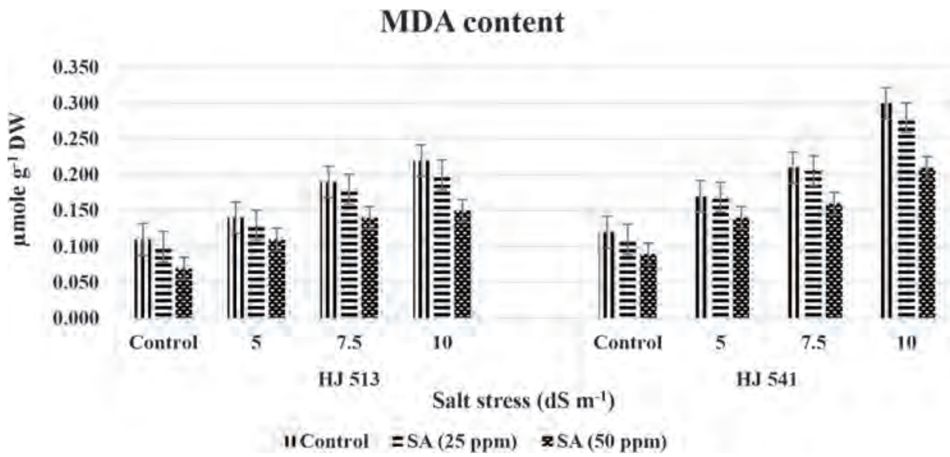

Fig. 1. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on MDA content in sorghum varieties.

the well-watered controls. The exogenous application of MeJA+SA resulted in a lower MDA, by 46 %, in maize plants under drought stress compared to drought-stressed plants without pre-treatment with exogenous hormones (Tayyab *et al*., 2020).

Hydrogen peroxide

A significant increment in H_2O_2 content was noticed with every increment of salt stress in both varieties at 40 DAS (Fig. 2). Values of H_2O_2 content increased 34.76, 52.39 and 68.27 in HJ 513 and 31.46, 47.87 and 63.73 in HJ 541 respectively at 5, 7.5 and 10 dS m^{-1} of salt levels over their respective control. Oxidative damage occurred inside the cell due to the production of ROS including superoxide radicals ($O_2^{\cdot-}$) and H_2O_2. Foliar application of salicylic acid (50 ppm) decreased the H_2O_2 content under stressed as well as in un-stressed conditions. But maximum decrease in H_2O_2 content was noticed at 5 dS m^{-1} of salt level *i.e.,* 50.2% in HJ 513 and 48.0% in HJ 541 with respect to their control. According to Tayyab *et al.* (2020), When compared to the well-watered controls, drought stress increased H_2O_2 by 131 percent. Exogenous application of MeJA+SA resulted in a 55 percent reduction in H_2O_2 concentrations in drought-stressed maize plants compared to drought-stressed plants without pre-treatment with exogenous hormones.

Total chlorophyll

It is observed from Fig. 3 that total chlorophyll content at 40 DAS decreased with the increasing levels of salt stress from control to 10 dS m^{-1} in both varieties. Per cent decrease was found maximum at at 10 dS m^{-1} of salt stress *i.e.,* 38% in HJ 541 and 33.9% in HJ 513 with respect to control. Values of chlorophyll content (SPAD units) decreased from control (44.59 and 42.63) to 10 dS m^{-1} (25.93 and 24.46) in HJ 513 and HJ 541, respectively (Fig. 4). In general, photochemical quantum

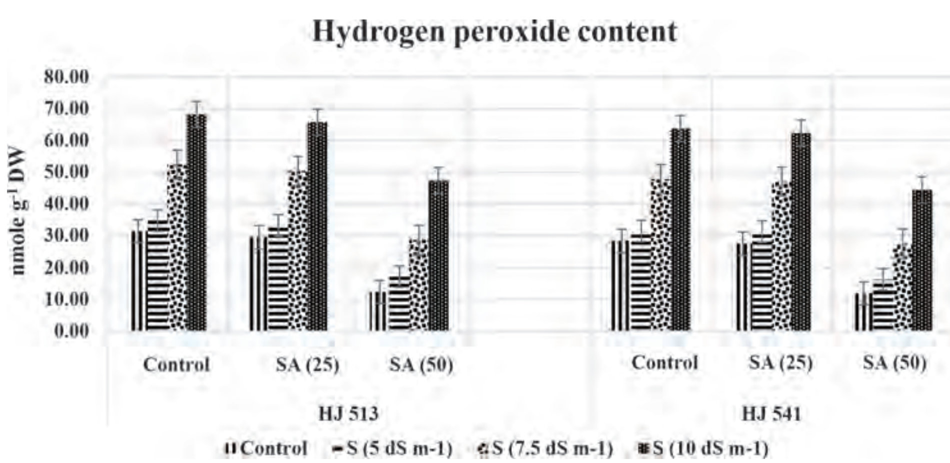

Fig. 2. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on H_2O_2 content in sorghum varieties.

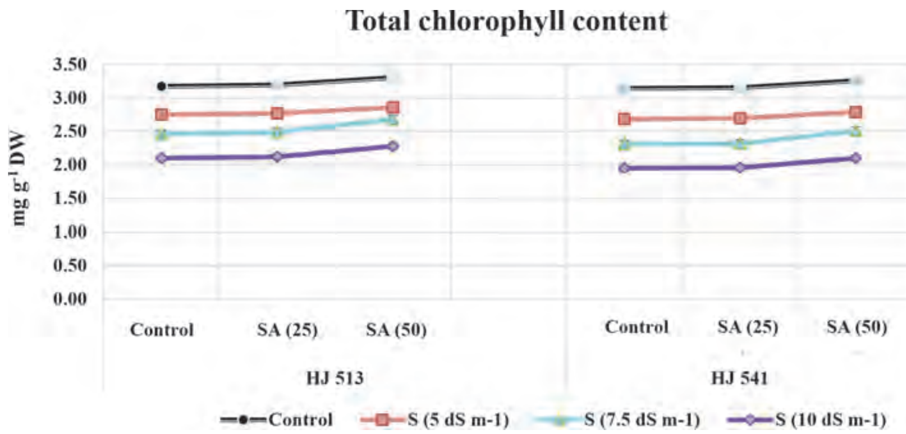

Fig. 3. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on total chlorophyll content in sorghum varieties.

yield at 40 DAS also decreased with the increasing levels of salt stress in both varieties (Fig. 5). Values of quantum yield decreased from control to 10 dS m^{-1} *i.e.,* 0.752 to 0.681 and 0.720 to 0.621, in HJ 513 and HJ 541 respectively. The reduction in total chlorophyll, chlorophyll content (SPAD units) and photochemical quantum yield under salt stress might be attributed to the oxidation of chlorophyll and other chloroplast pigments which are coupled with instability of the pigment protein complex (Tariq *et al*., 2011; Satpal *et al.*, 2020). These results are in accord with the observations of Wani *et al.* (2013), showed that NaCl concentration of 5.6 dS m^{-1} was more toxic that decrease the SPAD values by 41.9% and 50.0% in Varuna and RH-30, respectively. Decreased photochemical quantum yield with increasing salt stress was also confirmed by Ghassemi-Golezani and Lotfi (2015). Reduction in chlorophyll content *i.e.,* total chlorophyll and SPAD reading were also recorded in wheat cultivars under stress condition (Aldesuquy *et al.,* 2012; Devi *et al*., 2021).

Progressive increase in total chlorophyll was observed with the imposition of salicylic acid (25 & 50 ppm) in both varieties, but maximum increase was noticed at 50 ppm of salicylic acid in HJ 513 (8.9%) and HJ 541 (8.6%) at 7.5 dS m^{-1} of salt level with respect to their control. Enhancement in chlorophyll content was observed after application of two different doses of salicylic acid in both varieties, but maximum enhancement was noticed at 50 ppm of salicylic acid. Values of chlorophyll content increase from 25.93 to 34.69 mg g^{-1} FW in HJ 513 and 24.46 to 27.98 mg g^{-1} FW in HJ 541 at 10 dS m^{-1} of salt level after application of SA. On application of 50 ppm of salicylic acid resulted in progressive increase in quantum yield at each salt level as well as in control, but maximum increase was noticed from 0.710 to 0.736 and 0.659 to 0.676 in HJ 513 and HJ 541 respectively, at 7.5 dS m^{-1} of salt level. Exogenous application of SA, reduce the damages induced by salt stress. Our results were similar to Nimir *et al.,* (2015), both chlorophyll a and b contents decreased with increasing salinity and temperature levels. There were no significant differences between 0

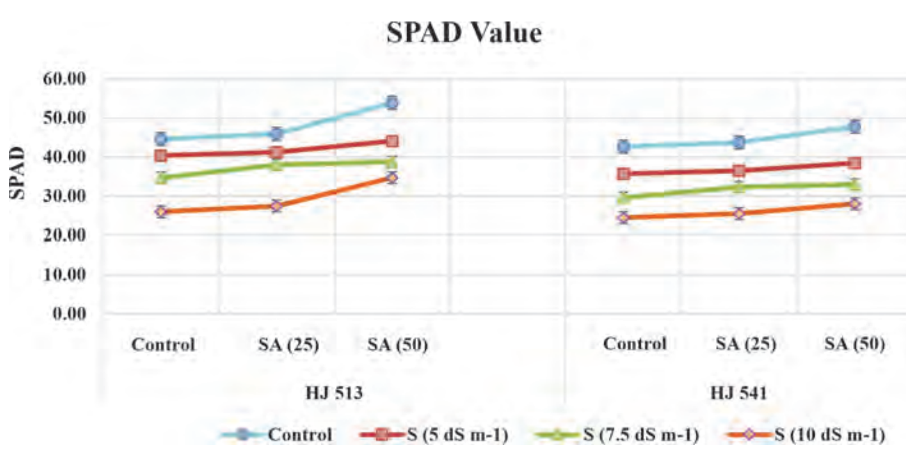

Fig. 4. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on SPAD value in sorghum varieties.

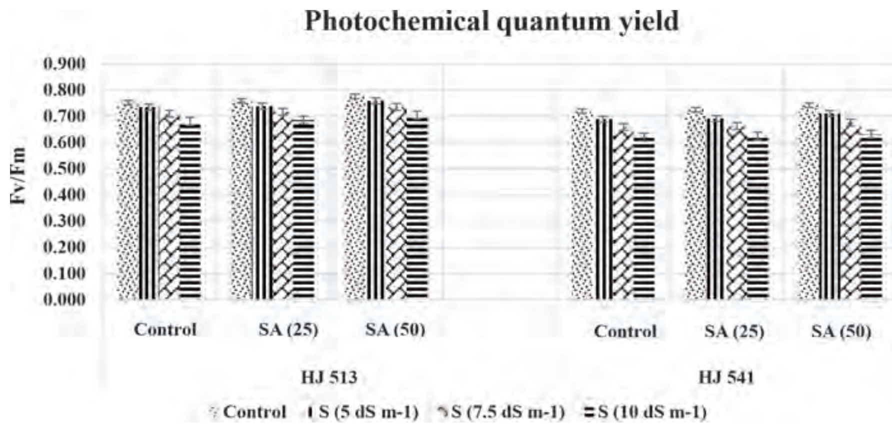

Fig. 5. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on photochemical quantum yield in sorghum varieties.

and 100 mM NaCl at 37^{0}C for chlorophyll a. However, 200 mM NaCl at 37^{0}C caused reductions by 30.8% and 62.3% in chlorophyll a and b content, respectively, compared with 0 mM NaCl. At 200 mM NaCl, salicylic acid increased chlorophyll b content by 7.9% over the control at the same salinity level.

Yield attributes and yield

Data presented in Fig. 6, 7, 8, 9 & 10 showed that decrease in panicle length, panicle weight, seed yield plant^{-1}, 100 seed weight and harvest index with every increment in levels of salt in both varieties respectively. More percent decrease in panicle length was observed at 10 dS m^{-1} of salt level *i.e.,* more in HJ 541 (59.4%) as compared to HJ 513 (44.7%) with respect to control. The higher reduction in panicle weight was observed with increasing salt levels *i.e.,* 29.7%, 42.6% and 47.8% in HJ 541 at 5, 7.5 and 10 dS m^{-1} of salt level than HJ 513, over their respective control. Decrease in seed yield plant^{-1} was slightly higher in HJ 541 as compared to HJ 513 at 10 dS m^{-1} of salt level *i.e.,* 26.7% and 26.3% respectively with respect to control. Maximum decrease in 100 seed weight was observed at 10 dS m^{-1} of salt stress *i.e.,* 12.8% in HJ 513 and 13.3% in HJ 541 with respect to control. Harvest index decreased more at 10 dS m^{-1} of salt level in both varieties. Reduction in yield attributes was more at higher level of salt (10 dS m^{-1}), because of adverse effect on the physiological processes and dry matter accumulation and ultimately reduce the seed yield of plant. Reduction in number of seed per plant was due to the reduced flower production and due to a smaller number of fertilizations. Our results are also in concomitant with the findings of Kumar *et al.* (2018), yield depends on the ability of the crops to assimilate and utilize the resources. Stress treatments had significant effect on 1000 seed weight. Stresses reduced yield by reducing the number of filled grains per panicle. Reduction in seed weight may possibly be due to decreased pollen viability or decreased receptivity of the stigmatic surface or both. Reduction in total grain yield /plant ranged from 4.59 to 1.38 under different treatments whereas total dry weight/plant from

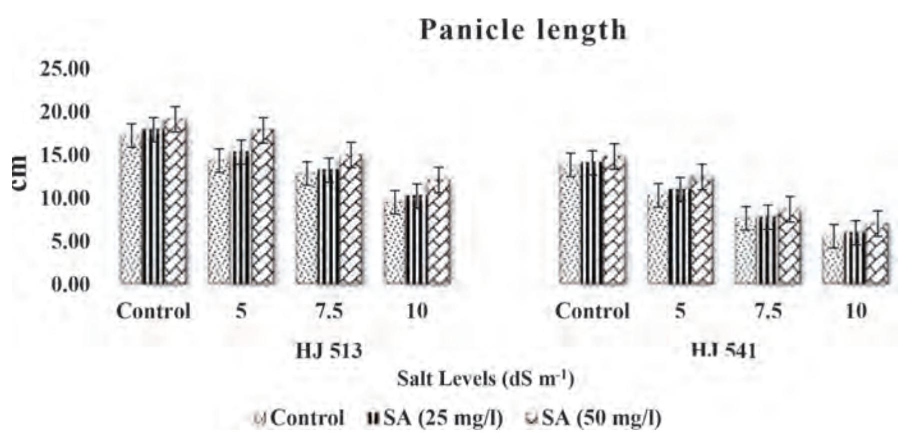

Fig. 6. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on panicle length in sorghum varieties.

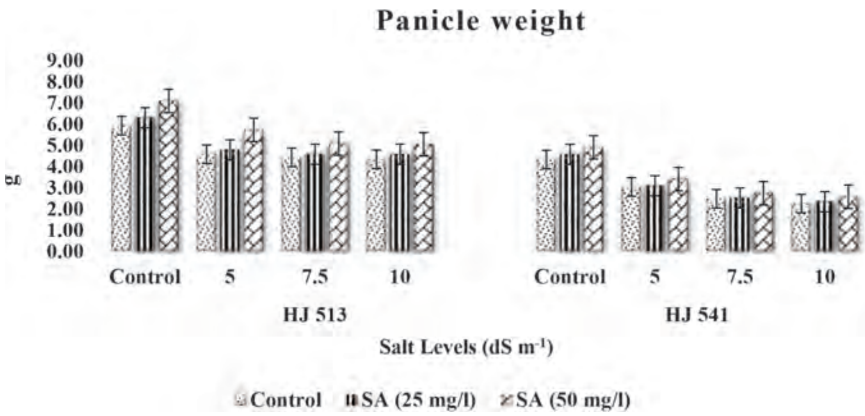

Fig. 7. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on panicle weight in sorghum varieties.

10.19 to 2.74 g/plant. At 10 dS m^{-1} of salt stress, only two genotypes i.e. SPV 2457 and CSH 22SS (C) could show seed setting whereas other genotypes couldn't survive (Devi *et al.,* 2018).

Exogenous application of SA (25 and 50 ppm) was significantly increasing the panicle length, panicle weight, seed yield per plant, 100 seed weight and harvest index. Increase in panicle length was 26.9% in HJ 513 and 25.8% in HJ 541 over their respective control at 10 dS m^{-1} of salt level. However, foliar application of SA increased the panicle weight in both varieties under stressed as well as in un-stressed conditions. Imposition of 50 ppm of SA enhanced the seed yield per plant under stressed condition and this enhancement was noticed more at 7.5 dS m^{-1} of salt level *i.e.,* 13% in HJ 513 and 12% in HJ 541. Per cent increase in 100 seed weight was more in HJ 513 (7.1%) as compared to HJ 541 (6.7%) at 5 dS m^{-1} with respect to their control. However, application of 50 ppm of SA significantly enhances the harvest index under stressed as well as in un-stressed conditions in both varieties. After foliar spray of SA, there was corresponding increase in yield attributes and yield under salt stress. Thus, chlorophyll content increased after application of SA under salt stress, this increase was reflected in terms of increased yield attributes and yield. According to Aneela *et al.* (2019), value of H_2O_2 content and lipid peroxidation (MDA content) decreased the yield under salt stress, but on application of salicylic acid, data regarding grain yield revealed that maximum grain yield (14.08 g pot^{-1}) was observed in treatment in combination with PGPR (13.81 g) as compared to control (9.23g),

Taking the above parameters into consideration, it can be concluded that salt stress reduced the physiological and yield parameters leading to ultimate reduction in productivity in both varieties. Reduction on slightly higher side was calculated in HJ 541 as compared to HJ 513, based upon increased MDA and H_2O_2 content and lesser photosynthetic pigment (chlorophyll) and yield attributes with increasing levels of salt stress from control to 10 dS m^{-1}. Deleterious effects were more pronounced at higher level of salt (10 dS m^{-1})

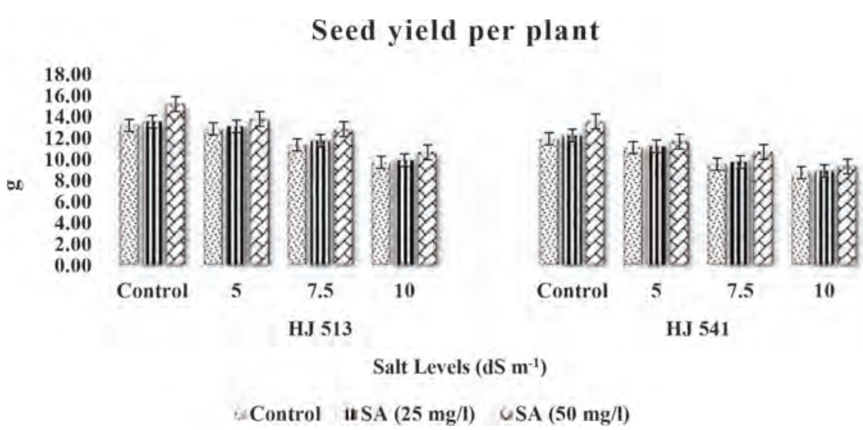

Fig. 8. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) seed yield per plant in sorghum varieties.

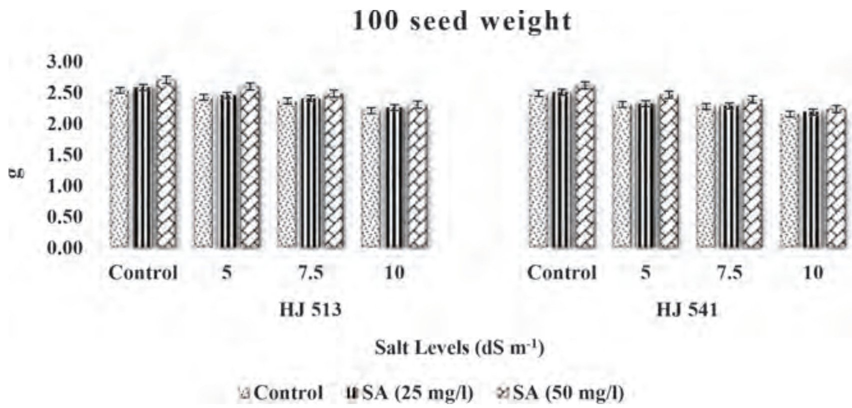

Fig. 9. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on 100 seed weight in sorghum varieties.

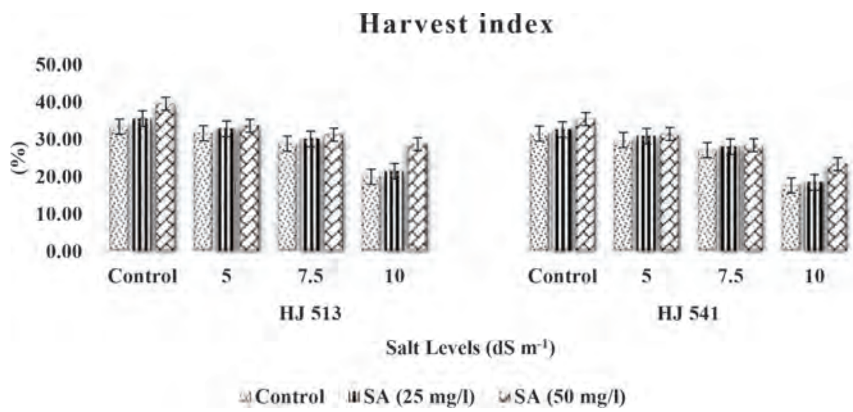

Fig. 10. Interactive effects of salt stress (Control, 5, 7.5 and 10 dS m^{-1}) and salicylic acid (Control, 25 and 50 ppm) on harvest index in sorghum varieties.

stress. Better ameliorating effect was seen at 50 ppm of SA based on the reduction in MDA and H_2O_2 content, improvement in photosynthetic pigment (chlorophyll), yield attributes and yield. The performance of sorghum variety HJ 513 was at par with HJ 541 under salt stress for most of the physiological parameters which indicates that both the varieties performed well under salt stress and further their performance improved with foliar application of 50 ppm SA. The SA treatments could ameliorate the negative effects of salt stress on the physiology of sorghum by reducing the MDA, H_2O_2 content and by enhancing the photosynthetic pigment. The foliar application of SA could offer an alternative for sustained production to the salt sensitive sorghum varieties.

Authors' contribution

Conceptualization of research work and designing of experiments (SD, S); Execution of field/lab experiments and data collection (MJ); Analysis of data and interpretation (S,R); Preparation of manuscript (MJ, S, SD, NK).

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