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Exogenous Application of Melatonin Mitigates Salinity Stress by Modulating the Photosynthetic Efficiency of *Sorghum bicolor* (L.)

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ABSTRACT: A field experiment was conducted during the years 2022 and 2023 to evaluate eight sorghum varieties for salinity tolerance based on their photosynthetic efficiency under saline conditions. The salinity under the field conditions varied between 8 to 10 dS m^{-1} in each subplot. Exogenous application of melatonin (0, 50, and 100 µM L^{-1}) was given at 35 and 50 DAS to mitigate the adverse effect of salinity stress. Among all the varieties, a significantly higher chlorophyll a and b content was observed in HJ 513 (3.9 and 0.91 mg g^{-1} FW) and HC 308 (3.6 and 0.81 mg g^{-1} FW) compared to other genotypes under study, respectively. Maximum chlorophyll stability index (CSI) was observed in HC 308 (96.9) followed by CSV 32F (96.0) with application of 100 µM L^{-1} melatonin. Melatonin concentration 100 µM L^{-1} was found most effective in mitigating the adverse effect of salinity and varieties HC 308, HJ 513 and HJ 541 performed relatively superior to other varieties which can be used in crop breeding programs aimed to improve salt tolerance in sorghum.

Keywords: *Sorghum bicolor*, salinity, melatonin, chlorophyll content, photosynthesis.

INTRODUCTION

Sorghum is an annual C_4 crop that belongs to the family *Poaceae* and is widely cultivated in arid and semi-arid parts of the world. It is the 5th most important crop after wheat, rice, maize and barley. Sorghum is mostly cultivated for both food and fodder purposes. The world's total sorghum production is approximately 62 million tonnes (USDA 2020). The largest growers of sorghum are the USA, India, China and Nigeria. India contributes over 9.45% of the world's sorghum production with Karnataka contributing 26% followed by Maharashtra (25%) and Rajasthan (14%). In Haryana, sorghum is grown as a fodder crop and the area of sorghum in Haryana is 40.3 thousand hectares, with a production of 21.3 thousand tonnes with an average yield of 528 kg per hectare (DOA Haryana 2020). Sorghum is usually rich in carbohydrates (75%), fat (3.4%), protein (8-15%) and somewhat amount of vitamin B1, calcium, iron and niacin. Sorghum seed is reported to have 22 mg calcium, 3.9 mg niacin, 3.8 mg iron and 0.18 mg riboflavin per 100 g of the seed. Many sorghum varieties have high flavonoids and phenolic content (antioxidants) which lowers the risk of heart-related diseases and cancer in humans (Dykes *et al.*, 2005). The average yield of sorghum in India is comparatively lower than in other countries due to a lack of management practices and several biotic and abiotic stresses. However, sorghum is considered moderately tolerant to various stresses like salinity and drought and can thrive under low rainfall conditions and considered tolerant up to 6 dS m^{-1} salinity stress but a salinity higher than that drastically affects the growth of the sorghum. The major abiotic stress that affects sorghum growth and yield is salinity stress.

Salinity stress is one of the major ecological abiotic stresses affecting agriculture production and growth. Approximately, 20% of the cultivable agricultural land is salinity affected primarily in arid and semi-arid parts of the world (Mukhopadhyay *et al.*, 2021). Around 6.73 million ha area in India, which is around 2.1% of the geographical area of the country, is salt-affected, of which 2.96 million ha is saline and the rest 3.77 million ha is sodic (Arora and Sharma 2017). Salinity stress results in the production of excess reactive oxygen species (ROS) inside the plant cells which disrupts major physiological processes such as photosynthesis and respiration by damaging the photosynthetic machinery and also disrupting the transfer of electrons through the electron transport chain (Abuzaid *et al.*, 2025; Kumari *et al.*, 2025). Salinity also offers ionic and osmotic stress inside the cells which causes major mineral deficiencies eventually affecting the key physiological processes (Liu *et al.*, 2023). Salinity results in the immediate stomatal closure which decreases the photosynthetic and assimilation rates in plants (Munns and Tester 2008). Therefore, it is necessary to adopt some management practices such as the reclamation of saline soil into usable land using chemicals, the use of plant growth regulators and the cultivation of tolerant genotypes which can yield higher under unfavorable conditions.

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous molecule present in plants, animals, and fungi. Melatonin is also known to have similar effects to that of auxin on plant growth and development under stress as well as unstressed conditions (Dandapat and Bordolui 2025). Melatonin plays an important role in plants by acting as an antioxidant and also by boosting the antioxidative activities under various abiotic stresses including salinity stress. Exogenous application of melatonin boots seed germination, photosynthetic rates and antioxidative enzymatic activities in plants under salinity stress (Ali *et al.*, 2024). Very limited information is available about the role of melatonin in combating the adverse effects of salinity on sorghum crops. Therefore, the current study was conducted to evaluate the effective role of melatonin in mitigating the adverse effect of salinity on sorghum crops and to identify the potential tolerant varieties that can be cultivated under saline areas with higher yields and can be used in crop breeding programs aimed at improving salt tolerance in forage crops.

MATERIAL AND METHODS

A. Experimental Material

The present study was conducted in the *Kharif* season during the two consecutive years (2022 and 2023). The seed of eight sorghum (HC 136, HC 171, HC 260, HC 308, HJ 513, HJ 541, CSV 32F, and CSV 35F) varieties were procured from the Department of Genetics and Plant Breeding, CCS HAU, Hisar, Haryana and were sown in the saline field area of Forage Research Farm, CCSHAU, Hisar, Haryana. The salinity in the field ranged between 8 to 10 dS m^{-1} during the experiment. Foliar treatment (0, 50, and 100 µM L^{-1}) of melatonin (N-acetyl-5-methoxytryptamine) was given at 35 days after sowing (DAS) and 60 DAS in all the sorghum varieties. Sampling was done 7-10 days after the foliar treatment. The crop was raised according to the standard package and practices of Haryana and the experiment was conducted using a Randomized Block Design (RBD).

B. Assessment of photosynthetic efficiency of sorghum

The efficiency of photosynthesis in the eight sorghum varieties was estimated by measuring the chlorophyll a and b content, thylakoid membrane damage (%), and chlorophyll stability index (%).

Chlorophyll content (mg g^{-1} FW): The chlorophyll a (Chl a) and b (Chl b) content was estimated in leaf tissue using the method given by Sawhney and Singh (2002). Test tubes containing 100 mg washed leaf (excluding veins) and 5 ml DMSO were incubated at 60ºC for one hour. Absorbance was measured at 480 and 645 nm for Chl. a and Chl. b respectively.

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

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

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

Chlorophyll Stability Index (CSI): It was measured in terms of total chlorophyll content and was calculated using the formula suggested by Hiscox and Isrealstam (1979):

CSI (%) = (Total chlorophyll under stress)/(Total chlorophyll under control) × 100

Thylakoid membrane damage (TMD%): The TMD was calculated in terms of relative stress injury and was estimated according to the method of Sullivan and Ross (1979). The electrical conductivity of the external medium was used to calculate the percentage of ion leakage into the external aqueous medium compared to the total ion concentration in the stressed tissue.

200 mg of leaves were stored in 20 ml test tubes with 10 ml de-ionized water at room temperature. The electrical conductivity (EC) of the solution was measured after 5 hours and labeled as EC1. Then the samples were placed in a boiling water bath for 50 minutes and the EC of the solution was measured again after cooling and labeled as EC2. The following formula was used to calculate the membrane injury:

RSI (%) = (EC1/EC2) × 100

Statistical Analysis: The data was analyzed statistically for ANOVA using randomized block design (RBD) using grapes software (version 1.0.0) using Tukey HSD test at 0.05 level of significance and data was expressed as mean ± standard errors. Treatments were compared using CD values at a 5% significance level.

RESULTS

Chlorophyll a content (mg g^{-1} FW): Data presented in Fig. 1 indicates that the exogenous application of melatonin had a significant effect on Chl a in all the sorghum varieties at both growth stages. Significantly highest Chl a content was reported with the application of 100 µM L^{-1} melatonin compared to 50 µM L^{-1} and control. Significantly highest Chl a was observed in HJ 513 (4.5 and 3.9 mg g^{-1} FW) followed by HC 308 (4.0 and 3.6 mg g^{-1} FW) at 45 and 60 DAS with the application of 100 µM L^{-1} melatonin compared to other genotypes under study, respectively.

Chlorophyll b content (mg g^{-1} FW): Exogenous application of melatonin significantly increased the chlorophyll b content in all the sorghum varieties (Fig. 2). Maximum increase was observed with the application of 100 µM L^{-1} melatonin. Significantly highest chlorophyll b content was observed in HC 308 (0.85 and 0.87 mg g^{-1} FW) and HJ 513 (0.9 and 0.91 mg g^{-1} FW) at 45 and 60 DAS with the application of 100 µM L^{-1} melatonin compared to other genotypes under study, respectively.

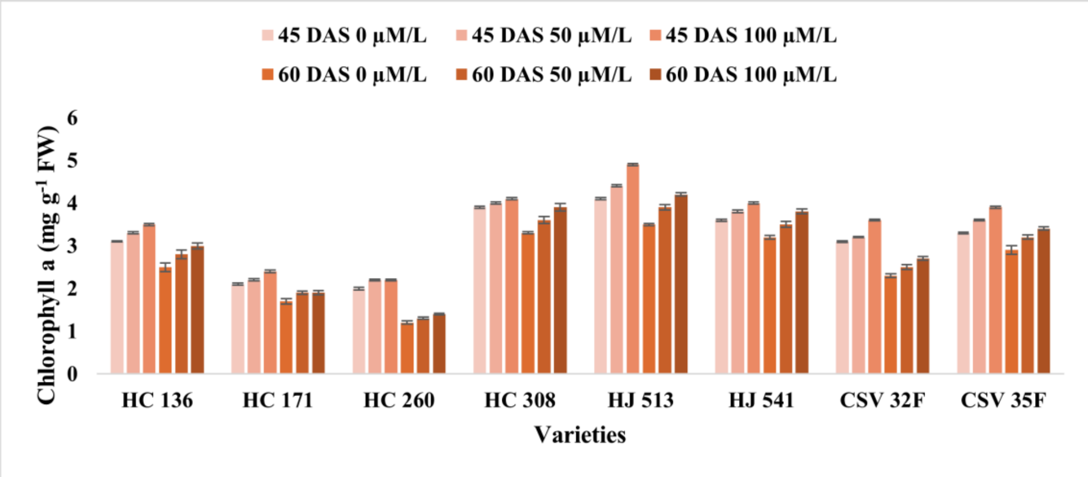

Fig. 1. Effect of foliar application of melatonin (0, 50 and 100 µM L^{-1}) on chlorophyll a content (mg g^{-1} FW) in the leaves of different sorghum varieties grown under salinity stress at 45 and 60 DAS.

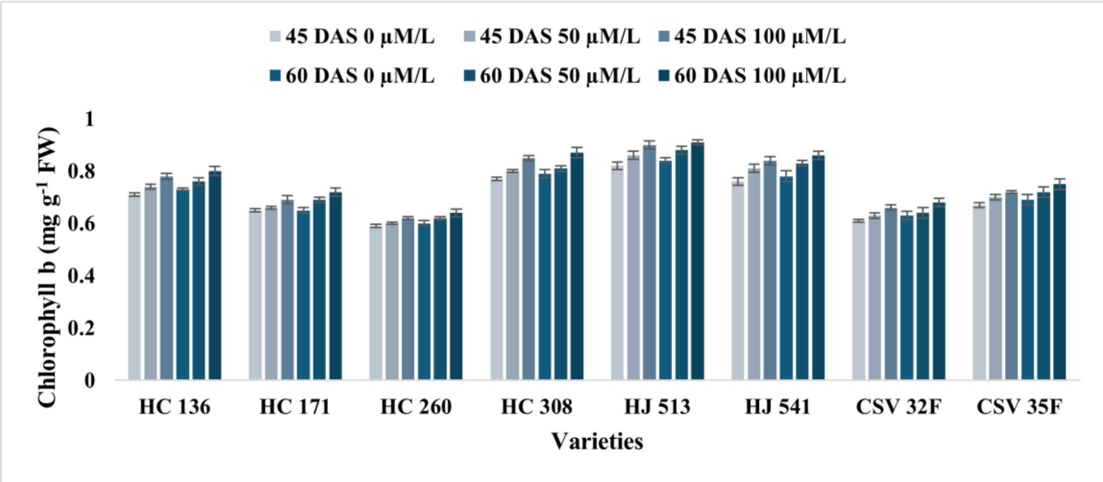

Fig. 2. Effect of foliar application of melatonin (0, 50 and 100 µM L^{-1}) on chlorophyll b content (mg g^{-1} FW) in the leaves of different sorghum varieties grown under salinity stress at 45 and 60 DAS.

Chlorophyll Stability Index (%): Fig. 3 shows the effect of foliar application of melatonin (0, 50, and 100 µM L^{-1}) on CSI of eight sorghum varieties grown under salinity stress. The stability of chlorophyll was found maximum at the vegetative stage of sorghum (45 DAS) compared to the reproductive stage of the plants (60 DAS) while chlorophyll in the plants was found more stable under the application of 50 µM L^{-1} melatonin compared to 100 µM L^{-1} application. Among the varieties, the significantly highest CSI was observed in HC 308 (96.9%) followed by CSV 32F (96.0%) while the lowest was observed in HC 260 (93.1%) and HJ 541 (93.3%) at 45 DAS with the application of 50 µM L^{-1} melatonin. At 60 DAS, maximum CSI was noticed in HC 260 (94.4%), and the minimum was noticed in HJ 513 (89.2%) and HC 171 (90.5%) with the application of 50 µM L^{-1} melatonin.

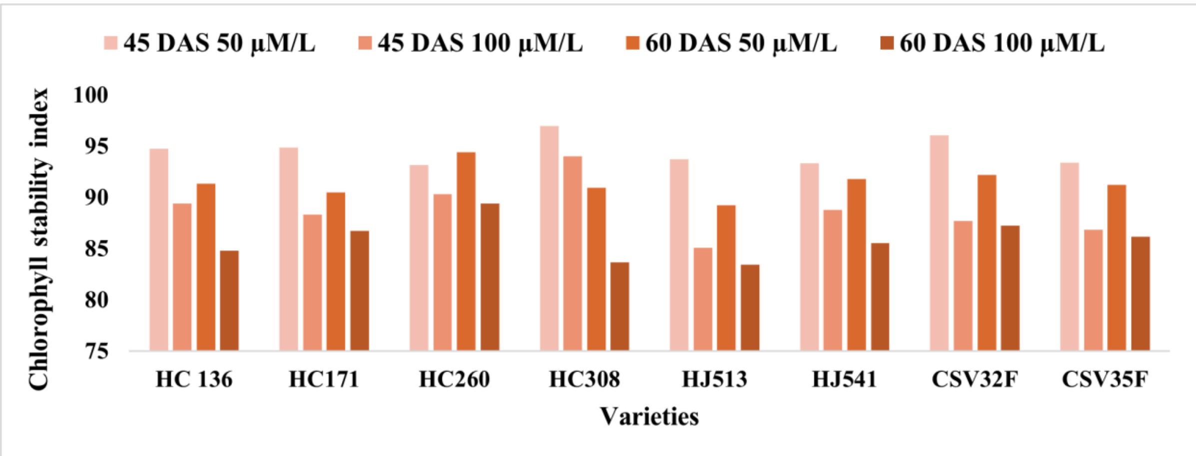

Fig. 3. Effect of foliar application of melatonin (50 and 100 µM L^{-1}) on chlorophyll stability index (%) in the leaves of different sorghum varieties grown under salinity stress at 45 and 60 DAS.

Thylakoid Membrane Damage (%): Results presented in Fig. 4 depicted that TMD increased progressively under salinity stress and the extent of stress injury showed high genetic variability in the leaves of all the observed varieties. Application of melatonin significantly decreased the TMD in all the sorghum varieties and application of 100 µM L^{-1} of melatonin was found most effective in mitigating the salinity stress. Foliar application of 100 µM L^{-1} resulted in a decrease in the TMD after the first and second foliar application. Among the varieties significantly lowest TMD was observed in HJ 513 (8.5 and 9.6 %) followed by HC 308 (8.8 and 11.6 %) and HC 136 (10.8 and 12.8 %) while the significantly highest in CSV 32F (26.8 and 33.6 %) and HC 260 (23.1 and 29.4%) at 45 and 60 DAS, respectively after application of 100 µM L^{-1} melatonin. The interaction between treatments and varieties was found statistically non-significant after the first foliar application and significant after the second foliar application.

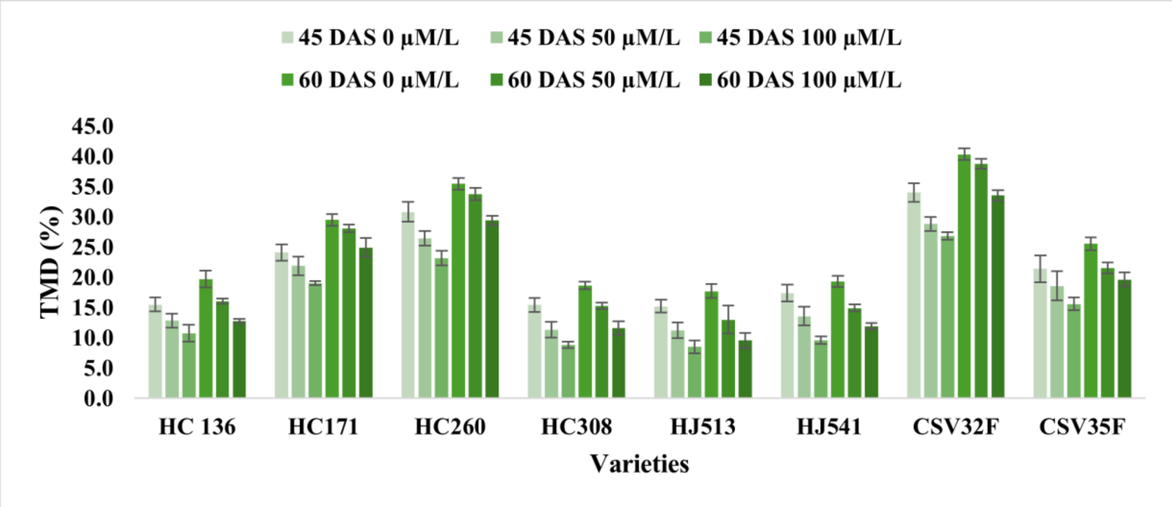

Fig. 4. Effect of foliar application of melatonin (0, 50, and 100 µM L^{-1}) on thylakoid membrane damage (%) in the leaves of different sorghum varieties grown under salinity stress at 45 and 60 DAS.

DISCUSSION

Salinity stress posed a serious effect on the growth and development of all the sorghum varieties. All the varieties performed differently under the saline conditions due to differences in their genetic potential. Tolerant varieties (HJ 513, HJ 541 and CSV 35F) were least affected compared to susceptible varieties (CSV 32F and HC 260) which were most affected (Fig. 1-4). Photosynthesis is the key physiological process that maintains life on Earth. Salinity stress negatively affects photosynthesis and hence decreases the growth and yield of the crops. Salinity disrupts the chloroplast ultrastructure and also results in the swollen thylakoid structures which ultimately results in a decrease in the photosynthetic pigment, chlorophyll (Wang *et al.*, 2016). An immediate effect of salinity stress includes the closure of stomata to prevent excess water loss which results in the decreased gaseous exchange and a decrease in the internal CO_2 concentration which also ultimately decreases the photosynthetic efficiency in the plants (Porcel *et al.*, 2015; Zhao *et al.*, 2023). Chlorophyll fluorescence indicates the quantum efficiency of PS II to convert light energy into chemical energy and hence indicates the photosynthetic efficiency of the plant. Chlorophyll fluorescence is also negatively affected due to ionic toxicity and overproduction of reactive oxygen species which causes photoinhibition and prevents photosynthetic electron transfer hence decreasing photosynthetic rates in plants (Wang *et al.*, 2021). Salinity stress also resulted in a higher TMD (%) due to salinity-induced photooxidation of the lipid bilayer which modifies the physical and chemical nature of the plasma membrane and hence increases the TMD in the sorghum varieties (Mahlooji *et al.*, 2018). Furthermore, salinity also results in the disruption of ionic homeostasis in plant cells which causes ionic leakage and destabilizes the chloroplast and thylakoid ultrastructures (Dastogeer *et al.*, 2020).

Exogenous application of melatonin was found effective in maintaining crop growth and development under various abiotic stresses including salinity stress. In our study, we found that melatonin application significantly improved the photosynthetic efficiency in all the sorghum varieties (Fig. 1-4). Melatonin improves photosynthesis in the sorghum varieties by increasing the stomatal conductance as a result of increased uptake of K^+ ions which is a key ion responsible for stomatal movements. Increased stomatal conductance increases the CO_2 influx inside the cells and increases carbon fixation (Barman *et al.*, 2019; Hanci and Tuncer 2020). Melatonin prevents chlorophyll degradation and chloroplast damage by disintegrating the ROS formed under salinity stress (Li *et al.*, 2017; Imran *et al.*, 2021). Melatonin prevents the degradation of chlorophyll pigment by peroxidases and chlorophyllases with the maintenance of enzyme pheophorbide a oxygenase and also by expression of genes that help in the biosynthesis of chlorophyll and carotenoids (Sharif *et al.*, 2018). Melatonin prevents swelling and maintains the ultrastructure of the thylakoid under salinity which further improves chlorophyll content in plants (Wang *et al.*, 2016). In addition to that melatonin also increases sucrose content inside the cell which acts as a substrate for photosynthesis therefore, indirectly increasing the photosynthetic rates in the leaves (Li *et al.*, 2017). Melatonin application can improve tolerance to plants under salinity by upregulating genes related to photosynthesis which increases the activity of RUBISCO, ATP synthase and genes improving carbon assimilation in the leaves (Antoniou *et al.*, 2017).

CONCLUSIONS

Based on the current investigation, it can be concluded that salinity stress adversely affected the photosynthetic efficiency of all the sorghum varieties. However, foliar application of both concentrations of melatonin (50 and 100 µM L^{-1}) significantly mitigated the adverse effect of salinity stress. All the varieties performed differently due to their difference in their genetic potential. Forage sorghum varieties HC 308, HJ 513, HJ 541 and CSV 35F performed relatively better compared to other varieties in terms of higher chlorophyll content, higher CSI and lower TMD (%) while varieties CSV 32F and HC 260 performed relatively poor compared to other varieties. Application of 100 µM L^{-1} melatonin was found most effective in mitigating the adverse effects of salinity compared to 50 µM L^{-1} and control. Conclusively, HJ 513, HJ 541 and HC 308 varieties have significant potential for higher forage production under saline areas and can be used in crop breeding programs for salt tolerance while foliar application of melatonin can also be potentially exploited for mitigating the adverse effect of salinity in various crops.

FUTURE SCOPE

Physiological attributes such as the estimation of chlorophyll content give an insight into the photosynthetic efficiency of sorghum under salinity stress. However, genetic studies such as proteomics coupled with physiological studies help in better understanding the melatonin and salinity-induced proteins that are responsible for better salt tolerance in tolerant varieties such as HC 308, HJ 513, HJ 541 and CSV 35F. Advanced tools like marker-assisted studies and genomic studies which investigate the expression of melatonin-induced genes for salt tolerance in different cereal crops and help in the development of robust agriculture and contribute towards a step ahead in sustainable agriculture and food security.

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