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EXOGENOUS APPLICATION OF SALICYLIC ACID IMPROVES PHYSIOLOGICAL PROCESSES OF MAIZE (*ZEA MAYS* L.) HYBRIDS UNDER LIMITED WATER CONDITIONS

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Abstract

Drought is one of the most important abiotic factors acting as a bar in the progress of agriculture around the world. Like other crops, cereals also have to endure the impact of limited water availability. A pot experiment was conducted to check the effects of exogenous application of salicylic acid (SA) on physiological processes of spring maize (DK-6525 and NK-8711). In this experiment, seed priming, foliar of SA and their combined effects of SA @ 100 mg L^{-1} were checked at vegetative as well as reproductive growth stages of maize. In this experiment control was no priming and no spray. Then hydro-priming and distilled water spray at vegetative as well as reproductive growth stages was applied to have a better comparison with SA application. Crop was raised till its maturity. Results of physio-chemical attributes showed that foliar application of SA at the vegetative growth stage of maize improved dry matter production per plant by 26% under moisture stress conditions along with the water relations and gas exchange parameters to a significant extent.

Key words: Water relations, Photosynthesis, Dry matter yield, Salicylic acid, Drought.

Introduction

Drought is the condition of suboptimal supply of water for a specific period in a particular region for the sustenance of human and plant life. Globally in general, and, arid and semi-arid regions in particular, have to face the consequences of drought stress (Chaves *et al.,* 2002; Nawaz *et al.,* 2015a,b). Owing to the severe food shortages, drought is affecting almost 70 million people throughout the Middle East, Central, East, Southern Africa, and parts of Asia (Anon., 2017). Population explosion increased global need of water for development of industries and agriculture. Struggle for producing more food for increasing world population resulting into unwise and uncalculated use of fresh water thus have caused a significant decrease in the global water availability (Ceccarelli *et al.*, 2010). Predictions are there that by 2025, one third of the global population will be facing the sever water shortages (United Nations Organization, 2014).

Maize occupies the third position among all the cereal crops of the world after rice and wheat. Increasing importance of maize in human food and nutrition could be estimated by its global average production of about 1067 million tons during the year 2016-2017. In addition to its use as food and feed for livestock and poultry, maize grains are also used in many other commercial and industrial products. In Pakistan, 35% of maize is sown in the rainfed areas which receive less than 200 mm rainfall annually (Zhang *et al*., 2014). In Pakistan maize has a significant share of 22% in value addition along with an active share of 0.5% in the Gross Domestic Product (GDP). During the year 2016 it was grown on an area of almost 1.14 million hectares, 0.3% less than the last year (Anon., 2016). Absence of each millimeter of water results into 15 kg less production of maize (Zhang *et al*., 2014. However, its cultivation in rainfed and areas with low moisture availability is decreasing which is forcing its import to fulfill the food, feed and industrial raw material demands of growing population of the country. There is an urgent need to focus either to develop maize varieties having low water demand or to develop some shotgun techniques like use of plant growth regulators (PGRs) or other chemicals for increasing drought tolerance potential of existing varieties.

To mitigate the negative effect of drought stress, different approaches have been reported in the literature. However, the permanent solution is the development of drought tolerant crop varieties. It is a lengthy process, so some shotgun approach could be adapted to have optimum crop yield under limited water supply. Among shotgun approaches, seed priming or foliar application of different PGRs, inorganic salts and fertilizers are recommended which play a significant role in reducing the adverse effects of abiotic stresses and improving the yield and production of crop plants (Ahmed *et al*., 2014). These chemicals play a very specific role in plants. Some are effective in increasing seed germination, some are growth promoters and others enable plants to adjust the changing environment (Ahmed *et al*., 2014). It has been found that salicylic acid (SA) plays vital role in the plant signaling by acting as a messenger and signal transducer (Gunes *et al*., 2007). It plays a vital role in plant growth, development and interaction with other plant growth substances for amelioration of abiotic and biotic stresses (Senaratna *et al*., 2000; Vlot *et al*., 2009). Ion uptake and transport, stomatal conductance, photosynthetic rate, and transpiration have also been found positively affected by SA (Khan *et al*., 2003). Moreover, SA has been found effective in the induction of resistance against various pathogens by promoting the signaling mechanism in plants (Alverez, 2000). Salicylic Acid is not only effective in the signaling but it also has a key role in the mitigation of oxidative stress which is otherwise detrimental for the plant health (Taiz & Zeiger, 2010). In the same way, SA is very influential in resistance induction in plants in the both limited as well as excess water stresses (Ghasempour & Kianian, 2007).

Foliar application and seed treatment with SA may increase the yield of various crops to a considerable extents (Hamid *et al*., 2010; Gunes *et al*., 2007; Karlidag *et al*., 2009) by mainly mitigating the adverse effects of a biotic stresses in general and drought stress in particular (Elwana & El-Hamahmyb, 2009). In recent years, a considerable focus on the ameliorative effects of SA has been studied for various abiotic stresses and reports have proved its effectiveness against abiotic stresses (Pal *et al*., 2002; Bhupinder & Usha, 2003; Sakhabutdinova *et al*., 2003; Shakirova *et al*., 2003). However, the effect of SA on physiological mechanisms and biochemical phenomena like photosynthetic attributes, mineral uptakes and membrane permeability are still unclear and less understood (Senaratna *et al*., 2000; Shakirova *et al*., 2003). The present study was carried out to investigate the role of salicylic acid application on physiological behaviour and growth of maize hybrids.

Materials and Methods

Pot experiment: The experiment was conducted at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan in the large plastic pots, each filled with 8.0 kg of well mixed loamy soil. Five seeds were sown in each pot and once the seedlings were established, only one plant was kept in each pot till maturity. Whole experiment was divided into two parts, one without water stress while the other was given the drought stress (60% field capacity. Nutrient requirements of the plants were fulfilled by the application of Hoagland's solution at prescribed rates. Salicylic acid (SA) was applied through seed priming and foliar spray independently or in combination. Foliar sprays of SA were applied at two growth stages i.e., first at vegetative growth stage (44 days after sowing and second at reproductive growth stage (88 days after sowing). The concentration of salicylic used was 100 mg L^{-1} which was selected from dose optimization experiment. In total, there were following 9 treatments in the experiment; No priming and no foliar spray (control), priming with distilled water, priming with 100 mg SA L^{-1}, distilled water spray at vegetative stage, foliar spray of 100 mg SA L^{-1} at vegetative stage, water spray at reproductive stage, foliar spray of 100 mg SA L^{-1} at reproductive stage, priming and foliar spray of 100 mg SA L^{-1} at vegetative stage, and priming and foliar spray of 100 mg SA L^{-1} at reproductive stage.

Measurements and calculations: The net photosynthetic rate (Pn, µmol CO_2 m^{-2} s^{-1}), rate of transpiration (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance ($g_{s,}$ mol m^{-2} s^{-1}) were measured using fully expanded middle leaf between 9.00-11.00 a.m. by portable infrared gas analyzer (LCA-4, Analytical Development Company, Hoddesdon, England).

For the measurement of leaf water potential (Ψw), the third fully expanded leaves from top of plants were selected from each treatment at early morning (06:00-8.00 a.m.) and water potential was recorded by "Scholander" type pressure chamber (Model 1000, PMS, Oregon-USA).

The leaves used for water potential measurements were frozen at -20°C for 7 days and then thawed to extract cell sap. Leaf osmotic potential (Ψs) was measured using an osmometer (Wescor 5520, USA).

The leaf turgor potential (Ψp) was calculated by the following formula:

$$\Psi p = \Psi w - \Psi s$$

From the top the third fully expanded leaf was collected for the measurement of relative water content (RWC). Soon after cutting at base of the lamina, leaves were sealed in plastic bags and quickly transferred to the laboratory. Fresh weight (FW) of each sample was recorded using a digital electrical balance and leaves were dipped in test tube containing distilled water for 24 h. Then leaves were taken out, wiped with the tissue paper and their turgid weight (TW) was recorded. The samples were dried at 65°C for 72 h and dry weight (DW) of each sample was recorded. Relative water contents were calculated using the following formula given by Cornic (1994);

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

where,
FW = Sample fresh weight
DW = Sample dry weight
TW = Sample turgid weight

Statistical analysis: Data collected in this experiment were subjected to Fisher's analysis of variance and Statistix-8.1 program was used for analysis. *Tukey's HSD* (Honest significant difference) test at 0.05 probability level was used to evaluate differences among the treatment means.

Results

Water potential: Data presented in the Fig. 1 indicates that SA application as foliar spray at the vegetative stage with solution concentration of 100 mg L^{-1} enabled the plants capable of obtaining maximum water potential under both normal and moisture stressed conditions. However, in the controlled conditions when the combinations of SA as seed priming and foliar spray at vegetative stage were applied it gave the second highest water potential in the both maize hybrids (DK-6525 and NK-8711). Minimum water potential was observed under controlled conditions in which neither priming nor the foliar spray was appliedlish.

Osmotic potential: Maximum osmotic potential was observed where foliar spray with SA at the vegetative growth stage of maize plant was applied. Plants in which seed priming was applied before sowing and those which were also sprayed with SA at their vegetative growth stage produced second highest values of osmotic potential (Fig. 2a). However under the drought stressed conditions, foliar spray at vegetative growth stage when compared with the control where no priming and foliar spray was applied exhibited the maximum osmotic potential. Drought tolerant maize hybrid (DK-6525) yielded maximum dry weight per plant when compared with drought sensitive maize hybrid (NK-8711) as shown in the Fig. 2(a, b).

Turgor potential: Under normal condition, DK-6525 showed highest turgor potential with treatment of SA at the rate of 100 mg L^{-1} at vegetative growth stage as compared to other treatments (Fig. 3a). NK-8711, a drought sensitive maize hybrid, also performed better in terms of higher turgor potential when sprayed with same treatment of SA at vegetative stages compared to control. Under drought stress condition, both maize hybrids gave considerably better turgor potential when sprayed with 100 mg L^{-1} SA at their vegetative growth stages. Conditions of no priming and no spray showed minimum turgor potential as shown in the Fig. 3(b).

Relative water contents: Foliar spray of SA with the concentration of 100 mg L^{-1} resulted in the maximum relative water contents under control as well as water stress conditions Fig. 4(a, b). DK-6525 being the drought tolerant maize hybrid performed relatively better as compared to NK-8711, a drought sensitive maize hybrids. However, where both treatments simultaneously applied at the vegetative growth stages of maize hybrids gave second highest relative water contents. Minimum values were shown in the both conditions where no treatments whether priming or foliar spray was applied (Fig. 4a, b).

Net photosynthetic rate: Foliar spray of SA with the selected dose (100 mg L^{-1}) showed a significant increase in the net photosynthetic rate in the both maize hybrids. As shown in the Fig. 5(a, b), DK-6525 in the control as well as drought stress conditions showed maximum net photosynthetic rates, when sprayed with SA at its vegetative growth stage. In the same way, NK-8711, though, a drought sensitive maize hybrid also showed better performance regarding net photosynthetic rate when sprayed with SA with the selected dose of concentration at its vegetative growth stage (Fig. 5b). In treatments of no priming and foliar spray application had the minimum net photosynthetic rate as shown in the Fig. 5(a, b).

Transpiration rate: Data presented in the Fig. 6(a, b) clearly indicates that when SA was sprayed at the vegetative stage with solution concentration of 100 mg L^{-1}, it improved transpiration rate of plants under both normal and drought stress conditions. However, under normal condition the combination of seed priming with SA and foliar application of SA at vegetative stage had the second highest water potential in the maize hybrid NK-8711 while DK-6525 showed second highest transpiration rate in the conditions where only seed priming with SA at the rate of 100 mg L^{-1} was done. In the drought stress conditions, combination of priming and foliar spray with SA gave the second highest transpiration rates in the both maize hybrids. Minimum transpiration rate was observed in the controlled conditions in which no priming and no foliar spray was done.

Stomatal conductance: The data shows that under normal condition, DK-6525 showed the highest stomatal conductance with treatment of SA at the rate of 100 mg L^{-1} at vegetative growth stage as compared to various treatment conditions as shown in the Fig. 7a. The drought sensitive hybrid NK-8711 also performed better in terms of stomatal conductance when sprayed with same treatment of SA as compared to various treatments and growth stages. In the drought stress condition, both maize hybrids had considerably higher stomatal conductance when sprayed with 100 mg L^{-1} SA at their vegetative growth stages. Under treatments of no priming and no spray lowest stomatal conductance was recorded Fig. 7b.

Dry weight per plant: Figure 8 indicates the results of SA application on maize hybrid regarding dry matter production when SA was applied at the rate of 100 mg L^{-1} at the vegetative growth stage of maize hybrid it affected the dry matter production significantly (Figs. 8a & 8b). However, when foliar and priming were done simultaneously it gave second best results in the production of dry matter per plant.

Discussion

Salicylic acid has been found reportedly as the elixir of plant life against all odds of abiotic stresses particularly to the drought. Data presented above indicated that at various places under drought conditions, when exogenous SA application was done, it caused a helpful manipulation of osmotic and turgor potential, most probably be due to the phenomenon of osmotic adjustment (Hura *et al.*, 2007; Machado & Paulsen, 2001). Relative water contents and turgor potential of maize cells are maintained by the exogenous application of SA as it increases the cell sap in the leaf lamella which also decreases the transpiration losses (Karlidag *et al.*, 2009; Rao *et al.*, 2013). This is possibly because SA plays an ameliorative effect on the membrane stability and CO_2 fixation along with keeping the light harvesting apparatus intact even in the unfavorable circumstances (Athar & Ashraf, 2005). Exogenous application of SA helps in producing more photosynthats because of the enhancement in photosynthetic rate, increased sap production in the leaf lamina and as a result higher relative water content is maintained (Sakhabutdinova *et al.*, 2003; He *et al.*, 2005). This is might be linked to the fact that SA plays an important role in the nitrate metabolism which is ultimately helpful for production of chlorophyll contents and energy production for almost all metabolic pathways going on in the maize plants for withstanding the adversities of drought. When the drought happens, transpirational water losses exceed the absorption through roots. Transpiration rate is usually increased in the abiotic stress particularly drought. In the drought sensitive plants, the mechanism of manipulating the rate of transpiration do not work according to the changing environment (Tas & Tas, 2007). That’s why these plants start wilting and ultimately die. Under drought stress, SA plays a significant reconstructive role in the maintenance of plant’s critical physiological functions under unfavorable conditions. It is evident from the literature that in many plants including maize SA helps plant mitigate drought adversities by enhancing root length for more water absorption and manipulating the stomatal conductance to regulate the transpiration losses of water (Khan *et al.*, 2003; Tas & Tas, 2007). Fariududdin *et al.*, (2003) reported that water use efficiency, transpiration rate, stomatal conductance and internal CO_2 were enhanced in *Brassica juncea* when SA was applied exogenously.

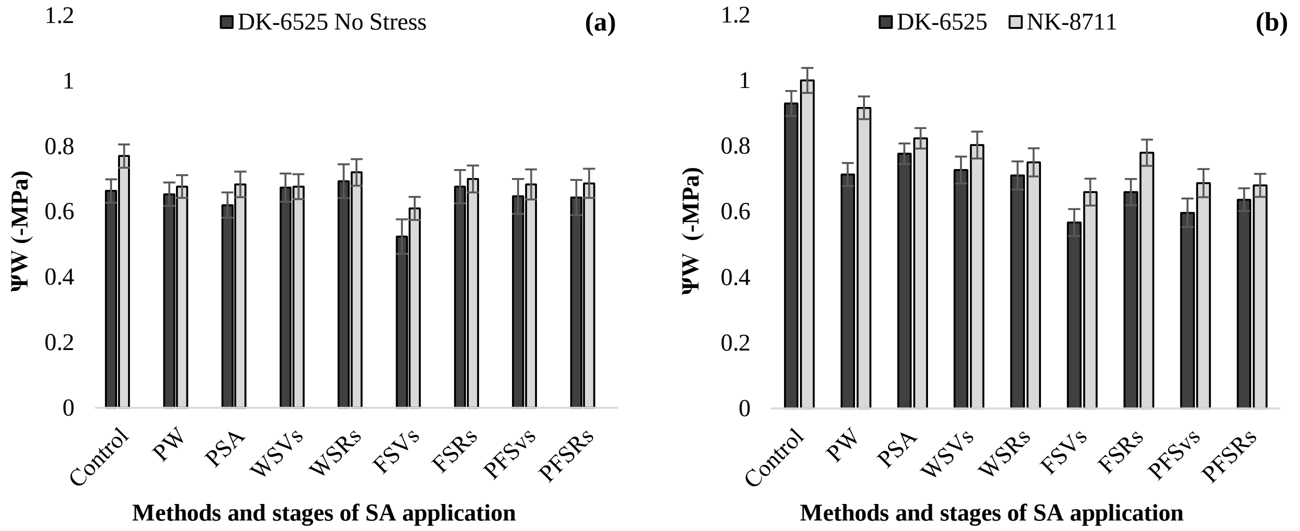

Fig. 1. Effect of seed priming and foliar spray of SA (100 mg L^{-1}) at vegetative stage on water potential (Ψ_W) of maize hybrids DK-6525 and NK-8711 under normal (a) and drought stress (b) condition. The error bars indicate standard error.

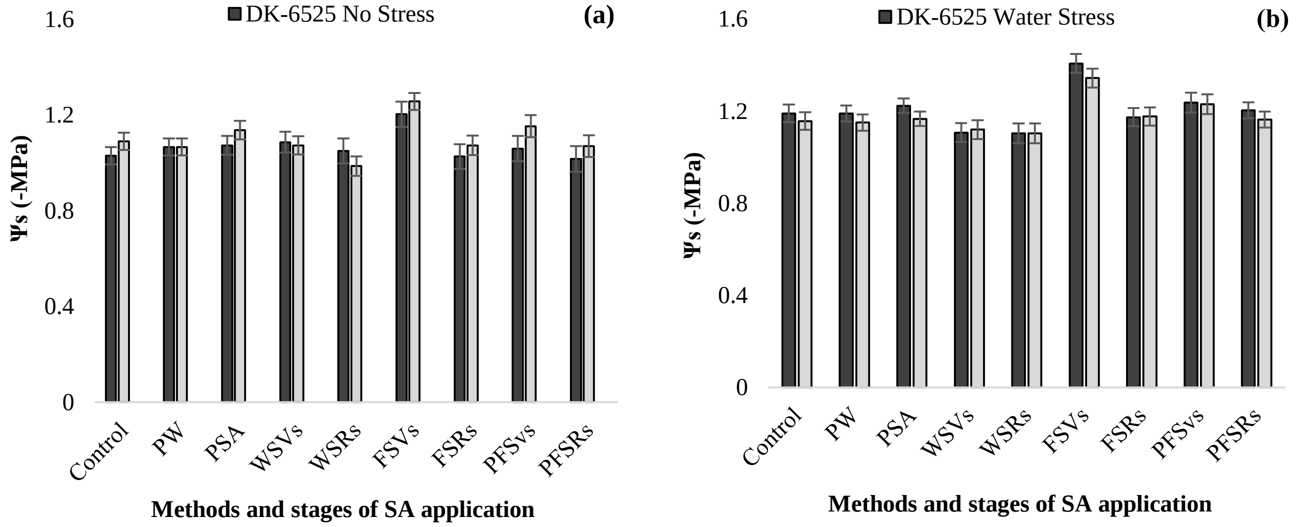

Fig. 2. Effect of seed priming and foliar spray of SA (100 mg L^{-1}) at vegetative stage on Osmotic potential (*Ψs)* of maize hybrids DK-6525 and NK-8711 under normal (a) and drought stress (b) condition. The error bars indicate standard error.

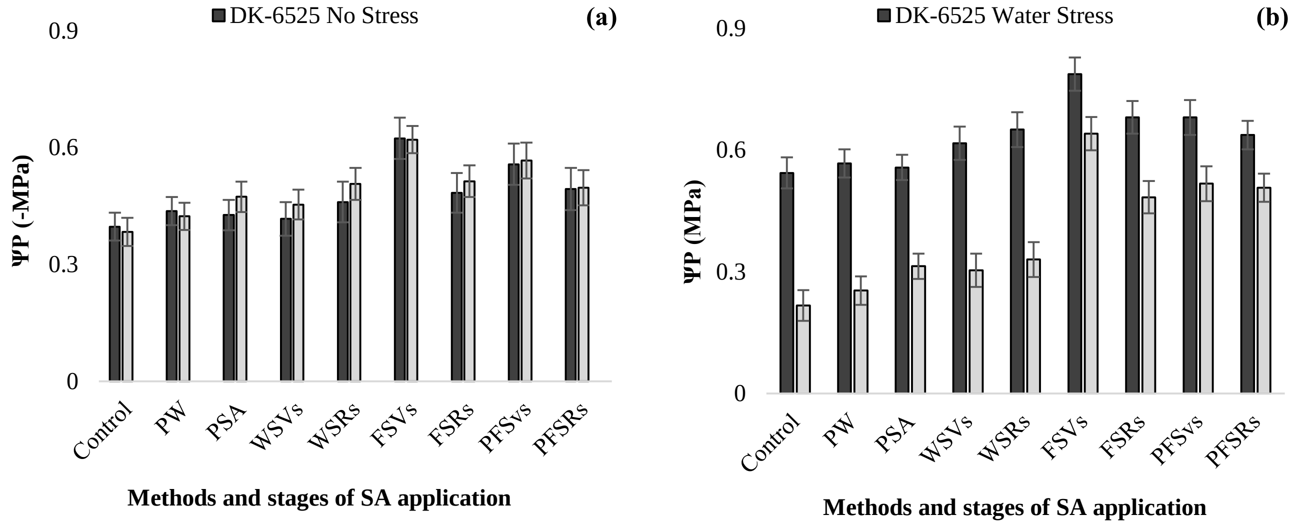

Fig. 3. (a and b) indicates that foliar spray of SA at the vegetative growth stage with concentration of 100 mg L^{-1} significantly affects the turgor potential (Ψ_P) of maize hybrids both in controlled and water deficit conditions.

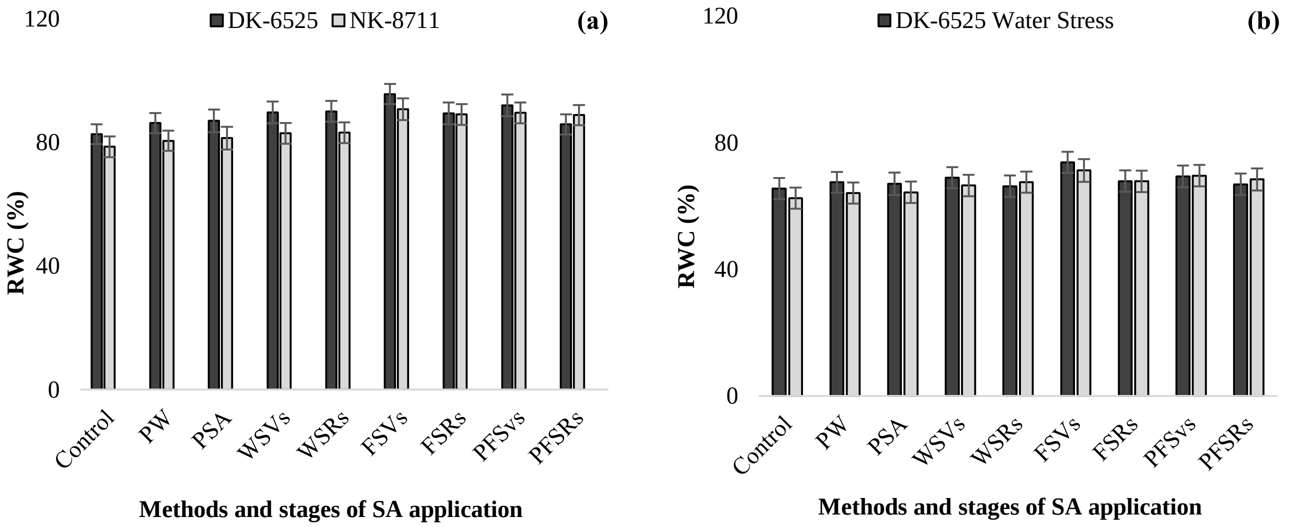

Fig. 4. (a and b) indicates that foliar spray of SA at the vegetative growth stage with concentration of 100 mg L^{-1} significantly affects the relative water contents (RWC) of maize hybrids both in controlled and water deficit conditions.

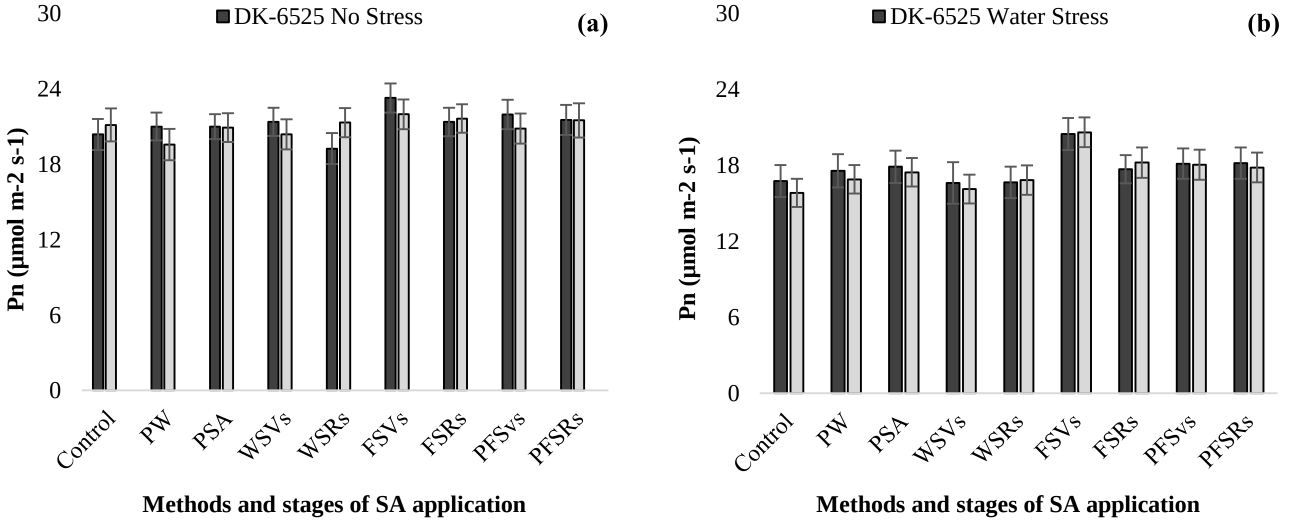

Fig. 5. Shows that foliar spray of SA at the vegetative growth stage with concentration of 100 mg L^{-1} significantly influences the net photosynthetic rate (*P*n) of maize hybrids both in controlled (a) and water deficit conditions (b).

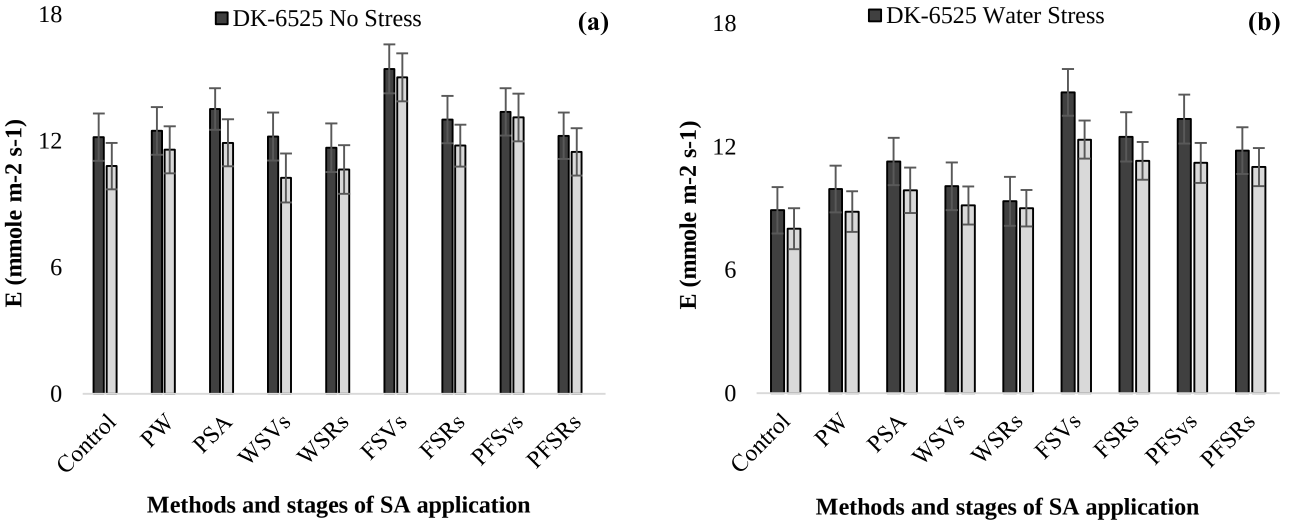

Fig. 6. Indicates that foliar spray of SA at the vegetative growth stage with concentration of 100 mg L^{-1} significantly affects the rate of transpiration (*E*) of maize hybrids both in controlled (a) and water deficit conditions (b).

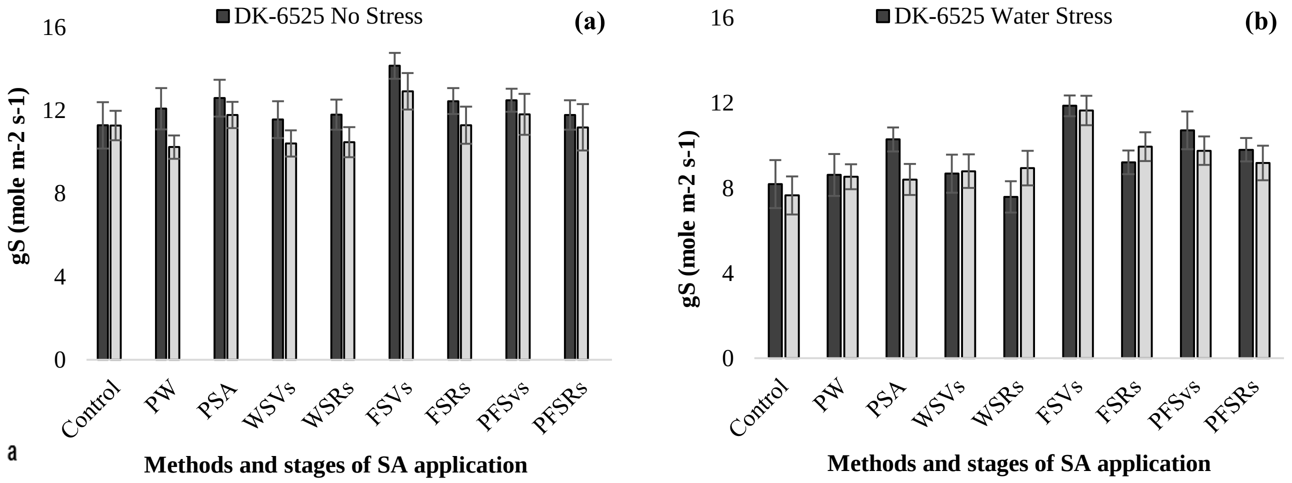

Fig. 7. (a and b) indicates that foliar spray of SA at the vegetative growth stage with concentration of 100 mg L^{-1} significantly affects the stomatal conductance of maize hybrids both in controlled and water deficit conditions.

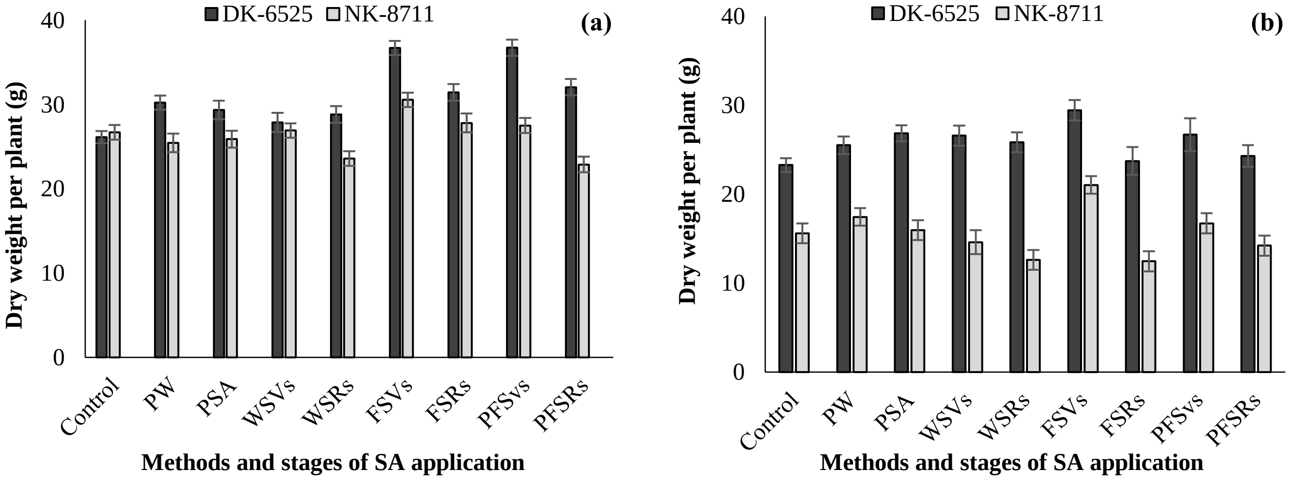

Fig. 8. (a and b) indicates that foliar spray of SA at the vegetative growth stage with concentration of 100 mg L^{-1} significantly affects the dry weight (g $Plant^{-1}$) of maize hybrids both in controlled and water deficit conditions.

Conclusion

Drought causes a significant loss to the maize plants if it is not addressed timely and properly. Foliar application of salicylic acid (100 mg L^{-1}) at the vegetative growth stage of maize hybrids ameliorated the adverse effects of drought and enhances the physiological characteristics which are a key indicator of better crop health and ultimately increased plant productivity.

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