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FOLIAR APPLIED SALICYLIC ACID AMELIORATES WATER AND SALT STRESS BY IMPROVING GAS EXCHANGE AND PHOTOSYNTHETIC PIGMENTS IN WHEAT

ZAHOOR AHMAD[1], EJAZ AHMAD WARAICH[2], RANA MUHAMMAD SABIR TARIQ[3], MUHAMMAD AAMIR IQBAL[4], SAJID ALI[5], WALID SOUFAN[6], MONTASER M. HASSAN[7], M. SOHIDUL ISLAM[8] AND AYMAN EL SABAGH[9]*

[1]University of Central Punjab, Bahawalpur Campus, 63100, Pakistan
[2]Department of Agronomy, University of Agriculture Faisalabad, Pakistan
[3]Department of Agriculture & Agribusiness Management, University of Karachi, Karachi, Pakistan
[4]Department of Agronomy, Faculty of Agriculture, University of Poonch Rawalakot, Rawalakot-12350 (AJK), Pakistan
[5]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
[6]Plant Production Department, College of Food and Agriculture Sciences, King Saud University,
P.O. Box 2460, Riyadh 11451, Saudi Arabia
[7]Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[8]Department of Agronomy, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh
[9]Department of Agronomy, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt
**Corresponding Author's Email: ayman.elsabagh@agr.kfs.edu.eg*

Abstract

Abiotic stresses adversly effect the growth and physiological processes in crop plants, which ultimately lead to significant reduction in the yield. Based upon emerging threats of salinity and drought stresses, present investigation was done to evaluate the effect of foliar applied salicylic acid (SA) on the growth, gas exchange attributes and leaf pigments in wheat under water and salt stress. The experiment was conducted in a warehouse upon three commercial wheat varieties (Galaxy-2013, Punjab-2011 & Millat-2011) treated with two levels of foliar application of SA (Control, SA @ 100 mg/L) under CRD-factorial arrangement which was replicated thrice. According to the results, all the growth attributes, pigments and gas exchange parameters were significantly ($p \leq 0.01$) affected by the salt and drought stresses, and foliar application of SA remarkably remediated the adverse effects of stresses and improved the studied traits in all wheat cultivars. Elevated levels of chlorophyll a (2.31 mg g^{-1} FW), chlorophyll b (3.24 mg g^{-1} FW) and total chlorophyll content (5.55 mg g^{-1} FW) were found in SA treated plants under both types of stresses. Similarly, the maximum leaf photosynthetic rate (5.33 µmol CO_2 m^{-2} s^{-1}), leaf transpiration rate (3.47 mmol H_2O m^{-2} s^{-1}) and stomatal conductance (0.85 mmol H_2O m^{-2} s^{-1}) were recorded by Glaxy-2013 under foliage applied SA (100 mg/L. In conclusion, wheat cultivar Glaxy-2013 surpassed all other cultivars by exhibiting significantly improved growth attributes salt and drought stresses.

Key words: Growth, Gas exchanges attributes, Pigments, Foliar A, Wheat.

Introduction

Drought and salinity are the leading causes globally for reducing the crop yields by 28-59%, depending upon the level of stress and the crop species (Wang *et al.*, 2017; Ahmad *et al.*, 2020). Vital biochemical processes occurring in plants including photosynthesis and transpiration are negatively affected owing to drought and salinity (Tiwari *et al.*, 2010; Waraich *et al.*, 2013; EL Sabagh *et al.*, 2019a). These stresses are directly linked to inflicting oxidative damage which led to cell death (Ahmad *et al.*, 2018a). Drought causes significant reduction in growth & physiology of crops (Naeem *et al.*, 2018; Bukhari *et al.*, 2020). Photosynthesis, in particular, is much more affected due to stomatal limitations. Waraich *et al.*, (2020a) reported that the closure of stomata is the first response of plants to drought. No doubt stomatal closure is essential to retain the available moisture of plants under stress, meanwhile, it also reduce the CO_2 absorption, inducing the extended stress for the longer time (Ahmad *et al.*, 2018b). However, salt stress exerts more drastic effects in terms of less productivity (Munns, 2002). Higher level of salts affects the physiological processes by modifying the ionic balance, water potential status, mineral nutrition and altered photosynthetic rate due to stomatal behavior (Hamid *et al.*, 2010; Zafar *et al.*, 2018).

Salicylic acid (SA) a hormone-like substance has been found to play a crucial role in boosting photosynthetic rate along with increasing stomatal conductance and transpiration (Khan, 2003; Arfan *et al.*, 2007; Akhtar *et al.*, 2013). Antioxidative protection and inhibiting Na^+ and Cl^- accumulation have also been recorded to be controlled by SA (Gunes *et al.*, 2006). A number of investigations have reported that SA is a useful substance to reduce the drastic impact of salinity (Shakirova *et al.*, 2003; Hamid *et al.*, 2010) and drought (Singh & Usha, 2003) stresses. The underlying mechanism of regulating the activities of antioxidants and ultimately making crop plants more tolerant to stresses favors exogenous application of SA to cope with abiotic stresses (Eraslan *et al.*, 2007; Hayat *et al.*, 2010; Kadioglu *et al.*, 2011). However, SA always needs to be optimized for different crops, mode of application and level of the stress induced.

Wheat (*Triticum aestivum* L.) is a staple food and a cash crop of Pakistan, contributing 2% to the national GDP and overall 9.9% to the value added in agriculture (Siddiqui *et al.*, 2019; Iqbal *et al.*, 2020). Area under wheat cultivation in Pakistan is 9204 thousand hectares showing an increase of 0.6% as compared to the previous year (Government of Pakistan, 2019). Wheat yield is much lower than its true potential (Iqbal *et al.*, 2018; Youldash *et al.*, 2020) which has compromised the food security of skyrocketing population, while emerging threat of climate change is expected to worsen the situation (Sorour *et al*., 2019; EL Sabagh *et al*., 2019). Regardless of the other contributing factors, water shortage and recurrent droughts are more significant cause of low wheat yield in Pakistan, but inadequate water or recurrent droughts contribute significantly. Thus, we made hypothesis that SA could alleviate stress inflicted by drought and salt. This experiment was aimed to find the effect of foliar SA on various morpho-physiological parameters of wheat, planted under drought and salt stress. Comparative analysis of the results was useful to understand the physiological mechanisms of the commercial wheat varieties that were altered by this phytohormone.

Materials and Methods

Present research examined the growth, chlorophyll contents and gas exchange attributes of three wheat varieties (Galexy-2013, Punjab-2011 and Millet-2011) under SA foliar (at two levels i.e. 0 and 100mg/L). Controlled environment was provided as wire house in the Department of Agronomy, University of Agriculture, Faisalabad, Pakistan, during November 2015. Each treatment was repeated thrice under CRD factorial layout.

The sandy soil was collected, air dried and sieved through 2mm mesh to make it ready for the experiment. It was filled in the pre-lined pots with polythene bags @ 500g each. Basic nutrient analysis indicated that contained 2mg Ammonium-N, 5mg nitrate-N, 8mg P, 39mg K and 4.6mg S per Kg of the sample.

A pre-plant drench of basal nutrient medium was applied at the following rates in ml/Kg of sand, water content were maintained at 27% (w:w). Seed from three wheat varieties was sieved (4-5mm), surface-sterilized with 1% (w:v) KClO for 2 min and washed with double-deionized water in triplicate. Later, the seed was sown at 2-cm depth in the pre-treated sandy soil @ 10 seed/pot. Thinning was done to five plants/pot at five-leaf stage.

The stresses were applied after seedling emergence. The irrigation was done by estimating the daily water loss from each pot on daily basis by physical balance. SA (@ 100mg/L) was applied at tillering stage as foliar spray after 25 days of sowing. Samples were collected after the completion of almost 40 days. The morphology, chlorophyll contents and gas exchange attributes were measured in this study.

Physical parameters: Shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight were recorded after harvest. Collected samples were dried at 70 C° for 2-3 days in the oven before dry weighting. Samples were same for both fresh and dry weights.

Chlorophyll contents: Chlorophyll contents (Chl a, Chl b) from harvested treatments were measured by using a method and formula described by Nagata & Yamashita (1992).

$$\text{Chlorophyll a (mg/100ml)} = 0.999\ A663 - 0.0989\ A645 \quad (1)$$

$$\text{Chlorophyll b (mg/100ml)} = -0.328\ A663 + 1.77\ A645 \quad (2)$$

Gas exchange attributes Transpiration rate (E), net CO_2 assimilation rate (A), and stomatal conductance (gs) were recorded by randomly selecting the well-expanded young leaves of three plants from each pot. All gas exchange attributes were measured according to the procedure describe by the Waraich *et al*., (2020).

Statistical analysis:

The data collected by following the standard procedures were statistically analyzed by Fisher's ANOVA by using "MSTAT-C" software and least significant difference (LSD) test was employed at 5% probability level (R Core Team, 2014).

Results

Growth parameters: The data regarding growth parameters indicated that foliar application of SA significantly ($p \leq 0.01$) enhanced the shoot-root length, their fresh and dry weights of wheat under different stresses. The water stress in addition to the salt stress critically affected the growth parameters in all wheat cultivars under control conditions. The performance of the variety Glaxy-2013 was the best as compare to other cultivars. However, the maximum shoot-root lengths (21.59cm, 45.72cm), shoot root fresh (2.73g, 1.87g) and dry weights (1.43g, 0.59g) were recorded from Glaxy-2013 which treated with SA @ 100mg/L at tillering stage under control conditions. The minimum shoot root lengths (13.01cm, 23.17cm) shoot-root fresh (0.63g, 0.50g) and dry weights (0.34g, 0.25g) were recorded from Millat-2011 under water and salt stress conditions without foliar application of SA (Fig. 1 a, b, c, d, e, f). All the other interactions such as SA × S × V were recorded to be non-significant.

Chlorophyll contents: The chlorophyll contents of wheat were depleted under water and salt stress but they were significantly ($p \leq 0.01$) improved by foliar applied SA (@ 100 mg/L) under same conditions. Glaxy-2013 treated with SA @ 100mg/L depicted a significant improvement in Chl *a* (2.31 mg g^{-1} FW), Chl *b* (3.24 mg g^{-1} FW) and total chlorophyll content (5.55 mg g^{-1} FW) under stress conditions, followed by Punjab-2011 and Millat-2011. The minimum amount of Chl *a* (1.99 mg g^{-1} FW), Chl *b* (2.01 mg g^{-1} FW) and total chlorophyll contents (4.00 mg g^{-1} FW) was observed in Millat-2011 under both stress conditions with no application of SA (Fig. 2 a, b, c).

Gas exchange parameters: The gas exchange parameters were significantly ($p \leq 0.01$) improved in wheat under salt stress, water stress and salt + water stress) where foliar SA was applied @ 100 mg/L or 0.1 g/L. All cultivars were affected by the stresses significantly. The maximum leaf photosynthetic rate (5.33 µmol CO_2 m^{-2} s^{-1}), leaf transpiration rate (3.47 mmol H_2O m^{-2} s^{-1}) and stomatal conductance (0.85 mmol H_2O m^{-2} s^{-1}) were recorded from Glaxy-2013 under control conditions, where foliar SA was applied @ 100 mg/L. The minimum leaf photosynthetic rate (0.94 µmol CO_2 m^{-2} s^{-1}), leaf transpiration rate (1.41 mmol H_2O m^{-2} s^{-1}) and stomatal conductance (0.23 mmol H_2O m^{-2} s^{-1}) was recorded from Millat-2011 without SA application under salt+water stress (Fig. 3 a, b, c). All the other interactions such as SA × S × V were non-significant.

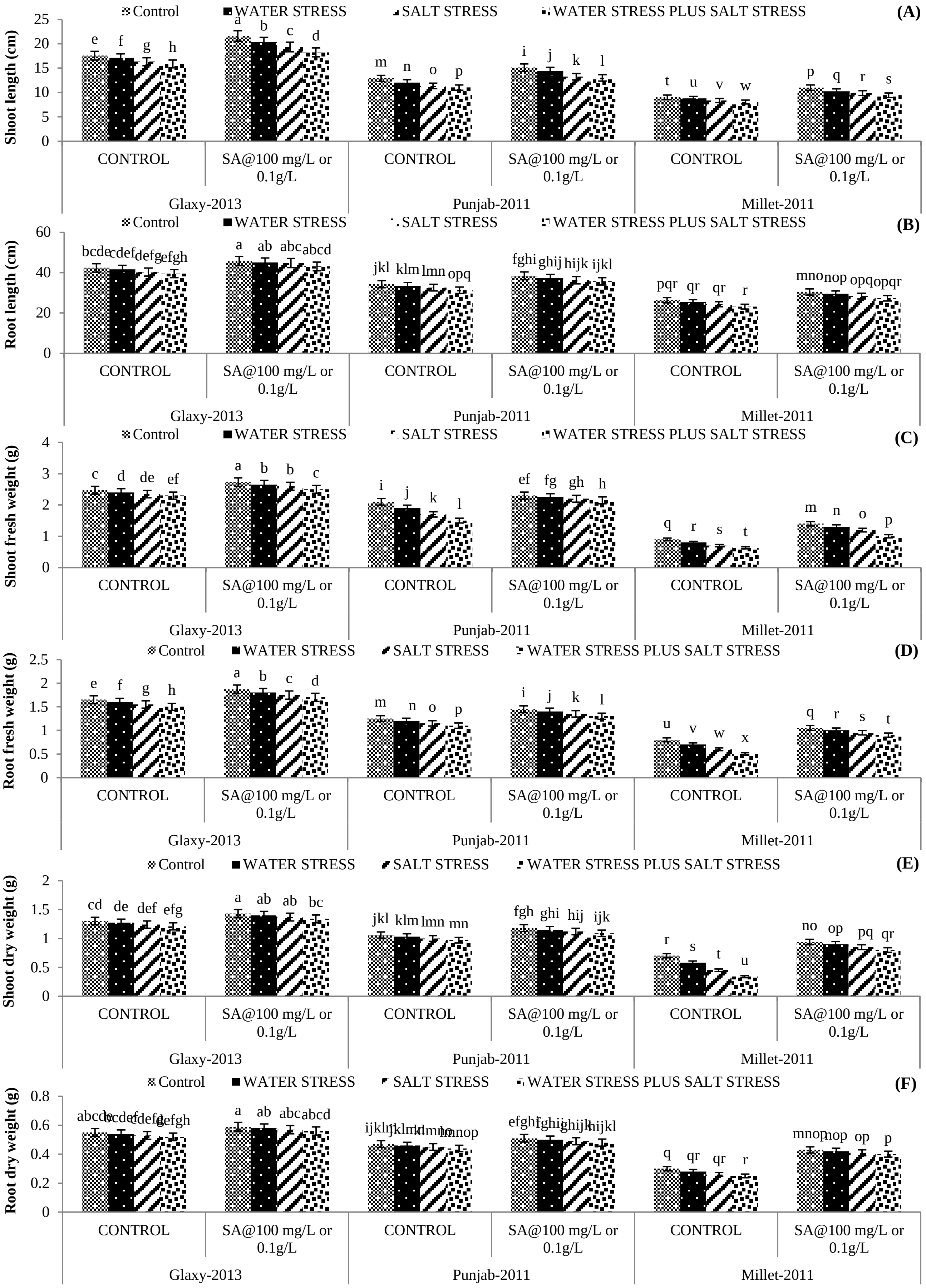

Fig. 1 (a, b, c, d, e, f): Effect of foliar SA application on root- shoot length (cm), root-shoot fresh and dry weight (g) of three wheat cultivars under water and salt stress conditions (mean values ± S.E).

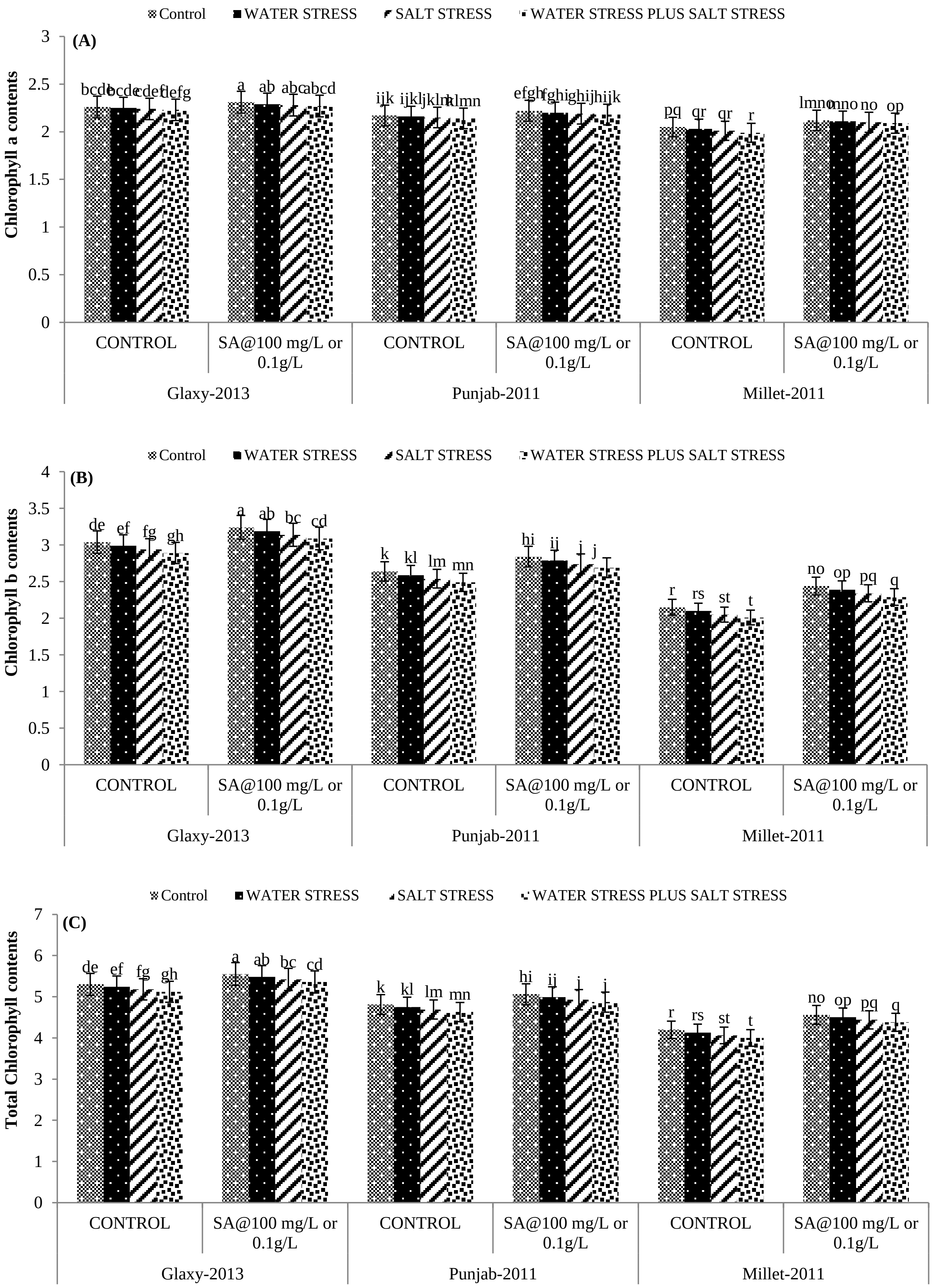

Fig. 2 (a, b, c): Effect of foliar SA application on chlrophyll *a*, chlorophyll *b* and total chlorophyll contents of three wheat cultivars under water and salt stress conditions (mean values ± S.E).

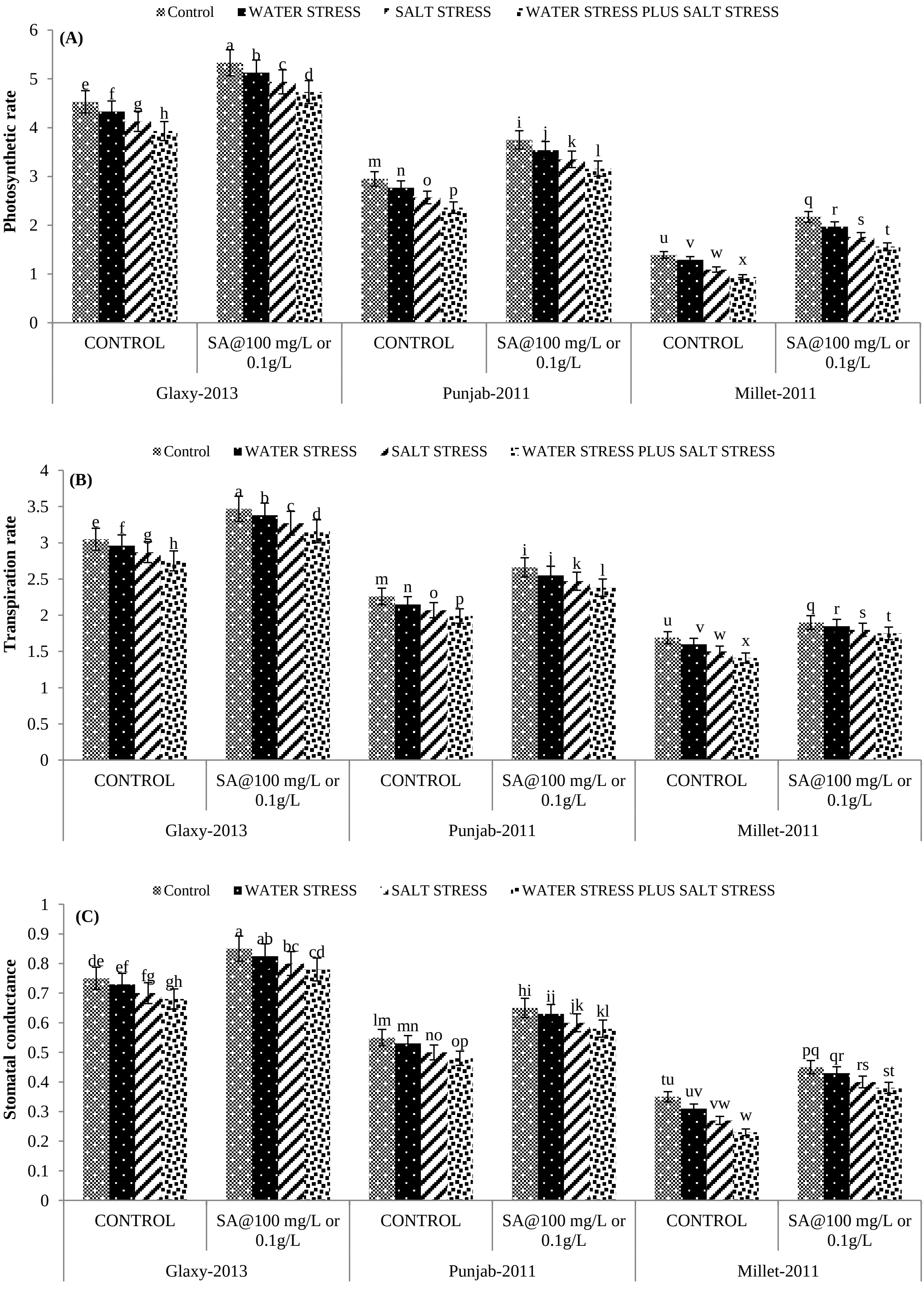

Fig. 3 (a, b, c): Effect of foliar SA application on photosynthetic rate, transpiration rate and stomatal conductance of three wheat cultivars under water and salt stress conditions (mean values ± S.E).

Discussion

The research findings of our trial explained that the morphological characteristics of wheat cultivars decreased under water and salt stresses, while foliar applied SA (100 mg/L) at tillering stage mitigated their adverse effects. The SA is an important phenolic compound, which regulates the plant growth and development (Horváth *et al*., 2007). Sayyari *et al*., (2013) reported earlier that water and salinity stress decrease the shoot-root fresh and dry weight of the plants. Another study supported our conclusion that application of SA enhanced the root and shoot fresh weights in mung bean (Akhtar *et al*., 2013).

According to the present studies, Chl a, Chl b and total chlorophyll (Chl a+b) were reduced in wheat under salt and water stress. This reduction is identified as a stress response of the host plant due to minimized rate of light absorption required for optimized photosynthesis (Pastenes *et al*., 2005). However, the foliar applied SA supported the photosynthetic process under water and salt stress. There was found an interaction between Chl a/b ratio, SA application and water stress. SA application caused to increase this index dramatically. Earlier reports also support this conclusion by explaining the supporting role of SA for increased Chl content and stomatal conductance (Singh & Usha, 2003; Idrees *et al*., 2010; Sharma *et al*., 2017). Additionally, SA promotes the synthesis of carotenoids and xanthophyll's (Moharekar *et al*., 2003). In the present study, Chl content have been found in positive correlation with SA in wheat. Chlorophyll (a, b, and total contents) were further correlated with net photosynthesis (Pn) & transpiration rate (E). It was demonstrated that chlorophylls have a key role as an intercepting antenna for light energy (Taiz & Zeiger, 2010). This suggests that the increase in Pn owing to SA treatment is largely due to Chl content.

The decrease of Pn induced by water deficit can be associated with different physiological parameters including stomata conductance. Foliar application of SA improved the Pn in wheat under water stress. Various studies support this conclusion for different crop (Stevens *et al*., 2006). Hamada & Al-Hakimi (2001) has shown that SA application in wheat support the photosynthetic process ultimately adding to its growth and productivity. However, some reports explained that SA might interfere the photosynthesis in wheat subjected to drought stress. Reason behind this phenomenon is the reduced stomatal conductance and transpiration rate (Németh *et al*., 2002; Szalai *et al*., 2016). Generally explaining the role of SA, it is quite effective for various physiological parameters of wheat, because the drought stress decreases Pn, Chl content and E, and SA mitigates its harmful effects (Wu *et al*., 2012). The exogenous application of SA was found effective in enhancing the Pn and E as well as productivity of corn & soybean (Khan, 2003), wheat (Hamid *et al*., 2010), mung bean (Akhtar *et al*., 2013) and tomato (Tariq *et al*., 2018).

Conclusion

It is concluded that salt and drought alone or combined seriously hampered the growth of wheat cultivars by adversely effecting the vital physiological. Study parameters included shoot-root fresh and dry weights, their lengths, stomatal conductance, chlorophyll (*a, b* and total contents) transpiration rate and net photosynthetic rate at tillering stage which were negatively affected by the stresses. Glaxy-2013 showed the maximum tolerance level under stress as compared to Punjab-2011 and Millat-2011. The foliar applied SA (100 mg/L or 0.1 g/L) at tillering stage improved all studied characteristics. Outstanding performance of Galaxy-2013 was recorded in terms of superior potential to cope with salt and drought stresses along with being more responsive to SA foliage application. However, more research is required for genetic mapping of these varieties for in depth analysis of genetic linkages and mechanisms of tolerance to abiotic stresses.

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