Pak. j. sci. ind. res. Ser. B: biol. sci. 2022 **65B**(1) 28-36

Augmenting the Tolerance Potential of Muskmelon (*Cucumis melo* L.) Using Salicylic Acid

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(received February 27, 2020; revised August 12, 2020; accepted September 18, 2020)

Abstract. Salicylic acid (SA) is considered an important plant hormone that controls many aspects of plant growth and development, as well as resistance to biotic and abiotic stresses. In current investigations, a pot experiment consisting of four different levels of SA (0, 1, 2 and 3 mM SA) were tested on growth and physiological attributes of muskmelon (*Cucumis melo* L.), grown at different salinity levels (0, 50, 100 mM NaCl). Results revealed that all the morphological and physiological attributes were significantly ($P<0.05$) adversely affected by salinity stress, whereas application of SA improved growth rate of muskmelon both in saline and non-saline conditions. SA treated plants showed higher total chlorophyll content, photosynthetic rate and stomatal regulations as compared to control plants. SA application reduced the salt deleterious effects by inhibiting toxic Na^+ ions accumulation in leaf and increased K^+/Na^+ ratio. Among the various applied concentrations, 2 mM SA increased shoot fresh weight by (30%), dry weight (34%), leaf area (25%) and K^+/Na^+ ratio by (84%) as compared to control plants. Thus, 2 mM SA concentration is concluded to be the best ameliorative treatment in salt stressed environments to enhance the muskmelon production.

Keywords: salinity, salicylic acid, *Cucumis melo* L., physiological attributes, K^+/Na^+ ratio

Introduction

Soil salinity is the imminent problem for agriculture and according to an estimate 50% of the arable land will be salt affected by the year 2050 (Machado and Serralheiro, 2017). High concentration of salt in soil obstructs plant growth and their metabolic activities. The distinct effects of salt on plant are osmotic and ionic stresses (Gupta and Huang, 2014). In addition to this, salt stress also exhibiting oxidative stress in plants. Salinity stress obstructs the physiological and metabolic processes in plant and consequently reduces the vegetative growth such as shoot root length, shoot root biomass and leaf area (Rahneshan *et al*., 2018). Resistance capacity of plant to salt stress is different due to differences in genetic makeup (Flower and Colmer, 2008). Most of the plant species do not have the ability to restrain the toxic salt ions going inside the body, which create osmotic pressure in cytosol. This disturbs the ionic homeostasis in plant and ultimately smashes the normal physiological and biochemical functions (Gupta and Huang, 2014). Under salt stress conditions, Na^+ and K^+ homeostasis has a critical role in progression and development of plant (Wakeel, 2013). Plants having the ability to maintain greater K^+/Na^+ ratio under salt stress conditions are measured as salt tolerant or *vice versa* (Khan *et al*., 2009). K^+ is an essential macronutrient and its role is vital in physiological procedures such as maintenance of membrane turgidity, stomatal conduction, activation of enzymes and osmotic pressure regulation (Rahneshan *et al*., 2018). Salinity stress also limit the photosynthetic process in plants and it may be related to stomatal or non-stomatal factors. The stomatal factor is related to stomata closure in leaf, while non-stomatal factors relates to chlorophyll production, photosynthetic electron transport and others (Liu *et al*., 2011).

Salicylic acid (SA) is the natural chemical compound has a significant role in regulation of plant growth development under salt stress (Jini and Joseph, 2017). It subsidizes the physiological processes and alleviates salinity stress for plants (Hayat *et al*., 2010). SA improves the mineral composition of plant (Khan *et al*., 2015) and such modifications increase the plant growth under salt stressed conditions (Nazar *et al*., 2015). Numerous studies describe the productive role of SA in plants under salt stress conditions (Yadu *et al*, 2017; Tufail *et al*., 2013). Exogenous SA has the feature to reduce toxic ions (Na^+) accretion in shoot and leaf and increase

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ratio of beneficial ions such as K^+ and Ca^{2+} in saline conditions (Elwan and El-Shatoury, 2014). In addition to this, SA fastens the uptake of other plant beneficial elements such as Mn, Ca, Cu, Fe, P and Zn and thereby reduces oxidative stress (Wang *et al*., 2011). Tufail *et al*. (2013) stated that application of SA to salt stress maize plants increased K^+/Na^+ and Ca^{2+}/Na^+ ratios, enhanced gas exchange attributes and therefore, obtained greater vegetative growth and yield. The ameliorative effects of SA for salt tolerance have been studied in some of the horticultural crops such as pea (Yadu *et al*., 2017), fenugreek (Babar *et al*., 2014) and tomato (Wasti *et al*., 2012). The efficacy of SA to plants depends upon various factors such as growth stage, the mode of application and the concentration of applied and endogenous SA levels (Hara *et al*., 2012).

Muskmelon is the important fruit crop of arid and semi-arid regions (Patil *et al*., 2014) and salinity is the most prevailing substance in such areas. In Pakistan, almost 80% of land is lying under arid and semi-arid climatic zones and this include southern and central Balochistan, Punjab southern areas, southern and northern Khyber Pakhtunkhwa, Sindh and Gilgit-Baltistan province (Shah *et al*., 2011). In this regard, the present study was under taken to mitigate salinity stress in muskmelon with optimal level of salicylic acid as a foliar application.

Materials and Methods

A pot experiment was conducted to assess the various levels of salicylic acid at different salinity levels in green house of National Agriculture Research Centre, Islamabad, during the year 2012. The experiment was conducted using completely randomized design (CRD) with factorial arrangement replicated thrice. Salt treatments used were (0, 50 and 100 mM NaCl) and different SA concentrations were (0, 1, 2 and 3 mM SA). Seeds of muskmelon genotype 'T-96' were obtained from Ayyub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Plastic pots of 3 L volume capacity filled with washed silica sand and ten (10) sterilized seeds in each pot were sown. Before sowing, the seeds were surface sterilized with 10% hypochlorite solution for 5 min and then rinsed with distilled water thoroughly. When seedlings achieved 2 to 3 leaf growth, they were thinned and maintained 3 uniform seedlings per pot. The seedlings were watered with half strength Hoagland solution for irrigation. The fifteen days old seedlings were subjected to various salts stresses (0, 50 and 100 mM NaCl). Two days later of salt application, the seedlings were foliar sprayed with SA of particular concentration and ensured full coverage. The total duration of this experiment was 32 days.

Vegetative growth attributes. Shoot and root length (cm) of three randomly selected plants from each treatment were measured with the help of ruler and averaged. Shoot fresh and dry weight (g) of three randomly selected plants from each treatment were weighed with the help of electronic balance and averaged. For dry weight shoot determination, it was oven dried at 70 °C for 72 h. Leaf area was calculated by leaf area meter (Li-Cor, model LI-3000A) UK and averaged.

Gas exchange attributes. The gas exchange parameters like photosynthetic rate (A) and stomatal conductance (g_s) were recorded through Infrared Gas Analyzer (IRGA, LCA-4, ADC, Hoddesdon, UK). The readings were taken from the fully expanded leaf of the muskmelon plant at 9:00-11:00 am in the full sunshine. Before taking the measurements, the IRGA instrument was calibrated and zero was adjusted during the data measurement period. All the attributes measured by IRGA were recorded three times for each treatment and then averaged.

Total chlorophyll content. Fresh leaf was grinded in mortar and pestle using 20 mL of 80% acetone. The sample was centrifuged at 2500 rpm for 15 min. The supernatant of sample was used for chlorophyll estimation. Readings were taken at A_{654} nm and A_{663} nm of wavelength for chlorophyll 'a' and 'b' and total chlorophyll was calculated by summation of chlorophyll 'a' and 'b', using spectrophotometer (Unico- UV 210 japan). Leaf chlorophyll content was calculated as described by (Arnon, 1949), and were counted three times for each treatment and then averaged.

Ionic attributes. Leaf Na^+ and K^+ content were determined by a method described by Chapman and Pratt (1961). Leaf samples (0.5 g) were oven dried at 65 °C for 48 h. The dried samples were kept in crucible and ashed in muffle furnace at 550 °C for 5 h. Subsequently, 2N HCl was added to the ashed samples and left for 1 h. Samples were diluted to 50 mL with distilled water and then filtered. Na^+ and K^+ contents were determined by flame photometer (Sherwood model 410, Japan) and the concentrations expressed as (% DW). The readings were taken three times for each treatment and averaged.

Statistical analysis. The data recorded were subjected to analysis of variance technique appropriate with factorial arrangement and means were calculated using least significant difference (LSD) test as suggested by Steel *et al*. (1997). Statistical analysis was performed using the SAS program version 9.1 (SAS Institute, Cary, NC).

Results and Discussion

Morphological attributes. The impact of both salinity stress and salicylic acid (SA) on shoot fresh and dry weight of muskmelon seedlings is given in Fig. 1. Shoot fresh and dry weight of muskmelons were significantly ($P \leq 0.05$) decreased with increasing of salt concentration. Therefore, maximum shoot fresh and dry weight were recorded at control concentration and minimum were observed at 100 mM salinity level. Foliar application of SA significantly ($P \leq 0.05$) enhanced shoot fresh and dry weight of muskmelon both in saline and non-saline conditions. Among the various applied SA concentrations, 2 mM SA gave the maximum shoot fresh and dry weight followed by 1 mM SA concentration. When calculated at 100 mM NaCl level, 2 mM SA concentration increased shoot fresh and dry weight by (30%) and (34%) respectively as compared to control. Further increase in SA concentration (3 mM SA) was not much effective and boosted the shoot fresh and dry weight by (10%) and (9%) respectively as compared to control. Salt stress and SA treatments also affected shoot and root length and is presented in (Fig. 2). Shoot and root lengths steeply and significantly ($P \leq 0.05$) decreased with increasing of salt concentration and therefore, minimum shoot and root length was noted at highest 100 mM NaCl levels. Various levels of exogenous SA substantially increased the length of shoot and root. However, 2 mM SA show the maximum shoot and root length both in normal and saline conditions. When tested at 100 mM NaCl level, 2 mM SA increased the shoot and root length by (29%) and (26%) respectively as compared to control plants. Similarly, leaf area was also significantly affected by salinity and SA levels and is given in (Fig. 3). Maximum leaf area was obtained in control (non-saline) and minimum was recorded under 100 mM NaCl level. The different SA dosages significantly enhanced leaf area under both saline and non-saline conditions. The 2 mM SA level was the most effective and enhanced leaf area by (25%) as compared to control plants. The present results showed that vegetative growth of muskmelon reduced when exposed to salinity stress. The reduction was severe with the increasing salt concentration and it was similar to those of earlier findings (Babar *et al*., 2014; Li *et al*., 2014). The reduction in plant growth due to salt stress is associated with low photosynthetic process and chlorophyll degradation, interruption of essential minerals translocation and accruate of toxic ions in cytosol. All these factors equally responsible and adversely affect the total biomass of plant under salt stress (Mahlooji *et al*., 2018). Our results showed that exogenous SA had a positive effect on muskmelon growth and tempted salinity stress for it. Exogenous SA significantly improved all the vegetative growth attributes both in normal and saline conditions and these findings are in

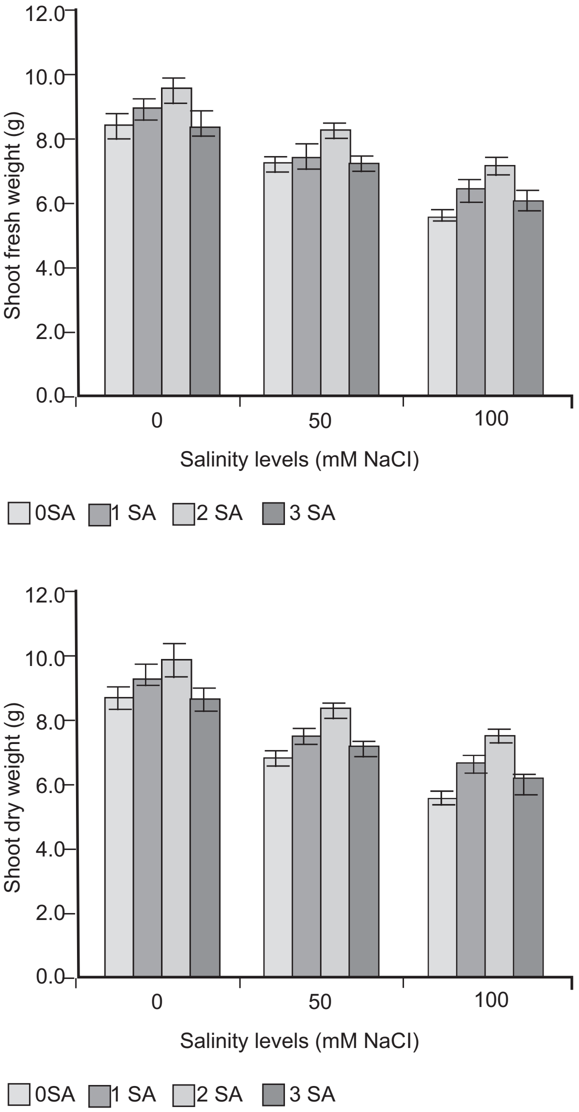

Fig. 1. Effect of salicylic acid (SA) on shoot fresh and dry weight of muskmelon at various salinity levels.

accordance of previous reports (Elwan and EL-Shatoury, 2014; Noreen and Ashraf, 2008), who acquired greater biomass for sunflower and cabbage plants both in saline and non-saline conditions when treated with SA. Bayat *et al*. (2012) described that exogenous SA increased shoot and root dry weight, leaf areas and plant heights of calendula under salinity stress conditions. Hussain *et al*. (2011) reported similar positive effect of SA to violet plants under salt stress and stated that SA treated plants displayed high N and relative water content and produced greater biomass. In addition to this, SA prestige to repair and promote antioxidant system in plants under salt stress conditions and increases plant growth rate (Li *et al*., 2014). All this valuable effects of SA to plant under salt stress ratifies the findings of the present study.

Biochemical attributes. Sodium (Na^+) is the toxic ion of salt and the results of present study showed that Na^+ content in leaf of muskmelon was substantially increased with increasing NaCl concentration (Fig. 4). Consequently, maximum leaf Na^+ content of muskmelon was recorded at 100 mM NaCl level. SA treatment significantly ($P \leq 0.05$) restrained Na^+ content and 2 mM SA concentrations gave the least Na^+ accumulation in muskmelon under salt stress conditions. When

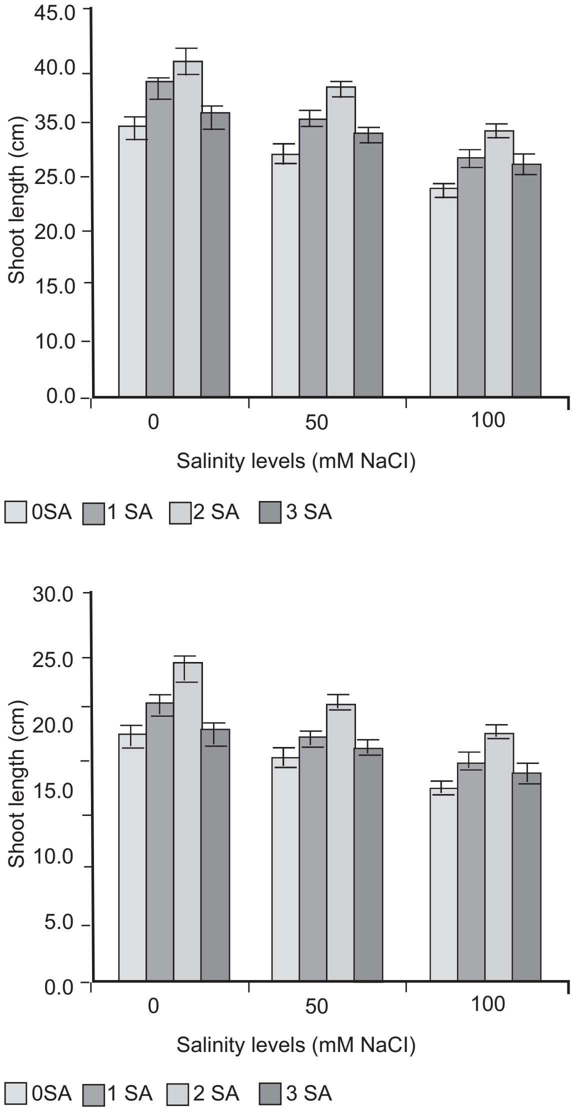

Fig. 2. Effect of salicylic acid (SA) on shoot/root lengths of muskmelon at various salinity levels.

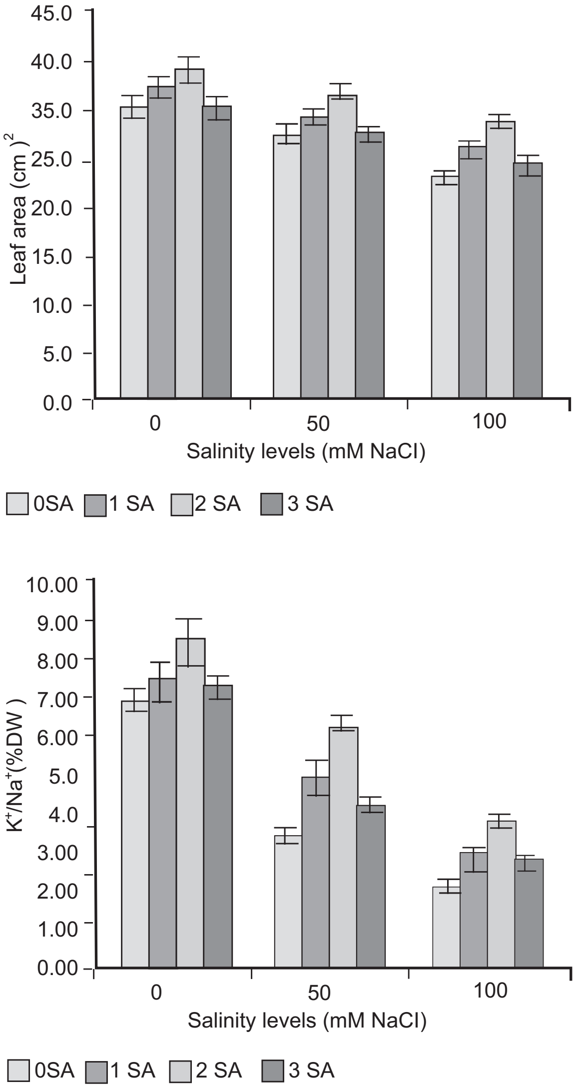

Fig. 3. Effect of salicylic acid (SA) on leaf area and K^+/Na^+ ratio of muskmelon at various salinity levels.

evaluated at 100 mM NaCl level, 2 mM SA reduced Na^+ by (31%) as compared to control plants. Further increase in SA concentration (3 mM SA) does not responded well in terms of Na^+ restriction as it reduced the Na^+ content merely by (11%) as compared to control. Leaf potassium (K^+) content was significantly affected by salinity stress and SA levels (Fig. 4). K^+ content of leaf gradually decreased with increasing of NaCl concentrations. Therefore, least K^+ content were recorded at highest 100 mM NaCl level. The various SA levels enhanced K^+ contents both in normal and saline conditions. Among the various SA levels, 2 mM SA treatments gave the excellent results regarding K^+ content increase in both saline and non saline plants. When assessed at 100 mM NaCl level, 2 mM SA increased K^+ content by (26%) as compared to control. Similarly, the present results showed that K^+/Na^+ ratio was also significantly ($P \leq 0.05$) affected by salinity and SA levels (Fig. 3). The K^+/Na^+ ratio of muskmelon decreased with increasing of salt stress and eventually minimum ratio was recorded at highest 100 mM NaCl level. The exogenous application of SA significantly ($P \leq 0.05$) improved K^+/Na^+ ratio both in saline and non-saline conditions. Among the various applied doses of SA, 2 mM SA showed pronounced results and enhanced K^+/Na^+ ratio both in non saline and saline environments. When assessed at highest 100 mM NaCl level, 2 mM SA increased the K^+/Na^+ ratio by (84%) as compared to control. Keeping the balance, ionic homeostasis in plants under salt stress is an essential and vital phenomenon to enhance plant growth (Wakeel, 2013). The results of present study showed that Na^+ content of muskmelon plants increased with increasing salinity stress and these results are in accordance with previous studies (Ibrarullah *et al.*, 2019; Rahneshan *et al.*, 2018). Higher accrual of Na^+ in plants under salt stress is always inversely proportional to plant growth rate (Li *et al.*, 2014). Na^+ is considered the most toxic ion of salts. In saline condition, Na^+ suppress the entry of beneficial ions such as K^+ and Ca^{2+} in plants (Simaei *et al.*, 2012) and such findings support the present results, as K^+ content decreased as the Na^+ increased in leaf of muskmelon. Different studies showed that exogenous SA reduced Na^+ accretion and increased K^+ and Ca^{2+} contents in plants under salt stress conditions (Shaki *et al.*, 2019; Parizi *et al.*, 2011) and these reports backing the present findings. Li *et al.* (2014) investigated the effect of SA on *Artemisia annua* L. plants under salt stress and their results revealed that supplemented SA induced salinity stress in plants by hindering Na^+ content and increased beneficial ions such as K^+, Ca^{2+} and Mg^{2+}. Such reports strongly support the present findings and validate the role of SA to encourage salt tolerance in plants. Potassium (K^+) is the essential macronutrient of plant and its role is vital in various physiological processes such as maintenance of membrane turgidity, stomatal conduction, activation of enzymes and osmotic regulation (Rahneshan *et al.*, 2018). The present results indicate that exogenous SA application increased K^+ content of muskmelon under both saline and non saline conditions. There are evidences that exogenous SA counter the Na^+ content and enhanced the accrual of K^+ in plants under salt

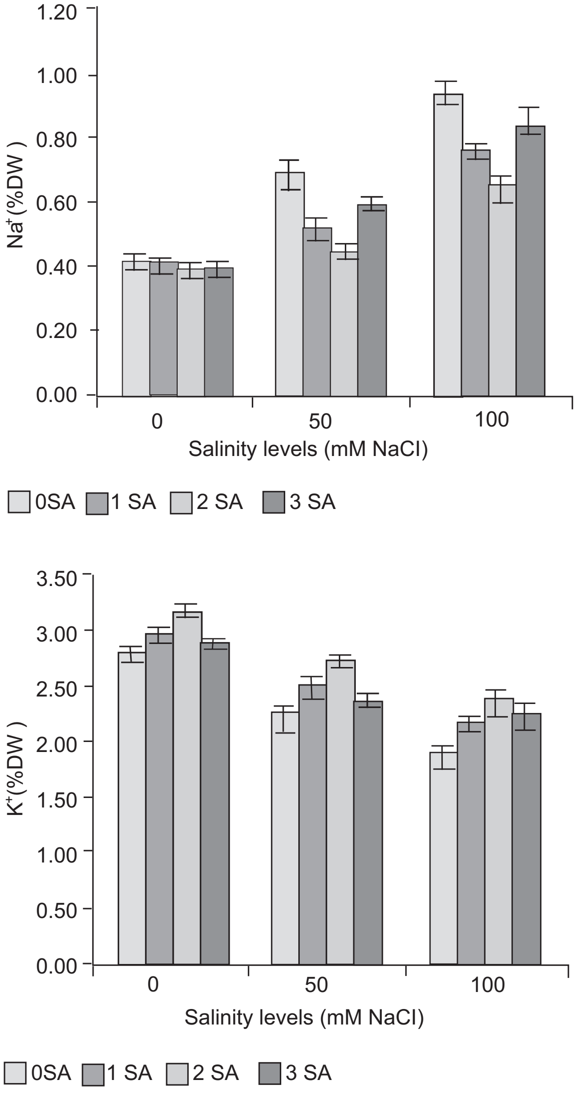

Fig. 4. Effect of salicylic acid (SA) on N^+ and K^+ content of muskmelon at various salinity levels.

stress conditions (Jini, and Joseph, 2017). The K^+/Na^+ ratio is the important criteria to see salt tolerance ability of any plant (Li *et al*., 2014) and present results showed that K^+/Na^+ ratio of muskmelon plants decreased with increasing of salinity stress. However, exogenous SA inverse the ionic homeostasis and increased K^+/Na^+ ratio of muskmelon in both saline and non saline environments and thereby increased the plant growth. Numerous reports confirmed that salinity stress decline K^+/Na^+ ratio of plants under salt stress conditions and plant growth rate depends upon this (Ibrarullah *et al*., 2019; Mahlooji *et al*., 2018). SA treatments recovered all these adverse effects of salt for plants and improve the K^+/Na^+ ratio in plants under salt stress conditions (Khan *et al*., 2015; Tufail *et al*., 2013). These outcomes validate the present findings and signify the ameliorative role of SA under salt stress conditions.

Physiological attributes. The present results showed that leaf photosynthetic rate and stomatal conductance of muskmelon were significantly affected by salinity stress and different SA levels (Fig. 5). Salinity stress considerably reduced the photosynthetic rate of muskmelon and it was more obvious with increasing salinity level. Eventually, the least photosynthetic rate was observed at highest 100 mM NaCl level. Exogenous foliar application of SA comprehensively increased photosynthetic rate of muskmelon both in saline and non saline conditions. Among the various applied SA levels, 2 mM SA showed the highest photosynthetic rate and followed by 1 mM SA level. 2 mM SA increased the photosynthetic rate by 43% at 100 mM NaCl level, as compared to control. Similarly, stomatal conductance was also significantly affected by salinity and SA levels (Fig. 5). Stomatal conductance significantly ($P \leq 0.05$) decreased with increasing of salinity stress. Therefore, minimum stomatal conductance was observed when plants exposed to highest (100 mM) NaCl stress. However, exogenous application of SA significantly improved the stomatal conductance of leaf both in saline and non saline conditions. Among the various tested levels of SA, 2 mM SA level increased the maximum stomatal conductance, and it was 20% higher as

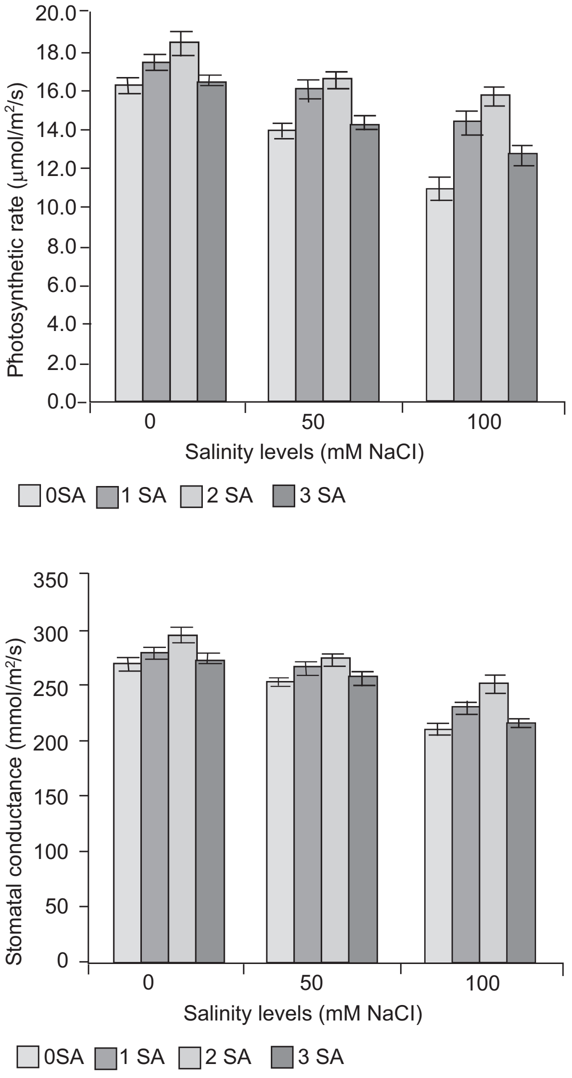

Fig. 5. Effect of salicylic acid (SA) on photosynthetic rate and stomatal conductance of muskmelon at various salinity levels.

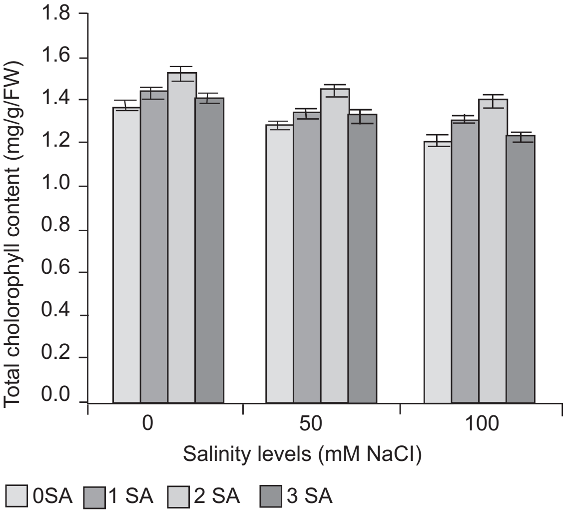

Fig. 6. Effect of salicylic acid on Total chlorophyll content of muskmelon at various salinity levels.

compared to control. Total chlorophyll content of leaf was also significantly affected by salinity stress and SA levels (Fig. 6). Salt stress decreased total chlorophyll content with salt increment. Various levels of SA significantly ($P \leq 0.05$) improved the total chlorophyll content both in stress and non-stress conditions. Among the various applied concentrations of 2 mM SA was the most effective and increased the total chlorophyll content by 15% as compared to control. Present results revealed that salinity stress causes acute decline in physiological functions of plant, resulted in reduced plant growth. Gas exchange traits such as photosynthetic rate and stomatal conductance in muskmelon leaf decreased with increasing of salt concentrations. These results are in accordance with previous findings (Ibrarullah *et al*., 2019; Liu *et al*., 2011), who reported that leaf photosynthetic rate and stomatal conductance of plant decreases when subjected to salinity stress. Ibrarullah *et al*. (2019) stated that leaf photosynthetic rate is directly link to plant biomass production, and its efficacy decrease with increase of salt stress. However, application of SA at different levels ameliorates negative effect of salinity by enhancing photosynthetic rate and stomatal conductance of muskmelon both in saline and non saline conditions. The stimulating role of SA related to photosynthetic and stomatal conductance in plant leaf under salt stress has also been reported by (Babar *et al*., 2014; Aftab *et al*., 2011) and support the SA salt stress alleviation phenomenon. Similar positive effects of SA on maize and sunflower under salinity stress has also been reported by Noreen *et al*. (2009) and Tuna *et al*. (2007) and elucidate that SA is helpful to activate the various physiological functions of plant.

Conclusion

Soil salinity is the main factor limiting crop yield in arid and semi-arid regions of the world. Crop plants are sensitive to salinity stress at all growth stages, however, early seedling growth is the most vulnerable and good stand of crop is depend upon its vigorous seedlings. The results of present study revealed that salinity stress adversely affected all the morphological and physiological attributes of muskmelon. However, exogenous applied SA significantly decreased the toxic effect of salt and improved the seedling growth in saline and non-saline environment. Hence, it is concluded that SA treatment is a valuable and cost effective technique and may be practiced in adverse saline conditions to improve muskmelon vegetative growth, and ultimately its fruit production.

Acknowledgement

Authors are thankful to the Higher Education Commission (HEC) of Pakistan for their financial support under the scheme of Indigenous Scholarship Program.

Conflict of Interest. The authors declare no conflict of interest.

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