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RESEARCH ARTICLE

Effects of Aerial Application of Salicylic Acid on Growth, Pigment Concentration, Ions Uptake and Mitigation of Salinity Stress in Two Varieties of Wheat (*Triticum aestivum* L.)

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ABSTRACT

The agricultural crops are greatly impacted by climate change, eco-edaphic factors and salt stress. This study was conducted to assess the negative effects of salts stress on two varieties of wheat crop i.e. S-24 and MH-97. It was aimed to determine the proportionate extent of stress alleviation induced by salicylic acid (SA) and its impact on biological yield, relative water content, chlorophyll content, Na^+/K^+ ions and other physiological and biochemical attributes in wheat varieties. The plants were challenged with salinity stress (200 mM NaCl) and the effects of foliar spray of SA (200 mg L^{-1}) were investigated. The shoot fresh weight was reduced by 30% under saline stress, but exogenously applied SA caused an increase in shoot fresh weight by 11.1%. The higher quantity of biological yield by 21.9% was obtained from variety MH-97 compared to variety S-24. The varieties differed greatly in maintenance of total soluble proteins, while a little affected by salt stress. The varieties S-24 and MH-97 maintained the quantity of total soluble proteins by 18.7 and 11.3 mg/g, respectively. The crop treated with SA at the rate of 200 mg L^{-1} produced 69.6% higher total soluble proteins compared to unsprayed crop. The varieties differed significantly in relation to total free amino acids. The variety MH-97 contained 18.1% higher content compared to variety S-24 under saline environment. The spray of SA enhanced the amount of total free amino acids from 12.29 to 19.42 mg/g under saline condition. The SPAD values decreased from 47.15 to 41.62 and 44.52 to 40.58 in varieties S-24 and MH-97, respectively grown on untreated and treated soils with 200 mM salinity. Relative water contents (RWC) were reduced by 16.1% under saline condition. The application of SA induced the uptake of K^+ ion from soil medium. The results have revealed that salt stress in wheat crop could be reduced by foliar spray of salicylic acid.

INTRODUCTION

The agricultural production in arid and semi-arid land is significantly affected by salinity stress. The accumulation of soluble salts poses devastating effects on the production of crop plants (Zhu, 2001). Approximately six million hectares of the irrigated land is affected by salinity in Pakistan (parc.gov.pk). Most of the cultivated land has salinity patches. The economic production of various crops in such area is a challenging task for the farmers because they have to face problems in seedlings establishment, restricted plant growth and development and substantial loss in quantity and quality of the product (Hartmann et al., 1990). There are different drastic effects of salinity stress on plants. The limited growth rate of plants in saline area is due to substantial absorption of the salts, particularly the Sodium ions (Na^+) and Chloride ions (Cl^-) by plant tissues. It results in restricted water absorption, damage to the root system, necrosis in leaves and progressive wilting that may lead to plant death (Husen et al., 2018; Ashraf, 2010). The uptake of greater amounts of toxic ions lowers the turgor potential of plants that is main driving force for sustaining the plant growth and development (Shabala et al., 2012; Farooq et al., 2010). The turgidity of leaves, relative water contents, accumulation of proteins, biomass production, and proportionate absorption and restriction of toxic ions with concurrent assimilation of K^+ by plant tissues influence greatly on crop productivity (Ashraf, 2010). The activity of photosystem I and II is negatively affected due to closure of stomata, which results in bringing various changes in the morphological, hormonal and physiological processes under salt stress conditions (Ashraf et al., 2011).

The different crop plants respond differently to the salinity stress. Their response is largely dependent upon the variations at the molecular, physiological and biochemical levels (Ashraf, 2004). It may vary in the accumulation level and appearance of toxic Na^+ and Cl^- ions, production of reactive oxygen species (ROS), absorption level of the essential nutrients and the balance of phytohormones required for normal growth of plants (Hayat et al., 2005; Dat et al., 2000). The ability of plant species to address drought condition under salt stress environment is adversely affected by the amount of generation of ROS. These factors cause enhancement in lipid peroxidation and deterioration of cell membrane structures (Joseph et al., 2010).

The salicylic acid (SA) is a ubiquitous endogenous compound in plants that can induce tolerance to salinity stress (Noreen et al., 2013; Ashraf, 2010); water stress (Ashraf et al., 2011; Singh and Usha, 2003); high temperature stress (Krasensky and Jonak, 2012) and generation of reactive oxygen species (ROS) (Noreen et al., 2009). The SA improves plants osmotic adjustment under stressful conditions (Jones and Turner 1980). It is responsible for regulating photosynthetic and evapotranspiration rate (Ahmad et al., 2013). It enhances the amount of chlorophyll contents (Noreen et al., 2013); induces the over-production of organic solutes (Ashraf, 2010) and restricts the assimilation of Na^+ and Cl^- ions (Noreen and Ashraf, 2008). It is helpful in longer retention of flowers (Hayat and Ahmad, 2007); in greater quenching of ROS by upregulating the antioxidant defense system (Sharma and Dubey, 2005) and in attaining higher turgor potential under stressful conditions (Mutlu et al., 2013). It helps in the ROS scavenging and enhances the capability of plants to overcome the deterioration of cell membrane leading towards improved osmotic adjustment in plants (Ajithkumar and Panneerselevam, 2014).

There are several reports of the SA induced mitigation of abiotic stresses in crop plants. Hamada and Al-Hakimi (2001) investigated alleviation of the drastic effects of salinity stress in Wheat seedlings by spraying 100 mg L^{-1} SA. El-Tayeb (2005) reported that increasing levels of NaCl in rooting zones resulted in the increased amount of soluble proteins in the roots of barley seedlings. Ilyas et al. (2017) studied the influence of SA and Jasmonic Acid on Wheat under drought stress. Babar et al. (2014) reported the mitigation of the negative effects of salinity in Fenugreek by foliar application of SA. Likewise, Nahrjoo and Sedaghathoor (2018) investigated the induction of salinity stress resistance in Rosemary by applying Jasmonic acid and SA and. Farouk and Arafa (2018) reported the use of sodium nitroprusside for the mitigation of salinity stress in Canola.

The Wheat (*Triticum aestivum* L.) is a very important food crop in the world that is cultivated on more than 8.66 million hectares land. Pakistan harvested 26.3 million tonnes of Wheat grains during the year 2017-18 (https://www.world-grain.com/articles/10791-pakistan-wheat-production-up-in-2017-18). Some of the agriculture land in Pakistan both irrigated and rainfed, is affected by salinity and/or sodicity to certain extent. The cultivation of Wheat in such area poses a challenging task to produce significant yield of good quality grains. Therefore, it is needed to look for possible measures to alleviate the drastic effects of salinity stress on crop plants for the sake of sustainable agriculture. Since SA is reported to be an active agent that strengthens plant tolerance level against various stresses, therefore, this study was designed to investigate the ability of SA to mitigate the salinity stress in two selected varieties of the Wheat crop under local environment.

MATERIALS AND METHODS

To investigate the response of two varieties of Wheat (S-24 and MH-97) towards foliar application of SA and its efficacy to mitigate salinity stress, a pot experiment was conducted in complete randomized design with four replications at the Botanic Garden, Institute of Pure and Applied Biology (IP&AB), Bahuddin Zakariya University Multan, Pakistan. Each pot (24.5 cm diameter and 28.0 cm deep) was filled with 12 Kg dry sand, irrigated with water and the seeds were sown. Irrigation was maintained with full strength Hoagland's nutrients solution (Epstein, 1972). After ten days of germination, thinning was practiced leaving five uniform seedlings in each pot. After 18 days of germination, the seedlings were challenged with salinity stress (200 mM NaCl) by administering first treatment of 50 mM NaCl along with full strength Hoagland's nutrients solution followed by same treatment for second, third and fourth time on every second day. The plants were regularly irrigated with nutrients solution every week. The plants were also sprayed with 100 ml deionized water to stabilize plants under salinity stress. After 26 days of germination, the plants were sprayed with Aqueous solution of SA (200 mg L^{-1}) supplemented with 0.1 % (w/v) Tween-20 at 08:00 to 10:00 AM in the morning.

After three weeks of the SA treatment, the total chlorophyll contents were estimated with the help of a portable SPAD chlorophyll meter by randomly selecting the fully expanded leaf from the apex. The relative water contents were measured by following the method of Jones and Turner (1980) by collecting fully expanded leaves from the top. Thereafter, the plants were harvested and the roots were washed with water. The shoots and roots samples were weighed by using a digital balance to record fresh weight. Afterwards these samples were oven dried at 70°C for 48 hours and the shoot and root dry mass was measured to determine the biological yield. The fresh plant material was used to determine the total free amino acids and total soluble proteins contents by employing the method of Bradford (1976). The plant material was used for the analysis of Sodium and Potassium ions concentration by following the methods of Ryan et al., (2001). The data were subjected to statistical analysis by using MSTAT-C computer Package (Cohart Software, Berkley, CA). The least significant differences (LSD) were calculated by using technique of Snedecor and Cochran (1980).

RESULTS

The results showed that the Wheat variety S-24 was relatively better in growth as compared to the variety MH-97 under normal condition. The salinity stress has significantly decreased the growth of both varieties however this decrease was relatively more in variety S-24. The fresh shoot weight was significantly affected by foliar spray of SA and salinity levels respectively. However, there was a little difference among wheat varieties, MH-97 and S-24. However, it was little affected by the interactive effects of varieties x SA levels, varieties x salinity regimes, SA levels x salinity and varieties x SA levels and salinity regimes (Fig. 1). The salinity stress reduced the fresh shoot weight by 40% and 28% in varieties S-24 and MH-97, respectively. Overall, the salt stress caused reduction in fresh shoot weight by 34%. However, the variety S-24 and MH-97 sprayed with SA produced 10% and 05% higher fresh shoot weight respectively under saline conditions, whereas SA spray resulted in 13% and 17% higher fresh weight in variety S-24 and MH-97 respectively under normal conditions (Fig. 1). The salinity stress has significantly affected ($P<0.001$) the dry shoot weight. The SA spray enhanced 15% and 09% shoot dry weight in variety S-24 and MH-97 respectively under saline condition. The various interactive combinations (varieties x SA levels, varieties x salinity regimes, SA levels x salinity regimes and varieties x SA levels and salinity regimes) also produced a little effect in improving the fresh shoot weight (Fig. 1). Furthermore, various SA level and varieties alone and/or interaction with salinity regimes did not show any significant differences amongst themselves in this parameter. Averaged across SA levels and salinity regimes 21.99% higher biological yield was obtained by variety MH-97 in comparison with variety S-24. The data further revealed that biological yield was reduced by 34.23% in crop grown under salt stress (Fig. 1). The salt stress and interaction of varieties x SA levels produced significant effect in reducing the biological yield, however, a little influenced by other interactive treatments. A higher quantity of biological yield by 15.16% was resulted from variety MH-97 in comparison with variety S-24. The spray of 200 mg L^{-1} SA resulted in the improvement of biological yield by 13.7% in comparison with unsprayed crop. Overall, the wheat varieties grown under salinity stress produced lower biological yield compared with under normal condition.

The amount of total soluble proteins (TSPs) was significantly decreased due to different varieties and SA levels, however, salts stress did not have any positive effect. The various interactive treatments (salinity regimes x varieties and salinity regimes x SA levels x varieties) caused significant effect in maintaining differential quantities of total soluble protein in leaf tissues (Fig. 2). The varieties produced pronounced effects in maintaining the differential quantities in the leaf tissues. An amount of 18.7 mg g^{-1} of the TSPs was accumulated in variety MH-97, while it was 11.25 mg g^{-1} in variety S-24. Thereby, variety S-24 accumulated 67.11% higher amount of TSPs in comparison with variety MH-97. Furthermore, the spray of SA proved to be good in maintaining greater amount of TSPs in wheat varieties. An improvement of 69.58% TSPs was observed by exogenous application of SA over the unsprayed crop. The interactive treatment of salinity regimes and SA levels also produced significant ($P<0.01$) effects in maintaining higher amount of TSPs. The varieties S-24 and MH-97 maintained 44.66% and 40.59% higher quantities of TSPs under salinity stress compared to non-saline conditions.

There were significant differences in total free amino acids (FAAs) due to effects produced by interaction of varieties to salinity regimes, while a little affected by spraying of SA. The differences in total FAAs were statistically non-significant by other interactive treatments. However, varieties behaved differently in response to various treatments. A higher value of FAAs by 18.1% was recorded in variety MH-97 compared with variety S-24. Overall the salinity stress caused significant ($P<0.001$) effect enhancing the number of total FAAs in both varieties. The total FAAs were increased from 12.29 to 19.42 mg g^{-1}, in crop grown on saline soil, showing an increase by 58.1% over non-saline soil (Fig. 2).

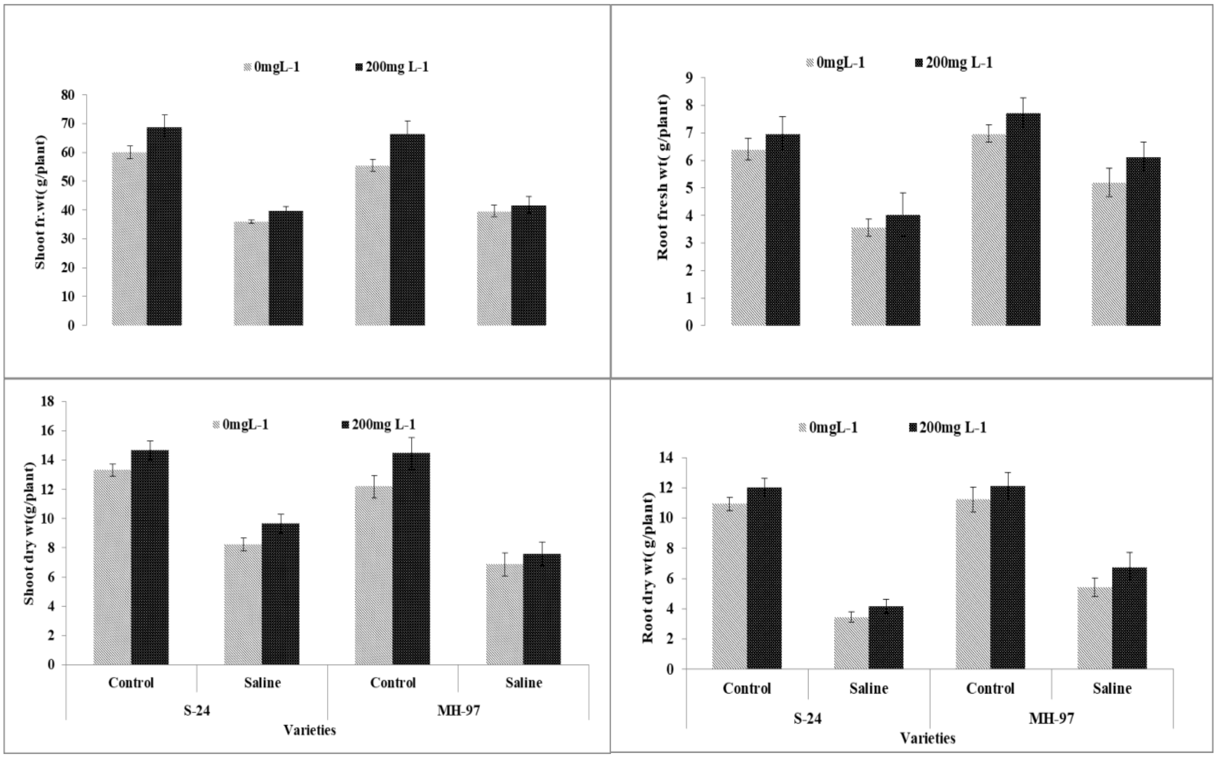

Fig. 1: The effects of foliar spray of salicylic acid on shoot and root fresh and dry weight of two wheat varieties grown under saline and non-saline conditions.

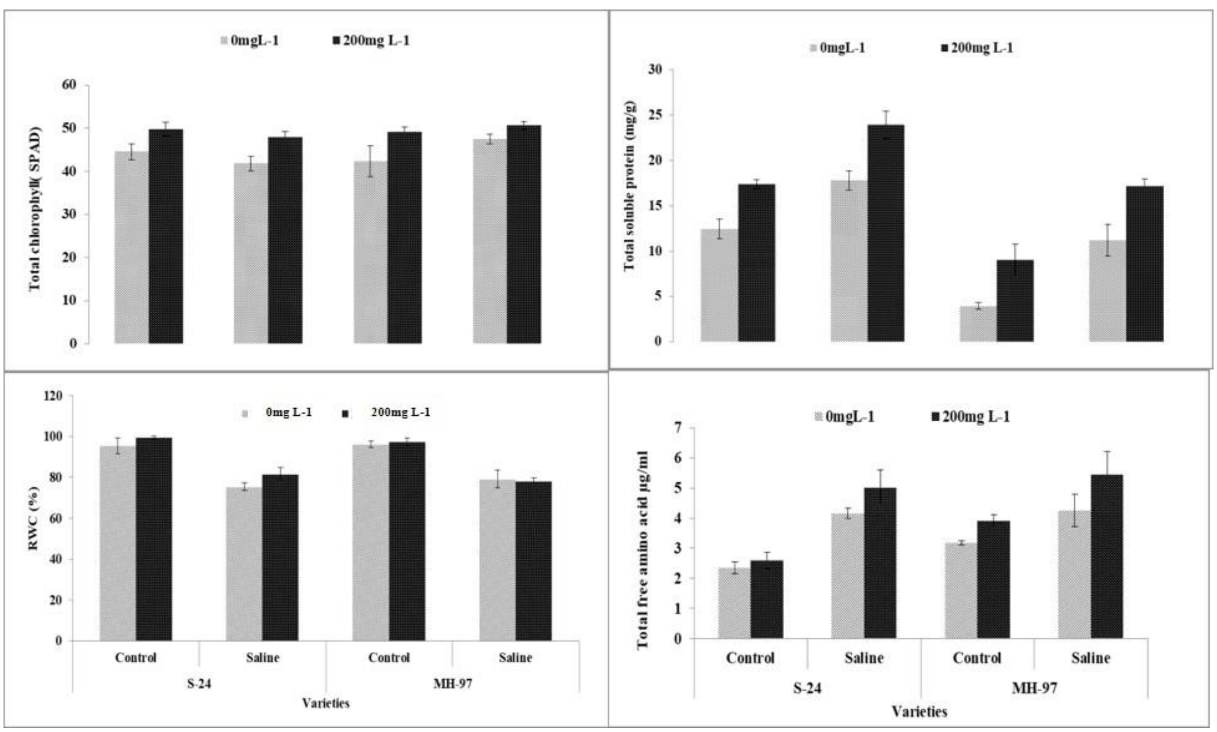

Fig. 2: The effects of foliar spray of salicylic acid on total soluble proteins, total free amino acids, total chlorophyll content and relative water contents of two wheat varieties grown under saline and non-saline conditions.

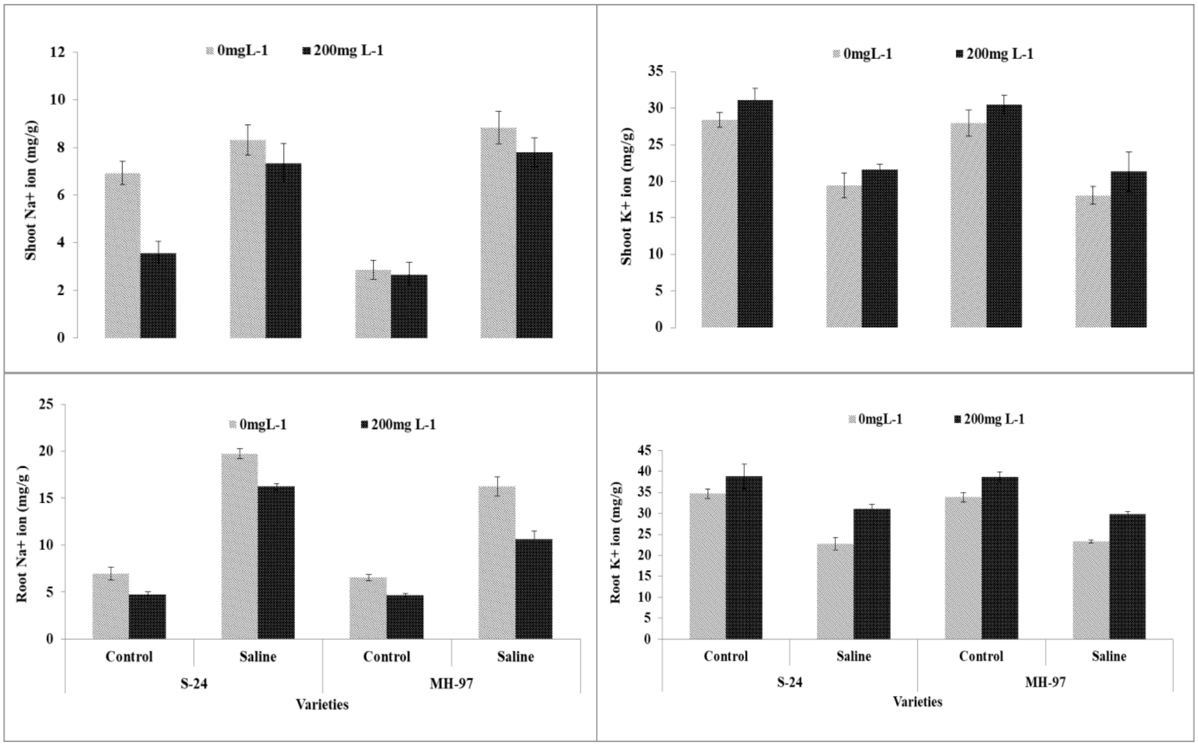

Fig. 3: The effects of foliar spray of salicylic acid on the Na^+ and K^+ ions concentration in shoot and root of two wheat varieties grown under saline and non-saline conditions.

The total chlorophyll content were affected significantly by various factors. However, it was a little influenced by various interaction treatments (varieties x SA levels, salinity regimes x SA levels and salinity regimes x SA levels x varieties). In variety S-24, the chlorophyll content was decreased from 47.15 to 41.62, while it was increased from 40.58 to 44.52 in variety MH-97 under normal and salt-treated soils. The relative water content (RWC) was affected significantly by various factors. The RWC was found higher by 3.34% in variety MH-97 compared to variety S-24. The spray of SA increased RWC by 19.57% over unsprayed crop. Contrarily, RWC was reduced by 16.1% under saline condition in comparison with salt treated soils (Fig. 2).

The varieties significantly differed in the concentration of Na^+ and K^+ in roots and shoots in response to SA levels and salinity. However, both varieties maintained similar values of K^+ content in their root tissues. The spray of SA caused improvement in K^+ concentration from 13.45 to 15.26 mg g^{-1} compared to untreated crop. There was decrease in K^+ concentration from 14.42 to 12.26 mg g^{-1} under non saline condition (Fig. 3). The Na^+ in root tissues were affected significantly due to varieties and salinity levels. While, it was a little influenced by foliar spray of SA. Moreover, it was also unaffected by various interactive treatments (varieties x SA levels, salinity regimes x varieties, salinity regimes x SA levels and salinity regimes x SA levels x varieties. The pattern of Na^+ in root tissues differed significantly ($P<0.01$) by various varieties. The root tissues of variety S-24 absorbed 16.96 mg Na^+ g^{-1} compared to variety MH-97 having 15.18 mg Na^+ g^{-1} (Fig. 3).

DISCUSSION

The adverse effects of salinity on agricultural production exceed far a greater proportion than any environmental factors. The agricultural production areas either irrigated and/or rainfed are equally vulnerable to salinity stress. The crop husbandry practices, including growing of salt tolerant crop species, soil amendments and spraying of plant growth regulators are of common practice in most of the areas. The exogenous spray of SA could alleviate stress in crop species caused by salinity (El- Tayeb, 2005).

The results of the present study indicated that various attributes of growth and accumulation of Sodium and Potassium ions were substantially affected by salinity. The varieties showed differential pattern of response to salt stress. The variation in response to salts stress was associated with genetic make-up of different crop species, composition of salt in the growth medium and management practices (Iqbal and Ashraf, 2005). Thereby, the production of biological yield varied greatly in response of salinity levels and treating the crop with salicylic acid. The maintenance of low osmotic potential under saline conditions reduces the absorption of water from rooting zone, thereby osmotic adjustment is not sustained (Ashraf, 1994). The plant species strive to adjust the situation for continuation of their life cycle. The stressful conditions induce anti-oxidant defense system and accumulate the organic osmolytes to counter the negative effects of external stress (Noreen et al., 2018; Li et al., 2018). The accumulation and over production of organic solutes maintain the osmotic adjustment for continuation of growth (Munns et al., 2000).

The results showed that exogenous application of SA resulted in improving the biological yield under saline as well as non-saline conditions. Various researchers reported that grain yield of wheat was improved by spraying of SA under salt stress conditions (El-Tayeb, 2005; Singh and Usha, 2003). Other than spraying of SA, the spray of glycine–betaine has also been found effective in reducing the negative effects on salinity *Brassica* species and spring cereals (Makela et al., 1996). The results of this study are collaborated with those of Khodary (2004) and El-Tayeb (2005) that SA enhanced growth rate and also counteracted the negative effects caused by salinity stress in various crop species. The response of crop species to SA vary greatly due to time of application, stage of crop growth, crop species and concentration of hormone being applied (Noreen et al., 2013). Various researchers (Singhvi et al., 2002; Jayachandran et al., 2001; Sivakumar et al., 2001;) reported that arable crops, namely rice and pearl millet responded positively in mitigating the deleterious effects of salinity by spray of SA ranging from 100 to 150 mg L^{-1} during vegetative growth.

The similar results were reported by Chandra et al. (2007) that deleterious effects of salts could be off-set through exogenous application of SA on various crop species. The amount of total soluble sugars and soluble proteins were increased in cowpeas plants, which accrued in safeguarding the cell membrane structures and maintaining the various functions involved in growth and development. It was attributed that maintenance of differential quantities of total soluble proteins and free amino acids were associated with inherited characteristics and eco-edaphic factors in production areas. Moreover, the varieties maintained similar quantities of chlorophyll content in their leaf tissues under both non-saline and salt-stressed environments. Contrarily, chlorophyll content was impacted to a greater extent in response to salt-stress or water-stress (Tas and Tas, 2007). The effects of water-stress and/or salt-stress could be reduced to a proportionate level by spraying SA (Zhang et al., 2004). It was further revealed that absorption of Na^+ by both crop varieties was increased with concurrent reduction in K^+ content under saline environment. It was reported that wheat cultivars absorb higher quantities of K^+ due to foliar spray of salicylic acid. However, the absorption and translocation of K^+ and Na^+ by barley crop was reduced in response to foliar spray of SA under saline conditions (El-Tayeb, 2005). Zhang et al. (1998) observed that spray of salicylic acid favored in absorption and translocation of higher quantities of K^+, with simultaneous reduction in Na^+ and Cl^- ions by plant tissues in response to salt stress. In conclusion, the exogenous application of SA proved to be highly effective in mitigating the drastic effects of salinity stress in both wheat varieties i.e. S-24 and MH-97. The exogenous application of SA improved antioxidant defensive mechanism and induced the tolerance mechanism of the plants under abiotic stress conditions. Thus, wheat crop can be sprayed with SA (200 mg L^{-1}) to reduce the salinity stress under arid environment.

Authors' contribution

SN conceived the idea, designed the project and wrote the manuscript; AS performed the experiments. UA helped in Lab-work; KHS participated in the design of the study and helped in conducting the experiments, data analysis and writing the manuscript. All authors read and approved the final manuscript.

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