Journal of Bioresource Management

Volume 8 | Issue 4 Article 4

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Recommended Citation

Aslam, Z., Ahmad, A., Ali, A., Sher, A., & Sarwar, M. (2021). Salinity Stress Mitigation by Foliar Feeding of Salicylic Acid on Maize (Zea Mays L.) grown under Hydroponic Culture, *Journal of Bioresource Management, 8* (4).
DOI: https://doi.org/10.35691/JBM.1202.0203
ISSN: 2309-3854 online
(Received: May 1, 2021; Accepted: Jul 23, 2021; Published: Oct 28, 2021)

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SALINITY STRESS MITIGATION BY FOLIAR FEEDING OF SALICYLIC ACID ON MAIZE (*ZEA MAYS* L.) GROWN UNDER HYDROPONIC CULTURE

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ABSTRACT

Salicylic acid (SA) is mainly associated with the regulation of plant growth particularly in salinity stress conditions and this signaling molecule exists in pivotal parts of plant. The objective of this research analysis was to overcome stress condition by distinct foliar treatments of Salicylic acid, for instance, 0 and 100 mM. Foliar applications of SA on maize were rendered on 10 days later than transplanting under a hydroponics experiment. Plants were grown under non-saline (S_0= 0 mM NaCl) and saline (S_1= 100 mM NaCl) conditions. Evaluation of biochemical, physiological and morphological attributes of maize was rendered after harvesting of plants. The experimental layout of Completely Randomized Design (CRD) under a factorial arrangement with three replications of each treatment was assigned for this study. According to our results it was confirmed that cultivation of maize under saline condition reduced the morphological, physiological and biochemical attributes of plant. However, exogenous application of SA on maize had a positive impact on the above mentioned traits under presence and absence of saline environment. Finally, it was justified that exogenous application of variable concentration of SA significantly improves whole parameters of maize cultivar.

Keywords: Biochemical, hydroponic culture, maize, salicylic acid, salinity stress.

INTRODUCTION

Maize (*Zea mays* L.) is categorized into C_4 plant species and it is a 3rd important cereal crop followed by wheat and rice (Araus et al., 2002). This cereal crop is widely grown for production of grain which is nutritionally consumed by human being and produce fodder as a dietary source for livestock consumption. The production of maize is more critical to abiotic factors and reduced by heat, drought, water logged soil, salinity condition, prolonged coldness and wind (Hara et al., 2011). Saline stress is a main constraint factor to reducing the productivity of crop. The uptake capability of water by the plant presumably reduce under the high concentration of salt resultantly decline the growth rates, additionally with a suitable metabolic alternations similar to those induced by drought condition.

Salinity is a main constraint factor that is associated with reduction of crop yields and decreases the usage of formerly cultivated lands. Salinity stress has adversely affected approximately 45 million hectare from 230 million hectare of irrigation lands (20 %) in the world (Ahmad et al., 2013). Accumulation of Salinity is enhancing in the irrigated canal regions of Pakistan. Elevated salt concentration in soil mainly associated in induction of ionic toxicity which resultantly changed the K^+/Na^+ ratios in cytosol, impact on plant growth, creation of osmotic pressure and expansion of plant cell. The disruption of ionic homeostasis lead to denature the structural and functional proteins in plant cells (Zahu, 2001). Scarcity of water availability is

most constraint issue that mainly causes reduction of crop yield and adverse effect on the majority of farmed regions all around the world. Plants correspond adversely under water scarcity and modify to salinity environment by distinct biochemical and physiological variations, involving phonological alternations (Golbashy et al., 2010).The accumulation of cytosol compatible organic osmolytes for instance, proline, betaines and polyols are associated in adaption of crop plants under salinity stress. Exogenous application or seed treatment of chemicals including salicylic acid, glycine betaine and kinetin may enhance the yield of various crops that will lead the enhancement of plant growth under stress induced condition and application of these chemicals mainly increase leaf area, chlorophyll contents, dry mass production, leaf area index and productivity attributes of crop (Khan et al., 2003; Elwana and El-Hamahmy 2009; Karlidag et al., 2009).

Plants generally correspond to stress conditions through the formation of signaling compounds. These signaling compounds are involved in activation of wide ranges of signaling transduction pathways, most of which mitigate the plant to overwhelm the stress conditions. Numerous signaling compounds such as Ethylene (ET), Jasmonic acid (JA), Salicylic Acid (SA) and Calcium (Ca) have been determined in plants. The functionality of SA in plant acts as a defense signal which was briefly explored in several plants (Klessig and Malamy 1994; Ganesan and Thomas 2001).

The physiological and biological roles of Salicylic acid (2-hydroxybenzoic acid) in plants, has qualified as a plant regulating hormone (Raskin, 1992), and it has been inferred as messenger or signal transducer in stress environments (Klessig and Malamy 1994). Comprehensive studies have been conducted to elaborate the molecular study of induced Systemic resistance (SAR) (Raskin 1992; Levin et al., 1994; Mur et al., 1996). Whereas, now knowledge regarding to growth regulator hormone (SA) associates in modification of physiological and biochemical processes, and signal regulating mechanism of plant resistance to stress conditions are still not studied (Senaratna et al., 2000; Shakirova et al., 2003). SA occurs naturally in plant which acts a plant regulator hormone. This plant regulator hormone influences several biochemical and physiological processes in plants it is an important tolerant and signals transduction molecule in stress environments. This signal molecule is contributing a pivotal role in the regulation of various physiological processes of plant and tolerates the salinity stress in maize crop (Arfan et al., 2007). Hence, the current study was strategically organized to evaluate the performance of SA in maize plant under salinity stress environments.

MATERIALS AND METHODS

Study Location

The experimental study regarding the Influence of salicylic acid on maize (*Zea mays* L.) grown under salinity stress was conducted in kharif season of 2019 at Green House, University of Agriculture Faisalabad at location of (altitude 184 m, latitude 31.40º N, longitude 73.05º E).

Experimental Design

Experimental design consists of CRD under factorial arrangement. Seeds of FH-988 hybrid cultivar maize were sown in iron trays with rinsed sand and water was provided on every day to regulate the optimum moisture condition for seedling growth of maize seeds. Eight days after sowing, two leaves appeared at seedling growth stage, transplanting of seedlings was conducted in thermpore sheet holes with the support of foam wrapped at junction of root and shoot.

Table 1: Composition of Hoagland's nutrient solution.

Reagents	Stock solution g/L 1M	ml stock/L for half strength Hoagland solution	ml stock/100L for half strength Hoagland solution
Macronutrients			
$MgSO_4.7H_2O$	246	1.0	100
KNO_3	101	2.5	250
KH_2PO_4	136	0.5	50
$Ca(NO_3)_2.4H_2O$	236	2.5	250
Micronutrients			
$MnCl_2.4H_2O$	1.81	0.5	50
H_3BO_3	2.86	0.5	50
Fe-EDTA	37.33	0.5	50
$H_2MoO_4.H_2O$	0.02	0.5	50
$CuSO_4.5H_2O$	0.08	0.5	50
$ZnSO_4.7H_2O$	0.22	0.5	50

pH of the Hoagland solution was adjusted at 6.0 - 6.5 with H_2SO_4 or NaOH

Every seedling was dangled on 100 L of iron tube with ½ concentration of Hoagland's nutrient solution (Hoagland and Arnon, 1950).

The nutrients solution was altered for one week. Later transplanting of four days, plant were exposed to salinity of (0 and 100 Mm) absent and present of NaCl in Hoagland's nutrient solution correspondingly. Different concentrations, 0 and 100 mM of SA were treated as foliar technique. 6-6.5 pH for solution was adjusted with NaOH or H_2SO_4 throughout the experiment on daily basis. Shoot and root length (cm) of ten seedlings from every experimental unit were assessed by incorporation of meter rod and data were averaged. Fresh and dry weight (g) of shoot and root were measured with the support of digital electrical balance.

Counting of number of leaves of every plant from each cultivar was rendered manually and data were averaged. Leaf area (cm^{-2}) was measured by Leaf Area Meter. Stem diameter (cm) measured by meter rod and average was taken. Water potential (-MPa) was measured by Pressure Chamber and average was taken. Osmotic potential (-MPa) was measured by Osmo-meter and average was taken. Chlorophyll contents of leaf of every plant from the whole cultivars were assessed by chlorophyll meter (SPAD value). Photosynthetic rate (µmole $m^{-2}s^{-1}$) was measured by Infra-Red Gas Analyzer (IRGA) and average was taken and determination of Relative water contents (RWC %) of plant leaf was performed regarding to following equation of recommended by Schonfeld et al., (1988).

RWC (%) = [(fresh weight - dry weight) / (turgid weight - dry weight)]*100

MSI (Membrane stability index) was evaluated regarding to the mentioned formula of Sairam et al., (2002), given below,

$MSI= (1-(C_1/C_2)) x100$

Samples of completely expanded leaf, root and stem were separated and fresh weights of each sample were recorded on oven dried weight. Digestion of each samples were done in 25 ml solution of 1 % HNO_3 on hot plate for 4 h at 85 °C. After digestion, digested mixture of 1 ml was further diluted to make 10 ml concentration for determination of K^+ (m mol g^{-1} dwt^{-1}) and Na^+ (m mol g^{-1} dwt^{-1}) quantities in each samples through flame photometer (Sherwood, UK, Model 360), regarding general method of USDA Laboratory Staff (1954). The superoxide dismutase and peroxide (µmol mg^{-1}

protein) activity was assessed to measure its capability to suppress the photo-reduction of nitro blue tetra zolium (NBT), regarding procedure of Giannopolitis and Ries (1977). The 3 ml of reaction solution included 50 µM phosphate buffer 13 mM methionine, 50 µM NBT and 1.3 µM riboflavin respectively.

Statistical Analysis

The statistical analysis of data was interpreted by incorporation of Fisher's analysis of variance (ANOVA) method. At 5 % probability level, data was evaluated for comparison of significant treatment means (Steel et al., 1997).

RESULTS

Our results represented in table 2 showed significant reductions in root and shoot length under salinity condition. Statistically minimum root (18.33 cm) and shoot length (60 cm) were observed under saline conditions without any foliar spray. However, under saline conditions foliar spray of salicylic acid @100 mM significantly improved root (27.88 cm) and shoot length (79.56 cm). An increase of 52.10 % and 32.6 % was recorded respectively by the foliar spray of salicylic acid. Data regarding root and shoot fresh weight is presented in table 2 which showed that statistically minimum root fresh weight (15.33 g) and shoot fresh weight (66.44 g) was observed under salinity conditions. Whereas, significantly higher root fresh weight (23.11 g) and shoot fresh weight (78.44 g) was observed by the foliar spray of salicylic acid. By applying foliar spray of salicylic acid, an increase of 50.75 % and 18.06 % was observed in root fresh weight and shoot fresh weight, respectively. Data pertaining root and shoot dry weight is presented in table 2 which showed that minimum root dry weight (1.95 g) and shoot dry weight (3.35 g) was observed under salinity conditions. Whereas, significantly higher root dry weight (3.30 g) and shoot dry weight (5.77 g) was observed by the foliar spray of salicylic acid. By applying foliar spray of salicylic acid an increase of 69.23 % and 72.24 % was observed in root dry weight and shoot dry weight, respectively.

Significantly minimum number of leaves (7.77 g) and leaf length (11.77cm) was observed under salinity exposure in table 3. However, foliar spray of salicylic acid proved to increase number of leaves (8.88) and leaf length (26.23 cm). Data regarding leaf area per plant and stem diameter is presented in table 3 which showed that statistically minimum leaf area per plant (342.2 cm^{-2}) and stem diameter (0.56 cm) was observed under salinity conditions. Whereas, significantly higher leaf area per plant (713.2 cm^{-2}) and stem diameter (1.01 cm) was observed by the foliar spray of salicylic acid. By applying foliar spray of salicylic acid, an increase of 108.42 % and 80.36 % was observed in leaf area per plant and stem diameter, respectively. Data pertaining leaf water and osmotic potential is presented in table 3 which showed that statistically more negative water potential (-1.25 MPa) and osmotic potential (-0.29 MPa) was observed under salinity conditions.

Whereas, foliar spray of salicylic acid tends to decrease the water potential (-0.21 MPa) and osmotic potential (-0.24MPa) towards zero According to our results significantly minimum chlorophyll contents (27.11 SPAD Value) and photosynthetic rate (11.55) was observed under salinity exposure in table (3, 4). However, foliar spray of salicylic acid proved to increase chlorophyll contents (29.44 SPAD Value) and photosynthetic rate (13.66). By applying foliar spray of salicylic acid, an increase of 8.59 % and 18.27 % was observed in chlorophyll contents and photosynthetic rate, respectively.

Table 2.: Effect of salinity stress and foliar spray of salicylic acid on growth parameters of maize

Treatments	Root length (cm)		Shoot length (cm)		Root fresh weight (g)		Shoot fresh weight (g)		Root dry weight (g)		Shoot dry weight (g)	
	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)
C0ntrol	34.22 b	18.33 a	90.78 b	60 d	28.77 b	15.33 d	90.56 b	66.44 d	3.97 b	1.95 c	7.50 b	3.35 d
Salicylic acid spray@100mM	42.88 a	27.88 c	119.0 a	79.56 c	35.33 a	30.11 c	102.89 a	78.44 c	5.77a	3.30 b	10.22 a	5.77 c

Table 3.: Effect of salinity stress and foliar spray of salicylic acid on growth and physiological parameters of maize

Treatments	Number of leaf		Leaf length (cm)		Leaf area per plant (cm^{-2})		Stem diameter (cm)		Water potential (-MPa)		Osmotic potential (-MPa)		Chlorophyll contents (SPAD Value)	
	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)
Control	9.88 b	7.77 c	30 b	11.77 d	934 b	342.2 d	1.12 b	0.56 d	0.47 c	1.25 a	0.19 c	0.29 a	35 b	27.11 c
Salicylic acid spray@100mM	14.44 a	8.88 bc	43.88 a	26.23 c	1038.7 a	713.2 c	1.30 a	1.01 c	0.27 d	0.74 b	0.21 c	0.24 b	41.11 a	29.44 c

Table 4: Effect of salinity stress and foliar spray of salicylic acid on physiological and biochemical parameters of maize

Treatments	Photosynthetic rate		Relative water contents (%)		Membrane stability index (%)		K^+ (m mol g^{-1} dwt^{-1})		Na^+ (m mol g^{-1} dwt^{-1})		Superoxide dismutase (µmol mg^{-1}protein)		Peroxide (µmol mg^{-1}protein)	
	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)	Non-saline (S_0)	Saline (S_1)
Control	16.33 b	11.55 d	82.87 c	73.55 d	80.88 c	71.55 d	171.33 a	121.22 c	26.33 c	99.77 a	161.56 b	74 d	13.44 b	6 d
Salicylic acid spray@100mM	21.33 a	13.66 c	92.44 a	86.44 b	89.88 a	84.44 b	137.78 b	101.89 d	21.11 c	67.33 b	200.78 a	121.56 c	19 a	10.22 c

Data regarding relative water content and membrane stability index is presented in table 4 which showed that statistically less relative water content (73.55 %) and membrane stability index (71.55 %) was observed under salinity conditions. Whereas, foliar spray of salicylic acid tends to increase the relative water content (71.55 %) and membrane stability index (84.44 %). Significantly maximum potassium (121.22 m mol g^{-1} dwt^{-1}) and sodium contents (99.77 m mol g^{-1} dwt^{-1}) was observed under salinity exposure (Table 4). However, foliar spray of salicylic acid proved to decrease potassium contents (101.89 m mol g^{-1} dwt^{-1}) and sodium contents (67.33 m mol g^{-1} dwt^{-1}). By applying foliar spray of salicylic acid, a decrease of 18.97 % and 48.18 % was observed in potassium and sodium content, respectively. Data pertaining superoxide dismutase and peroxidase is presented in table 4 which showed that statistically less superoxide dismutase (74 µmol mg^{-1}protein) and peroxidase (6 µmol mg^{-1}protein) contents was observed under salinity conditions. However, application of SA tends to increases the enzymatic concentration of superoxide dismutase at (121.56 µmol mg^{-1}protein) and peroxidase at (10.22 µmol mg^{-1}protein).

DISCUSSION

Current experimental trial was conducted to evaluate the salinity in maize cultivar by exogenous application of Salicylic Acid spray. SA has a major contribution in control of salinity and can be utilized as plant growth regulator to elevate the plant growth in saline stress environments (Mohamed and Ahmed, 2010). The significant reduction in shoot length was owing to the accumulation of excessive salt concentration which also considerably impacts on length of root (Gulzar et al., 2003). In current study, root and shoot length was seriously impacted owing to salinity stress. Sudden decline in dry and fresh weight under prune of salinity was owing to toxic effect of ions (Zhu, 2002). Root and shoot length of maize cultivars is alleviated by elevating the concentrations of salt (Pessarakli and Kopec, 2009). Similar results were also reported by Gill and Singh (1989) in rice. The application of salicylic acid significantly increased the number of leaf, leaf length, and leaf area and stem diameter. However, imposing salinity significantly decreased the number of leaf, leaf length, and leaf area and stem diameter. These results are in accordance with those reported by Pirlak and Esitken, 2004.

The results showed that salinity significantly decreased the water potential as well as osmotic potential. These results are in accordance with those reported by Chinnusamy and Zhu (2003) who concluded that salinity reduced the osmotic potential as compared to untreated control which might result in decreased water availability. Salicylic acid efficiently increased the osmotic potential under salinity that is necessary to restore the turgor pressure. Chinnusamy and Zhu (2003) reported that survival of plants depend on maintaining a positive turgor pressure, which is essential for growth of cells, expansion and stomata opening.

Like other researchers findings of our study showed that chlorophyll content was significantly decreased by salinity and foliar spray of salicylic acid improved chlorophyll content. These finding are in accordance with Parida and Das (2005) who concluded that salt stress decreased the chlorophyll content in leaves of many crops. The increase in chlorophyll content in maize by applying salicylic acid confirmed the results of Gunes et al., (2007). It has been explicitly justified that foliar application of salicylic acid had positive impact on RWC in salinity condition. Water balance in plant is maintained by the transpiration loss of water and the difference of water absorbed

from the soil. The transpiration rate elevates the water absorption rate in salinity condition resultantly decreased relative water contents (RWC) (Tas and Tas, 2007) and causing in reduction of cell turgor pressure. Abscisic acid reduced the stomatal opening which resultantly regulate high relative water contents under saline condition and relatively maximum growth of roots was reported (Makoto et al., 1990), lead to regulate cell turgidity, photosynthetic rate and chlorophyll content (Keyvan, 2010).

Membrane stability (MSI) is main constituent factor of salinity control. High membrane stability is indicated by lower amount of ion leakage from cell membrane (Jaleel et al., 2007). Exogenous spray of Salicylic acid mainly enhances the Ca^{+2} accumulations which could regulate the integrity of membrane (Khan et al., 2010). The effect of abiotic stresses is reduced by application of Salicylic Acid which resultantly leads to the improvement of antioxidant system and this is essential to decrease the oxidative breakdown and leakage of ions (Yusuf et al., 2008).

Treatment of appropriate concentration of 100 ppm salicylic acid to the plants under salinity stress may incorporate to regulate K^+ ions in leaf and inhabiting salinity to damage the photosynthesis. These findings are lined with Zahir et al., (2000) who found that application of SA noticeably increased the no. of shoots and roots (Karlidag et al., 2009). Higher concentration of Sodium ions (Na^+) cause ionic toxicity in plants and translocation of ionic Na^+ in cell membrane is an integral factor for the evaluation of tolerance or sensitive of maize genotypes under salinity stress (Khan, 2001). Excessive concentration of Na^+ ions in root, leaf and stem of maize crop exhibited negative impacts over the development of salinity susceptible hybrids. These results are similar to previous researcher's work who has reported that maximum amount of ionic Na^+ is owing to higher concentration of salinity (Munns et al., 1995). Storage of Potassium (K^+) ions in plant is significant for tolerance of salt in plant. Regulation of higher concentration of K^+ ions in stem, leaf and root are supportive for the better performance of hybrids in salinity stress environment (Santa-Cruz et al., 1999).

The explorations of current study exhibited that tolerance of salinity is owing to the maximum ionic ratio of K^+/Na^+ in plants. The imposing salinity resulted in significantly higher concentration of superoxide dismutase and peroxidase in leaves of plants. The salicylic acid foliar spray to plants grown in saline conditions resulted in increased superoxide dismutase and peroxidase concentrations. Similar results are reported by Garratt et al., 2002 who concluded that plants containing high concentrations of antioxidants showed significant resistance to oxidative damage due to activated oxygen species. Current investigation showed that salicylic acid improved the antioxidant system of maize plants by increasing the superoxide dismutase and peroxidase concentration in leaves. The results are in confirmatory with that of Chen et al., (1997) who find out that superoxide dismutase and peroxidase activity increases in response to salicylic acid application.

CONCLUSION

It has been conclusively recorded that higher concentration of salt (NaCl) is associated in induction of salinity stress which drastically suppress the physiological, morphological and biochemical characters of maize cultivars. However, exogenous application of SA spray at concentration of 100 mM reduced the salinity stress effects. The exogenous treatment of SA was too valuable for plants grown under un- saline conditions. This signal molecule associated in accumulation of K^+ ions in plant and elevated the chlorophyll contents and reduced the accumulation of Na^+ ions in

plants. Salycylic acid is noticed as a main protectant molecule under salinity stress of maize. Therefore, exogenous supplementation of this hormone effectively reduced the salinity stress in plants. Moreover, further research analysis is mandatory for attaining compressive outcome for practical aimbition. Exogenous treatment of this chemical is an efficient way to restrict the adverse effects of salinity in plants.

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