

Exogenously Applied GA₃ Promotes Plant Growth in Onion by Reducing Oxidative Stress Under Saline Conditions

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ABSTRACT

Onion (*Allium cepa* L.) is a biennial crop of high commercial value in Pakistan. Onion is considered as salt sensitive plant species. The present investigation was carried out to investigate the effect of salinity on onion and its alleviation through exogenously applied gibberellic acid (GA₃; 100 mg L⁻¹). Foliar application of GA₃ (100 mg L⁻¹) was applied on onion seedlings grown under three levels (0, 2 or 4 dS m⁻¹) of salinity after 45 days of sowing. Results revealed that growth parameters and total soluble

protein (TSP) contents declined with increase in soil salinity level. While, antioxidant enzyme activities (CAT, SOD and POD) were increased with salinity. However, exogenously applied GA₃ significantly enhanced the plant growth and TSP in onion seedlings. Interestingly, CAT, SOD and POD concentration decreased with GA₃ application which depicts stress alleviation in saline stressed onion plants due to GA₃. It was concluded that the growth of onion could be enhanced to some extent by the application of GA₃ under salinity stress.

Keywords: Allium cepa, PGRs, Saline stress, Antioxidative response, Yield

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1. Introduction

Onion (*Allium cepa* L.) is a biennial crop, and it belongs to *Alliacea* family (Adamicki & Kepka 1974). It is the one of most popular vegetables in the daily diet. Onion is an important commercial crop for the economy of Pakistan (FAO 2012). Salinity has adverse effect on growth and production of agricultural crops (Munns & Tester 2008). Studies showed that due to the saline toxicity every aspect of physiological and biochemical of plant is effect. (Khan & Panda 2008). Plants face two basic problems under saline conditions. Firstly, excessive salt lower down the osmotic potential in soil solution which reduces uptake of water in plant. Secondly, maximum Na⁺ and Cl⁻ ions uptake diverts the absorption rate of essential minerals and ascribe toxicity to plants (Tester & Davenport 2003). Specific ion toxicities damaged the tissues of transpiring leaves which occur due to boron, sodium, and chloride accumulation. This accumulation of adverse ions may reduce protein synthesis, photosynthesis, and inactivate enzymes, and also damaged chloroplasts as well as other plant organelles (Taiz & Zeiger 2002).

Onion lacks tap root system and root hairs. Most of the root system is confined within top 20-25 cm of soil. Plant growth rate is half of other vegetables such as cauliflower and cabbage (Brewster 1994). Onion is more vulnerable to salinity than other vegetables, particularly the seedling emergence stage (Brewster 1994). Threshold electrical conductivity (EC) level for onions is 1.2 dS m⁻¹ at 25 °C and each unit change in EC cause about 16% reduction in yield (Allen et al. 1998).

Gibberellic acid (GA₃) affects production by increasing the stem length and internodes of onion plant. It restricts senescence by change in lipid peroxidation and adjusts high level of cellular antioxidants like superoxide dismutase and catalase (Dhindsa et al. 1982). It also promotes the growth of the plant, promote cell division and cell extension (Olszewski et al. 2002; Ubeda et al. 2009). Sarkar et al. (2018) reported that GA₃ at 60 mg L⁻¹ increased bulb weight over the control under normal conditions. GA enhances the water use efficiency of tomato plants at low salinity level by reducing stomatal resistance (Maggio et al. 2010). So, in the present study, it was hypothesized that GA₃ can alleviate salt stress in onion. Therefore, the present work was taken up under field conditions to determine the impact of different level of salinity on growth of onion and alleviation of stress by GA₃.

2. Material and Methods

The Phulkara genotype which is local variety was selected for this experimental study. Seeds were obtained from Punjab Food Corporation Faisalabad, Pakistan. The experiment was carried out in the vegetable experimental area of Institute of Horticultural Sciences (IHS), University of Agriculture Faisalabad, Pakistan in the year 2018 and 2019.

Seed sowing was done in soil pots containing 10 kg soil. Before the sowing of onion seeds, the electrical conductivity (EC) of the soil from representative area was calculated and compared to the results of real EC dS m⁻¹ of soil. Then artificially salinity was caused by adding the measured quantity of salt (NaCl) in soil and mixed well according to get the desired salinity dS m⁻¹. The calculations were calculated according to the Rayment & Higginson (1992) method. Measured quantity of salt was added to the soil and mixed well by the soil mixer to get the homogeneous mixture.

Then the experimental area was cleaned and layered with polythene tunnel sheet to avoid the leaching of salt in other soil. Inorganic fertilizers were supplied properly at the rate of 50 kg ha⁻¹ K as potassium sulphate, 50 kg ha⁻¹ of N as urea and 80 kg ha⁻¹ of P as di-ammonium phosphate. The six treatments were maintained as; (T_0) control; (T_1) GA₃: GA₃100 mg L⁻¹; (T_2) salinity level: 2 dS m⁻¹; (T_3) salinity level: 2dS m⁻¹ + Gibberellic acid: GA₃100 mg L⁻¹; (T_4) salinity level: 4 dS m⁻¹; (T_5) salinity level: 4 dS m⁻¹ + Gibberellic acid: GA₃100 mg L⁻¹) was applied after 45 days of sowing days. Onion plants were harvested after 135 days of GA₃ application and different growth parameters were analyzed. Samples were saved in -20 °C for biochemical analysis.

2.1. Growth parameters

The plant height was calculated by the scale from the tip of that plant to the base on ground. The length of the leaf blade was measured from the base of leaf of observing plant to the tip. It was done by using a scale meter. Evacuated plants were washed with clean water, straightened and after that its root length was estimating by utilizing a tape meter in centimeters and the average was taken for each replicate. The diameter of onion bulb was calculated at right angles to longitudinal axis at the widest form of the bulb of arbitrarily chosen plants in each plot by using veneer caliper (model 141) (Demisie & Tolessa 2017).

Plants chosen from each treatment were harvested at the end of the experiment. Roots were removed from that plants then they were washed with water to expel the dirt and soil. Then root weight was measured by using weighing balance. Root dry weight was estimated by putting the sample in oven at 65 °C for 72 hr to dry the samples. At that point, weight was ascertained by adjust and the normal mean for each sample was figured.

2.2. Germination percentage

Data was recorded for germination on daily basis after one week of sowing seeds till 14 days. Germination percentage was then calculated by using the following formula:

Germination percentage =
$$\frac{\text{no.of germinated seeds}}{\text{Total no of seeds sown}} \times 100$$

2.3. Biochemical analysis

Fresh samples were collected from the experimental area and stored at -20 °C. All the samples were crushed in 50 mL of 100 mM sodium phosphate (pH 7) buffer containing 0.5% (w/v) polyvinyl pyrrolidone and 1 mM ascorbic acid and homogenize mixture was prepared. After preparation of mixture sample were placed at 4 °C for 5 min. The collected mixture was filter by using filter paper and centrifuged it for 15 min at 5000 RPM and the supernatant was collected.

Peroxidase (POD) was determined by using the method of (Onsa et al. 2004). In this method 4-methylecatechol was used as a substrate. To check the activity of POD reaction mixture was prepared by using 4-methylcatechol, 10 mM sodium phosphate buffer, 5 mM H_2O_2 and 500 μ L of total volume of 3 mL crude extract of the sample at room temperature. By using spectrophotometer absorption was measured at 420 nm.

Superoxide dismutase (SOD) activity was determined by using the method of (Kumar et al. 2012). Reaction mixture was prepared by using 0.2 mM EDTA, 12 mM L-methionine, 50 mM buffer of sodium phosphate (pH 7) .10 uM riboflavin, 50 μ M NBT and 100 μ L of final volume of 3 mL of the crude extracted sample. The reaction mixture was incubated into the white light for 15min at room temperature. After incubation of 15 min absorption was observed at 560 nm by using spectrophotometer.

Catalase (CAT) activity was measured by using the method of Aebi 1983. To calculate the CAT activity spectrophotometer was used. The reaction mixture was prepared by using 30 mM H_2O_2 100 mM sodium phosphate buffer (pH 7) and 100 uL crude extract of sample by volume of 3 mL. Absorption was calculated at 240nm at room temperature in spectrophotometer.

The soluble protein (TSP) was measured according to Coomassie Brilliant Blue G-250 Staining Method (Sedmak & Grossberg 1977).

2.4. Statistical analysis

All experiments were conducted in triplicate with two factorial randomized complete block design (RCBD) and all results were expressed as the average \pm standard error of the measurements. Statistix 8.1 software was used for statistics.

3. Results and Discussion

Data about growth parameters (plant height, root fresh and dry weight, leaf blade length, root length, bulb diameter and germination percentage) of treated and untreated plants of onion seedlings are shown in Table 1. The foliar spray of GA₃ 100 mg L⁻¹ showed significant effect on plant height, root fresh and dry weight, leaf blade length, root length, bulb diameter and germination percentage under salinity (Table 1). Previously, Ali et al. (2015) also found that application of GA₃ has positive impact on growth and yield of onion. However, salinity stress reduced the growth parameters as compared to the control conditions (El-Shaieny 2015; Nasri et al. 2017). As the salinity increased, there was a gradual decline in all growth parameters of onion (Stia-Baba et al. 2010). Similarly, results showed that when salinity level increased the plant growth declined as compared to control (Table 1). The decrease in plant growth in saline stress might be as a result of that salinity removes the potassium ions via plant roots, which generates physiological discrepancy because potassium ion is essential for the synthesis of proteins and metabolism (Chen et al. 2007). However, exogenously applied GA₃ enhanced all the growth parameters under salinity stress as compared to their respective controls (Table 1). Similarly, Chauhan et al. (2019) reported that GA₃ enhanced the plant growth under the salinity condition. It might be due to the effect of GA₃ which partially diminishes the toxic effect of salinity by increasing anti-oxidative, vigor, accumulation of osmolytes, and enzyme activities (Neelambari et al. 2018). Another study reported that decline in growth of plant under saline stress because of osmotic stress (Hakim et al. 2010).

Table 1- Effect of Gibberellic acid (GA₃, 100 mg L⁻¹) and salinity (0, 2, 4 ds m⁻¹) on plant height, leaf blade length, root length, bulb diameter, root fresh weight, root dry weight and germination percentage of onion (phulkara variety) plants

Treatments	Plant height (cm)	Leaf blade length (cm)	Root length (cm)	Bulb diameter (cm)	Root fresh weight (g)	Root dry weight (g)	Germination percentage (GP%)
Control	46.7±1.10ab	38.6±1.37b	12.5± 0.77ab	$3.8 \pm 0.52 b$	$56.6 \pm 2.71 \text{b}$	$6.43 \pm 0.41 b$	$77.7 \pm 3.2b$
GA ₃	50.4± 1.15a	47.7± 1.08a	$14.3{\pm}0.72a$	5.5± 0.33a	68.1±2.11a	8.33±0.31a	88.8± 3.2a
2 dS m ⁻¹	$43.7{\pm}~0.92b$	$36.3 \pm 1.21 \text{bc}$	$11.6{\pm}~0.50b$	$3.5 \pm 0.21 \text{b}$	$41.5{\pm}2.06d$	$4.03{\pm}0.42c$	66.6± 3.2c
$2 \text{ dS } \text{m}^{-1} + \text{GA}_3$	$45.1{\pm}1.82b$	37.9±1.19bc	$12.3{\pm}0.70ab$	$3.7{\pm}~0.29b$	$46.9{\pm}~1.62{c}$	$4.8 \pm 0.34c$	83.3±3.2ab
4 dS m ⁻¹	$34.7{\pm}~1.99{c}$	33.6± 1.35c	$7.4 \pm 0.81c$	$2.5 \pm 0.28c$	33.6± 2.19e	$2.9{\pm}~0.55d$	$44.4{\pm}3.2d$
4 dS m^{-1} + GA ₃	$44.8{\pm}~1.23b$	36.7±1.55bc	$11.8{\pm}0.40b$	$3.3 \pm 0.08 \text{b}$	36.2± 2.97e	$3.1{\pm}0.34d$	$75.9 \pm 4.8 \text{bc}$

Each data values are represented as mean and \pm SD of three replications and different lower case letters are representing the significant difference between treatments and same lower-case letters represent the no significant difference by according to LSD test (P \leq 0.05)

Results of biochemical assays depict that salinity stress significantly have increased the activity of antioxidant enzymes (CAT, POD and SOD) as compared to control conditions (Figures 1-3). When the salinity was increased then the activity CAT, POD and SOD was increased maximum in onion. While foliar application of GA₃ further enhanced the activity of CAT, POD and SOD under salinity stress as compared to their respective control. The basic function of POD in plants is to break down the hydrogen peroxide (H_2O_2) which is very toxic and reactive element (Botella et al. 1994). Saline stress condition might be causing the univalent reduction in O_2^- which produce hydrogen peroxide. In the salt stress condition, the level of H_2O_2 was reduced which was the damage of plant defence system due to the higher concentration of POD. Sancho et al. (1996) also stated same consequences in his study and recognized this to variations in the mechanical characteristics of cell wall which in turn, might be connected to the salinity adjustment mechanism. The enhancement of POD with GA₃ under salinity stress (Figure 1) might be due to that GA₃ increases the gibberellins which stimulates the decrease in hydrolytic enzymes and sugars (Mathew & Murray 1968). GA₃ have competition with saline conditions via improving the membrane permeability of plant cell and adjust the level of nutrients in cell. Eventually this leads to increase the growth, and GA₃ also has induced physiochemical variations which are responsible for the influence of salt tolerance (Amal & Mohamed 2014).

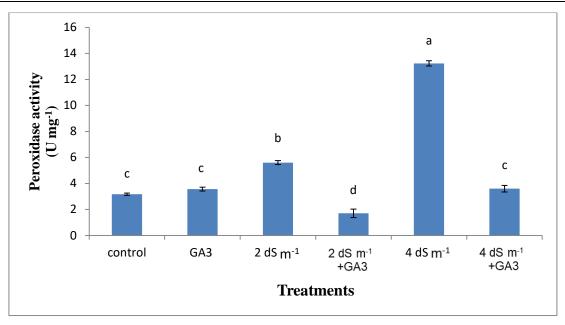


Figure 1- Effect of Gibberellic acid (GA₃, 100 mg L⁻¹) and salinity (0, 2, 4 dS m⁻¹) on peroxidase activity (POD) of onion seedlings. Each data values are represented as mean and <u>+</u>SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

Superoxide dismutase (SOD) is an important antioxidant which scavenges the reactive oxygen species (ROS) and it is activated under stress conditions. This is the reason that SOD enhanced in saline stress condition (Figure 2). It gives the initial line of protection against the noxious effects of stress. SOD eliminates superoxide radicals by catalyzing the dismutation of superoxides and reduces it to peroxide which is also oxidized by another antioxidant POD (Gill & Tuteja 2010). Foliar spray of GA₃ increased SOD activity to some extent because it increased the gibberellins which stimulate the decrease in hydrolytic enzymes and sugars (Mathew & Murray 1968). The SOD decreased in saline treated plants by foliar treatment of GA₃ (100 mg L^{-1}) as compared to control (Figure 2). It is due to that foliar application of GA₃ improves the membrane of plant cell and adjusts the level of nutrients in the cells (Chakrabarti & Mukharji 2003). Eventually this leads to decrease in SOD, and GA₃ induces physiochemical variations which are responsible for the influence of salt tolerance (Amal & Mohamed 2014).

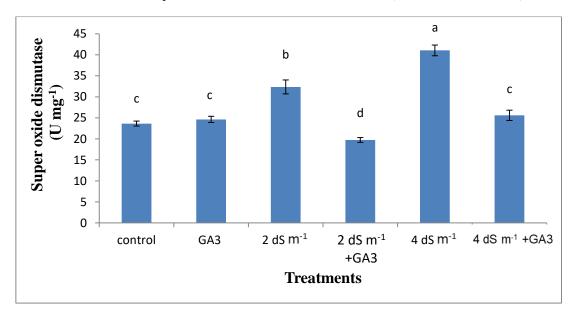


Figure 2- Effect of Gibberellic acid (GA₃, 100 mg L⁻¹) and salinity (0, 2, 4 dS m⁻¹) on superoxide dismutase activity (SOD) of onion seedlings. Each data values are represented as mean and <u>+</u>SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

Catalases (CAT) also plays important role in plant during the saline stress condition. During the saline stress condition, the CAT level increased as compared to control (Figure 3). Eyidogan & Oz (2007) observed that CAT level was increased in the

leaves of *C. arietinum* under saline conditions. In stress conditions, isoforms of CAT presents on different chromosomes which regulate independently (Scandalias 1990). CAT helps the plants to fight against oxidative stress that improves the plant growth (Polidoros & Scandalios 1999). Exogenous application of GA₃ improved the membrane permeability of plant cell and adjusts the level of nutrients in cell. This leads to decrease in CAT and also GA₃ induce physiochemical variations which are responsible for the influence of salt tolerance (Chakrabarti & Mukharji 2003; Amal & Mohamed 2014).

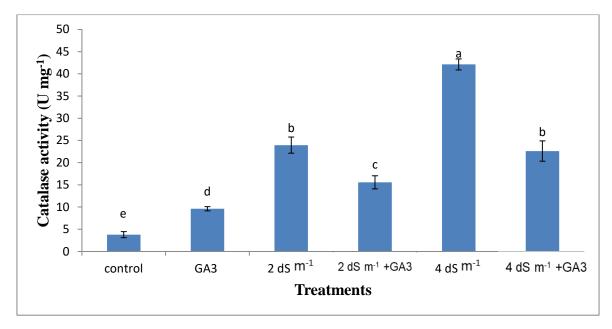
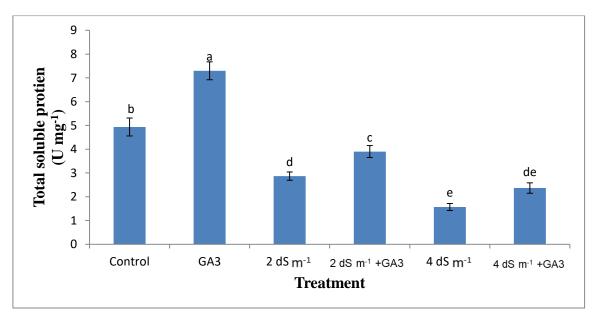
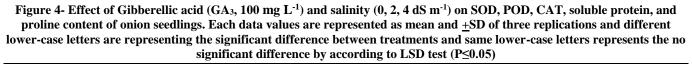


Figure 3- Effect of Gibberellic acid (GA₃, 100 mg L⁻¹) and salinity (0, 2, 4 dS m⁻¹) on catalase activity (CAT) of onion seedlings. Each data values are represented as mean and \pm SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

Total soluble proteins (TSP) has been observed lower when the plants are grown under saline toxic soils (Zhang et al. 2009), some proteins which have defensive mechanism, stimulated the plant growth under salinity and tolerate against salt stress (Aghaei et al. 2008). Significant effects of GA_3 and NaCl and its interaction was observed on TSP contents in onion (Figure 4). TSP contents firstly increased at low salinity levels with GA_3 and then the contents of TSP decreased at high concentrations of salinity level (Figure 4). The similar results were also found by Jiao et al. (2019) in caster bean as the concentration of GA_3 increased, the TSP content first increased and then decreased.





4. Conclusions

Our findings revealed that the salinity stress significantly reduced plant growth parameters in onion seedlings. However, exogenously applied GA₃ significantly enhanced the plant growth and by reducing oxidative stress. Interestingly, a decline was observed in antioxidant enzyme activities (SOD, POD and CAT) with the application of GA₃ in saline stress conditions. Although these activities were increased to maximum level at salinity stress alone treatment. It is concluded from our study that we can enhance the growth of onion plant in saline stress with the foliar application of GA₃. Less is known about the mechanism of GA₃ in onion under salinity stress. Hence, to check the mechanism and role of GA₃ under saline conditions in different plant species more study is required.

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