



**Full Length Article**

## Gibberellic Acid Amended Antioxidant Enzyme and Osmotic Regulation to Improve Salt Tolerance of Okra at Early Growth Stage

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### Abstract

Seed germination and emergence are most staple stages for crops establishment. However, these stages are severely inhibited under salinity stress. External application of regulating substances might be an effective method to alleviate the suppression effects. But the associated physiological mechanism is still in controversial. Therefore, this research evaluated the amendment effects of gibberellic acid (GA<sub>3</sub>) on plant growth, antioxidant enzymes and osmotic regulation of okra (*Abelmoschus esculentus* L.) under salinity stress. The results showed that increasing NaCl level from 0 to 120 mM, growth related parameters like plant height, leaf area, dry weight of root, stem and leaf were significantly decreased. Enzyme activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) were slightly or significantly increased at 60 mM NaCl level, but significantly reduced at the 120 mM NaCl level. The osmotic regulation substances including soluble proteins (SP) and proline (Pro), significantly increased under both 60 and 120 mM NaCl levels. Compared with 300 μM GA<sub>3</sub>, presoaking seeds with 150 μM GA<sub>3</sub> had the best effects on increasing growth-related parameters. The activity of POD and SOD, together with Pro and SP increased prominently at 150 μM GA<sub>3</sub>. These results suggest that using optimum concentration of GA<sub>3</sub> for priming seeds is an alternative way to improve early seedling growth of okra under saline conditions. © 2019 Friends Science Publishers

**Keywords:** Okra; NaCl stress; Gibberellic acid; Germination; Growth; Antioxidant enzyme

### Introduction

Okra (*Abelmoschus esculentus* L.) is an annual herbaceous plant and important source for carbohydrates, fat, vitamins and various minerals, which grown in most parts of tropics and during summer in the warmer parts of temperate regions (Oyelade *et al.*, 2003). During the past decades, the acreage of okra has been progressively increased world widely. Although okra is classified as semi salt-tolerant vegetable crop, the productivity is highly limited due to the salinization of soil and irrigated water in salt affected regions (Oyelade *et al.*, 2003; Akram *et al.*, 2017).

Salinity is defined as the amassment of water-soluble salts in the top layer of soil that drastically affects crop production (Flowers *et al.*, 1977). Currently, more than 7% of land area and over 5% of cultivated lands are affected by salinity in the world (Islam *et al.*, 2013). It has become one of the worldwide major abiotic problems limiting crop growth and production, especially in arid area where soils are often affected by salinity (Munns and Tester, 2008).

Salinity soils usually contain high contents of Na<sup>+</sup> which is a major threat to crop growth by exert various abiotic stresses, such as osmotic stress, ionic stresses, or their combination (Yeo, 1998; Farooq *et al.*, 2015). Under salt condition, some physiological processes are disturbed (Parida and Das, 2005). The ROS (reactive oxygen species), such as O<sub>2</sub><sup>-</sup> (superoxide radical), OH<sup>•</sup> (hydroxyl radical), H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) and singlet oxygen, are massively generated and accumulated in crop plants suffering from salt stress (Dionisio-Sese and Tobita, 1998; Farooq *et al.*, 2017; Naqve *et al.*, 2018). These ROS seriously disturb metabolism balance by imposing oxidative damage to nucleic acids, lipids and protein. Fortunately, the whole system of antioxidant enzymes in plants are activated to protect the plants avoid being injured from ROS. SOD (Superoxide dismutase) is a key scavenger of O<sub>2</sub><sup>-</sup> and its biological function result in producing H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>. Then the H<sub>2</sub>O<sub>2</sub> is resolved by POD (peroxidases) or be directly resolved into harmless H<sub>2</sub>O and O<sub>2</sub> by CAT (catalases).

In addition, salt stress can change the expression levels

of plant hormones (Farooq *et al.*, 2015). The balance of plant hormones can be disturbed under salinity stress (Farooq *et al.*, 2017). Exogenous hormones can be applied at proper concentrations to maintain the endogenous hormone balance in plants and also be a beneficial pathway to promote germination and seedling growth under abiotic stress (Datta *et al.*, 1998; Zhou *et al.*, 2014). Among them, gibberellic acids (GA<sub>3</sub>) play a key role in adjusting plant growth and developmental events, such as germination, hypocotyl elongation, stem lengthening, leaf expansion, floral initiation, flowering and grain filling (Swain and Singh, 2005; Jaleel *et al.*, 2009). The GA<sub>3</sub> also play a crucial role in plants defense reactions to stresses (Srivastava and Srivastava, 2007). A large number of studies have shown that application of suitable concentrations of GA<sub>3</sub> significantly increased the seed germination and seedling growth (Kaur *et al.*, 1998), improved the plant height, leaf area, dry weight, nutrient uptake and grain yield of wheat and castor bean under saline conditions (Zhou *et al.*, 2014). However, to date, the effects of GA<sub>3</sub> on the germination, early growth and antioxidant enzymes of okra have rarely been reported. We hypothesized that the application of GA<sub>3</sub> at suitable concentrations might also be used to increase germination and early growth of okra. The study is critical importance to improve early seedlings growth and production of okra under salt saline conditions.

Therefore, the purpose of this study was to: (1) investigate the interactive effects of exogenous application of GA<sub>3</sub> and salinity on germination and early growth, osmotic substance and antioxidant enzymes of okra plants and (2) ascertain the appropriate GA<sub>3</sub> concentration for early growth of okra grown under salinity environments.

## Materials and Methods

### Plant Material

The study was conducted in Crop Stress Physiology Laboratory of Yangzhou University, Jiangsu Province, China in 2016. Two local grown varieties YK and LV were used in this study. The seeds of two okra cultivars were provided by Yancheng Academy of Agricultural Sciences, Jiangsu Province, China. The seeds were harvested in 2015 and carefully stored in brown bags at 4°C to maintain the germination potential. Before experiment, the germination percentage of the seeds was over 90%. The uniform-sized seeds were chosen, sterilized with 0.1% HgCl<sub>2</sub> for 30 min, and then carefully washed with deionized water. Prior to the study, prepared fine sand was used to seeding media. The sand was washed with deionized water till clean and sterilized in a heated dryer at 80°C for 48 h.

### Experimental Design

This study consisted of two experiments. The first experiment was conducted to compare the two varieties

(YK and LV) in terms of germination at the three NaCl concentration of 0 (control), 60 and 120 mM. This experiment was set in a 2 × 3 factors by completely randomized design with four replicates. The seeds of each treatment were sown at a seeding depth of 2.5 cm in a stainless steel tray (40, 30 and 4.0 cm in length × width × depth), filled sands with 3 cm depth and irrigated with 1/2 Hoagland solution containing NaCl at the corresponding concentration. One tray with 30 seeds and 3 trays per replication. All trays were putted in germinators (Model GXZ, Jiangnan Instrument Co., Ltd., Ningbo, Zhejiang, China) keeping 25°C and 70% RH for 12 d. To avoid moisture deficiency, deionized water was added to trays at a 2 d interval to compensate for at the evaporation loss. From this experiment, the variety LV was found more tolerant to NaCl in terms of germination as compared with the variety YK. Hence, the variety LV was used in the second experiment for further study.

The second experiment was conducted to investigate the effect of GA<sub>3</sub> and NaCl on early seedling growth and anti-oxidant enzymes under salinity stress. The experiment was arranged in a 3 × 3 factorial (three GA<sub>3</sub> concentration: 0 (control), 150 and 300 μM; three NaCl concentration: 0 (deionized water as control), 60 and 120 mM) completely randomized design with three replicates. Okra seeds, same treated as in the first experiment, were soaked by deionized water (control) or GA<sub>3</sub> solutions at different concentration levels at 25°C for 12 h. Then the seeds were placed in stainless steel trays (the same as in the first experiment) containing 1/2 Hoagland solution and finally placed in the germinator for 48 h to accelerate germination. The uniform germinated seeds in each tray were carefully chosen for seedling culture in plastic cylinder pots (10 cm diameter × 9 cm height, without holes at the bottom). There were 27 pots for this experiment, three pots for each treatment (one pot as a replicate). Each pot was put into 0.4 kg sand and then sown 3 cm depth with 30 germinated seeds. After that, each pot was added with 90 mL 1/2 Hoagland solution at different NaCl levels. All pots were randomly placed in growth chamber (Model PYX-300G-B, Jiangsu Co., Ltd., Jiangsu, China) for 12 days. The growth chamber was maintained 30/25°C (day/night) and photoperiod of 12 h and 600 μmol m<sup>-2</sup> s<sup>-1</sup> light intensity. The relative humidity was determined at 65% using an automatic water spray system (Model OK-TS4, Zhengzhou OKQ Instrument Co., Ltd., Zhengzhou, China). Both experiments in this study were conducted twice under the same environmental conditions.

### Observations and Measurements

For the first experiment, the number of germinated seeds of each tray was counted on the 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> d after seeding, and germination was calculated as the quantity of germinated seeds as a percent of the total sown seeds. For the second experiment, in each treatment and replication, 15 seedlings were randomly chosen on the 12<sup>th</sup> d after seeding

for plant height (PH) determination. Then, 5 out of the 15 seedlings were selected and washed carefully. Then, the plants were separated into root, leaf and stem. The leaf area (LA) was measured with leaf area meter (LI-3000A, Lincoln, NE, USA). After that all samples were put in oven dried at 70°C till constant weight to biomass measurement. Finally, the remained 10 seedlings were harvested and their leaves were cut, washed with distilled water, immersed by liquid nitrogen for 15 min, then stored in freezer (-80°C) to assay SOD, POD, CAT, proline and soluble protein. The activity SOD, CAT and POD were referenced Janmohammadi *et al.* (2012) and Xu and Ye (1989). The contents of soluble protein and proline were determined according to Zhou *et al.* (2014).

### Statistical Analyses

Analysis of variance (ANOVA) was performed with Statistix 9.0 (Analytical Software, FL, USA), the mean values were compared based on the least significant difference (LSD) test at  $P < 0.05$ .

## Results

### Germination

The variety, salinity, and their interaction had significant effects on the seeds germination of okra ( $P < 0.01$ ). The germination percentage of YK was significantly lower than LV. On the 12<sup>th</sup> d, the germination percentage of LV was 7.6, 15.6 and 29.8% higher than YK under each salt stress. As compared with control, the germination of YK on the 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> d was declined 12.7 and 35.7%; 23.1 and 33.2% and 18.6 and 28.6% at 60 and 120 mM NaCl, respectively. For LV, 60 and 120 mM NaCl reduced germination by 18.5 and 33.3%; 20.0 and 21.5% and 12.6 and 19.8%, respectively on the 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> d after seeding (Fig. 1).

### Seedling Growth

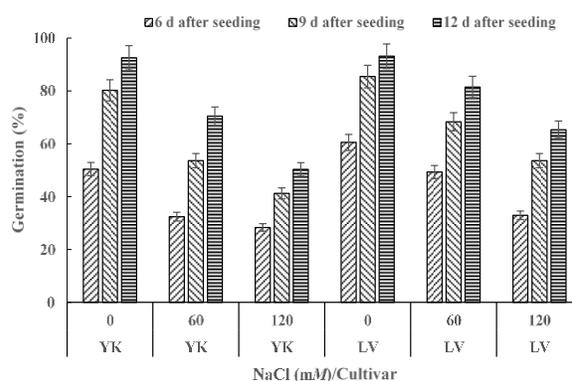
The NaCl, GA<sub>3</sub> and their combinations produced significant effects on most growth of okra seedlings as indicated by the plants morphological characters (Table 1). Seedling growth significantly inhibited with the increase of NaCl concentrations in seeding media. As compared to non-NaCl level, PH, LA, dry weight of root, stem and leaf were reduced by 20.6 and 34.6%; 20.3 and 37.3%; 36.3 and 43.3%; 16.8 and 28.3% and 17.1 and 38.6% at 60 and 120 mM NaCl levels, respectively. As for dry weight, NaCl stress had the biggest inhibitive effects on root growth, followed by stem and leaf (Fig. 2, 3 and 4).

Amendment of GA<sub>3</sub> showed most effective in improving growth of okra plants in saline conditions. As compared with non-GA<sub>3</sub> treatment, 150 μM GA<sub>3</sub> increased

**Table 1:** Analysis of variance of the effects of salinity and GA<sub>3</sub> amendment on growth and physiological parameters of okra seedlings in a controlled study

Dependent variable	Independent variable		
	Salinity	GA <sub>3</sub>	Salinity × GA <sub>3</sub>
Plant height	**	**	**
Leaf area	**	**	**
Dry root weight	**	**	**
Dry stem weight	**	**	**
Dry leaf weight	**	**	**
SOD	**	**	*
POD	**	*	n.s.
CAT	*	**	*
Soluble protein	**	**	n.s.
Proline	**	**	n.s.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; n.s. means not significant



**Fig. 1:** Germination of okra seeds of two varieties (YK and LV) as influenced by NaCl

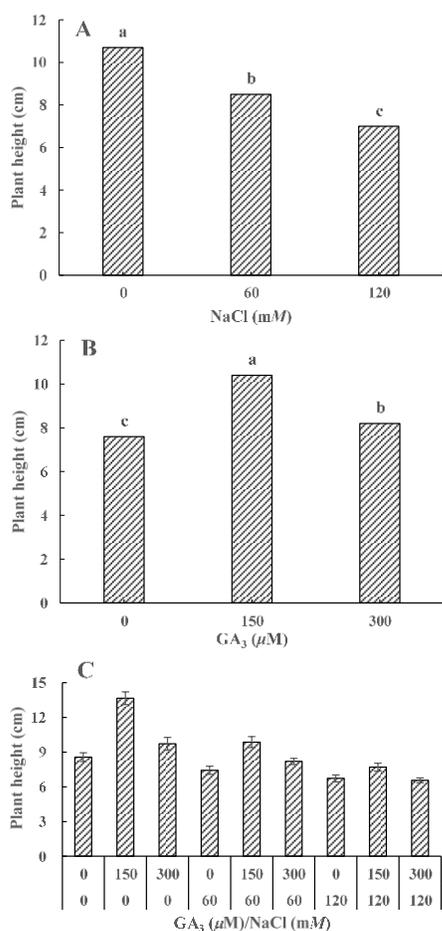
Error bars associated with data point indicate variability in germination percentage

PH, LA, dry weight of root, stem and leaf by 36.8, 22.5, 28.8, 43.8 and 34.9%, respectively. The 300 μM GA<sub>3</sub> increased PH, root weight, stem weight, and leaf weight by 7.9, 30.3, 23.4 and 5.5%. The amendment of GA<sub>3</sub> had the best growth effects on root, followed by stem and with least effects on leaf area and weight (Fig. 2, 3 and 4).

### Physiological Parameters

The SOD activity increased by 26.5% at the 60 mM NaCl level but declined by 30.1% at the 120 mM NaCl level compared to 0 mM NaCl. The application of GA<sub>3</sub> increased SOD activity by 44.9 and 28.8% at the 150 and 300 μM GA<sub>3</sub>, respectively. For control, the application of 300 μM GA<sub>3</sub> performed in increasing SOD activity. However, at the 60 and 120 mM NaCl treatment, the best effects on increasing SOD activity was produced by 150 μM GA<sub>3</sub> (Fig. 5A).

The POD activity was less affected under 60 mM NaCl treatment but prominently reduced by 32.7% at the 120 mM NaCl level compared to control. Compared with the non-GA<sub>3</sub>, POD activity significantly increased at 150 μM GA<sub>3</sub>, but that less affected at 300 μM GA<sub>3</sub> level. At



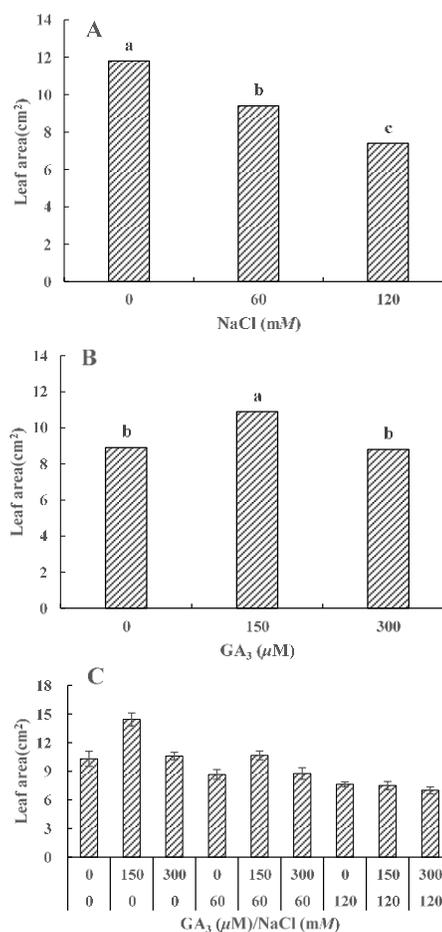
**Fig. 2:** Plant height of okra seedling as influenced by NaCl (A) and GA<sub>3</sub> (B), and their combination (C). Within each sampling date, bars with different letters are significantly different at the 0.05 probability level by an ANOVA-protected LSD test

all the NaCl levels, 150  $\mu\text{M}$  GA<sub>3</sub> had the best effects on increasing POD activity (Fig. 5B).

The CAT activity was slightly increased at the 60 mM NaCl treatment but contrary slightly decreased at the 120 mM NaCl level. At 150 and 300  $\mu\text{M}$  GA<sub>3</sub> increased CAT activity by 48.1% and 74.3% compared with non-GA<sub>3</sub>, respectively. At 0 and 60 mM NaCl treatment, 300  $\mu\text{M}$  GA<sub>3</sub> produced the best effects in increasing CAT activity. However, at 120 mM NaCl level, 150  $\mu\text{M}$  GA<sub>3</sub> had the best effects on increasing CAT activity (Fig. 5C).

The soluble protein contents increased by 13.0% and 16.8% at 60 and 120 mM NaCl levels compared with the control (Fig. 6A). Compared with other GA<sub>3</sub> levels, soluble protein was showed the highest value at the 150  $\mu\text{M}$  GA<sub>3</sub>, with 26.6% higher than the no GA<sub>3</sub> treatment (Fig. 6C). In this regard, the application of 150  $\mu\text{M}$  GA<sub>3</sub> showed maximum values for both the NaCl levels (Fig. 6E).

The proline contents significantly increased with

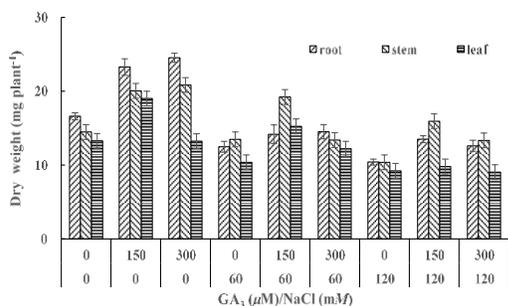


**Fig. 3:** Leaf area of okra seedling as influenced by NaCl (A) and GA<sub>3</sub> (B) and their combination (C). Bars with different letters are significantly different at the 0.05 probability level by an ANOVA-protected LSD test

increasing NaCl level and GA<sub>3</sub> amendment. Compared with control, proline contents increased by 62.9% and 72.9% at 60 and 120 mM NaCl levels (Fig. 6B). For 150 and 300  $\mu\text{M}$  GA<sub>3</sub>, proline contents increased by 49.2% and 79.5% as compared with the no GA<sub>3</sub> treatment (Fig. 6D). At all the NaCl treatments, the highest proline was recorded at the 300  $\mu\text{M}$  level (Fig. 6F).

## Discussion

Germination is the base and key phases in the growth cycle of crops, which determine crop establishment and yield gain. In present study, the germination of okra seeds was significantly reduced both at the 60 and 120 mM NaCl levels, which is consistent with the study of Shahid *et al.* (2011), where a significant decrease in seeds germination of okra under NaCl stress was found. Salinity may have multiple effects on seed germination process, such as it changes seeds water imbibition caused by lower osmotic



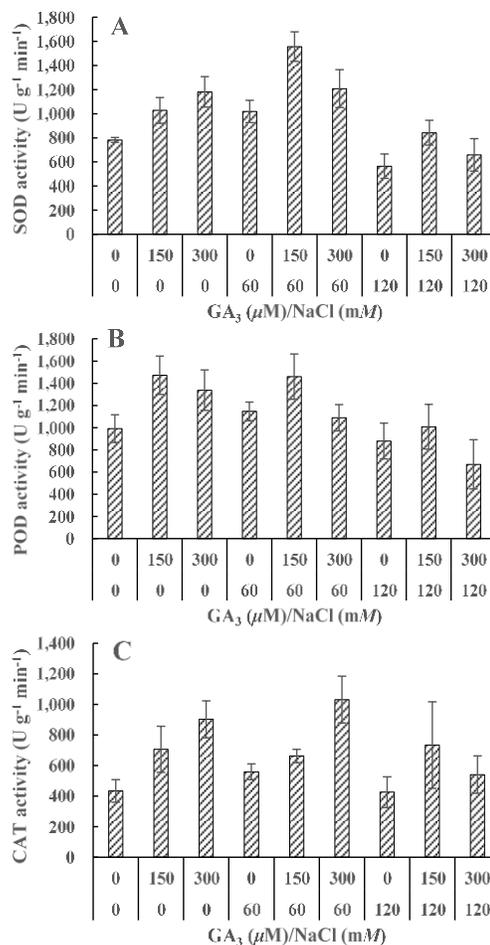
**Fig. 4:** Dry weight of okra seedling as influenced by NaCl and GA<sub>3</sub>. Bars with different letters are significantly different at the 0.05 probability level by an ANOVA-protected LSD test

potential in germination media (Khan and Weber, 2008), reduces the utilization of seed reserves (Othman *et al.*, 2006), causes toxicity that alters the enzyme activity of nucleic acid metabolism, disorders protein metabolism (Dantas *et al.*, 2007) and disturbs hormonal balance (Khan and Rizvi, 1994).

In the present study, okra plants exhibited significant decrease in each growth parameters, such as PH, LA and dry weight of root, stem and leaf, under salt stress. This depressed growth of okra plants under NaCl stress could be attributed to low water potential and toxic effect of Na<sup>+</sup> and Cl<sup>-</sup> ions present in NaCl in the rooting media (Silveira *et al.*, 2009). These reductions in growth may occur immediately after crop plants are exposed to saline stress because of osmotic changes outside the root zone. The lower water potential in saline seeding media is partially responsible for lower cell turgor in roots, usually causing reduction in cell elongation and cell division, and thus resulting into reduced plant growth (Greenway and Munns, 1980). Later on, reduced growth is caused by salt accumulation in leaves bringing about toxicity (Munns and Tester, 2008).

The inhibitive effect of NaCl on okra seedling growth was significantly lessened by GA<sub>3</sub>, especially at 150 μM GA<sub>3</sub>. The promotional effects of GA<sub>3</sub> on seedling growth were also recorded in maize (Tuna *et al.*, 2008) and in wheat (Ashraf *et al.*, 2002) under saline stress conditions. The enhanced plant growth of salt-stressed seedlings with suitable GA<sub>3</sub> treatment in our study was partially contributed to the positive effects of GA<sub>3</sub> on encouraging cell division and growth (Kaur *et al.*, 1998), reducing stomatal resistance, and enhancing water use efficiency (Maggio *et al.*, 2010).

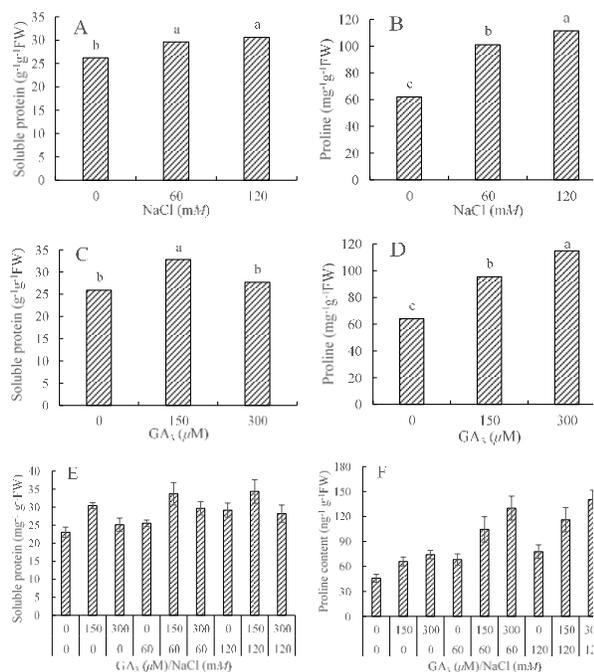
The SOD is the family of metalloenzymes and is widespread in cellular component of plant. It catalyzes the scavenging of O<sub>2</sub><sup>-</sup> to molecular oxygen and H<sub>2</sub>O<sub>2</sub>. The production of SOD in plants cells is usually the first step of defense against the toxic oxygen radicals, especially under stress conditions (Dionisio-Sese and Tobita, 1998). In this study, it was observed that SOD activity significantly increased at 60 mM NaCl level (Fig. 5A). Similar results were found in wheat (Sairam and



**Fig. 5:** The activity of SOD, POD and CAT of the leaves of okra seedling as influenced by NaCl and GA<sub>3</sub>. A: SOD. B: POD. C: CAT. Bars with different letters are significantly different at the 0.05 probability level by an ANOVA-protected LSD test

Srivastava, 2002), cotton (Meloni *et al.*, 2003) and castor bean (Zhou *et al.*, 2014). The increase in SOD could increase the ability of plant to scavenge O<sub>2</sub><sup>-</sup> radicals under salt stress. However, at the level of 120 mM NaCl, the activity of SOD was significantly declined (Fig. 5A). Similar result was also recorded in castor bean (Zhou *et al.*, 2014). In the present study, SOD activity was significantly improved by 150 μM GA<sub>3</sub> and 300 μM GA<sub>3</sub>. This observation was reversed in the study of Barman and Chakrabarti (2009), who reported that SOD was decreased in barley under salt stress by application of GA<sub>3</sub>.

POD plays a key role in scavenging H<sub>2</sub>O<sub>2</sub> in crops. Higher level of POD enhanced it to scavenge more H<sub>2</sub>O<sub>2</sub> and protect plants against environmental stresses more effectively. In this study, compared with the non-NaCl control, POD activity was less affected at the 60 mM NaCl level, but was significantly reduced at the 120 mM NaCl level, indicating that okra seedlings plants were protected by enhanced POD activity at the 60 mM NaCl level but were



**Fig. 6:** The content of soluble protein and proline of the leaves of okra seedling as influenced by NaCl, GA<sub>3</sub>, and their combination. A, and B: the major effects of NaCl on the content of soluble protein and proline. C and D: the major effects of GA<sub>3</sub> on the content of soluble protein and proline. E and F: the interactive effects of NaCl and GA<sub>3</sub> on the content of soluble protein and proline. Within each sampling date, bars with different letters are significantly different at the 0.05 probability level by an ANOVA-protected LSD 0.05 test

damaged by reduced POD activity at the 120 mM NaCl level (Fig. 5B). Similar results were showed by Manchandia *et al.* (1999), who reported that POD activity was transiently increased by up to two to four-folds in cotton callus tissue under the stress of 250 mM NaCl, but when the salt stress was exceeded the threshold level, POD activity was drastically reduced.

CAT can reduce high concentration of H<sub>2</sub>O<sub>2</sub> by resolving it directly into O<sub>2</sub> and H<sub>2</sub>O (Dionisio-Sese and Tobita, 1998). Enhanced CAT activity was frequently reported when plants were treated with salinity stress (Kukreja *et al.*, 2005; Janmohammadi *et al.*, 2012). However, in our study, the CAT activity was not changed under 60 and 120 mM NaCl levels, but was significantly increased by GA<sub>3</sub> amendment (Fig. 5C). The enhanced CAT activity by GA<sub>3</sub> amendment was beneficial for okra plants to be more efficient in breaking H<sub>2</sub>O<sub>2</sub> into oxygen and water.

Total protein synthesis is to be inhibited under salt stress. Soluble protein generally diminished in response to salinity (Wasti *et al.*, 2012). On the other hand, Abd and Baki (1996) reported that N-contents and high protein content in some glycophytic plants were caused by high salt concentrations. In the current study, soluble protein was

significantly increased by 150 μM GA<sub>3</sub>, indicating that GA<sub>3</sub> at this level played a beneficial role in stabilizing protein synthesis (Fig. 6C).

Proline plays important roles in stabilizing subcellular structures, scavenging free radicals and buffering cellular redox potential under salt stress. It considers as an index of environmental stress (Hasanuzzaman *et al.*, 2013). In most plants, salinity may cause proline increase in leaves, stems and roots (Ahmad *et al.*, 2010; Ahmad, 2010). In the present study, higher Pro content was showed under 60 and 120 salinity stress as compared with the non-NaCl control (Fig. 6B). The accumulated Pro might attribute to the increase in Pro biosynthesis, the decline in protein utilization and the hydrolysis of proteins (Charest and Phan, 1990). In our study, we observed proline content was significantly increased when 150 and 300 μM GA<sub>3</sub> was applied.

## Conclusion

By increasing NaCl levels, germination of the two okra cultivars of YK and LV was significantly reduced. The cultivar LV was more tolerant to NaCl than YK in terms of germination. NaCl stress significantly inhibited seedling growth by reducing plant height, leaf area, dry weight of root, stem and leaf. The activity of SOD, POD, CAT and the content of soluble protein and proline were also significantly decreased. The amendment of GA<sub>3</sub> lessened the inhibitive effects of NaCl stress by enhanced the activity of SOD, POD and CAT and the content of soluble protein and proline, which resulted in increasing plant height, leaf area, dry weight of root, stem and leaf. This study illustrates that it is feasible to use GA<sub>3</sub> at appropriate concentrations to enhance early seedling growth of okra plants grown under NaCl conditions.

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