

RESEARCH ARTICLE

Exogenous application of glutamic acid promotes cucumber (*Cucumis sativus* L.) growth under salt stress conditions

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ABSTRACT

Salinity is expected to be the major destructive abiotic stress that causes ionic and oxidative damage leading to growth reduction and ultimately plant death. Glutamic acid (GA) is an α -amino acid that is used by almost all living beings in the biosynthesis of proteins. Therefore, in the present study, we tried to investigate the effect of foliar application of glutamic acid (GA) on cucumber (*Cucumis sativus* L.) under altered salinity levels. Cucumber seedlings were grown in plastic pots under greenhouse conditions by applying four levels of salinity (0, 3 dS/m, 6 dS/m and 12 dS/m) and two levels of foliar applied GA (0, 10 mM). Salinity was induced by mixing the salt and soil before seed sowing; however, exogenous GA was applied when the vine length was reached up to maximum height. Morphological characters showed disruptive response under saline conditions especially in indigenous cultivar (local cucumber represented as V1). Enhanced activities of superoxide dismutase ($0.29 \mu\text{g}^{-1} \text{FW}$), guaiacol peroxidase ($3.51 \mu\text{g}^{-1} \text{FW}$) and ascorbate peroxidase ($0.39 \mu\text{mol AsA} \cdot \text{mg}^{-1} \text{Chl} \cdot \text{min}^{-1}$) were observed in salt-stressed cucumber leaves. Both varieties showed unusual behavior for malondialdehyde in decreasing manner with increasing salinity levels ($2.0333 \mu\text{mol g}^{-1} \text{FW}$ at 12dS/m in local cultivar; while, $1.98 \mu\text{mol g}^{-1} \text{FW}$ at 12dS/m in hybrid cultivar SSC-228). However, exogenously applied GA played a beneficial role in promoting all morphological parameters under stress with increasing scavenging abilities against reactive oxygen species. Foliar application of GA improved plant defense mechanism with minimum destruction. Remarkable calculations showed that under salt stress, GA improved plant stress tolerance against salinity by maximizing the growth rate.

Keywords: *Cucumis sativus*; Salinity; Glutamic acid; Antioxidant machinery; Plant growth

INTRODUCTION

On earth, sodium is assumed to be the sixth most abundant element (Rodriguez et al., 2009) that's why, sodium salt is becoming dominant in saline areas (Tavakkoli et al., 2010). This may lead to loss of arable land for crop cultivation (Wang et al., 2003). There is need to develop agricultural practices to fulfill the food demand of 9.5 billion people up to 2050. Feasible means must be used to cope with this recent challenge by cultivating salt-tolerant cultivars (Van et al., 2016). Salt stress adversely effects on plant growth and development by excessive ion uptake mechanism. It usually occurs due to the influence of toxic ions, disrupting biochemical activities of plant life. Under salt stress, Na^+

is observed to be the main cause of ion-specific damage (Trajkova et al., 2006). Higher accumulation of sodium ions may lead to inhibition of growth by impairing various cellular mechanisms (Saqib et al., 2005). Due to salinity, plants may face osmotic and ionic stresses, resulted in lower growth with minimum productivity rate.

Plasma membrane is assumed to be the primary site, mainly damaged by salt. To observe salt stress and tolerance levels, membrane permeability is the most sensitive test (Mansour and Salama, 2004). Due to which, salt stressed or salt sensitive cultivars can be generally differentiated by plasma membrane stability. Under the saline effect of sodium chloride, impairment of root and shoot cells have been observed. Salt stress tolerance is generally observing to be

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correlated with higher cell membrane stability (Meloni et al., 2003; Sudhakar et al., 2001). So, lipid peroxidation mainly occurs as a result of abiotic stresses (Elkahoui et al., 2005). Malondialdehyde (MDA) is mainly the decomposition of membrane fatty acids, so MDA concentration generally shows lipid peroxidation under abiotic stress. Due to the accumulation of certain organic and inorganic solutes, plants usually decrease their osmotic potential (Liu and Staden, 2001).

Oxidative stress is generally followed by lipid peroxidation, inactivation of enzymes, and inhibition of protein synthesis and disruption of membrane system (Tanou et al., 2009; Zhu et al., 2004). Generally, Na^+ accumulation in plants impairs enzymatic processes by causing K^+ deficiency (Blumwald, 2000). As a result of which, many enzymes remain inactive under saline conditions (Tester and Davenport, 2003). Due to inactivity of enzymes, growth is adversely affected with low yield (Miller et al., 2010). A lot of chemicals, general species of *Bacillus bacterium* or some specific amino acids are generally used to lessen the negative effect of oxidative stress, caused by the accumulation of sodium and chloride ions. Recent studies showed that foliar application of poly- γ -glutamic acid in major crops enhanced plant potential against oxidative stress by acting as the best bio-control agent (Shih and Van, 2001; Chen et al., 2005). Barbosa et al. (2010) testified the positive role of glutamic acid against abiotic stress in muskmelon by improving growth and stress tolerance. Production of reactive oxygen species was also inhibited by glutamic acid application (Miyashita and Good, 2008). Hellmann et al. (2000) analyzed the positive response of glutamate on proline accumulation in case of wheat crop. Synthesis of these amino acids serves as a precursor for the best nitrogen uptake efficiency of watermelon (Luo et al., 2013). Hence, glutamate is proved to be the best growth regulating precursor under biotic or abiotic stresses. So, the glutamic acid may promote the growth of cucumber under salt conditions. In plants, protein is considered to be the most frequently accumulated osmolytes under saline conditions, and often deliberated to be involved in stress resistance mechanisms. Additionally, it is usually accepted that the plants improved the accretion of certain osmolytes such as protein, proline, glycine betaine and soluble sugars under salt stress.

Cucumber, a highly cultivated crop, is more sensitive to saline environment. It has been nominated as a salt sensitive crop. A definite decline was observed concerning plant height, fresh and dry weight and biochemical activities. Growth of cucumber is badly affected by saline conditions *i.e.* plant height (Chartzoulakis, 1992), stem diameter (Al-Harbi, 1994), total leaf area and expansion rate (Chartzoulakis, 1994), fresh weight and dry mass

production (Jones et al., 1989). Therefore, it is of great interest to determine if foliar glutamic acid application could confer resistance in cucumber plants to salt stress. As, glutamic acid empowers the nutrient uptake capacity even under stressed conditions by providing a feasible environment for a plant to grow well, for example in cucumber (Wang et al., 2008) cabbage (Xu et al., 2014) and wheat (Xu et al., 2013). Moreover, glutamic acid also plays a major role against pathogenic attack and abiotic stresses (Jin et al., 2019). That's why; an experiment was carried out to amplify the crucial role of growth regulator under the susceptible cucumber cultivars (indigenous and hybrid cultivar represented as V1 and V2, respectively) concerning the fact that how glutamic acid behaves antagonistically against abiotic stresses, how glutamic acid plays role in scavenging mechanisms against reactive oxygen species. By analyzing general growth and biochemical parameters of both cucumber cultivars, efficiency of glutamic acid was observed. Predominantly, the objective of this study was based on determining the efficacy of glutamic acid influencing the rate of plant growth and biochemical assays under salt stress.

MATERIALS AND METHODS

Plant materials and treatments

The experiment was conducted at Institute of Horticulture Sciences, University of Agriculture Faisalabad, Pakistan. Two cucumber (*Cucumis sativus* L.) cultivars indigenous as V1 and SSC-228 as V2 were used in this study. Before experiment, different soil parameters were analyzed including pH, electrical conductivity, N, P, K analysis, calcium and magnesium ions, chlorides, carbonates and bicarbonates. Four levels of salinity (0, 3dS/m, 6dS/m and 12dS/m) were maintained and salinity was induced by mixing the relevant amounts of salt and soil before seed sowing. A little amount of sand was also used at sowing pots for the soften sprouting of radicals. Consistent water irrigation and desired dose of relevant fertilizers were added at regular intervals of time. After 2-3 days, seed germination was observed in all pots. As cucumber is a vining plant, so when the vine length was reached up to maximum height, a foliar spray of "Glutamic acid (10 mM)" was applied to the specified pots. After 1 week of PGR application, harvesting was done. Morphological characters were measured and sample for biochemical analysis were collected and preserved at -80°C .

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. After

detachment of roots, evacuated plants were washed with clean water, straightened and after that its root length was estimated by utilizing a tape meter in centimeters and the average was taken for each replicate. Then root weight was measured by using weighing balance. Root dry weight was estimated by putting the samples in oven at 65°C for 72 hr. At that point, weight was ascertained and the normal means for each sample was figured.

Determination of antioxidant enzyme activities

Frozen cucumber leaves were ground by adding BPS buffer solution with 7.8 pH. Prepared homogenates were centrifuged at 4°C for twenty minutes at 8000-13000rpm. Resulted supernatants were used for determining different antioxidant enzyme activities.

Superoxide dismutase (SOD) activity was assessed by following the method of Ries and Giannopolitis (1977) by constrain the photochemical reduction of nitro blue tetrazolium. Reaction solution consisted of the constituent amounts of NBT, riboflavin, EDTA and methionine. For enzyme activity assay, reaction solution was placed at dark for 15 minutes. SOD activity was measured by spectrophotometer by adjusting wavelength at 560nm.

Scobbba et al. (2001) method was followed to measure peroxidase (POD) enzyme activity by using required amounts of H₂O₂ and guaiacol. POD activity was observed by adjusting absorbance level at 470nm. Following the method of Kato and Shimizu (1987), catalase (CAT) activity was assessed by using H₂O₂ and buffer solution. Activity was analyzed at 240nm due to decline of extinction of hydrogen peroxide (H₂O₂).

Determination of H₂O₂ concentration and MDA contents

Hydrogen peroxide (H₂O₂) concentration was determined according to Patterson et al. (1984). Reaction solution was made by adding required amounts of acetone, thiosulphate, ammonia solution and H₂SO₄. Absorbance values were quantified using standard curve generated from known concentrations of H₂O₂. Moreover, malondialdehyde (MDA) was also performed by succeeding the protocol of Heath and Packer (1968). To perform this, leaf tissue was homogenized in trichloroacetic acid (TCA) solution. Prepared homogenate was centrifuged for 5 minutes at 15,000g. At 95°C, mixture was heated for 5 minutes and cooled in ice bath. For determining MDA content, absorbance level was adjusted at 532 nm.

SPAD index

Generally, SPAD index value was calculated to manipulate the influence of different cultivars concerning developmental stages. In certain plants, SPAD meter measures the

difference between the transmittance of a red (650 nm) and an infrared (940 nm) light throughout the leaf. Furthermore, by using three biological and technical replications a non-destructive method was used to estimate the chlorophyll content with full accuracy (Neufeld et al., 2006). Chlorophyll meter was used to analyze chlorophyll pigments.

Total soluble proteins

The basic principle of Bradford assay is that the general binding of protein molecules with the coomassie dye under acidic conditions usually results in a color change, which actually measures the presence of the amino acid. Following Bradford (1976) method, soluble protein content was measured. For this purpose, bovine serum albumin was generally used. By using phosphate buffer leaf sample were to be homogenized and centrifuged at 4°C and pipetted in spectrophotometer tubes. Using a spectrophotometer, absorbance level was adjusted as 595nm.

Statistical analysis

The experiment was laid down using a complete randomized design (CRD). In this study, values were reported on the basis of means of three replications. By using two-way ANOVA and Tukey's test, statistical analysis was performed.

RESULTS

Effect of glutamic acid on cucumber growth under salt stress

In this study, both cucumber varieties showed disruptive growth with high lipid peroxidation level (Table 1). High values of plant height, fresh weight, plant, root and shoot fresh weights were observed in SSC-228 (V2) under salt stress showing its salt tolerance capacity (Table 1). Furthermore, the highest plant, shoot and root dry weights were observed in SSC-228 (V2) under the exogenous application of glutamic acid (Table 2). Recorded results showed the distinct changes in morphological and biochemical enzymatic activities induced by glutamic acid under saline conditions, either separately or in combination. All of the morphological parameters including plant, root and shoot weights and SPAD index were reduced due to abiotic stress at all levels comparably to control one. However, exogenously applied glutamic acid alone had a constructive effect on the plant scavenging mechanisms against stress. Antioxidant activities (SOD, POD and CAT) were positively affected by exogenous glutamic acid with decreased MDA concentration.

Antioxidant enzymatic activities

Recorded data showed that antioxidant enzyme activities (SOD, POD) increased with the increasing

Table 1: Effects of different treatments of NaCl and glutamic acid on number of plants per pot, plant fresh weight, shoot fresh weight and root fresh weight

Index	Cultivars	No of plants per pot	Plant fresh weight (g)	Shoot fresh weight (g)	Root fresh weight (g)
Control	V1	4.75±0.08 ^a	0.39±0.01 ^f	0.32±0.08 ^a	0.065±0.14 ^{cd}
	V2	4.75±0.04 ^b	0.70±0.08 ^b	0.47±0.004 ^b	0.02±0.004 ^f
10mM Glu	V1	5.15±0.004 ^{cd}	0.49±0.006 ^a	0.41±0.004 ^{cd}	0.05±0.12 ^{bc}
	V2	6.2±0.009 ^a	0.77±0.026 ^a	0.5±0.009 ^a	0.27±0.004 ^a
3dS/m NaCl	V1	3.98±0.007 ^h	0.36±0.004 ^g	0.28±0.007 ^h	0.07±0.085 ^d
	V2	5.4±0.002 ^{de}	0.61±0.004 ^d	0.40±0.02 ^{de}	0.20±0.006 ^{fg}
6dS/m NaCl	V1	2.87±0.008 ^{ij}	0.31±0.002 ^{ij}	0.24±0.008 ^{ij}	0.07±0.04 ^e
	V2	4.9±0.006 ^f	0.51±0.004 ^e	0.36±0.006 ^f	0.14±0.004 ^{gh}
12dS/m NaCl	V1	2.34±0.006 ^k	0.28±0.004 ^k	0.19±0.006 ^k	0.08±0.08 ^{ef}
	V2	4.5±0.006 ^{ij}	0.32±0.006 ^{hij}	0.23±0.006 ^{ij}	0.09±0.06 ^{hi}
3dS/m NaCl + 10 mM Glu	V1	4.2±0.015 ^g	0.39±0.004 ^f	0.31±0.015 ^g	0.075±0.06 ^{cd}
	V2	5.8±0.005 ^c	0.65±0.006 ^c	0.425±0.05 ^c	0.24±0.04 ^{cd}
6dS/m NaCl +10 mM Glu	V1	3.35±0.09 ⁱ	0.34±0.002 ^{gh}	0.24±0.009 ⁱ	0.09±0.04 ^f
	V2	5.12±0.04 ^{ef}	0.51±0.002 ^e	0.38±0.004 ^{ef}	0.12±0.002 ^f
12dS/m NaCl +10 mM Glu	V1	3.2±0.05 ^j	0.30±0.02	0.22±0.005 ^j	0.08±0.06 ^{ef}
	V2	4.82±0.07 ⁱ	0.34±0.01 ^{hi}	0.24±0.007 ^{ij}	0.1±0.08 ^f

Each data values are represented as means and ± 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 Tukey's test ($P \leq 0.05$).

Table 2: Effects of different treatments of NaCl and glutamic acid on leaf length, leaf width, plant dry weight, shoot dry weight and root dry weight

Index	Cultivars	Leaf length (cm)	Leaf width (cm)	Plant dry weight (g)	Shoot dry weight (g)	Root dry weight (g)
Control	V1	8.7±0.12 ^e	7.9±0.14 ^{cd}	0.21±0.01 ^{cd}	0.11±0.04 ^f	0.10±0.014 ^a
	V2	11±0.45 ^{bc}	9.05±0.06 ^a	0.29±0.01 ^a	0.16±0.01 ^b	0.13±0.007 ^a
10mM Glu	V1	11.6±0.12 ^{ef}	8.3±0.12 ^{bc}	0.24±0.07 ^{bc}	0.16±0.04 ^{bc}	0.08±0.006 ^b
	V2	12.7±0.32 ^{cd}	9.±0.14 ^{ab}	0.30±0.01 ^a	0.19±0.01 ^a	0.11±0.02 ^a
3dS/m NaCl	V1	8.0±0.4 ^{fg}	7.12±0.075 ^d	0.18±0.004 ^{ef}	0.10±0.007 ^{fg}	0.08±0.004 ^b
	V2	9.9±0.33 ^{ef}	8.72±0.04 ^b	0.24±0.006 ^b	0.15±0.01 ^{bcd}	0.09±0.004 ^b
6dS/m NaCl	V1	7.5±0.17 ^h	5.72±0.04 ^e	0.15±0.011 ^g	0.08±0.006 ^{gh}	0.07±0.002 ^c
	V2	8.5±0.18 ^g	8.22±0.047 ^b	0.20±0.02 ^{de}	0.12±0.004 ^{def}	0.07±0.004 ^c
12dS/m NaCl	V1	6.62±0.2 ^{ab}	4.62±0.08 ^{ef}	0.12±0.002 ^h	0.065±0.006 ^{hi}	0.05±0.004 ^d
	V2	7.37±0.17 ^a	7.31±0.06 ^h	0.15±0.009 ^g	0.11±0.004 ^f	0.04±0.006 ^e
3dS/m + 10mMGlu	V1	8.37±0.06 ^{ef}	7.35±0.003 ^{cd}	0.16±0.001 ^{fg}	0.13±0.004 ^{cd}	0.03±0.004 ^{de}
	V2	10.22±0.62 ^c	9.5±0.006 ^a	0.23±0.004 ^{bc}	0.16±0.002 ^b	0.07±0.001 ^c
6dS/m +10mMGlu	V1	7.8±0.25 ^g	5.5±0.04 ^f	0.18±0.009 ^f	0.10±0.0002 ^f	0.07±0.002 ^c
	V2	8.9±0.05 ^{de}	8.61±0.04 ^b	0.21±0.004 ^d	0.14±0.007 ^{bcd}	0.06±0.002 ^d
12dS/m 10mMGlu	V1	6.72±0.24 ^{gh}	5.25±0.06 ^{ef}	0.14±0.006 ^{gh}	0.05±0.008 ^f	0.08±0.002 ^b
	V2	7.4±0.01 ^{efg}	7.37±0.01 ^{cd}	0.18±0.004 ^{ef}	0.125±0.005 ^{ef}	0.05±0.012 ^d

Each data values are represented as means and ± 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 according to Tukey's test ($P \leq 0.05$).

salt concentration. Both varieties respond likely but comparatively to indigenous (V1), SSC-228 (V2) showed much tolerance level to saline conditions (Fig. 1A and B). Higher values of these activities were observed when glutamic acid was applied separately or under saline conditions in V2 cultivar. According to our outcomes, exogenously applied glutamic acid increased the activities of SOD (0.273 μ g⁻¹ FW in V1 and 0.23 μ g⁻¹ FW in V2)

and POD (3.47 μ g⁻¹ FW in V1 and 3.24 μ g⁻¹ FW in V2) that contributed to lessen the adverse effect of oxidative stress in germinating cucumber and thereby improved germinating rate under saline conditions.

Under saline conditions, both varieties showed no significant difference in recorded readings regarding catalase (CAT) activity. No obvious differences were noted

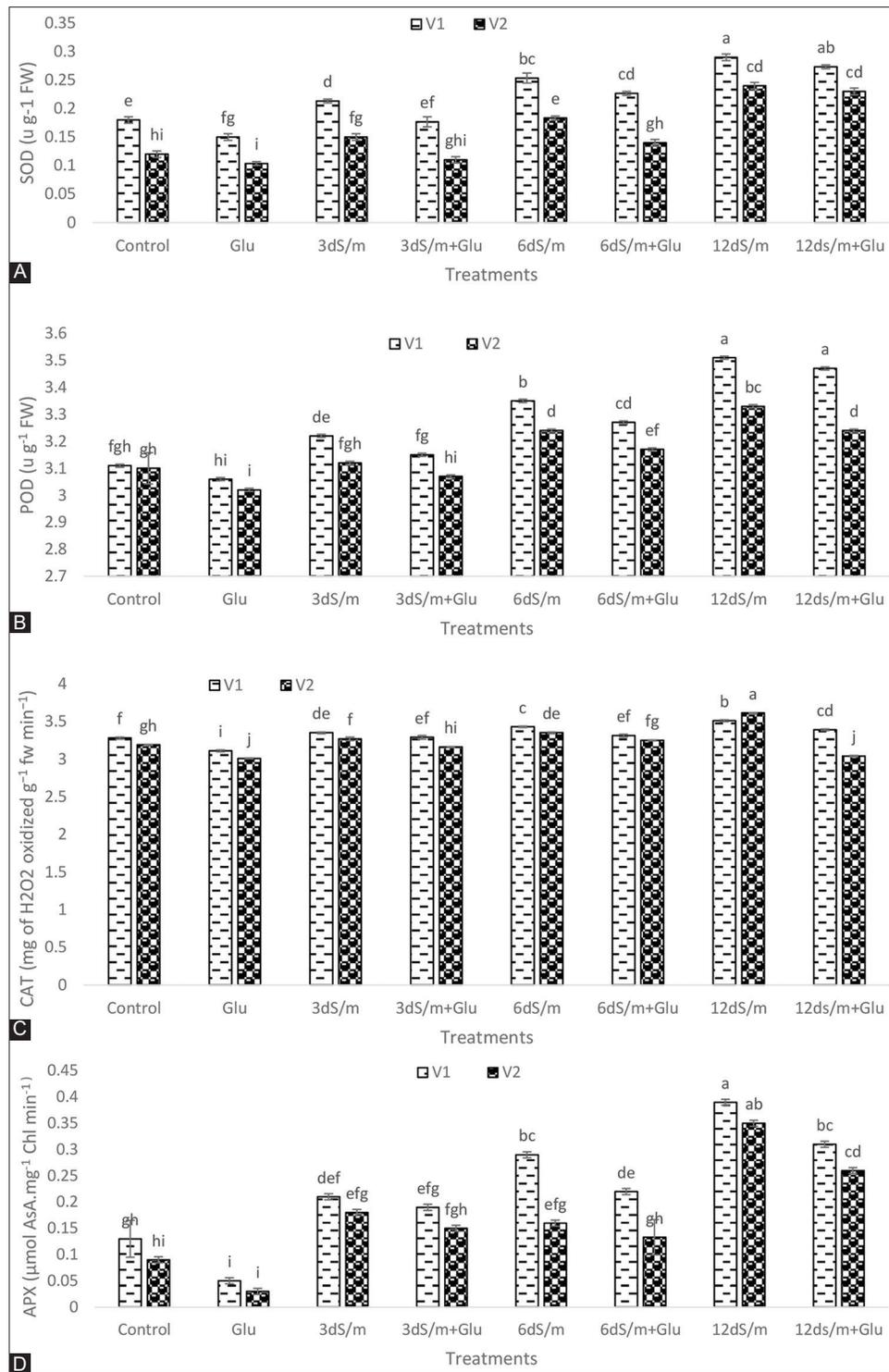


Fig 1. Effects of treatments of glutamic acid(0,10mM) and NaCl (0, 3, 6, 12dS/m) on Asuperoxide dismutase (SOD),B peroxidase (POD), Ccatalase (CAT) and Dascorbate peroxidase(APX) in Cucumis sativus L. Each data values are represented as means and $\pm\text{SD}$ of three replications and different lower case letters are representing the significant difference between treatments and same lower case letters represent the no significant difference by according to Tukey's test ($P \leq 0.05$).

with “NaCl” or “NaCl and glutamic acid”. At control condition, both varieties respond alike (3.28 and 3.19 $\text{mg g}^{-1}\text{FW min}^{-1}$ in V1 and V2, respectively) but with increasing salt concentration, an effective increase was observed in CAT activity (3.51 and 3.61 $\text{mg g}^{-1}\text{FW min}^{-1}$ in V1 and

V2, respectively). At highest salinity level, an abrupt change was noticed especially in V2 by exceeding value up to 3.61 $\text{g}^{-1}\text{FW min}^{-1}$ (Fig. 1C). Besides this, V2 also showed unusual behavior when glutamic acid was applied under saline environment.

Effect of glutamic acid on MDA and H₂O₂ content of cucumber under salt stress

Interestingly, MDA and H₂O₂ content showed significant decrease with increasing salt concentration in both varieties. Increasing salt concentration matched a cause to slow down malondialdehyde activity as upto 2.03 and 1.98 $\mu\text{mol g}^{-1}$ FW in V1 and V2, respectively. As MDA usually marks from lipid peroxidation that is usually inhibited by ion accumulation. While exogenously applied glutamic acid promoted un-saturation of fatty acids so ultimately enhanced MDA under saline conditions. A noticeable decrease was perceived especially in V2. Cucumber cultivars exhibiting NaCl alone displayed the minimum values as 2.03 and 1.98 $\mu\text{mol g}^{-1}$ FW in V1 and V2, respectively while a significant increase was observed when glutamic acid was applied under saline conditions. Recorded data frequently considered SSC-228 (V2) to be the most resistant cultivar (Fig. 2A and B). Ascorbate peroxidase activity (APX) was also examined by using H₂O₂ solution at 290nm absorbance level (Fig. 1D). Results showed high resistance for V2 by exceeding upto 0.31 and 0.26 $\mu\text{mol AsA.mg}^{-1}$ Chl min^{-1} in V1 and V2, respectively against oxidative stress assuming it to be more tolerant.

SPAD index

Glutamic acid treated plant leaves showed higher values for chlorophyll a by exceeding up to 36.1 and 37.36 $\mu\text{g cm}^{-2}$ in V1 and V2 (Fig. 2C and D) while salt ultimately became a reason for the reduction of chlorophyll content. Indigenous (V1) cultivar affected badly under salt conditions than the SSC-228 (V2). It was detected that glutamic acid could advance the growth of cucumber crop by increasing their chlorophyll content. Saline conditions suppressed the development of photosynthetic pigment by absorbing less light energy (32.2 and 34.3 $\mu\text{g cm}^{-2}$ in V1 and V2, respectively). Hence, glutamic acid is proved to be a best precursor under salt environment. It is precisely predictable that carotenoids play a defensive part against photo-oxidation by dispersing the surplus energy of excitation.

Total soluble proteins

In plants, protein is considered to be the most frequently accumulated osmolytes under saline conditions, and often deliberated to be involved in stress resistance mechanisms. Additionally, it is usually accepted that the plants improved the accretion of certain osmolytes such as protein, proline, glycine betaine and soluble sugars under salt stress (Fig. 3B)

DISCUSSION

In this study, foliar glutamic acid was applied to induce salt tolerance in cucumber seedlings. Results delineated that cucumber seedlings growth was significantly affected due

to salinity by impairing its morphological characters. As, it severely affects the plant growth parameters by disrupting their metabolic activities (Nasri et al., 2017). Similar results have been found in tamarind tress (Gebauer et al., 2004), paspalum (Lee et al., 2005), ziziphus (Meena et al., 2003), lettuce, peppers (Lycoskoufis et al., 2005) and cucumber (; Trajkova et al., 2006). Due to similar effect of salt on the growth of shoot and root, shoot/root ratio remained unaffected. This result is not consistent with the results found in lettuce. In accordance to Chauhan et al. (2019), salinity abruptly minimizes the germination percentage of onion 44% when salinity dose was adjusted as 4dS/m. Comparatively, applied PGR responds positively towards growth and morphological characters of cucumber seedlings by maximizing its plant weight, leaf length and number of leaves per plant. Plant scavenging system also developed positively under PGR application. Foliar use of such plant growth regulators enhances the growing abilities of plant against pathogenic and abiotic stress (Ghani et al., 2021). Besides this, a definite increase in chlorophyll pigments was observed too.

It is acknowledged that antioxidant enzymes such as SOD, POD and CAT perform a substantial role in scavenging reactive oxygen species in salt-stressed plants (Tseng et al., 2007; Tuna et al., 2008). CAT and POD are identified to scavenge H₂O₂ either by disintegrating it in to H₂O and O₂ or by transmitting its oxidative power to another substrate that is consequently, oxidized. Higher values of enzymatic CAT and POD have been investigated as a crucial for anti-oxidative defense mandatory for salt tolerance in rice and sugar beet. As mentioned in earlier studies, the main function performed by peroxidase (POD) is to breakdown the adverse bonding of H₂O₂ that is very severe for plant life (Neelambari et al., 2018). Applied glutamic acid did not show any positive change in APX because they can cause oxidative damage to the plant cell more rapidly than scavenging against ROS. Moreover, this enzyme is more stable at its optimum pH 6.0-9.0 so applied PGR didn't affect positively. As, MDA is generally assumed to be in association with aging therefore, it didn't show a prominent and significant relation with SOD and POD activities under saline conditions. Protein is assumed to be one generally collected osmolytes in plants under saline conditions, and habitually considered to be intricate in stress resistance mechanisms (Tanou et al., 2009).

As a result of salt stress, some unfavorable effects had also been observed in plants regarding their growth and photosynthesis. Consequently, we assumed that stress is a major factor that impairs cytoplasmic membrane due to which transport of ions usually fails across cell membrane. H₂O₂ formation is generally catalyzed by POD activity, therefore its increasing value is a sign of

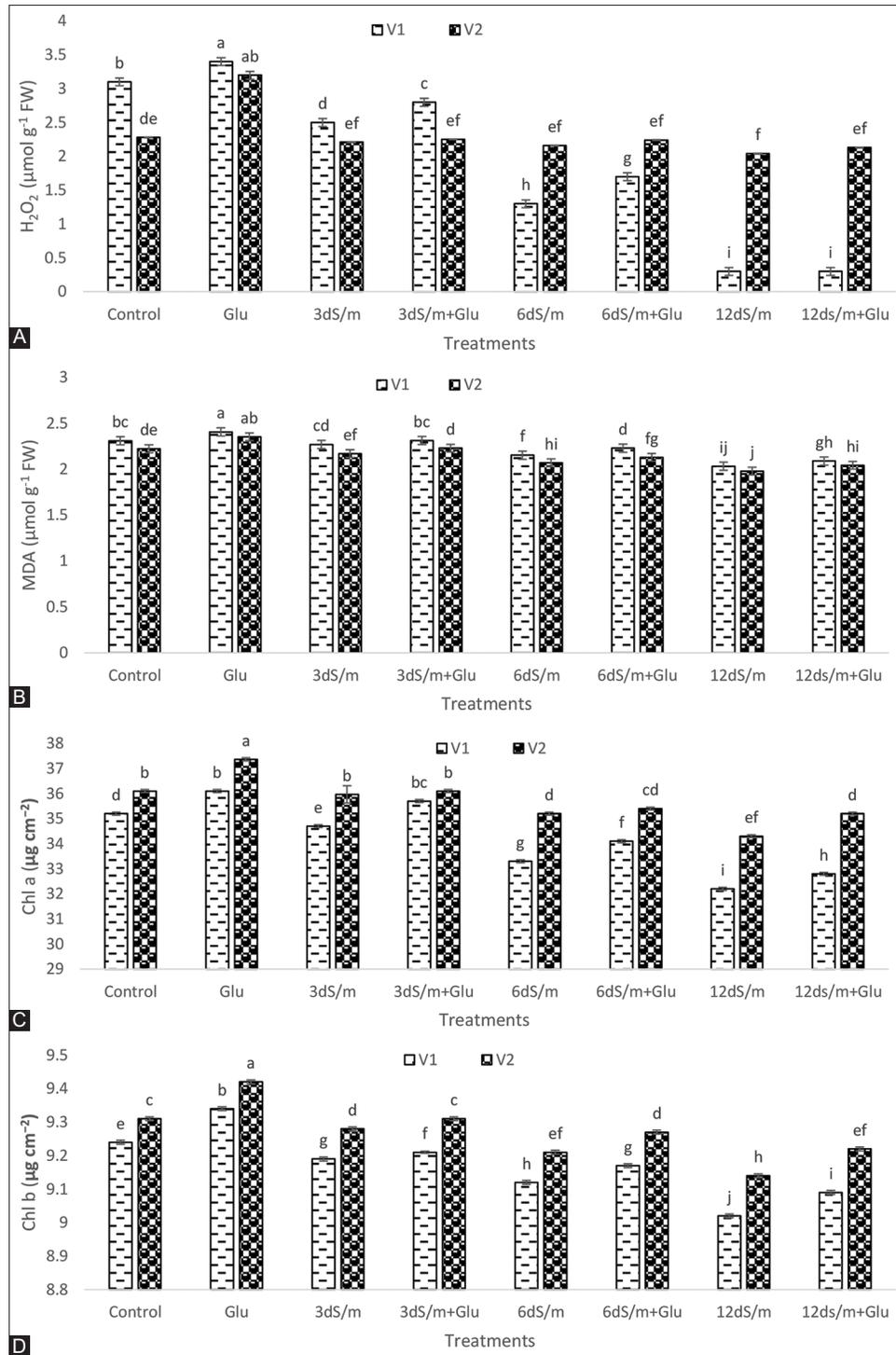


Fig 2. Effects of treatments of glutamic acid(0,10mM) and NaCl (0, 3, 6, 12dS/m) on A hydrogen peroxide (H_2O_2), B malondialdehyde (MDA), C chlorophyll a and D chlorophyll bin Cucumis sativus L. Each data values are represented as means and \pm SD of three replications and different lower case letters are representing the significant difference between treatments and same lower case letters represent the no significant difference by according to Tukey's test ($P \leq 0.05$).

unfavorable and improper growth. Related results have been found in rice under salt stress, where a strong relation was established between inhibition of root growth and POD activity. Prolonged salt stress leads towards Na^+ accumulation in all crop plants that leads to

detrimental growth by causing ionic toxicity and osmotic stress (Zhu et al., 2016).

On the beginning of salt stress, the reduced rate of photosynthesis is not the one reason of growth reduction

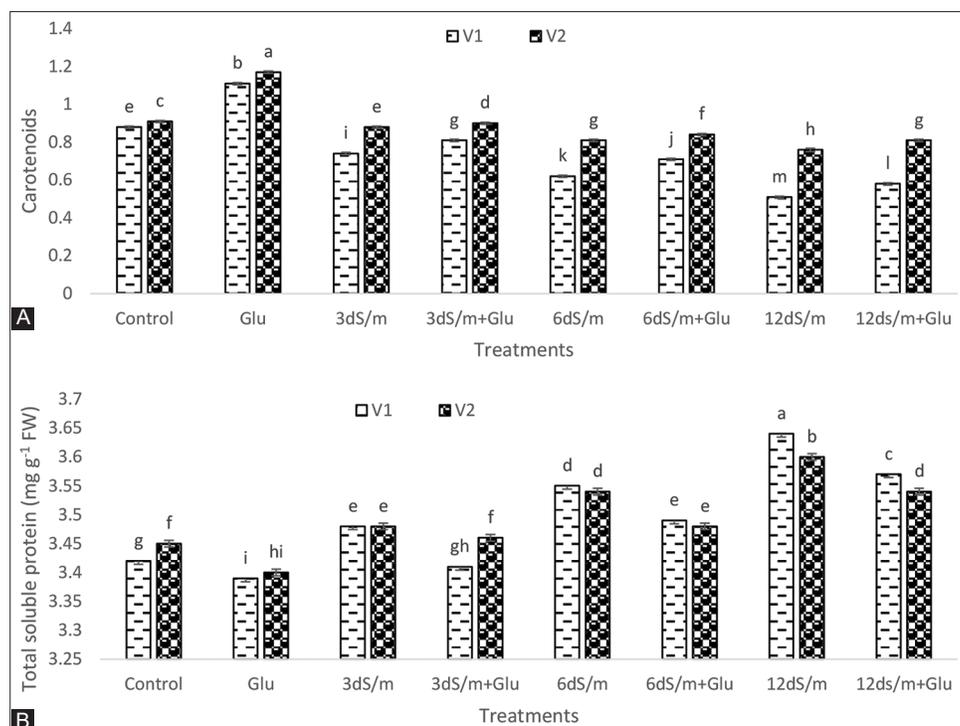


Fig 3. Effects of treatments of glutamic acid(0,10mM) and NaCl (0, 3, 6, 12dS/m) on A carotenoids and B total soluble proteins in *Cucumis sativus* L. Each data values are represented as means and \pm SD of three replications and different lower case letters are representing the significant difference between treatments and same lower case letters represent the no significant difference by according to Tukey's test ($P \leq 0.05$).

because a rapid change occurs in case of leaf expansion (Fricke et al., 2004;). This proposes that salinity impacts cucumber growth by minimizing the rate of leaf area and photosynthesis. As, NaCl stress can persuade a modification in leaf water status and a decrease in stomatal conductance (Kholova et al., 2009). Besides, extreme accumulation of sodium and chlorides ions is toxic and may disturb the veracity of the photosynthetic machinery (Aghaleh et al., 2009;). Such poisonous ion accumulation badly impaired photosynthetic capacity of plant by disrupting its chlorophyll pigment (chlorophyll) and hence reduced growth while exogenously applied PGR promoted photophosphorylation reactions by absorbing more sun light, required for synthesizing its own food.

Vegetative growth is also accomplished with the salt stress by impairing water and nutrient uptake system (Chartzoulakis, 1990). Plant height and all other morphological characters showed negative response to abiotic stress. The present study is also supporting to such general facts under saline conditions. Osmotic stress is basically assumed to be much harmful for plant growth and all other processes. As a result of which relative water content disturbs. Under saline conditions, all morphological characters of cucumber seedlings were severely affected and to nullify this toxic effect an exogenous PGR was used that promoted plant height in association to all other morphological characters. Membrane stability is also affected so photosynthetic

machinery is going to be damaged by minimizing chlorophyll content (Ashraf and Bhatti, 2000). Under saline conditions, reduced chlorophyll pigment effects electron transport chain and also both photo systems (Sudhir et al., 2005) that ultimately relates to reduction in productive yield (Meloni, 2003).

The inhibitory effect of salt stress was controlled using a plant growth regulator, glutamic acid. Its significant effect was easily being examined due to its high polarizability rate. As, it is being used as a best neurotransmitter so helps a lot in plant defending mechanism involving all types of abiotic stresses (Shaieny, 2015). In the present study, a significant increase was noticed in proline content due to foliar applied PGR. This PGR ultimately became a reason for both varieties to bear salt conditions. An earlier study about wheat resulted positively in response to glutamate under NaCl stress. All the morphological characters were also positively examined. Higher values of SPAD were also investigated by the use of glutamic acid in pumpkin (Fan et al., 2013; Goncalves et al., 2008).

CONCLUSIONS

Salt stress triggered the seed germination and growth of cucumber leaves. This suppression could be reversed by using exogenous spray of glutamic acid. Exogenously

addition of glutamic acid significantly enhanced SOD, POD, CAT and APX activities while reduced MDA, H₂O₂ content and concentration of total soluble proteins in salt-stressed cucumber leaves. Glutamic acid may act to alleviate the adverse effect of salt stress in cucumber leaves by minimizing membrane lipid peroxidation. Recorded morphological data completely supported the V2. So, the defensive role of glutamic acid under salt stress may be at least moderately attributed to its ability to arbitrate the ROS-scavenging enzymes and/or direct scavenging of the superoxide anion. Consequently, hybrid cultivar SSC-228 (V2) was assumed to be the best salt-tolerant cultivar.

ETHICS DECLARATIONS

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author Contributions

PI, MAG and BA have performed the experiment. MS, QI and KZ have analysis the experiment. MA, AN, KLC and JA have physical data and draft. MAG and BA have designed the experiment.

REFERENCES

- Aghaleh, M., V. Niknam, H. Ebrahimzadeh and K. Razavi. 2009. Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biol. Plant.* 53: 243-248.
- Al-Harbi, A. R. 1994. Effect of manipulating nutrient solution salinity on the growth of cucumber *Cucumis sativus* L. grown in NFT. *Acta Hortic.* 361: 267-273.
- Ashraf, M. Y. and A. S. Bhatti. 2000. Effect of salinity on growth and chlorophyll content in rice. *Pak. J. Ind. Res.* 43: 130-131.
- Barbosa, J. M., N. K. Singh, J. H. Cherry and R. D. Locy. 2010. Nitrate uptake and utilization is modulated by exogenous g-aminobutyric acid in *Arabidopsis thaliana* seedlings. *Plant Physiol. Biochem.* 48: 443-450.
- Blumwald, E. 2000. Sodium transport and salt tolerance in plants. *Curr. Opin. Cell Biol.* 12: 431-434.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.
- Chartzoulakis, K. S. 1990. Effects of saline irrigation water on germination, growth and yield of greenhouse cucumber. *Acta Hortic.* 287: 327-334.
- Chartzoulakis, K. S. 1992. Effect of NaCl salinity on germination, growth and yield of greenhouse cucumber. *J. Hortic. Sci.* 67: 115-119.
- Chartzoulakis, K. S. 1994. Photosynthesis, water relation and leaf growth of cucumber exposed to salt stress. *Sci. Hortic.* 59: 27-35.
- Chauhan, A., B. A. Abu-Amarah, A. Kumar, J. S. Verma, H. A. Ghramh, K. A. Khan and M. J. Ansari. 2019. Influence of gibberellic acid and different salt concentrations on germination percentage and physiological parameters of oat cultivars. *Saudi J. Biol. Sci.* 26: 1298-1304.
- Chen, X., S. W. Chen, M. Sun and Z. N. Yu. 2005. High yield of poly-γ-glutamic acid from *Bacillus subtilis* by solid-state fermentation using swine manure as the basis of a solid substrate. *Bioresour. Technol.* 96: 1872-1879.
- Elkahoui, S., J. A. Hernandez, C. Abdely, R. Ghir and F. Limam. 2005. Effects of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. *Plant Sci.* 168: 607-613.
- El-Shaieny, A. H. 2015. Seed germination percentage and early seedling establishment of five (*Vigna unguiculata* L. (Walp)) genotypes under salt stress. *Eur. J. Exp. Biol.* 5: 22-32.
- Fan, X. X., Z. G. Xu, X. Y. Liu, C. M. Tang, L. W. Wang and X. L. Han. 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Sci. Hortic.* 153: 50-55.
- Fricke, W., G. Akhiyarova, D. Veselov and G. Kudoyarova. 2004. Rapid and tissue specific changes in ABA and in growth rate response to salinity in barley leaves. *J. Exp. Bot.* 55: 1115-1123.
- Gebauer, J., E. K. Siddig, A. A. Saleh and G. Ebert. 2004. *Tamarindus indica* L. seedlings are moderately salt tolerant when exposed to NaCl-induced salinity. *Sci. Hortic.* 103: 1-8.
- Ghani, M. A., A. Mushtaq, K. Ziaf, B. Ali, M. M. Jahangir, R. W. Khan, M. Azam and A. Noor. 2021. Exogenously applied GA3 promotes plant growth in onion by reducing oxidative stress under saline conditions. *J. Agric. Sci.* 27: 122-128.
- Goncalves, B., C. M. Correia, A. P. Silva, E. A. Bacelar and A. Santos. 2008. Leaf structure and function of sweet cherry tree (*Prunus avium* L.) cultivars with open and dense canopies. *J. Sci. Hortic.* 116: 381-387.
- Heath, R. and L. Packer. 1968. Photo oxidation in isolated chloroplast. 1. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125: 189-198.
- Hellmann, H., D. Funk, D. Rentsch and W. B. Frommer. 2000. Hypersensitivity of an *Arabidopsis* sugar signaling mutant toward exogenous proline application. *Plant Physiol.* 122: 357-368.
- Jin, L., Y. Cai, C. Sun, Y. Huang and T. Yu. 2019. Exogenous l-glutamate treatment could induce resistance against *Penicillium expansum* in pear fruit by activating defense-related proteins and amino acids metabolism. *Postharvest Biol. Technol.* 150: 148-157.
- Kato, M. and S. Shimizu. 1987. Chlorophyll metabolism in higherplants. 7. Chlorophyll degradation in senescing tobacco-leaves phenolic-dependent per oxidative degradation. *J. Bot.* 65: 729-735.
- Kholova, J., R. K. Sairam, R. C. Meena and G. C. Srivastava. 2009. Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzymes activity. *Biol. Plant.* 53: 249-256.
- Lee, G., R. N. Carrow and R. R. Duncan. 2005. Growth and water relation responses to salinity stress in halophytic seashore *Paspalum* ecotypes. *Sci. Hortic.* 104: 221-236.
- Liu, T. and J. V. Staden. 2001. Growth rate, water relations and ion accumulation of soybean callus lines differing in salinity tolerance under salinity stress and its subsequent relief. *Plant Growth Regul.* 34: 277-285.
- Luo, J., H. Li, T. Liu, A. Polle, C. Peng and Z. B. Luo. 2013. Nitrogen

- metabolism of two contrasting poplar species during acclimation to limiting nitrogen availability. *J. Exp. Bot.* 64: 4207-4224.
- Lycoskoufis, I. H., D. Savvas and G. Mavrogianopoulos. 2005. Growth, gas exchange, and nutrient status in pepper (*Capsicum annuum* L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. *Sci. Hortic.* 106: 147-161.
- Mansour, M. M. F. and K. H. A. Salama. 2004. Cellular basis of salinity tolerance in plants. *Environ. Exp. Bot.* 52: 113-122.
- Meena, S. K., N. K. Gupta, S. Khandelwal and E. V. D. Sastry. 2003. Effect of sodium chloride on the growth and gas exchange of young *Ziziphus* seedling rootstocks. *J. Hortic. Sci. Biotechnol.* 78: 454-457.
- Meloni, D. A., M. A. Oliva, C. A. Martinez and J. Cambraia. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* 49: 69-76.
- Miller, G., N. Suzuki, S. Ciftciyilmaz and R. Mittler. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* 33: 453-467.
- Miyashita, Y. and A. G. Good. 2008. Contribution of the GABA shunt to hypoxia-induced alanine accumulation in roots of *Arabidopsis thaliana*. *Plant Cell Physiol.* 49(1): 92-102.
- Nasri, N., S. Maatallah, L. Saidi and M. Lachaal. 2017. Influence of salinity on germination, seedling growth, ion content and acid phosphatase activities of *Linum usitatissimum* L. *J. Anim. Plant Sci.* 27: 517-521.
- Neelambari, M. C. and S. S. Ganesh. 2018. Curative effect of ascorbic acid and gibberellic acid on wheat (*Triticum aestivum* L.) metabolism under salinity stress. *Int. J. Curr. Microbiol. Appl. Sci.* 7: 522-533.
- Neufeld, H., A. H. Chappelka, G. L. Somers, K. O. Burkey, A. W. Davison and P. Finkelstein. 2006. Visible foliar injury caused by ozone alters the relationship between SPAD meter readings and chlorophyll concentrations in cut leaf coneflower. *Photosynth. Res.* 87: 281-286.
- Patterson, B. D., E. A. Macrae and I. B. Ferguson. 1984. Estimation of hydrogen peroxide in plants using titanium (IV). *Anal. Biochem.* 139: 487-492.
- Ries, S. and C. Giannopolitis. 1977. Superoxide dismutases. 1. Occurrence in higher plants. *Plant Physiol.* 59: 309-314.
- Rodriguez-Rosales, M. P., F. J. Galves, R. Huertas, M. N. Aranda, M. Baghour, O. Cagnac and K. Venema. 2009. Plant NHX cation/proton antiporters. *Plant Signal Behav.* 4: 265-276.
- Saqib, M., J. Akhar and R. H. Qureshi. 2005. Na⁺ exclusion and salt resistance of wheat (*Triticum aestivum*) in saline water logged conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. *Plant Sci.* 169: 125-130.
- Scebba, F., L. Sebastiani and C. Vitagliano. 2001. Activities of antioxidant enzymes during senescence of *Prunus armeniaca* leaves. *Biol. Plant.* 44: 41-46.
- Shih, I. L. and Y. T. Van. 2001. The production of poly- (γ-glutamic acid) from micro-organisms and its various applications. *Bioresour. Technol.* 79: 207-225.
- Sudhakar, C., A. Lakshmi and S. Giridarakumar. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.* 161: 613-619.
- Sudhir, P. R., D. Pogoryelov, L. Kovacs, G. Garab and S. D. Murthy. 2005. The effects of salt stress on photosynthetic electron transport and thylakoid membrane proteins in the cyanobacterium *Spirulina platensis*. *J. Biochem. Mol. Biol.* 38: 481-485.
- Tanou, G., A. Molassiotis and G. Diamantidis. 2009. Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. *Environ. Exp. Bot.* 65: 270-281.
- Tavakkoli, E., P. Rengasamy and G. K. McDonald. 2010. High concentrations of Na⁺ and Cl⁻ ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J. Exp. Bot.* 61: 4449-4459.
- Tester, M. and R. Davenport. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.* 91: 503-527.
- Trajkova, F., N. Papadantonakis and D. Savvas. 2006. Comparative effects of NaCl and CaCl₂ salinity on cucumber grown in a closed hydroponic system. *Hortic. Sci.* 41: 437-441.
- Tseng, M. J., C. W. Liu and J. C. Yiu. 2007. Enhanced tolerance to sulfur dioxide and salt stress of transgenic Chinese cabbage plants expressing both superoxide dismutase and catalase in chloroplasts. *Plant Physiol. Biochem.* 45: 822-833.
- Tuna, L. A., C. Kaya, M. Dikilitas and D. Higgs. 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environ. Exp. Bot.* 62: 1-9.
- van-Ittersum, M. K., L. G. J. van-Bussel, J. Wolf, P. Grassini, J. van-wart, N. Guilpart, L. Claessens, H. De-Groot, K. Wiebe, D. Mason-D'croz, H. Yang, H. Boogaard, P. A. J. van-Oort, M. P. van-Loon, K. Saito, O. Adimo, S. Adjei-Nsiah, A. Agali, A. Bala, R. Chikowo, K. Kaizzi, M. Kouressy, J. H. J. Makoi, K. Ouattara, K. Tesfaye and K. G. Cassman. 2016. Can sub-Saharan Africa feed itself? *PNAS.* 113: 14964-14969.
- Wang, Q., S. Chen, J. Zhang, M. Sun, Z. Liu and Z. Yu. 2008. Co-producing lipopeptides and poly-γ-glutamic acid by solid-state fermentation of *Bacillus subtilis* using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects. *Bioresour. Technol.* 8: 3318-3323.
- Wang, W., B. Vinocur and A. Altman. 2003. Plant responses to drought, salinity and extreme temperatures towards genetic engineering for stress tolerance. *Planta.* 218: 1-14.
- Xu, Z., P. Lei, X. Feng, X. Xu, J. Liang, B. Chi and H. Xu. 2014. Calcium involved in the poly (γ-glutamic acid) mediated promotion of Chinese cabbage nitrogen metabolism. *Plant Physiol. Biochem.* 80: 144-152.
- Xu, Z., P. Lei, X. Feng, X. Xu, H. Xu, H. Yang and W. Tang. 2013. Effect of poly (γ-glutamic acid) on microbial community and nitrogen pools of soil. *Res. J. Agric. Sci.* 63: 657-668.
- Zhu, X. C., F. B. Song, S. Liu and F. L. Liu. 2016. Role of arbuscular mycorrhiza in alleviating salinity stress in wheat (*Triticum aestivum* L.) grown under ambient and elevated CO₂. *J. Agron. Crop Sci.* 202: 486-496.
- Zhu, Z., G. Q. Wei, J. Li, Q. Q. Qian and J. Q. Yu. 2004. Silicon alleviates salt stress and increases antioxidant enzyme activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Sci.* 167: 527-533.