

RESEARCH ARTICLE

Optimizing efficacy of turnip growth through foliar application of glutamic acid under saline conditions

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ABSTRACT

Salinity is assumed to be a distressing abiotic factor that mainly disrupts crop quality and yield by impairing plant cell mechanisms. Due to ion accumulation, salinity stress results in lowering growth rate and water uptake. This issue is being solved by the use of several plant growth regulators. Plant growth regulators have been proven to increase plants' ability to withstand against stress. In this study, turnip (purple top cultivar) was subjected to four distinct levels of salt (0, 4, 8, and 12 dS/m), as well as one level of gibberellic acid, in order to assess the function of exogenously applied plant growth regulator glutamic acid (GA) (10 mM). Results revealed that salt stress slowed plant growth and decreased the amount of chlorophyll in turnip leaves. Application of salt alone resulted in a considerable decline in biochemical characteristics. However, in salt-stressed conditions, exogenous application of GA improved the antioxidant activity, chlorophyll contents and plant growth in the turnip leaves. Moreover, results depict that under salt stress vitamin C decreased; however, exogenous application of GA enhanced the Vit. C in turnip plants. Further, the uptake of salt content in turnip roots and leaves was significantly lowered by the application of GA. Additionally, under salt stress; GA dramatically controlled the quantity of phenolic compounds in turnip.

Keywords: *Brassica rapa*, Salinity, Glutamic acid, Morphological and biochemical assay, Reducing and non-reducing sugar

INTRODUCTION

Turnip (*Brassica rapa* L.), a dicotyledonous vegetable crop, basically belongs to cruciferous family. Generally instigated in Europe and it was taken to Asia throughout Greek time (Liang et al., 2006). Fleshy natured roots and leaves are chiefly composed of vitamin A, C and E (Parveen et al., 2015), that's why; it is also used as salad and pickles (Nielsen et al., 2008). There are a lot of biotic and abiotic stresses which disrupt plants quality, nutritious value, and yield rate (Shi et al., 2011). Among all, salinity is assumed to be an alarming threat for worldwide production rate. Irrigated saline water is also disordering food security (Abogadallah, 2010). Due to high ionic accumulation, there is a major decrease in plant growth, development, metabolic activities, and water uptake (Reynolds et al., 2005).

Salt stress is generally considered to be a major abiotic stress in all regions of the world. About 20% cultivated land of the world is under salt stress. Some plants are salt-tolerant; while, some are salt-sensitive, it's totally depends upon growth response of certain crops. Salt stress distracts plant at various cellular levels by impairing ionic uptake. Turgor loss and dehydration happens due to the increasing salt level (Murphy et al., 2003). Salt stress exerts detrimental effects on plant growth and development which ultimately reduce the crop productivity (Ashraf, 2004). Only roots of plants are directly in contact to the saline medium. As a result of which, uptake process of ions and water is badly affected. Salt stress leads to the impairment of many physiological processes such as nutrient uptake. For example, higher concentration of Na⁺ in soil diminishes the Ca²⁺ activity by lowering its availability to crop plants (Barnawal et al., 2017).

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Glutamic acid, generally connected by amid linkages, plays a super role concerning agricultural point of view. It was reported as a source for boosting cucumber's dry weight as well as to increase tolerance level against abiotic stresses (Xu et al., 2013). Previous studies showed that the plant growth and nitrogen assimilation were appreciably enhanced by glutamic acid (Ling et al., 2015). Furthermore, it became a major cause for increasing tolerance level and resistance of oilseed rape seedlings against salt stress. As genetic engineered technologies help the plant to ameliorate their metabolic activities in saline conditions, like this exogenous application of certain solutions or plant growth regulators are also being used to moderate abiotic stress circumstances. Moving towards such approaches, glutamic acid has been reported to improve plant metabolic activities under salt stress conditions (Shih and Van, 2001). It also acted as a precursor in attaining the crucial role of proline and amino butyric acid, which in turn used to protect plants during different environmental stresses (Evers, 1999). Glutamic acid shows great potential regarding agricultural use due to its biocompatible and biodegradable properties (Ashiuchi, 2011; Bajaj and Singhal, 2011; Sung, 2015). GA also can amplify the production of Chinese cabbage, cucumber and wheat in both physiological and reproductive growth stage (Dunn et al., 2006; Persson et al., 2006, Iqbal et al., 2021). It also plays a perspective role concerning water availability, nutrient uptake and plant metabolism (Xu et al., 2013). Turnip primarily reacts as a salt tolerant crop at germination period, comparatively later different stages of growth. Though, a remarked diminution in turnip growth is accounted under salt stress (Francois, 1984). To overcome all disruptive causes, different studies reported different methods i.e., foliar application of plant growth regulator. Thus, it's a great curiosity to conclude if exogenous application of glutamic acid could present resistance in turnip plant against salt stress. Predominantly, the aim was to determine glutamic acid's efficacy in influencing plant growth and metabolic assessments under salt stress. This study's main objective was to measure the amount of chlorophyll in turnip seedlings' leaves as well as the effectiveness of glutamic acid in promoting seedling growth in the phase of salt stress.

MATERIALS AND METHODS

Plant materials and treatments

To influence the major role of glutamic acid under different saline conditions, a pot experiment was designed at Vegetable Research Area, Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. For this purpose, seven different treatments with four replications were laid out. Treatments were comprised as T_0 = Control (without salt and GA), T_1 = 10 mM glutamic

acid, T_2 = 4 dS/m NaCl, T_3 = 8 dS/m NaCl, T_4 = 12 dS/m NaCl, T_5 = 4 dS/m NaCl+10 mM glutamic acid, T_6 = 8 dS/m NaCl+10 mM glutamic acid and T_7 = 12 dS/m NaCl+10 mM glutamic acid. Before sowing, salinity was developed in each pot (pot height 28cm and pot diameter 19cm) regarding related subsequent treatments. There were 6 kg of amended soil in each pot. Before being directly sown in the pot, turnip seeds were sterilized by soaking in 70% ethanol for 1 minute, sodium hypochlorite for 5 minutes, and then five times in distilled water. In each pot, three turnip plants were raised. All seedlings were sprayed with GA after 21 days of seed germination, with the exception of T_2 , T_3 , and T_4 (10mM). 9 a.m. to 10 a.m. saw the application of the foliar spray. Distilled water was sprayed over the plants in the control pots. Solution of GA was prepared in distilled water. GA has previously been demonstrated to accelerate plant development in turnip plants at a dosage of 10 mM. Each pot sprayed of 200 mL solution of GA in total. While GA was being applied to other plants, they were enclosed in polyethylene bags. Samples for biochemical assays were collected and stored at -80°C , while morphological parameters were assessed when plant length reached its maximum height (40–45 days following seed germination).

Morphological characters

The height of plant was determined by using scale from the plant's tip to its base on the ground. Shoot and root length of the plant was measured by using measuring scale. Shoot fresh and dry weight was taken on weighing balance. Initially shoots of plants were taken cleaned by distal water and fresh weight was calculated, for the dry weight plant shoot, samples were dried in sun and the placed them in oven at 65°C for 48hr after drying their weight was taken. Root diameter was taken by using Vernier caliper. Root fresh and dry weight was taken by using weighing balance (A& D, K9222350 by Japan). Firstly fresh samples of roots were taken washed properly by distal water and weight calculated, then these samples before oven dry placed in directly under sun and then in oven at 65°C for 72hr.

Biochemical enzymatic activities

The procedure that was used to assess the superoxide dismutase (SOD) activity was as follows of Zhang et al. (2008) by constraining nitro blue tetrazolium undergoes photochemical reduction. The Peroxidase (POD) activity was analyzed by Zhou and Leul (1999). The catalase (CAT) activity was examined by using the procedure of Aebi (1955), on the spectrophotometer, the H_2O_2 absorbance was measured at 270 nm. The H_2O_2 content was determined by the technique of Nakana and Asada, (1981) and Using a spectrophotometer, its absorbance was determined at 390 nm (Bio Tek Instruments, Inc., Winooski, VT, USA).

Determination of Malondialdehyde (MDA) and Total Soluble Solids (TSS)

The amount of MDA in the leaf tissue was determined by using the thiobarbituric acid (TBA) procedure to evaluate the lipid peroxidation of the membrane oxidative damage (Zhou and Leul 1998).

According to the Official Analytical Chemistry Association (Latimer, 2016), total soluble solids (TSS) were determined by reading directly in Brix by the use of digital refractometer. Sample drop placed or put on the prism of the instrument and recorded the value by the digital refractometer (Spectroquant ® Move DC)).

Determination of Chlorophyll content, Titratable acidity (TA), Vitamin C and Total sugar

Chlorophyll concentration was measured by following the method of Arnon (1949). All the samples were placed under photo spectrometer and absorbance was calculated at 630nm. The titration with 0.1 N NaOH was used to measure the titratable acidity in 100 g distilled water in 100 mL phenolphthalein, expressed as a percentage of citric acid in the fruit (Sadler, 2010). To check the amount of reducing and non-reducing sugar and total sugar was prepared. A specified indicator was also used for this purpose to assume the general determination of total sugar content. Prepared solution was titrated against the particular indicator, phenolphthalein (Ghani et al., 2020). Vitamin C will be determined by according as described method by Ruck (1963). Filtered aliquot (5mL) will be treated against dye (2, 6-dichlorophenolindophenol) until pink colour will be achieved. Vitamin C will be calculated by using formula given below:

$$\text{Ascorbic acid (mg/100g)} = \frac{R_1 \times V}{R \times W \times V_1} \times 100$$

Statistical analysis

Calculated data is expressed as the mean \pm standard deviation. Statistics 8.1 software was used for statistical analysis via one-way ANOVA method, complete randomized design (CRD) followed by tukey's test ($p < 0.05$).

RESULTS

Effect of glutamic acid on turnip growth under various levels of salt stress

The turnip cultivar's dry and fresh weight of roots, and growth of plant were all significantly lower when the plants were exposed to salt. However, with the addition of 12dS/m NaCl, the decline was, however, more prominent in the plants (Tables 1 and 2). Application of 10mL GA increased the plant biomass, as well as under salt stress condition, exogenous application of glutamic acid dramatically increased the plant's fresh and dry biomass.

The dry and fresh weight of root, root diameter, root and shoot length, dry and fresh weight of shoot weight of the turnip cultivar were all considerably increased by the foliar application of GA at 10mL (Table 1). The application of salt stress alone gradually decreased fresh and dry biomass of plant parts. The plants exposed to combine treatment of salt and GA prominently showed that GA decreased the effect of salt and improved the plant height and growth root length and root diameter. However, where the maximum concentration of 12dS/m salt was used along with GA 10 mL less growth was found and vice versa (Tables 1 and 2).

Antioxidant enzymatic activities

The antioxidant enzyme activities (POD, SOD, APX and CAT) were significantly affected by the various salt and glutamic acid treatments of turnip cultivar (Fig. 1). The results showed that at application of GA alone, the SOD and APX enzyme activities were increased in the leaves of the turnip cultivar (Fig.1A, 1D). The SOD, POD, CAT, and APX concentrations in the leaves of turnip plants were dramatically reduced when there was more salt present in the soil. However, with an increase in salt concentration, SOD and APX activity in the soil of the turnip plants showed a decreasing trend. (Fig. 1A and 1D). The POD and CAT activity in the turnip leaves was enhanced by the exogenous application of glutamic acid in combination with various salt concentrations. (Fig. 1B and 1C).

Effects of glutamic acid on the MDA and TSS under salt stress.

The variations in total soluble salts and malondialdehyde (MDA) content are shown in Fig. 3. Data indicated a considerable increase in the MDA contents enhanced in turnip plants under different salt treatments. However, exogenously application of glutamic acid considerably reduced the MDA concentrations when used alone, and when GA and salt were given together at a concentration of 12 dS/m, the MDA content was higher compared to the corresponding control. (Fig. 2A). Furthermore, according to this research data, turnip cultivars exposed to salt stress at a higher concentration of 12dS/m salt in soil had significantly higher TSS concentrations. Minimum TSS content was found where exogenous application of 10mM GA was applied (Fig. 2C).

Chlorophyll content

The impact of different salt and GA concentrations on chlorophyll content are presented in Fig. 2. The turnip cultivar's chlorophyll concentration was considerably reduced by salt treatment alone (Fig. 2B). In comparison to where the alone salt treatment was made, the combine and alone GA 10mM + 4dS/m salt foliar application considerably increased the leaf chlorophyll content in

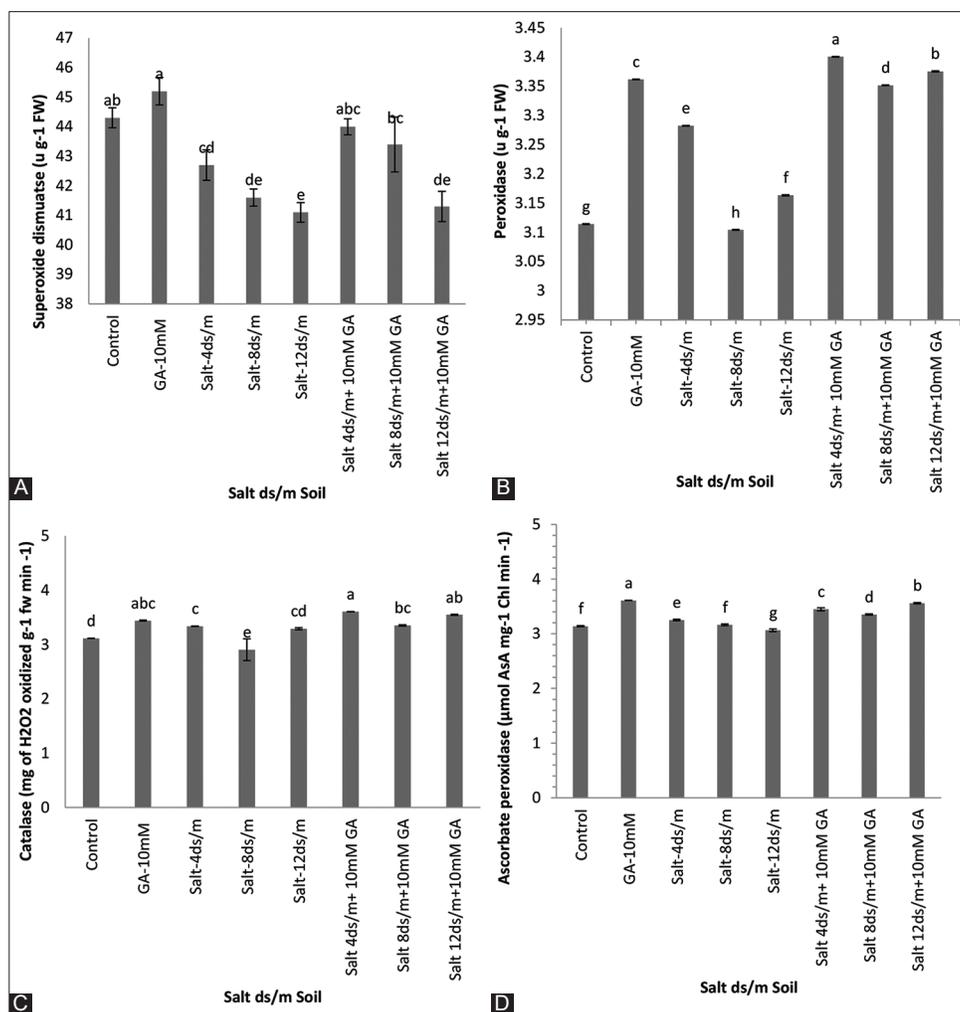


Fig 1. Effect of different treatments of salinity and glutamic acid on superoxide dismutase (A), peroxidase (B), catalase (C) and ascorbate peroxidase (D). Values are the mean \pm standard error ($n=4$).

turnip. The higher values were found at 10mM GA when turnip was exposed to exogenous GA (Fig. 2B).

Titrateable acidity (TA), Total sugar and Vitamin C

Reducing and non-reducing sugar of different treatments were measured in this study. Overall results showed significant difference among treatments (Fig. 3A). Results of reducing sugar of salt stress along with the application GA showed highest range at 8dS/m salt + 10mM GA, on other hand application of GA alone decreased the reducing sugar contents (Fig. 3A). Non-reducing sugar results indicated that in the combined application of salt and GA show more non-reducing sugar contents at 8ds/m salt + 10mM GA (Fig. 3B). Results for total sugar showed a range in combine treatment was highest as compared to other salt and GA alone application (Fig. 3C). Results for titrateable acidity (TA) are notably important, both for analysis and for their interaction. When the growth regulator was applied under 8dS/m of salt level, the highest TA concentrations were found. However, maximum TA activity was exhibited at GA

+ 8dS/m salt. Salt concentration steadily reduced the amount of TA in the solution, although combined application revealed a rising order with respect to salt stress. On the other hand, turnip cultivars showed minimal TA concentrations when the level of salt stress was at its highest (Fig. 3D). The salted treatment and exogenous application of GA have the different Vit. C content in turnip. The total Vit. C content in those plants on which alone GA application was done, which was 0.7 mg kg^{-1} , while on other hand Vit. C content in salted plants were ranged from 0.38 mg kg^{-1} to 0.5 mg kg^{-1} . The highest Vit. C content was observed in combined application of salt and GA which was 0.76 mg kg^{-1} at 8dS/m salt + 10mM GA as compared to other treatments (Fig. 2D). The results showed that the effect of salt stress decreased Vit. C contents but application of GA improved Vit. C content.

DISCUSSION

In this experiment, exogenous GA was used to increase salt tolerance in a turnip cultivar. Results showed that salt

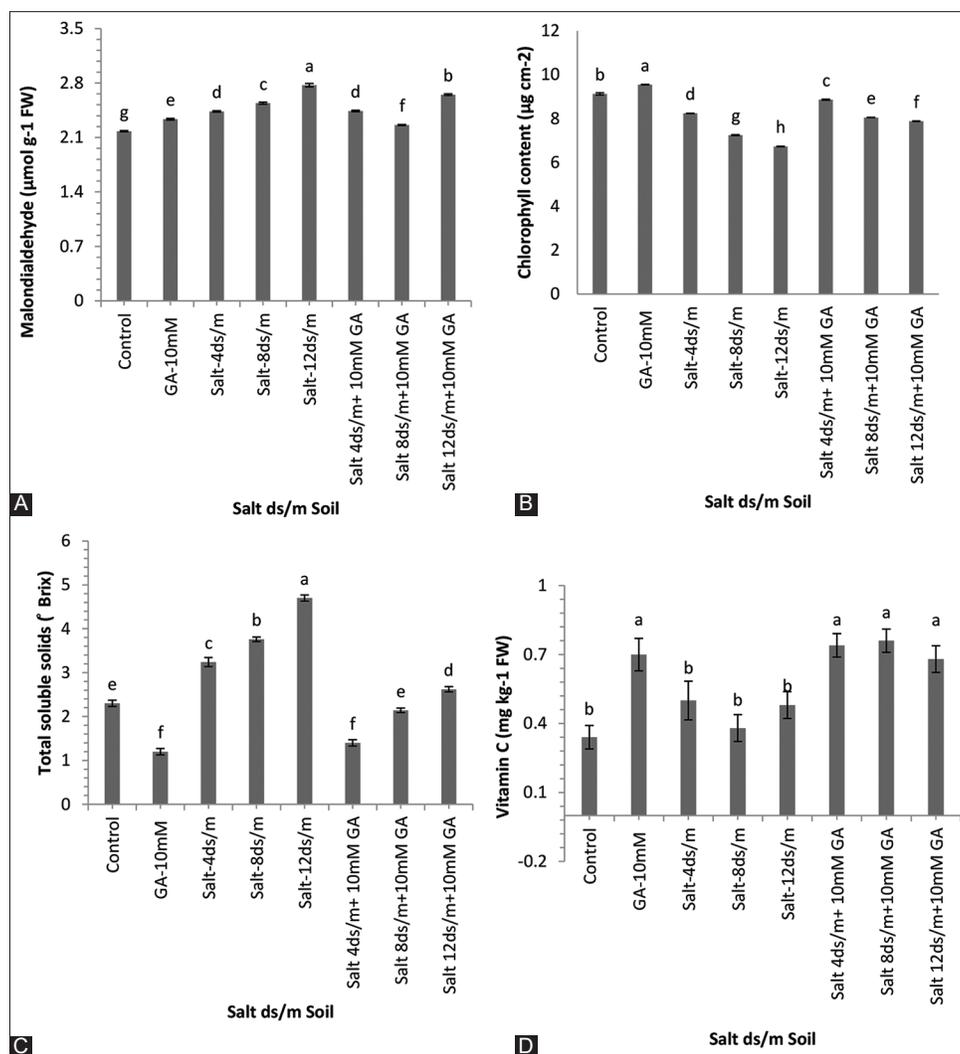


Fig 2. Effect of different treatments of salinity and glutamic acid on malondialdehyde (A), chlorophyll content (B) total soluble solids (C) and vitamin C (D). Values are the mean \pm standard error (n=4).

has a considerable negative impact on the growth of turnip plants by morphologically altering them (plant height, shoot and root length, shoot and root dry and fresh weight). Previously, similar findings in *Brassica napus* have already been found (Ali et al., 2014). Moreover, Huang et al. (2010) also studied about the effect of salt stress on *Brassica napus* and concluded that salt adversely affected the growth and number of leaves per plant. The concentration of salt adversely affected the shoot and root biomass, as studied by Francois (1984). Another study concluded that at lower level of salt, more root and shoot length was observed. Plant growth was more severely hampered by the higher salt concentration. Five turnip cultivars (Peela, Neela, Purple top, Golden bal, and desi) were subjected to salt stress, and Noreen et al. (2010) compared the phenotypic changes in these five cultivars. The results showed that plant bio mass plant dry and fresh weight, root dry and fresh weight was significantly decreased when plants exposed under salt stress. Another study of jia et al. 2020 also concluded the

same results where turnip plant under salt stress decreased their plant biomass but with the application of PGRs plant resist against salt stress.

The effect of salinity on the root length was determined on the early and bulb production stages of turnip. Application of glutamic acid during salt stress significantly showed positive response towards root length. This study is also in line with the results of Shahbazi et al. (2011) who observed that by adding more salt concentration root length of turnip plants showed negative behavior.

Present study concluded that alone salt stress did not produce healthy bulb of turnip, so the fresh weight was lower than control as well as glutamic application. Increased fresh weight was also observed in the previous study by Maheshwari et al. (2009) that coincided with our results regarding the reduction of root length by the application of salt. The amplified turnip root was influenced more

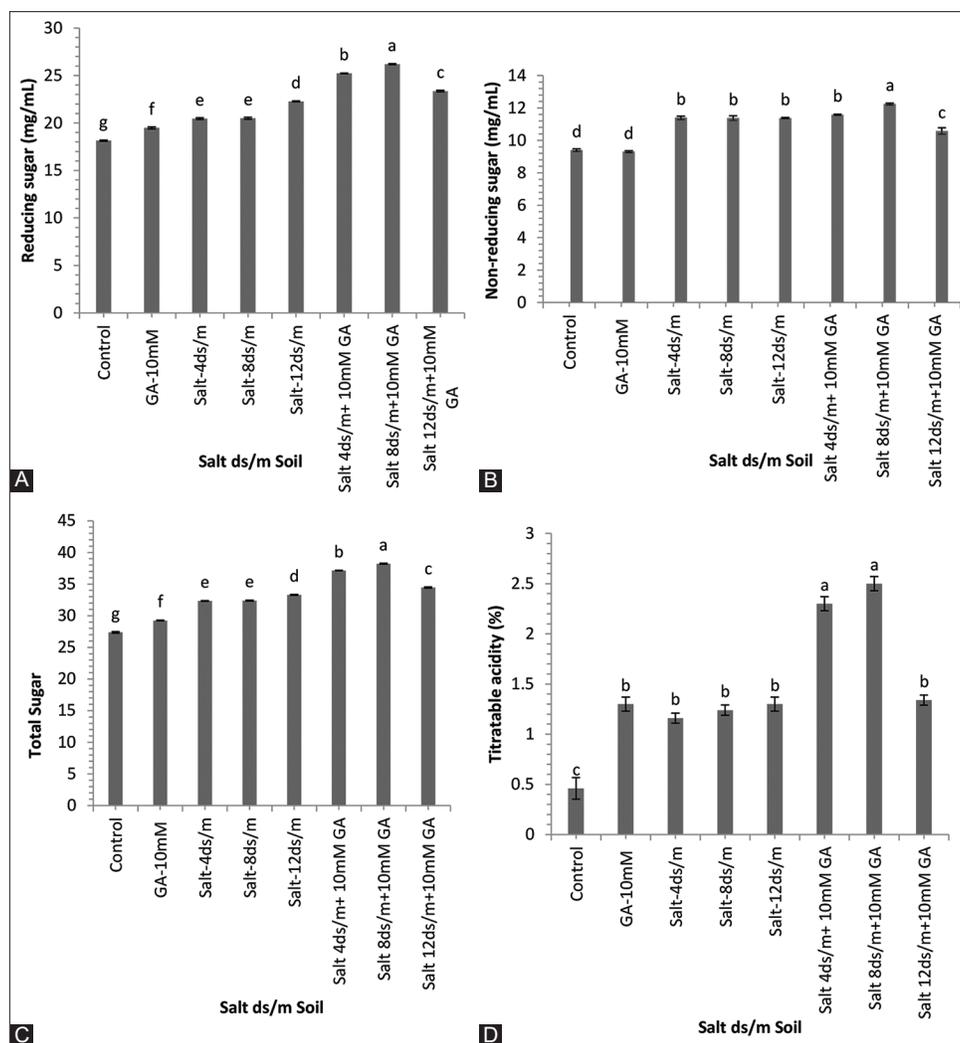


Fig 3. Effect of different treatments of salinity and glutamic acid on reducing sugar (A), non-reducing sugar (B), total sugar (C) and titratable acidity (D). Values are the mean \pm standard error (n=4).

Table 1: Effect of different treatments of salinity and glutamic acid (GA) on the root diameter (mm), root length (cm), root fresh weight (g), root dry weight (g), plant dry weight (g) and plant fresh weight (g) of turnip plant

| Treatments | Root diameter (mm) | Root length (cm) | Root fresh weight (g) | Root dry weight (g) | Plant dry weight (g) | Plant fresh weight (g) |
|-----------------|---------------------------------|--------------------------------|----------------------------------|-------------------------------|-------------------------------|---------------------------------|
| Control | 23.7 \pm 0.53 ^{abc} | 13.5 \pm 0.22 ^{ab} | 14.168 \pm 1.56 ^{ab} | 3.59 \pm 0.68 ^{ab} | 6.26 \pm 0.55 ^{ab} | 42.06 \pm 0.82 ^{ab} |
| Glu (10mL) | 26.82 \pm 0.50 ^a | 14.7 \pm 0.2 ^a | 16.042 \pm 1.27 ^a | 5.65 \pm 0.40 ^a | 7.46 \pm 0.45 ^a | 44.32 \pm 1.0061 ^a |
| 4dS/m NaCl | 21.928 \pm 1.27 ^{bc} | 12.9 \pm 0.4 ^{ab} | 10.118 \pm 0.40 ^{abc} | 3.62 \pm 0.70 ^{ab} | 5.66 \pm 0.11 ^b | 40.97 \pm 0.23 ^{abc} |
| 8dS/m NaCl | 19.63 \pm 0.79 ^{cd} | 11.9 \pm 0.71 ^{bc} | 11.962 \pm 0.24 ^{bc} | 2.87 \pm 0.33 ^b | 5.35 \pm 0.25 ^b | 38.88 \pm 0.88 ^{bcd} |
| 12dS/m NaCl | 17.13 \pm 0.76 ^d | 9.3 \pm 0.37 ^d | 8.188 \pm 0.39 ^d | 3.01 \pm 0.39 ^b | 5.15 \pm 1.14 ^b | 38.13 \pm 1.18 ^d |
| 4dS/m NaCl+Glu | 25.86 \pm 1.49 ^{ab} | 13.9 \pm 0.33 ^a | 14.034 \pm 0.51 ^{ab} | 4.72 \pm 0.51 ^{ab} | 6.48 \pm 0.42 ^{ab} | 43.16 \pm 0.35 ^a |
| 8dS/m NaCl+Glu | 23.47 \pm 0.79 ^{abc} | 13.04 \pm 0.56 ^{ab} | 12.948 \pm 0.34 ^{abc} | 3.63 \pm 0.34 ^{ab} | 5.48 \pm 0.21 ^b | 40.86 \pm 0.64 ^{abc} |
| 12dS/m NaCl+Glu | 21.34 \pm 0.40 ^c | 10.2 \pm 0.25 ^{cd} | 12.932 \pm 0.70 ^{cd} | 2.48 \pm 0.49 ^b | 5.29 \pm 0.29 ^b | 37.80 \pm 1.29 ^{cd} |

^{a-d}Different lower-case letters are denoting the significant difference between treatments by Tukey's test ($P \leq 0.05$). Each data values are represented as means and \pm SD of four replications

than different tissues by saltiness, likely on the grounds that high convergences of salt particles encompassing the roots made neighborhood parchedness and drove root harm, restraining its development and improvement. In concurrence with the current work, Kholova et al., 2009, inferred that turnip tops are essentially more salt open

mined than roots. Another investigation revealed that for every unit of expansion in saltiness (over the saltiness limit) root and shoot biomass creations diminished 9% and 5% individually. Interestingly, the impact of saltiness on the last germination rate in the current investigation was critical. As opposed to other plant species, saltiness

Table 2: Effect of different treatments of salinity and glutamic acid (GA) on shoot length (cm), plant height (cm), shoot fresh weight (g) and shoot dry weight of turnip plant

| Treatments | Shoot length (cm) | Plant height (cm) | Shoot Fresh weight (g) | Shoot dry weight (g) |
|-----------------|-------------------------|--------------------------|--------------------------|---------------------------|
| Control | 20.5±2.22 ^{ab} | 49.3±2.26 ^{ab} | 34.66±3.68 ^{ab} | 6.04±0.37 ^{ab} |
| Glu (10mL) | 37.2±1.98 ^a | 51.9±1.9 ^a | 38.24±3.10 ^a | 7.14±0.515 ^a |
| 4dS/m NaCl | 34±0.70 ^{ab} | 46.9±0.84 ^{abc} | 31.68±3.04 ^{ab} | 5.162±0.094 ^{bc} |
| 8dS/m NaCl | 31.2±1.24 ^{ab} | 43.1±0.71 ^{cd} | 28.16±3.11 ^{ab} | 4.81±0.19 ^{bc} |
| 12dS/m NaCl | 29.5±0.5 ^b | 38.8±0.2 ^d | 24.27±1.08 ^b | 4.61±0.17 ^c |
| 4dS/m NaCl+Glu | 35.2±1.15 ^{ab} | 49.1±0.97 ^{ab} | 33.69±3.44 ^{ab} | 6.08±0.36 ^{ab} |
| 8dS/m NaCl+Glu | 32±1.8 ^{ab} | 45.05±1.59 ^{bc} | 30.93±2.84 ^{ab} | 5.25±0.08 ^{bc} |
| 12dS/m NaCl+Glu | 31±0.44 ^{ab} | 41.2±0.33 ^{cd} | 27.33±1.77 ^{ab} | 5.11±0.12 ^{bc} |

^{a-d}Different lower-case letters are denoting the significant difference between treatments by Tukey's test ($P \leq 0.05$) Each data values are represented as means and+SD of four replications

had no critical impact on Ca^{2+} at various development stages. Root diameter also affected by the high salt ratio. Due to the high uptake of salt, metabolic reaction slowed down and ultimately affected the root diameter of turnip. Antioxidant enzymes activities were enhanced significantly with the application of glutamic acid under salt stress. Our findings suggested that glutamic acid controls the activity of these enzymes either directly or indirectly; as a result, this molecule functions as a scavenger against stressful situations (Gill et al. 2016). In the current study, activities of POD significantly enhanced in turnip with salt addition. The CAT activity gradually decreases with increase of salt ratio. Similar findings were made with wheat, which likewise showed improved APX and CAT contents. Due to glutamic acid's potential role as an antioxidant, these findings suggested that additional ROS should be excluded. Zhu et al., 2016, noticed that chlorophyll content of turnip was reduced significantly by high salinity dose.

Different concentrations of TSS content were reported in different families of vegetables (Rokaya et al., 2016). TSS are considered an crucial quality trait for the tomato that stated its nutrient content (Ghani et al., 2020). Glutamic acid showed high range of TSS against the salt stress. The majority of acids in food are organic acids, with acetic, tartaric, citric, lactic and malic acids being the most prevalent types (George, 2010). Previously, Mizoguchi et al. (2005) also found that salt application reduced the sugar contents in turnip plants. There has been evidence of sugar accumulation in plants under salinity stress condition, which allowed the plants to adapt osmotically. (Rolland et al., 2002).

CONCLUSION

In the present study, non-reducing sugar decreased by higher level of salinity but combine and alone application of glutamic acid retarded the elevation of stress and increased level of non-reducing sugar content. This study had shown that salinity reduced the growth and affected

the quality of turnip, especially fruit color and ascorbic acid concentrations. Additionally, it was shown that turnip seedlings under salt stress grew more slowly and had less chlorophyll in their leaves. Turnip seedlings would increase the activities of antioxidant enzymes to eliminate reactive oxygen and adjust the contents of TSS, sod, POD, CAT, MDA, and H_2O_2 to regulate plant growth in order to lessen the effects of salt stress. The findings of this study imply that salt stress causes turnip seedling growth to be inhibited. According to our findings, "glutamic acid" was very salt stress tolerant.

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

MAG, MMA and BA have performed the experiment. JI, QI, SN, and KZ have analysis the experiment. MAG, AN, FS and MZ have physical data and draft. MAG, RWKQ and BA have designed the experiment.

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