Journal of Plant Growth Regulation (2023) 42:3257–3273
https://doi.org/10.1007/s00344-022-10788-x

Alleviation of Salt Stress in *Solanum tuberosum* L. by Exogenous Application of Indoleacetic acid and L-Tryptophan

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Received: 5 January 2022 / Accepted: 3 September 2022 / Published online: 16 September 2022

Abstract
Salinity is a major abiotic stress factor that limits crop production and is an ever-present threat to agricultural sustainability and world's food security. Plant hormones are known to play critical role in regulating plant response to stress by up-regulating the proteins and antioxidant enzymes. The present study aimed at alleviation of salt stress in potato cv. Cardinal by exogenous application of various concentrations of indoleacetic acid (IAA; 17.142, 22.875 or 28.570 µM) and its precursor L-tryptophan (0, 1, 5, 10 or 15 µM) under in vitro conditions. Salt stress was imposed by four NaCl (0, 40, 60 or 80 mM) concentrations. Two modes of IAA application were tested, i.e., added directly to Murashige and Skoog (MS) medium or pretreatment of nodal explants procured from in vitro-raised potato plants. Tryptophan was added directly into the medium containing various concentrations of NaCl. Results were recorded for morphological (shoot and root length, number of shoots and roots and nodes and fresh weight) and biochemical parameters (protein content, peroxidase and superoxide dismutase activities) after 30 days of NaCl and IAA/tryptophan treatment. Salinity caused significant reduction in all growth parameters as well as in all biochemical attributes. Exogenously applied IAA and L-tryptophan alleviated these detrimental effects of salinity by enhancing potato growth, protein content and antioxidant enzyme activities in normal as well as NaCl-stressed plants. Direct incorporation of IAA into the medium was found to be a better approach as compared to its pretreatment to the nodal explants. This study has shown up-regulation of proteins, POD, and SOD activities by using IAA and L-tryptophan indicating their possible involvement in the scavenging process of reactive oxygen species in potato growing under salt stress.

Keywords Antioxidant enzymes · Indoleacetic acid · Potato · Proteins · Salt stress · Tryptophan

Introduction

Potato (*Solanum tuberosum* L.) is an economically important crop of the Solanaceae family. Its significance as food crop cannot be overemphasized. It is free of cholesterol and rich in carbohydrates, proteins, micronutrients, various vitamins, minerals, and dietary antioxidants (Beals 2019). Potato starch is also widely used by various industries as an adhesive, binder, texture agent and filler and can also be processed to produce fuel-grade ethanol (FAO 2008). Globally, potato is grown on 180,000 sq km with annual production of 323 million (FAO 2021). The world potato demand is expected to increase rapidly in future. In Pakistan, despite a dramatic increase in the area and production of potato, current national average tuber yield is far less than the actual potential of the genotypes and it is mainly due to non-availability of stress tolerant potato genotypes and lack of certified seed (Priegnitz et al. 2020).

Salinity is one of the major abiotic stresses known limiting crop productivity. Potato is moderately salt-sensitive and salinity levels as low as 2.3 dS m^{-1} are known to reduce both growth and tuber yield (Katerji et al. 2003). Potato cultivars and wild species respond to elevated levels of NaCl and Na_2SO_4 differently (Sajid and Aftab 2012) and thus become good candidates to provide better insight for studying abiotic stress tolerance mechanisms in higher plants.

Salinity affects plant growth and development considerably. It is a known fact that about 20% of agricultural land and 50% of cropland in the world is affected by salt stress (Islam et al. 2019). An estimated total area of salt-affected soils (saline and/or sodic) globally is 831 million hectares

Handling Editor: Branka Salopek Sondi.

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extending over almost all the major continents (FAO 2021). In Pakistan, out of the total cultivated area, about 6.30 million hectares of land are salt affected (Sajid and Aftab 2014; Syed et al. 2021). Sodium chloride (NaCl) is considered to be the most common cause (Li et al. 2006). Soil salinity limits the caloric and nutritional potential of agricultural production and thus proves to be a real threat to the world's food security. Salinity stress, in general is due to osmotic stress as well as ionic imbalance (Ali et al. 2022). Salinity affects the plant growth by lowering leaf water potential that leads to reduced turgor pressure, badly affects growth and in turn crop productivity. Roots are the first organ to be affected by salinity. High NaCl concentration causes a decline in root cell expansion and proliferation. Additionally, salt stress also causes an oxidative stress due to fast production of reactive oxygen species (ROS; Panda and Upadhyay 2004). These ROS (like superoxide radical (O_2^-), hydroxyl radical (HO^-) or singlet oxygen (O_2^1) harm photosynthetic components (Munir et al. 2021), cause enzyme inhibition, DNA/RNA damage, membrane lipid peroxidation and protein oxidation (Isayencov et al. 2019). Antioxidant enzymes are the key players in the scavenging system of ROS (Khilji et al. 2022). Superoxide dismutase (SOD) is a main scavenger of O_2^- resulting in the synthesis of hydrogen peroxide (H_2O_2). Catalase (CAT), ascorbate peroxidase (APX) and peroxidases (POD) are responsible for the breakdown of H_2O_2. The scavenging of ROS by increased activity of antioxidant enzymes may thus improve salt tolerance (Al Kharusi et al. 2019; Khalid and Aftab 2020).

Exogenous application or pretreatment with various inorganic or organic compounds like potassium chloride (KCl), sodium chloride (NaCl), sodium sulphate (Na_2SO_4), hydrogen peroxide (H_2O_2), Polyethyleneglycol, mannitol, sorbitol, glycine betaine, proline, ascorbic acid, has already been tested and found to be an efficient method to enhance the tolerance level of various plant species against abiotic stresses (Ashraf and Foolad 2005; Wahid et al. 2007; Ejaz et al. 2012; Abdel Latef et al. 2021). However, reports on the use of PGRs like indole-3-acetic acid or gibberellic acid (GA_3) and their precursors for amelioration of stress tolerance are scarce.

The phenomenon of plant adaptation to salinity stress is perhaps principally hormonally controlled that triggers activation of stress response mechanisms responsible for regaining ionic equilibrium (Hasegawa et al. 2000; Zhu 2001; Pedranzani et al. 2003; Ruggiero et al. 2004). The re-establishment of hormonal equilibrium under stress perhaps plays a key role in the survival of plants (Quamruzzaman et al. 2021). Indole-3-acetic acid (IAA) mediates a wide range of growth and developmental responses and detoxify the ROS to enhance the division of cells (Kaya et al. 2018). Several researchers have reported that exogenous application of IAA increases crop yield and evidence suggests that vigorously growing plants may cope with salinity stress in much a better way possibly by delaying the beginning of the salinity tolerance threshold (Dalton et al. 2000; Munns et al. 2006; Chauhan et al. 2009). IAA can also alleviate the adverse effects of salt stress on root system architecture by induction of rooting. IAA stimulates stomatal opening and water movement in roots (Saberi et al. 2021) and is thought to play role under stresses (salinity and drought) that affect turgor. Researchers also reported that salt stress causes reduction in endogenous IAA levels in plants (Dunlap and Binzel 1996; Wang et al. 2001). Further, IAA has also been reported to regulate antioxidant enzymes in stressed plants to neutralize the ROS (Junghans et al. 2006; Shiraz et al. 2021). Exogenous application of IAA has thus shown promise in alleviating the adverse effects of salt stress in wheat (Datta et al. 1997), soybean (Sarkar et al. 2002), mung bean (Chakrabarti and Mukherji 2003), and groundnut (Senthil et al. 2005).

L-tryptophan (L-TRP) is a well-known precursor of IAA in higher plants and soil microorganisms (San-Francisco et al. 2005). It is suggested that L-tryptophan has an even better effect on plant growth and yield as compared to pure auxins (Zahir et al., 2000; Hozayn et al. 2020). L-tryptophan is an amazing amino acid that acts as an antioxidant or activates the phytohormones. It may also act as an osmolyte, ion transport regulator, modulates stomatal opening and detoxify harmful effects of heavy metals (Rai, 2002). Studies have been conducted to evaluate the influence of exogenous application of L-tryptophan on plant growth, development, and stress tolerance (Hussein et al. 2014; Bakry et al. 2016; El-Gamal et al. 2016).

Perhaps no work has been reported on the interactive effect of IAA and L-tryptophan on in vitro grown potato under salt stress. Considering all the above information, in the present study it was hypothesized that exogenously applied IAA and L-tryptophan alleviate the detrimental impacts of salt stress by increasing the activities of antioxidant enzymes and protein contents which ultimately may improve the growth of potato plants. Furthermore, work was also carried out to find out the most suitable concentration and effective mode of application of these compounds.

Materials and Methods

Plant Material

In vitro-raised potato plants were obtained from Plant Developmental and Regenerative Biology Laboratory, Institute of Botany, University of the Punjab, Lahore, Pakistan. Such in vitro-raised plants were further proliferated on Murashige and Skoog (MS 1962) basal medium. Single nodal segments (ca. 1.0 cm long) from such 30 day-old in vitro-raised potato plants were used as explant source and further grown on MS medium containing various combinations of IAA and L-tryptophan (Sigma Aldrich, St. Louis, Missouri, United States) under a range of salt stress to study the growth response and alleviating effect of these compounds in potato.

Application of IAA and NaCl to Plants

MS medium was supplemented with four different concentrations of IAA, i.e., 0, 17.142, 22.875 or 28.570 µM and four different concentrations of NaCl (0, 40, 60 or 80 mM; control, S_1, S_2, or S_3). All media were supplemented with 30 g sucrose. The pH of the media was adjusted to 5.7–5.8. The agar (Oxoid, Hampshire, England) was added at $7 gL^{-1}$ concentration. Media were autoclaved at 121 °C and 15 lb $inch^{-2}$ for 15 min for sterilization. Appropriate quantities of filter-sterilized IAA were added to autoclaved media at around 50 °C, gently mixed and poured in pre-sterilized culture vessels (Pyrex; 150 × 25 mm) under aseptic conditions. Single nodal explant (1.0 cm) was inoculated on respective IAA and NaCl-containing culture vessels and were wrapped again with polypropylene sheets. In another experiment, single nodal explants (1.0 cm) of potato were dipped separately in four different IAA concentrations (0, 17.142, 22.875 or 28.570 µM) in sterilized conical flasks for 15 min under dark in order to avoid photo-degradation of IAA. These flasks were placed on an orbital shaker (OPTIMA OS-752) at 100 rpm. These IAA-pretreated nodal explants were transferred to the culture vessels containing 10 mL agar-solidified MS medium supplemented with four different NaCl concentrations. The cultures were maintained at 25 ± 2 °C under 16-h photoperiod (35 µmol m^{-2} s^{-1}) provided by cool white fluorescent tube lights (Philips Ltd., Karachi, Pakistan).

Application of L-TRP and NaCl to Plants

In case of L-tryptophan, a preliminary experiment was conducted to study the effect of L-TRP on the growth of potato. In this experiment, eleven concentrations of L-TRP (0, 10, 30, 50, 100 nM, 1, 10, 20, 30, 40, or 60 µM) were used. Required volumes of L-TRP from its mM or µM stocks were added directly into the MS medium before adjusting the pH. After 15 days, based on visual observation, two concentrations (1 and 10 µM) that were supporting better growth response were selected for further experimentation. Keeping in mind the observations of previous experiment, another experiment using five concentrations of L-TRP (0, 1, 5, 10, or 15 µM; control, T_1, T_2, T_3 or T_4) and four concentrations of NaCl (0, 40, 60, or 80 mM) was planned to study the interactive effect of salt stress and L-TRP. Nodal segment of 1 cm was inoculated on respective concentration of salt and L-TRP. These cultures were placed under 16-h photoperiod (35 µmol m^{-2} s^{-1}) at 25 ± 2 °C.

Data Collection for Morphological Attributes of IAA and L-TRP Treated Plants Grown Under Stress

In all above-mentioned experiments, data for growth and biochemical attributes were collected after 30 days of explants inoculation. For this, polypropylene sheets were removed, and plants were taken out of the culture vessels carefully and after removing medium from the roots, number of roots, shoots and nodes were counted. Estimation of fresh weight of plants was carried out by weighing them on fractional electric balance (Scientech-5220). Root and shoot length were measured by using Image J program (Rosband, W.S., image J, US National Institute of Health, Bethesda, MD, USA, http://rsbweb.nih.gov/ij/download.html). For shoot and root length, three longest shoots or roots were measured, and their mean was calculated.

Biochemical Investigation of IAA and L-TRP Treated Plants Grown Under Salt Stress

For biochemical studies, quantitative analyses of soluble protein contents and antioxidant enzymes (peroxidase and superoxide dismutase) were carried out. All analyses were performed on Hitachi U-1100 UV/VIS spectrophotometer. Fresh plant material (1.0 g) was taken and crushed in ice-chilled pestle and mortar with 0.1 g PVP (*PolyVinyl Polypyrrolidone*), 0.5% (v/v) Triton X-100 and 2 mL of 0.1 M phosphate buffer (pH 7.2). The slurry so obtained was centrifuged (Sorval RB-5 refrigerated super speed) for 30 min at 14,000 rpm at 4 °C. The supernatant so obtained was collected and stored at − 20 °C for biochemical analyses. Biuret method of Racusen and Johnstone (1961) was employed for the quantitative estimation of soluble protein contents whereas method proposed by Luck (1974) was adopted for the quantitative estimation of peroxidase activity (E.C 1.11.1.7). Superoxide dismutase (SOD) activity (E.C 1.15.1.1) during the present study was assayed by the method proposed by Maral et al (1977) with slight modifications.

Experimental Plan and Data Analysis

All experiments were run as completely randomized block design. In the first experiment, four different IAA concentrations were incorporated into the MS medium supplemented with four NaCl levels. There were sixteen treatments or media combinations and each treatment had six replicate culture vessels with one explant per vessel thus making a total of 96 culture vessels for all the treatments per experimental run. The experiment was repeated thrice. In 2nd experiment, explants were pretreated with four different IAA concentrations and then inoculated on MS medium supplemented with four NaCl levels. Again, there were sixteen treatments or media combinations each with five replicate culture vessels thus making a total of 80 culture vessels for one experimental run.

In order to study the effect of L-TRP on the growth of potato plants, five L-TRP concentrations were incorporated into the MS medium supplemented with four NaCl levels. There were twenty treatments or media combinations. In this case each treatment had ten culture vessels thus making a total of 200 culture vessels for the whole experiment. Two-way analysis of variance was performed using SPSS 22.0.0. Standard error of the mean values was calculated for each treatment. Duncan's multiple range test was performed to separate means at 0.05% level of probability.

Results

Effect of Various Levels of NaCl and IAA Added in MS Medium on Growth Attributes of Potato

The present investigation was carried out to see the effect of various concentration [0, 40 mM (S_1), 60 mM (S_2) and 80 mM (S_3) NaCl] and IAA [0, 17.142 µM (IAA_1), 22.875 µM (IAA_2) and 28.570 µM (IAA_3)] on various growth parameters of potato cv. Cardinal under in vitro conditions. Data presented in Table 1 indicate the effect of salt stress on various growth parameters of potato cv. Cardinal on MS medium at day 30. Salt stress significantly reduced the shoot length where highest reduction (from 5.05 to 2.80 cm) was recorded at 80 mM NaCl. Likewise, impact of IAA was significant and highest (8.79 cm) shoot growth was promoted by the application of 17.142 µM in comparison to other treatments. Moreover, interaction between salinity level and IAA when added in MS medium

Table 1 A comparison of growth parameters of *S. tuberosum* L. cv. Cardinal maintained on MS medium supplemented with various levels of IAA and NaCl at day 30

Treatments MS medium supplemented with		Shoot length (cm)	Root length (cm)	Number of shoots	Number of roots	Number of nodes	Fresh weight of plant (g)
NaCl (mM)	IAA (µM)						
0	0	5.05 ± 0.56^{f}	5.69 ± 0.41^{cd}	1.17 ± 0.09^{f}	4.33 ± 0.67^{cd}	12.39 ± 0.86^{b}	0.11 ± 0.01^{d}
0	17.14 (IAA_1)	8.79 ± 0.68^{a}	6.99 ± 0.26^{a}	0.85 ± 0.13^{g}	6.94 ± 1.78^{a}	13.11 ± 0.64^{a}	0.36 ± 0.03^{a}
0	22.875 (IAA_2)	7.65 ± 0.72^{b}	5.95 ± 0.23^{c}	1.22 ± 0.08^{f}	4.89 ± 1.49^{b}	12.11 ± 1.39^{bc}	0.31 ± 0.03^{ab}
0	28.57 (IAA_3)	7.55 ± 0.78^{bc}	6.07 ± 0.25^{bc}	0.90 ± 0.08^{g}	3.78 ± 1.76^{d}	10.22 ± 1.64^{c}	0.28 ± 0.03^{b}
40 (S_1)	0	3.89 ± 0.50^{e}	4.25 ± 0.73^{c}	1.67 ± 0.16^{d}	2.17 ± 0.37^{g}	9.44 ± 0.76^{de}	0.09 ± 0.01^{de}
40	17.142	6.34 ± 0.97^{c}	5.64 ± 0.49^{cd}	1.50 ± 0.19^{e}	4.44 ± 1.76^{c}	9.67 ± 1.23^{d}	0.27 ± 0.04^{b}
40	22.875	5.14 ± 0.59^{d}	5.89 ± 0.61^{c}	2.28 ± 0.33^{c}	4.72 ± 0.95^{bc}	7.89 ± 1.29^{f}	0.21 ± 0.02^{bc}
40	28.570	4.91 ± 0.56^{e}	6.02 ± 0.64^{bc}	1.56 ± 0.22^{de}	3.50 ± 0.82^{e}	7.33 ± 1.04^{fg}	0.13 ± 0.03^{d}
60 (S_2)	0	2.96 ± 0.26^{g}	2.42 ± 0.62^{g}	2.28 ± 0.20^{c}	1.00 ± 0.27^{h}	9.39 ± 1.35^{de}	0.08 ± 0.01^{e}
60	17.142	3.62 ± 0.35^{f}	5.93 ± 0.56^{c}	2.20 ± 0.23^{c}	3.56 ± 0.97^{d}	9.00 ± 1.05^{e}	0.18 ± 0.02^{c}
60	22.875	3.55 ± 0.44^{fg}	5.11 ± 0.69^{de}	2.44 ± 0.28^{bc}	3.00 ± 0.74^{f}	7.72 ± 1.18^{f}	0.18 ± 0.05^{c}
60	28.570	3.51 ± 0.48^{fg}	5.19 ± 0.67^{d}	2.61 ± 0.29^{ab}	2.11 ± 0.63^{g}	7.33 ± 0.82^{fg}	0.10 ± 0.01^{de}
80 (S_3)	0	2.80 ± 0.25^{gh}	0.64 ± 0.33^{h}	2.50 ± 0.26^{b}	0.28 ± 0.14^{i}	7.56 ± 0.75^{e}	0.07 ± 0.01^{e}
80	17.142	2.71 ± 0.36^{gh}	6.31 ± 0.51^{b}	2.28 ± 0.29^{c}	1.28 ± 1.04^{gh}	7.96 ± 1.30^{f}	0.12 ± 0.02^{d}
80	22.875	2.60 ± 0.29^{h}	6.84 ± 0.58^{ab}	2.83 ± 0.27^{a}	1.06 ± 0.26^{h}	6.11 ± 0.92^{g}	0.11 ± 0.01^{d}
80	28.570	2.32 ± 0.19^{h}	4.52 ± 0.82^{e}	2.72 ± 0.37^{a}	1.50 ± 0.54^{gh}	6.06 ± 0.73^{g}	0.10 ± 0.01^{de}
Effect of salt with 15 & 272 *df*		*	*	*	*	*	*
Effect of IAA with 15 & 272 *df*		*	*	**	*	**	*
Effect of salt × IAA with 15 & 272 *df*		**	**	*	*	***	**

The data were recorded at day 30 of initial culture to respective media, and all the growth parameter values are means (± SE) from 18 replicate cultures. Means within a column followed by the same letter do not differ significantly according to Duncan's multiple range test. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***), Significant at $P \leq 0.001$ according to two-way ANOVA with *df* mentioned against each was also significant and a pronounced increase (6.34 cm) was observed when plants were grown on medium containing 17.142 µM IAA under 40 mM salt stress conditions. Overall, shoot growth with 60 or 80 mM NaCl has also shown the same trend with maximum increase in shoot length in medium supplemented with 17.142 µM IAA than the other treatments. Root length was also greatly influenced by various NaCl treatments. A consistent sharp decline in root length was observed with raising NaCl concentration in MS medium. Maximum decrease (0.64 cm) in root length was observed at 80 mM NaCl than the other tested concentrations. When IAA was added in MS medium, it supported an increase in root length from 5.69 to 6.99 cm at 17.142 µM IAA. As a result of incorporation of IAA there was not only an increase in root length but visually root growth was also more vigorous as compared to the control (Fig. 1). As regards to interaction between salinity and IAA, when added in MS medium has also shown a significant ($P \leq 0.05$) and pronounced effect on plants. At 80 mM salt level, addition of 22.875 µM IAA resulted in a maximum (6.84 cm) increase in root length compared with other tested IAA levels. A gradual increase in shoot number was observed from 1.17 (control) to 1.67, 2.28, and 2.50 at 40, 60, or 80 mM NaCl concentrations, respectively. Shoots were observed in the form of a bunch (large number of shoots with short internodal distance) at 80 mM NaCl level. When MS medium was supplemented with IAA, shoot number was reduced from 1.17 to 0.85 at 17.142 µM IAA. At 40 mM salt level, when MS medium was supplemented with different concentration of IAA, maximum reduction (from 1.67 to 1.50) in shoot number was observed at 17.142 µM. At 60 and 80 mM salt, incorporation of IAA resulted in a slight decrease in shoot number and maximum decrease (i.e., 2.20 and 2.28

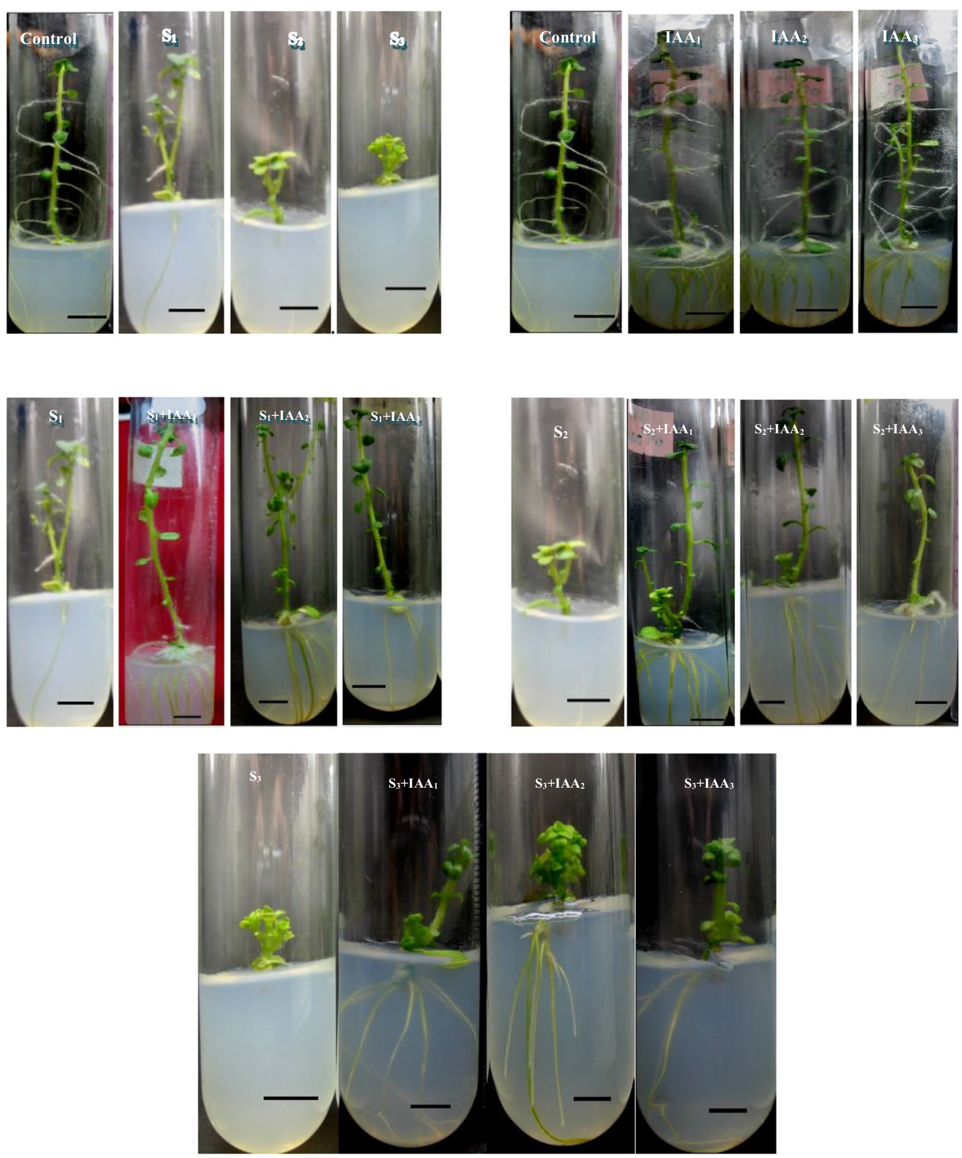

Fig. 1 Comparative growth of potato plants (cv. Cardinal) in MS medium supplemented with various concentrations of NaCl and IAA. Culture vessels are presenting 0 (control), 40, 60 and 80 mM (control, S_1, S_2, S_3) NaC1 alone and in combination with different concentrations of IAA (17.142, 22.875, and 28.570 µm; IAA_1, IAA_2 and, IAA_3) (Bar = 1 cm)

respectively) was observed at 17.142 µM. Another growth parameter which was significantly affected by NaCl was root number that decreased the most from 4.33 (control) to 0.28 at 80 mM salt. On the other hand, supplementation of MS medium with different concentrations of IAA resulted in increased root number and maximum increase was observed at 17.142 µM IAA. By the addition of salt (40, 60 or 80 mM) in the MS medium, number of nodes also significantly decreased while incorporation of IAA had a pronounced effect on their number and maximum increase (13.11) in number of nodes was observed at 17.142 µM IAA. Moreover, interaction between salinity level and IAA when added in MS medium was also significant ($P \leq 0.001$) and pronounced increase (9.44 cm) was observed in plants grown on medium containing 17.142 µM IAA under 40 mM salt stress conditions. A gradual decrease from 0.11 to 0.09, 0.08, and 0.07 g in fresh weight was observed at 40, 60 and 80 mM NaCl level, respectively. However, IAA treatment has shown a significant effect on fresh weight of potato as in case of other growth parameters. Interaction of salt and IAA was also significant ($P \leq 0.01$). In few cases (IAA_2 and IAA_3; 17.142 and 22.875 µM) occasional callus induction was also observed.

Effect of NaCl and IAA Added in MS Medium on Biochemical Attributes of Potato

Salt stress significantly decreased the protein content where highest decrease (from 0.69 to 0.31 mg/g) was recorded at 80 mM NaCl. Likewise, impact of IAA was significant ($P \leq 0.05$), and highest (0.76 mg/g) protein content was observed by the application of 17.142 µM in comparison to other IAA treatments (Table 2). Moreover, interaction between salinity level and IAA, when added in MS medium was also significant and pronounced increase (0.55 mg/g of tissue) was observed when plants were grown on medium containing 17.142 µM IAA under 60 mM salt stress conditions. Salt stress also affected antioxidant enzyme activities of potato plants significantly. When different concentrations of NaCl were added in MS medium, peroxidase activity has generally shown a decreasing trend with increasing concentration of salt and maximum decrease was observed at 80 mM salt. Incorporation of IAA in MS medium resulted in an increase in peroxidase activity from 43.23 to 54.63 units/mg of protein at 17.142 µM as compared to other IAA treatments. Interaction of IAA and salt was also significant ($P \leq 0.001$) with maximum increase (43.74 units/mg of protein) at 40 mM salt with 17.142 µM IAA treatment.

Table 2 A comparison of biochemical parameters of *S. tuberosum* L. cv. Cardinal maintained on MS medium supplemented with various levels of IAA and NaCl at day 30

Treatments MS medium supplemented with		Protein contents (mg/g)	Peroxidase Activity (units/mg of protein)	SOD Activity (units/mg of protein)
NaCl (mM)	IAA (µM)			
0	0	0.69 ± 0.04^{ab}	43.23 ± 0.02^{b}	48.37 ± 4.72^{f}
0	17.142 (IAA_1)	0.76 ± 0.03^{a}	54.63 ± 0.08^{a}	143.85 ± 18.11^{a}
0	22.875 (IAA_2)	0.64 ± 0.02^{ab}	43.48 ± 0.02^{b}	104.71 ± 2.13^{b}
0	28.570 (IAA_3)	0.54 ± 0.04^{b}	40.19 ± 0.06^{c}	90.07 ± 11.79^{c}
40 (S_1)	0	0.55 ± 0.03^{b}	39.04 ± 0.01^{cd}	33.98 ± 2.41^{gh}
40	17.142	0.43 ± 0.01^{c}	43.74 ± 0.03^{b}	78.80 ± 3.98^{d}
40	22.875	0.42 ± 0.03^{c}	38.59 ± 0.01^{cd}	82.13 ± 0.82^{cd}
40	28.570	0.40 ± 0.02^{bc}	36.42 ± 0.05^{df}	59.73 ± 5.82^{ef}
60 (S_2)	0	0.44 ± 0.02^{c}	32.21 ± 0.01^{e}	30.25 ± 10.09^{gh}
60	17.142	0.55 ± 0.03^{b}	42.35 ± 0.03^{bc}	52.52 ± 3.59^{f}
60	22.875	0.48 ± 0.02^{bc}	41.45 ± 0.02^{c}	66.46 ± 7.50^{e}
60	28.570	0.44 ± 0.02^{c}	41.26 ± 0.02^{c}	35.19 ± 3.69^{g}
80 (S_3)	0	0.31 ± 0.02^{e}	22.16 ± 0.01^{f}	25.79 ± 0.70^{h}
80	17.142	0.43 ± 0.03^{c}	32.31 ± 0.03^{e}	51.91 ± 1.27^{f}
80	22.875	0.41 ± 0.02^{de}	30.47 ± 0.09^{ef}	42.39 ± 9.66^{fg}
80	28.570	0.33 ± 0.03^{e}	22.28 ± 0.01^{f}	35.88 ± 2.25^{g}
Effect of salt with 15 & 272 *df*		*	*	*
Effect of IAA with 15 & 272 *df*		*	*	*
Effect of salt × IAA with 15 & 272 *df*		*	**	***

The data were recorded at day 30 of initial culture to respective media, and all values are means (± SE) from 15 replicate cultures. Means within a column followed by the same letter do not differ significantly according to Duncan's multiple range test. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***), Significant at $P \leq 0.001$ according to two-way ANOVA with *df* mentioned against each

Superoxide dismutase (SOD) activity followed the same trend as peroxidase activity and maximum decrease was observed at 80 mM salt level. IAA significantly enhanced (143.85 units/mg of protein) the SOD activity. Likewise, interaction of IAA and salt has also shown a significant ($P \leq 0.001$) effect to enhance the SOD activity.

Effect of Pretreatment of Different IAA Concentrations on Growth of Potato under Salt Stress

Data presented in Table 3 indicates that shoot length generally decreased with rise in NaCl concentration (40, 60 or 80 mM) in MS medium as compared to control plants. Maximum decrease (1.88 cm) in shoot length was observed at 80 mM NaCl concentration. Conversely, pretreatment of nodal explants with different concentrations of IAA has shown an increase in shoot length from 4.14 (control) to a maximum (5.35 cm) at IAA_1 (17.142 µM). However, this increase in shoot length was comparatively less as in case of IAA added directly into the medium. Similarly, interaction of salt and IAA in various treatments had a significant effect on plants and maximum increase in shoot length was observed in case of explants treated with 17.142 µM IAA at all the tested salt concentrations. Root length was observed to have decreased from 5.78 (control) to 4.64, 2.82, and 1.45 cm at 40, 60, and 80 mM NaCl level, respectively. When plants were pretreated with IAA, shoot root length increased significantly ($P \leq 0.05$). IAA pretreatment has also affected positively to root length in the presence of all tested levels of NaCl and maximum root length was recorded at 40 mM NaCl with 17.142 µM IAA pretreatment. The growth trend in terms of shoot number was in contrast as compared to other growth parameters. An increase in number of shoots was recorded by increasing concentrations of salt. There was a significant ($P \leq 0.01$) decrease in the number of roots at 40, 60 and 80 mM NaCl but IAA pretreatment to plants supported an increase in the number of roots and maximum increase (9.53) was observed at 17.142 µM IAA. Similarly, interaction of salt and IAA pretreatment to plants increased the number of roots and maximum increase was observed with 17.142 µM IAA pretreatment at 80 mM salt. Pretreatment of different IAA levels to nodal explants improved the number of roots but this improvement was less as compared

Table 3 A comparison of growth parameters of IAA-pretreated & non-pretreated nodal explants of *S. tuberosum* cv. Cardinal maintained on MS medium supplemented with various NaCl levels at day 30

Treatments		Shoot length (cm)	Root length (cm)	Number of shoots	Number of roots	Number of nodes	Fresh weight of plant (g)
MS medium supplemented with NaCl (mM)	Explants pretreated with IAA (µM)						
0	0^{η}	4.14 ± 0.51^{bc}	5.78 ± 0.54^{b}	1.13 ± 0.09^{e}	6.73 ± 0.77^{c}	9.87 ± 0.75^{d}	0.110 ± 0.02^{c}
0	17.142(IAA_1)	5.35 ± 0.46^{a}	6.58 ± 0.41^{a}	1.00 ± 0^{df}	9.53 ± 1.03^{a}	10.20 ± 0.81^{cd}	0.122 ± 0.02^{b}
0	22.875(IAA_2)	4.33 ± 0.37^{b}	5.88 ± 0.48^{b}	1.80 ± 0.44^{d}	7.67 ± 1.10^{b}	9.80 ± 0.91^{d}	0.117 ± 0.01^{bc}
0	28.570(IAA_3)	4.13 ± 0.52^{bc}	5.66 ± 0.58^{b}	0.93 ± 0.07^{f}	7.93 ± 1.00^{b}	8.13 ± 1.05^{fg}	0.133 ± 0.02^{a}
40 (S_1)	0^{η}	3.48 ± 0.28^{c}	4.64 ± 0.96^{c}	1.80 ± 0.22^{d}	2.00 ± 0.38^{ef}	8.33 ± 1.44^{f}	0.087 ± 0.01^{de}
40	17.142	2.89 ± 0.32^{d}	6.12 ± 0.95^{ab}	1.87 ± 0.17^{d}	3.27 ± 0.53^{d}	10.27 ± 0.92^{cd}	0.100 ± 0.01^{d}
40	22.875	2.41 ± 0.31^{e}	4.48 ± 1.08^{cd}	1.53 ± 0.22^{de}	1.53 ± 0.35^{fg}	9.53 ± 0.97^{de}	0.100 ± 0.01^{d}
40	28.570	2.79 ± 0.49^{de}	4.48 ± 0.84^{cd}	1.53 ± 0.24^{c}	2.67 ± 0.49^{e}	9.87 ± 1.38^{d}	0.095 ± 0.01^{de}
60 (S_2)	0^{η}	2.45 ± 0.28^{de}	2.82 ± 0.77^{fg}	2.07 ± 0.28^{ab}	1.67 ± 0.44^{f}	11.33 ± 1.55^{bc}	0.090 ± 0.01^{de}
60	17.142	3.19 ± 0.32^{cd}	3.93 ± 0.94^{d}	2.87 ± 0.22^{b}	2.53 ± 0.56^{e}	11.53 ± 1.09^{b}	0.098 ± 0.02^{de}
60	22.875	2.29 ± 0.25^{f}	3.44 ± 0.94^{e}	2.40 ± 0.21^{bc}	1.67 ± 0.35^{f}	10.80 ± 1.47^{c}	0.100 ± 0.01^{d}
60	28.570	2.39 ± 0.29^{e}	2.98 ± 0.82^{f}	2.13 ± 0.09^{c}	0.87 ± 0.26^{h}	9.07 ± 1.27^{e}	0.072 ± 0.01^{e}
80 (S_3)	0^{η}	1.88 ± 0.17^{fg}	1.45 ± 0.16^{h}	4.87 ± 0.24^{a}	0.53 ± 0.22^{hi}	11.93 ± 0.69^{b}	0.075 ± 0.01^{e}
80	17.142	2.94 ± 0.28^{d}	2.35 ± 0.95^{fg}	2.27 ± 0.23^{bc}	1.07 ± 0.27^{g}	12.20 ± 1.32^{a}	0.077 ± 0.01^{e}
80	22.875	2.43 ± 0.28^{e}	1.97 ± 0.77^{g}	1.73 ± 0.18^{d}	0.67 ± 0.23^{hi}	10.60 ± 0.99^{c}	0.093 ± 0.01^{de}
80	28.570	2.00 ± 0.35^{f}	0.58 ± 0.23^{i}	1.60 ± 0.29^{de}	0.47 ± 0.19^{i}	9.13 ± 0.91^{e}	0.065 ± 0.01^{f}
Effect of salt with 15 & 224 *df*		*	*	*	*	**	*
Effect of IAA with 15 & 224 *df*		*	**	*	*	*	*
Effect of salt × IAA with 15 & 224 *df*		*	*	*	***	*	***

$^{\eta}$Pretreated with double distilled water, Growth parameter values are means (±SE) from 15 replicate cultures. Means within a column followed by the same letter do not differ significantly according to DMRT. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***), Significant at $P \leq 0.001$ according to two-way ANOVA with *df* mentioned against each to IAA added in medium with different concentrations of NaCl. An increase in fresh weight of plants with IAA pretreatment to potato plants was also significantly positive and maximum increase (0.133 g) was observed at 28.57 µM IAA. Interaction of salt and IAA has also shown an increasing trend as compared to non-pretreated plants under salt stress conditions (Fig. 2).

Effect of Pretreatment of Different IAA Concentrations on Biochemical Parameters of Potato

It is obvious from the data shown in Table 4 that protein contents decreased significantly (0.50, 0.45, and 0.42 mg/g) at 40, 60, and 80 mM NaCl levels, respectively, as compared to control (0.61 mg/g) plants. However, protein contents increased from 0.61 mg/g (control) to 1.46, 1.26 and 0.93 mg/g of plant tissue with the pretreatment of 17.142, 22.875, and 28.57 µM IAA, respectively. Likewise, interaction of salt and IAA also affected protein contents significantly ($P \leq 0.05$) and maximum increase (1.32 mg/g) was observed in plants pretreated with 22.875 µM IAA and 40 mM salt. An overall increasing trend was observed in case of antioxidant enzyme activities by increasing concentrations of IAA pretreatment. Peroxidase and SOD activities were maximum (36.24 and 37.86 units/mg of protein, respectively) at 17.142 µM IAA pretreatment as compared to non-pretreated (32.20 units/mg of protein) plants. IAA pretreatment to plants has also shown significant ($P \leq 0.001$) positive effect under various salt stress conditions. Maximum peroxidase activity (31.93 units/mg of protein) and SOD activity (35.35 units/mg of protein) was observed by

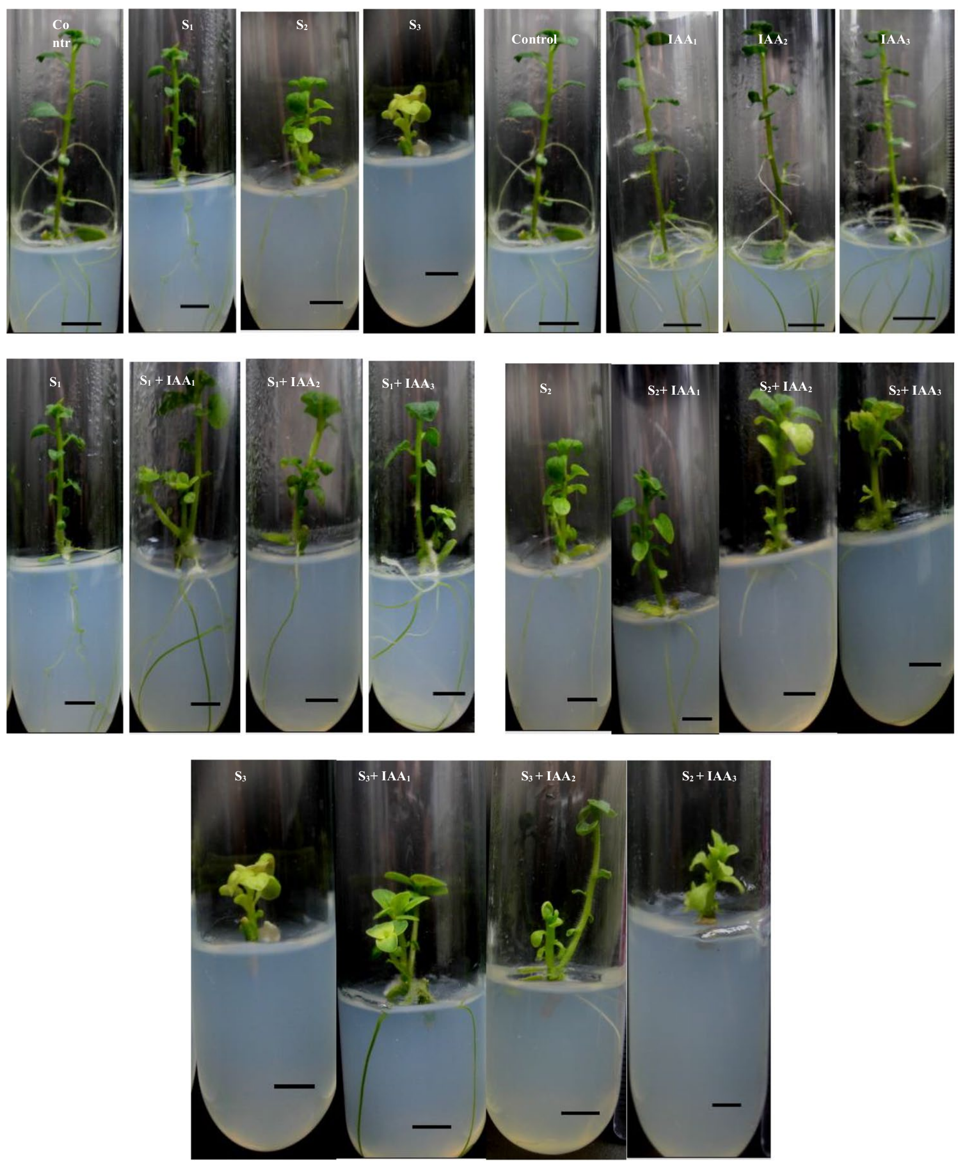

Fig. 2 Comparative growth of potato plants (cv. Cardinal) pretreated with different concentrations of IAA i.e., 17.142, 22.875 and 28.570 µM (IAA_1, IAA_2, and, IAA_3) inoculated on MS medium supplemented with various concentrations of NaC10 (control), 40, 60, and 80 mM (control, S_1, S_2, S_3) (Bar = 1 cm)

Table 4 A comparison of biochemical parameters of IAA-pretreated or non-pretreated nodal explants of *S. tuberosum* cv. Cardinal maintained on MS medium supplemented with various NaCl levels at day 30

Treatments		Protein contents (mg/g)	Peroxidase Activity (units/mg of protein)	SOD Activity (units/mg of protein)
MS medium supplemented with NaCl (mM)	Explants pretreated with IAA (µM)			
0	0[η]	0.61 ± 0.06^{f}	32.20 ± 0.04^{c}	32.66 ± 1.30^{cd}
0	17.142 (IAA_1)	1.46 ± 0.02^{a}	36.24 ± 0.03^{a}	37.86 ± 1.34^{a}
0	22.875 (IAA_2)	1.26 ± 0.20^{c}	34.19 ± 0.04^{b}	33.96 ± 2.62^{c}
0	28.57 (IAA_3)	0.93 ± 0.04^{de}	33.28 ± 0.03^{bc}	25.34 ± 8.47^{e}
40 (S_1)	0[η]	0.50 ± 0.04^{g}	30.20 ± 0.04^{bc}	29.41 ± 4.07^{d}
40	17.142	0.93 ± 0.03^{de}	31.93 ± 0.04^{c}	35.35 ± 0.34^{b}
40	22.875	1.32 ± 0.22^{b}	29.31 ± 0.06^{cd}	32.22 ± 1.85^{cd}
40	28.57	0.48 ± 0.02^{g}	28.24 ± 0.02^{d}	34.77 ± 3.00^{bc}
60 (S_2)	0[η]	0.45 ± 0.02^{g}	26.20 ± 0.03^{e}	28.15 ± 2.06^{de}
60	17.142	1.20 ± 0.04^{cd}	26.27 ± 0.05^{e}	27.29 ± 1.59^{e}
60	22.875	1.08 ± 0.04^{d}	25.22 ± 0.01^{ef}	26.76 ± 1.36^{ef}
60	28.57	0.57 ± 0.04^{fg}	24.20 ± 0.15^{f}	25.25 ± 5.87^{e}
80 (S_3)	0[η]	0.42 ± 0.05^{gh}	24.19 ± 0.03^{e}	22.73 ± 2.28^{ef}
80	17.142	1.07 ± 0.06^{d}	25.16 ± 0.02^{ef}	29.86 ± 2.10^{d}
80	22.875	1.00 ± 0.02^{d}	25.00 ± 0.03^{ef}	22.36 ± 2.02^{f}
80	28.57	0.76 ± 0.1^{e}	23.182 ± 0.02^{f}	17.99 ± 0.41^{g}
Effect of salt with 15 & 224 *df*		*	*	*
Effect of IAA with 15 & 224 *df*		*	*	*
Effect of salt × IAA with 15 & 224 *df*		*	***	**

[η] Pretreated with double distilled water. The data were recorded at day 30 of initial culture to respective media, and all values are means (±SE) from 15 replicate cultures. Means within a column followed by the same letter do not differ significantly according to Duncan's multiple range test. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***), Significant at $P \leq 0.001$ according to two-way ANOVA with *df* mentioned against each

17.142 µM IAA pretreatment with 40 mM salt (Table 4). Overall, when IAA was added in medium or provided as pretreatment, all the biochemical parameters have shown an increasing trend, but the increase was more pronounced in case of IAA being added in the medium rather than its pretreatment. Hence, a clear difference was observed in both modes of application.

Effect of Different Tryptophan Concentrations Added in MS Medium on in Vitro Growth of Potato

Data presented in Table 5 indicate the effect of various concentrations of L-TRP on different growth parameters of potato plants. All growth parameters studied during this work have shown best results at 10 µM L-TRP. Maximum root and shoot lengths i.e., 9.18 and 7.64 cm were observed when 10 µM L-TRP was added in MS medium. Average number of roots and nodes/plant was also maximum at 10 µM L-TRP, while the number of shoots was maximum (1.8) at 30 nM. Fresh weight of plants also enhanced at 10 µM L-TRP as compared to control potato plants. Hence, for further experimentation 1, 5, 10, 15 µM L-TRP was selected to see its effects on salt stressed potato plants under in vitro conditions.

Data presented in Table 6 reveals that increasing concentrations of NaCl have significant ($P \leq 0.05$) effect on the growth of potato plants. Shoot length decreased from 4.36 to 2.03 cm by increasing the salt concentration from 0 to 80 mM. When different concentrations of L-TRP were added to MS medium (i.e., treatments T_1, T_2, T_3, and T_4; 1, 5, 10, and 15 µM L-TRP), shoot length increased significantly and maximum increase (5.22 cm) was observed at 15 µM L-TRP (T_4). Moreover, interaction between salinity level and L-TRP using MS medium also resulted in significant ($P \leq 0.05$) and pronounced increase (4.58 cm) of shoot length on medium containing 15 µM L-TRP under 40 mM salt stress. At 60 mM NaCl, shoot length increased from 3.83 to 4.58 cm with the addition of 15 µM L-TRP. At still higher salt level (80 mM), shoot length also increased at all tested tryptophan concentrations and maximum increase (2.90 cm) was observed at 80 mM + 15 µM L-TRP (Fig. 3). Another important parameter which was greatly affected by the salt treatment was root length, i.e., it sharply reduced from 5.83 to 1.71, 0.70 and 0 cm with the addition of 40, 60, and 80 mM NaCl, respectively. When L-TRP was added to MS medium, root

Table 5 A comparison of growth parameters of *S. tuberosum* L. cv. Cardinal maintained on MS medium supplemented with various concentrations of L-tryptophanat day 30

Treatments	Shoot length (cm)	Root length (cm)	Number of Shoots	Number of Roots	Number ofNodes	Fresh weight of plant (g)
Control (without Tryptophan)	5.58 ± 0.96[de]	5.81 ± 0.51[c]	1.00 ± 01[c]	4.17 ± 1.05[de]	13.33 ± 1.02[d]	0.12 ± 0.02[bc]
10 nM	5.18 ± 0.35[e]	4.80 ± 1.07[d]	1.60 ± 0.2[ab]	4.60 ± 1.40[d]	9.60 ± 0.88[g]	0.11 ± 0.02[c]
30 nM	5.48 ± 0.70[de]	5.17 ± 0.92[bc]	1.80 ± 0.4[a]	4.20 ± 0.70[cd]	11.40 ± 1.17[ef]	0.13 ± 0.01[bc]
50 nM	5.83 ± 0.66[de]	5.59 ± 0.33[c]	1.00 ± 0.2[c]	6.20 ± 0.70[cd]	14.40 ± 0.84[ef]	0.12 ± 0.02[bc]
100 nM	5.89 ± 0.85[d]	4.64 ± 0.69[d]	1.00 ± 0.2[c]	4.33 ± 1.15[d]	12.33 ± 0.84[e]	0.19 ± 0.02[b]
1 µM	7.30 ± 0.79[b]	5.07 ± 0.40[bc]	1.50 ± 0.34[b]	9.50 ± 1.06[b]	15.67 ± 1.15[b]	0.25 ± 0.03[a]
10 µM	9.18 ± 1.27[a]	7.64 ± 0.29[a]	1.33 ± 0.21[bc]	11.33 ± 2.97[a]	16.33 ± 0.92[a]	0.24 ± 0.03[a]
20 µM	6.38 ± 1.05[c]	5.53 ± 0.30[c]	1.50 ± 0.22[b]	8.83 ± 1.90[bc]	14.83 ± 1.01[c]	0.25 ± 0.03[a]
30 µM	4.51 ± 0.56[fg]	6.05 ± 0.78[b]	1.00 ± 0.1[c]	2.83 ± 0.79[e]	12.83 ± 0.48[de]	0.11 ± 0.01[c]
40 µM	5.07 ± 1.21[e]	4.86 ± 1.05[d]	1.00 ± 0.63[c]	6.60 ± 1.82[c]	11.20 ± 2.39[f]	0.13 ± 0.03[bc]
60 µM	4.87 ± 1.14[f]	4.75 ± 0.98[d]	1.33 ± 0.52[bc]	6.67 ± 2.11[c]	12.00 ± 1.83[e]	0.15 ± 0.04[b]
Effect of L-TRP with *df* 10 & 55	*	*	**	*	*	**

The data were recorded at day 30 of initial culture to respective media, and all the growth parameter values are means (± SE) from 6 replicate cultures. Means within a column followed by the same letter do not differ significantly according to Duncan's multiple range test. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***) according to two-way ANOVA

length was increased, and maximum increase (6.88 cm) was observed using T_3 (10 µM L-TRP). Shoot number generally increased by increasing the concentration of NaCl from 0 to 80 mM. With the addition of L-TRP in MS medium containing various salt concentrations, shoot number decreased significantly ($P \leq 0.01$). Root number was also negatively affected by various salt treatments, and it was significantly increased with the addition of L-TRP in MS medium. By the addition of 40, 60 and 80 mM salt in MS medium, number of nodes was observed to have decreased considerably. However, when MS medium was supplemented with L-TRP, number of nodes increased gradually, and maximum increase (13.57 cm) was observed using T_4 (15 µM L-TRP) as compared to control (10.57 cm). Similarly, fresh weight was also significantly influenced by NaCl and L-TRP treatments. With the addition of different concentrations of salt, fresh weight generally decreased which gradually increased with the addition of different concentrations of L-TRP.

Effect of Various Levels of Salt and L-Tryptophan on Biochemical Parameters

As evident from the data in Table 7, the protein content decreased from 1.65 to 1.49 mg/g, 0.97 and 0.69 mg/g of tissue at 40, 60, and 80 mM NaCl. Supplementation of media with L-TRP resulted in an increasing trend in protein content and maximum increase was observed at 15 µM of L-TRP treatment. Likewise, interaction of salt and L-TRP was also significant ($P \leq 0.05$). Peroxidase and SOD activity was also greatly influenced by the NaCl treatments while incorporation of different concentrations of L-TRP enhanced the antioxidant enzyme activity. Maximum peroxidase and SOD activity (23.19 and 39.02 units/ mg of protein, respectively) was observed when 15 µM of L-TRP was added in 40 mM salt containing medium.

Discussion

Salinity is an ever-increasing threat to agricultural sustainability. Salt stress induces several changes in cellular growth, division, and enzymatic activities. The present study aimed at alleviation of salt stress by exogenous applications of various concentrations of IAA and L-TRP. Various growth and biochemical parameters including enzymatic antioxidants were studied under normal and NaCl-stressed conditions to investigate the effect of IAA and L-TRP treatments. On exposure to salt stress, growth retardation is the most profound response of salt-sensitive plants (Zhao 1993). During the present investigation, various NaCl concentrations were used to induce salt stress in in vitro-grown potato plants. A progressive decrease in morphological parameters (shoot and root length, number of roots and nodes and fresh weight) was observed with a gradual increase of salt in MS medium. However, the number of shoots increased by increasing salt levels. Further drastic effect of salt was observed at its highest level (80 mM) where plantlets showed reduction in shoot length with an increased shoot number and very small root formation. The results are in agreement with the findings of earlier researchers *e.g.*, Khalid and Aftab (2020) who

Table 6 A comparison of growth parameters of *S. tuberosum* L. cv. Cardinal maintained on MS medium supplemented with various levels of L-tryptophan and NaCl at day 30

TreatmentsMS supplemented with		Shoot length (cm)	Root length (cm)	Number of shoots	Number of roots	Number of nodes	Fresh weight (g)
NaCl (mM)	L-TRP (µM)						
0	0	4.36 ± 0.59^{b}	5.83 ± 0.66^{b}	1.00 ± 0.10^{de}	3.14 ± 0.59^{b}	10.57 ± 0.1^{c}	0.18 ± 0.01^{b}
0	1 (T_1)	4.98 ± 0.39^{ab}	5.42 ± 0.35^{bc}	1.00 ± 0.01^{de}	5.57 ± 0.72^{ab}	12.33 ± 0.68^{b}	0.11 ± 0.01^{de}
0	5 (T_2)	4.35 ± 0.47^{b}	5.86 ± 0.58^{b}	1.00 ± 0.02^{de}	5.83 ± 0.40^{a}	12.00 ± 1.02^{bc}	0.12 ± 0.01^{d}
0	10 (T_3)	5.01 ± 0.16^{ab}	6.88 ± 0.25^{a}	1.10 ± 0.03^{d}	5.57 ± 0.43^{ab}	13.00 ± 0.31^{ab}	0.12 ± 0.02^{d}
0	15 (T_4)	5.22 ± 0.22^{a}	6.57 ± 0.31^{ab}	1.24 ± 0.03^{cd}	5.86 ± 0.94^{a}	13.57 ± 0.65^{a}	0.23 ± 0.01^{a}
40 (S_1)	0	3.83 ± 0.34^{c}	1.71 ± 0.36^{fg}	1.17 ± 0.14^{cd}	0.50 ± 0.29^{f}	7.50 ± 0.29^{fg}	0.17 ± 0.01^{b}
40	1	3.57 ± 0.13^{cd}	1.29 ± 0.58^{gh}	1.37 ± 0.11^{c}	1.17 ± 0.26^{d}	8.71 ± 0.99^{e}	0.18 ± 0.01^{b}
40	5	3.59 ± 0.29^{cd}	3.86 ± 1.02^{d}	1.20 ± 0.1^{d}	1.00 ± 0.38^{d}	10.00 ± 0.69^{d}	0.10 ± 0.04^{de}
40	10	4.36 ± 0.14^{b}	4.32 ± 1.03^{cd}	1.00 ± 0.2^{de}	1.57 ± 0.37^{cd}	10.17 ± 0.55^{cd}	0.08 ± 0.01^{ef}
40	15	4.58 ± 0.41^{ab}	4.78 ± 0.91^{c}	1.90 ± 0.1^{c}	1.71 ± 0.47^{c}	10.57 ± 0.37^{c}	0.20 ± 0.03^{ab}
60 (S_2)	0	3.46 ± 0.39^{cd}	0.70 ± 0.37^{hi}	1.91 ± 0.02^{c}	0.43 ± 0.20^{fg}	8.00 ± 0.58^{f}	0.10 ± 0.01^{de}
60	1	2.43 ± 0.17^{d}	0.72 ± 0.21^{hi}	1.00 ± 0.03^{de}	0.80 ± 0.26^{de}	8.00 ± 0.95^{f}	0.07 ± 0.01^{f}
60	5	1.98 ± 0.06^{ef}	0.70 ± 0.58^{hi}	1.14 ± 0.14^{cd}	0.17 ± 0.14^{g}	8.00 ± 0.58^{f}	0.10 ± 0.01^{de}
60	10	3.46 ± 0.32^{cd}	0.95 ± 0.80^{h}	1.50 ± 0.28^{ab}	0.57 ± 0.20^{f}	9.86 ± 0.67^{de}	0.13 ± 0.01^{d}
60	15	3.58 ± 0.32^{cd}	3.37 ± 1.06^{de}	2.00 ± 0.03^{b}	1.00 ± 0.31^{d}	10.00 ± 0.90^{d}	0.15 ± 0.02^{c}
80 (S_3)	0	2.03 ± 0.19^{e}	0.00 ± 0	2.71 ± 0.29^{a}	0.00 ± 0.0	6.57 ± 0.37^{h}	0.06 ± 0.01^{f}
80	1	2.06 ± 0.11^{e}	1.01 ± 0.76^{h}	1.43 ± 0.20^{ab}	0.43 ± 0.20^{fg}	6.00 ± 0.53^{hi}	0.07 ± 0.04^{f}
80	5	2.72 ± 0.19^{d}	1.33 ± 0.68^{g}	1.29 ± 0.18^{cd}	0.71 ± 0.18^{e}	8.00 ± 1.62^{f}	0.08 ± 0.01^{ef}
80	10	2.22 ± 0.25^{de}	1.91 ± 0.98^{f}	1.14 ± 0.14^{cd}	0.57 ± 0.20^{f}	7.29 ± 0.57^{fg}	0.09 ± 0.01^{e}
80	15	2.90 ± 0.37^{d}	2.23 ± 0.95^{e}	1.00 ± 0.1^{de}	0.71 ± 0.29^{e}	8.57 ± 0.84^{e}	0.09 ± 0.01^{e}
Effect of salt with 19 & 180 *df*		*	*	*	*	*	*
Effect of L-TRP with 19 & 180 *df*		*	*	*	*	*	*
Effect of salt × TRP with 19 & 180 *df*		*	*	**	**	*	***

The data were recorded at day 30 of initial culture to respective media, and all values are means (± SE) from 10 replicate cultures. Means within a column followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***), Significant at $P \leq 0.001$ according to two-way ANOVA with *df* mentioned against each

reported a similar response of in vitro-grown potato cv. Cardinal under NaCl stress. Similarly, reduction in growth of in vitro-grown plants under salt stress (0–4%) was also reported by Gupta and Huang (2014). Reduction of growth due to insufficient water uptake is a common indicator of salt stress reported by Munns (2002) and Rehman et al. (2022). One of the main reasons of this salt-induced growth retardation might be the adverse effect of salt on rooting that plays a major role in uptake of water and nutrients to the plants. Salt stress also reduces leaf expansion due to decreasing turgor pressure in leaf cells. The repressive effect of salt stress on plant growth might also be attributed to the decline in endogenous levels of plant growth regulators.

IAA is a plant hormone that promotes plant growth, vascular tissue development, cell expansion and elongation, maintains apical dominance and regulates phototropic and gravitropic behavior (Moore 1989). In the present study, exogenous application of IAA to in vitro plants either added in medium or as explants pretreatment helped to improve plant growth and to alleviate the adverse effect of salt stress. All tested concentrations of IAA significantly enhanced the plant growth by increasing shoot and root length, number of root and node and fresh weight in non-stressed as well as NaCl-stressed plants as compared to the control (without salt and IAA treatment). Possibly this increase in plant growth and enhanced vigor under non-stressed conditions might have helped the plants to better cope under stress condition, probably by delaying the onset of threshold for salinity tolerance (Dalton et al. 2000). In the present study, 17.142 µM IAA was found to be best the concentration for the enhancement of most of the studied growth parameters under normal and stressed conditions. However, in non-stressed plants, more increase in shoot number was observed on higher dose of IAA (i.e., 22. 875 µM) perhaps due to the positive role

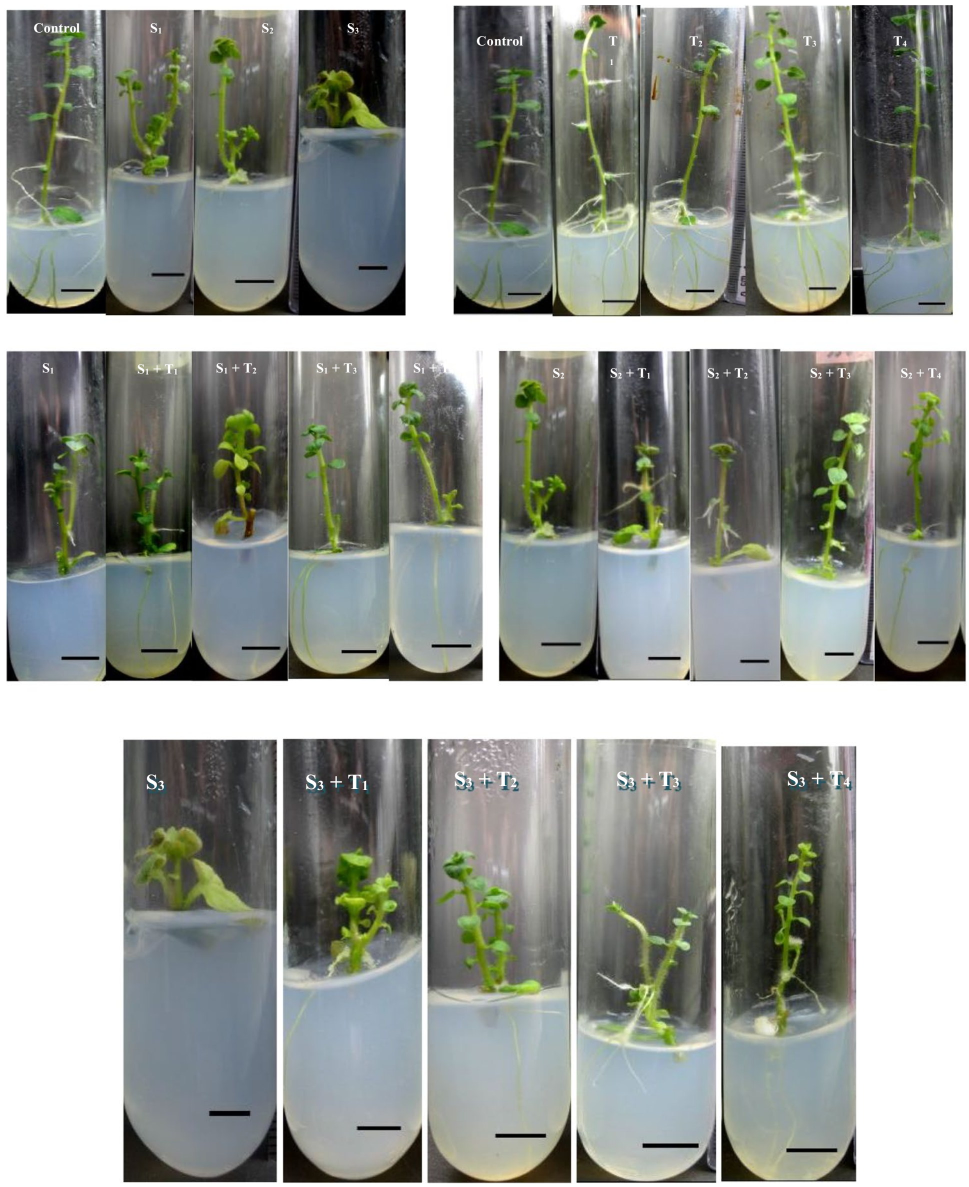

Fig. 3 Comparative growth of potato plants (cv. Cardinal) in MS medium supplemented with various concentrations of NaC1 and L-Tryptophan. Culture vessels are presenting 0 (control), 40, 60, and 80 mM (control, S_1, S_2, S_3) NaC1 alone and in Combination with L-Tryptophan (1, 5, 10, and 15 µM; T1,.T2, T3, and T4) (Bar = 1 cm)

of IAA in shoot growth. Several earlier studies have also reported the involvement of IAA in the process of lateral and adventitious shoot formation (Meuwly and Pilet 1991; Calenza et al. 1995; Arteca 1996; Rahman et al. 2002). San-Franscisco et al. (2005) reported the role of pretreatment of IAA in enhancement of plants' height, dry weight, mineral uptake, endogenous IAA content and polyamine content in *Capsicum annuum.* They suggested that better growth of IAA-treated plants under stress conditions is the capability of plants to convert putrescine into spermidine and spermine is directly related to its ability to perform better under stress conditions (Bouchereau et al. 1999). These findings are also in line with those of Chakrabarti and Mukherji (2003) and Egamberdieva (2009) who demonstrated the positive effect of IAA on seed germination and seedling growth under NaCl stress.

Two modes of IAA application (i.e., incorporation into the medium or pretreatment of in vitro-grown tissues) were also evaluated in this study. While comparing the effects of both, better growth parameters were observed when IAA was incorporated directly to the medium. It may possibly be due to the availability of IAA to the plants throughout the plant growth, so that a plant might itself manage the uptake of IAA according to its need. In few cases, callus formation was also observed on basal cut ends when 22.875 and 28.570 µM IAA concentrations were incorporated into the medium during the present investigation. This callusing response, however, has not been documented in the literature.

Amongst the various molecules involved in IAA biosynthesis, L-TRP is a good precursor of IAA (Abbas et al. 2013). Various workers reported the conversion of L-TRP into IAA

Table 7 A comparison of biochemical parameters of *S. tuberosum* L. cv. Cardinal maintained on MS medium supplemented with various levels of L-tryptophan and NaCl at day 30

Treatments MS medium supplemented with		Protein content (mg/g)	Peroxidase Activity (units/ mg of protein)	SOD Activity (units/ mg of protein)
NaCl (mM)	L-TRP (μM)			
0	0	1.65 ± 0.16^{ab}	22.17 ± 0.03^{b}	38.54 ± 0.80^{cd}
0	1 (T_1)	1.46 ± 0.13^{bc}	20.20 ± 0.01^{d}	40.42 ± 0.27^{c}
0	5 (T_2)	1.60 ± 0.07^{b}	20.10 ± 0.01^{de}	43.30 ± 1.17^{b}
0	10 (T_3)	1.71 ± 0.16^{ab}	23.18 ± 0.01^{ab}	45.90 ± 0.41^{ab}
0	15 (T_4)	1.80 ± 0.07^{a}	24.22 ± 0.02^{a}	46.50 ± 1.18^{a}
40 (S_1)	0	1.49 ± 0.15^{bc}	21.13 ± 0.02^{c}	32.81 ± 0.18^{g}
40	1	0.89 ± 0.11^{e}	20.83 ± 0.01^{cd}	37.51 ± 0.59^{de}
40	5	1.57 ± 0.07^{b}	22.14 ± 0.01^{b}	38.01 ± 0.74^{d}
40	10	1.60 ± 0.09^{b}	20.17 ± 0.01^{b}	38.92 ± 0.67^{cd}
40	15	1.74 ± 0.24^{a}	23.19 ± 0.02^{ab}	39.02 ± 0.99^{c}
60 (S_2)	0	0.97 ± 0.13^{de}	20.08 ± 0.01^{de}	30.15 ± 0.37^{gh}
60	1	0.54 ± 0.10^{fg}	20.10 ± 0.02^{de}	32.26 ± 0.50^{g}
60	5	0.97 ± 0.09^{de}	20.30 ± 0.02^{d}	34.02 ± 0.28^{f}
60	10	1.38 ± 0.14^{c}	20.96 ± 0.01^{cd}	35.96 ± 0.60^{ef}
60	15	1.69 ± 0.11^{ab}	21.11 ± 0.03^{c}	37.19 ± 0.37^{de}
80 (S_3)	0	0.69 ± 0.16^{f}	18.05 ± 0.01^{e}	27.89 ± 0.41^{i}
80	1	0.81 ± 0.11^{ef}	20.03 ± 0.01^{de}	29.32 ± 0.48^{h}
80	5	1.45 ± 0.16^{c}	20.96 ± 0.01^{cd}	29.35 ± 0.18^{h}
80	10	1.06 ± 0.17^{d}	20.07 ± 0.01^{de}	31.24 ± 0.25^{gh}
80	15	1.65 ± 0.06^{ab}	21.90 ± 0.05^{bc}	36.45 ± 0.30^{e}
Effect of salt with 19 & 180 *df*		*	*	*
Effect of L-TRP with 19 & 180 *df*		*	*	**
Effect of salt × L-TRP with 19 & 180 *df*		*	***	*

The data were recorded at day 30 of initial culture to respective media, and all values are means (± SE) from 10 replicate cultures. Means within a column followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test. *Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, (***), Significant at $P \leq 0.001$ according to two-way ANOVA with *df* mentioned against each

in different plant species (San-Francisco et al. 2005). Present study indicated that all tested L-TRP levels significantly enhanced the plant growth by increasing the in vitro growth parameters, i.e., shoot and root length, number of roots and nodes and fresh weight of potato plants, and also proved helpful in counteracting the deleterious effects of salt stress. In sugar beet, Hozayn et al (2020) reported increased plant height, dry weight, mineral uptake, endogenous IAA and polyamine content in response to L-TRP. These results are also in line with the findings of Abdel-Monem et al (2010) who reported increased plant growth, seed and oil yield, level of IAA, photosynthetic pigments, total carbohydrate, and protein content by L-TRP application under salt stress. During this study, among all the tested levels of L-TRP, 15 μM showed the best response regarding the studied parameters. The possible reason of this growth increment and salt stress alleviation might be related to the auxin-like effects of L-TRP and ultimately its conversion into IAA within the plant. However, further in vitro studies to check the endogenous IAA levels in plants treated with L-TRP are needed to provide conclusive evidence.

Proteins are amongst the potential biochemical indicators of salinity tolerance. In this study, a progressive decrease in soluble protein contents after exposure to 40, 60, and 80 mM NaCl was observed. These results are in accordance with the findings of Ejaz et al (2012) who also found decreased levels of protein content in potato under salt stress. The negative effect of salinization on proteins might be attributed to the osmotic effect (Yurekli et al. 2004) or due to decreased availability of amino acids and denaturation of the enzymes involved in the amino acid and protein synthesis under saline conditions (Sapre et al. 2022). Another possibility could have been the loss of potassium (K^+) under salt stress, as K^+ is necessary for protein synthesis (Ayala-Astorga and Alcaraz-Melendez 2010).

To survive under stressful environment, plants accumulate proteins that protect cells from such stress effects (Wang et al. 2003). During the present study, exogenous

IAA (supplemented to the medium or given as pretreatment) increased the protein contents in either non-stressed or NaCl-stressed potato plants as compared to the non-pretreated control ones. This increased protein content might have helped the plants in maintaining growth under stress. These results corroborate the findings of Agastian et al (2000) and Fidalgo et al (2004) who reported stress-induced proteins to play a key role in stress tolerance. High protein content in plants accumulated under stress conditions may provide a storage form of nitrogen (Singh et al. 1987) and possibly play a role in osmotic adjustment (Parida et al. 2004; Ashraf and Harris 2004). In this study, increased protein contents in response to IAA treatment might also be due to the stimulating effect of auxin on the activation of K^+ uptake channels (Claussen et al. 1997) which might facilitate the plants to withstand the harmful effects of salt stress through osmotic adjustments or by balancing ionic homeostasis. Addition of L-TRP (1–15 µM) to the MS medium as well as to the medium containing various concentrations of salt also increased the protein content in non-stressed as well as salt-stressed potato plants. This might be due to conversion of L-TRP into IAA or else as an amino acid enhancing protein synthesis directly.

Salt stress favors the production of reactive oxygen species (ROS) and cause oxidative stress (Parida and Das 2005). These ROS react with vital biomolecules and cause pigment co-oxidation, lipid peroxidation, membrane destruction, protein denaturation and DNA mutation (Mangal et al. 2022). Salt tolerance is generally attributed to high constitutive or up-regulated activities of antioxidant enzymes or ROS-scavenging enzymes (Kaur et al. 2022). In the present investigation, application of exogenous IAA (added to the medium or given as pretreatment) as well as exogenous supplementation of L-TRP alleviated the oxidative damage by increasing the superoxide dismutase (SOD) and peroxidase (POD) activities in normal as well as NaCl-stressed plants of potato as compared to control. Similar increase in the activity of SOD have been reported by Rahnama and Ebrahimzadeh (2005) and Milanovic et al. (2019) in salt tolerant potato and wheat cultivars, respectively. In another study, Senthil et al (2005) reported a similar increase in POD activity under salt stress in response to IAA. Similar increase in POD activity was also suggested to play a pivotal role in scavenging H_2O_2 in salt tolerant potato cultivar (Kumari et al. 2015). Results of the present study are also supported by the findings of some earlier researchers (Rahnama et al. 2003; Meloni et al. 2003; Sami et al. 2021) who suggested that increased activities of antioxidant enzymes confer the plants greater resistance against stress induced damage.

During this study, activities of these antioxidant enzymes were higher by exogenous application of IAA to plants than the control and NaCl-stressed plants (both modes of application) but comparatively more increase was observed when IAA was supplemented to the medium. This fact may also justify relatively better growth parameters of plants where IAA was supplied in the medium rather than pretreated. In case of L-TRP, higher antioxidant activities were observed at highest tryptophan dose (15 µM), which in turn was reflected in better growth parameters at the same concentration of L-TRP.

Our findings suggest up-regulation of antioxidants with exogenous application of IAA and L-TRP that in the first instance were down-regulated in response to salt stress. SOD functions as first line of defense against ROS, but its end product is the toxic H_2O_2 (Ulfat et al. 2021). POD might further provide a selective advantage in defense and play a role in scavenging H_2O_2. Higher POD activity decreases H_2O_2 level in cells and increase the stability of membranes and CO_2 fixation as several enzymes of the calvin cycle within chloroplast are sensitive to H_2O_2 (Kaya et al. 2018).

Conclusion

Salt stress severely inhibited the growth of potato cv. Cardinal plants by decreasing all the studied growth and biochemical parameters under in vitro conditions. Exogenously applied IAA and its precursor L-TRP played a significant role in alleviation of adverse effects of salt stress. Response of IAA on supplementation to the medium was more profound in regard to growth and stress alleviation than IAA pretreatment. IAA and L-TRP probably alleviated the salt-induced damage by maintaining endogenous hormone level and by increasing the activities of antioxidant enzymes, which in turn increased potato growth and conferred resistance to plants to withstand salt stress conditions. However, these in vitro findings necessitate further experimentation under both glasshouse as well as field conditions to draw a clear picture of this alleviating behavior of the tested biomolecules.

Author Contributions MG Performed the experiments and prepared the manuscript, ZAS Co-supervised and proofread the manuscript and FA supervised and proofread the manuscript.

Declarations

Conflict of interest Authors declare that there is no conflict of interest.

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