In Vitro Cellular & Developmental Biology - Plant (2020) 56:377–389
https://doi.org/10.1007/s11627-019-10047-x

PLANT TISSUE CULTURE

Effect of exogenous application of IAA and GA_3 on growth, protein content, and antioxidant enzymes of *Solanum tuberosum* L. grown *in vitro* under salt stress

Arifa Khalid[1] · Faheem Aftab[1]

Received: 22 April 2019 / Accepted: 13 December 2019 / Published online: 13 January 2020 / Editor: Jessica Rupp

Abstract

Indole-3-acetic acid (IAA) and gibberellic acid (GA_3) are essential for the growth and development of plants. In the present study, the ameliorative potential of these phytohormones on growth, protein content, and antioxidant enzymes was investigated in *in vitro*-grown *Solanum tuberosum* L. cultivars 'Cardinal' and 'Desiree' under salt stress. A 4 × 3 factorial combination of 0, 40, 60, or 80 mM NaCl with 0, 7, or 14 μM IAA, or 0, 14, or 21 μM GA_3, were added to Murashige and Skoog (MS) basal medium, followed by inoculation of nodal explants or callus cultures. The data for root and shoot number and length, number of nodes and leaves, fresh weight of plants, increase or decrease in fresh weight of callus cultures, total soluble protein, and superoxide dismutase (SOD) and peroxidase (POD) activities were recorded after 30 d. The growth of both callus cultures and nodal explants subjected to NaCl stress was substantially reduced compared with the control. Both IAA and GA_3 successfully alleviated the harmful effects of salt stress on all of the growth parameters studied. Salt stress resulted in decreased protein content, which increased when the media also contained phytohormones. The activities of SOD and POD were increased with either IAA or GA_3 under NaCl stress. Therefore, the exogenous application of both IAA and GA_3 not only played a positive role in terms of *in vitro* potato growth but also significantly affected the biochemical parameters tested.

Keywords Antioxidants · GA_3 · IAA · Protein · Salt stress

Introduction

Soil salinity is a major factor that limits crop yield. It is becoming a global problem and affects about 20% of the irrigated agricultural land (Zhu 2001). During salinity stress, not only do the plants experience the shortage of water but they also undergo ion disequilibrium, which may disrupt homeostasis and lead to oxidative damage (Karimi *et al.* 2005; Gill and Tuteja 2010). Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen ($^1O_2$), and hydroxyl radical ($^{\cdot}OH$), are normally produced as by-products during plant cell metabolism and support the biosynthesis of complex organic molecules (Foyer and Shigeoka 2011). Under stress conditions, the production of ROS increases, which disrupts a balance that normally exists between the production of ROS and their detoxification. This off-balance (more ROS at a particular given time) not only inactivates various enzymes but also causes damage to vital cellular macromolecules, such as lipids, proteins, and DNA, which poses a serious threat to the existence of plants. Under these circumstances, cells may produce enhanced copies of antioxidative enzymes such as superoxide dismutase, catalase, peroxidase, and nitrate reductase, to scavenge surplus ROS in an improved manner and streamline cellular metabolism and plant growth under stress.

Phytohormones play a crucial role in the growth and development of plants by regulating many processes. Phytohormones may enhance stress tolerance and minimize the yield loss of plants caused by abiotic stress (Ilias *et al.* 2007). Various studies have determined the roles of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) in plants under stress conditions (Amzallag *et al.* 1990). Alone or in combination, they improve plant growth by either improving germination or reducing oxidative damage by controlling

✉ Faheem Aftab
faheem.botany@pu.edu.pk

[1] Department of Botany, University of the Punjab, Lahore 54590, Pakistan

activities of antioxidative enzymes (Kaur *et al.* 2000; Senthila *et al.* 2005; Shah and Ahmad 2007).

Potato is the fourth most important crop by volume of production after maize, wheat, and rice (FAO 2008). Potato is considered to be moderately salt tolerant but is sensitive during tuber bud initiation, which leads to a decrease in the average tuber size (Teixeira and Pereira 2007). The acquisition of salt tolerance in potato plants may help to improve its growth and ultimately crop yield. It is important to blend various approaches to develop a successful experimental plan that results in improved yield using biotechnology methods such as genetic engineering and chemical modifications. The use of plant tissue culture techniques has also been used to screen stress-tolerant varieties of various crops (Tal 1983). Plant tissue culture can provide an opportunity to manipulate *in vitro* cultures reproducibly under a desired set of experimental conditions. Exogenous application of various biomolecules including PEG, glycine-betaine, proline, sorbitol, mannitol, ascorbic acid, brassinosteroids, IAA, and GA_3 have been reported to induce stress tolerance in plants (Datta *et al.* 1998; Al-Hakimi and Hamada 2001; Ashraf and Fooland 2005; Qasim *et al.* 2006; Anuradha and Rao 2007; Chauhan *et al.* 2009; Khalid and Aftab 2016; Kaur and Gupta 2018). Exogenous application of IAA and GA_3 that affects various growth parameters and antioxidant enzyme activities has not been thoroughly reported in potato plants grown *in vitro* under salt stress. The purpose of the present study was to evaluate the impact of selected levels of NaCl on growth and development of potato plants and to determine the effect of the exogenously applied growth regulators IAA and GA_3 on various growth and biochemical parameters and their response to stress. The study also aimed to determine the relationship (if any), between the activities of superoxide dismutase and peroxidase, and the growth of calluses/plants under stress.

Materials and Methods

Plant material *Solanum tuberosum* L. tubers (cvs. Cardinal and Desiree) were obtained from the Seed Centre, University of the Punjab, Lahore. They were planted in 8 × 12 cm pots and grown in a greenhouse at 27°C, during October 2014. After 2 wk, 10-cm-long shoots were cut and used as the explant source for additional experiments. The shoots were initially disinfected by washing with detergent (Unilever Karachi, Pakistan) to remove any adhered dust particles. Next, the shoots were rinsed three times with distilled water and kept in a solution of 0.7% (*v/v*) sodium hypochlorite (Unilever, Karachi, Pakistan), and 0.1% (*v/v*) Tween 20 (Sigma-Aldrich, St Louis, MO) for 5–10 min in an Erlenmeyer flask (500 mL, Pyrex, Corning Inc., Corning, NY). The shoots were washed three times with sterile (autoclaved) distilled water in a laminar air-flow cabinet to get rid of traces of the sodium hypochlorite. Murashige and Skoog (MS) medium (Murashige and Skoog 1962) was used for shoot induction and additional culture maintenance. The medium was prepared by weighing appropriate quantities of the individual required chemicals (Sigma/Merck grade) for the preparation of stock solutions and later mixing the solutions according to the requirements. Sucrose (30 gL^{-1}) and agar (0.7% (*w/v*); Oxoid, Hampshire, UK) were added after adjusting the pH of the medium to 5.7. The medium was autoclaved for 15 min at 121°C (103.42 kPa), and 10 mL of the medium was aliquoted in each of the culture tubes (25 × 160 mm; Pyrex). After trimming the chlorinated shoot ends, 8-mm-long single-node cuttings were placed in each culture tube and incubated for a 16-h photoperiod (40 μmol m^{-2} s^{-1} photon flux density cool-white fluorescent light, Philips, Pakistan), at 25 ± 2°C for shoot induction. For callus induction, the internodes were excised from the disinfected shoots and inoculated on MS medium supplemented with 1 mM 2,4-dichlorophenoxyacetic acid (2,4-D; Sigma-Aldrich) and incubated at 25 ± 2°C in the dark for 60 d.

Treatment outline and experimental design A 4 × 3 factorial combination of NaCl (0, 40, 60, and 80 mM) and each growth regulator, which included IAA (0, 7, and 14 μM; Sigma-Aldrich) and GA_3 (0, 14, and 21 μM; Sigma-Aldrich) was used. The specific levels of IAA/GA_3 were selected based on the results of a pilot experiment (Khalid and Aftab, unpublished). MS medium with NaCl treatments (0, 40, 60, and 80 mM) were autoclaved and cooled-down to around 55°C, before the addition of respective levels of filter-sterilized IAA/GA_3 solutions (dissolved in ethyl alcohol and diluted with distilled water). Ten culture tubes were used for each treatment. *In vitro*-grown 30-d-old potato plants cvs. Cardinal and Desiree were excised from the culture tubes, and 1 cm long single nodes were cut for inoculation. The culture tubes were kept at 25 ± 2°C for a 16-h photoperiod (40 μmol m^{-2} s^{-1} photon flux density cool-white fluorescent light) for 30 d, and morphological and biochemical parameters were evaluated. For studies on calluses, the callus induction medium was supplemented with respective IAA/GA_3 concentrations and later inoculated with pre-weighed calluses. They were kept in the dark at 25 ± 2°C. The data for callus morphology and fresh weights were recorded after 30 d of inoculation. The experiment was repeated three times, over a period of 8 mo with the same number of replicates for each experiment. Data were pooled together from these experiments for subsequent analyses.

Morphological and biochemical analyses Data were obtained for both growth and biochemical parameters for plantlets, which included root and shoot number and length, number of nodes/leaves, fresh weight (FW), total soluble proteins, and activities of superoxide dismutase (SOD) and peroxidase (POD), after treatment for 30 d. For morphological analysis, plants were uprooted from the culture vessels and root and shoot lengths were determined. Fresh weight was recorded by weighing the whole plants on an electric balance

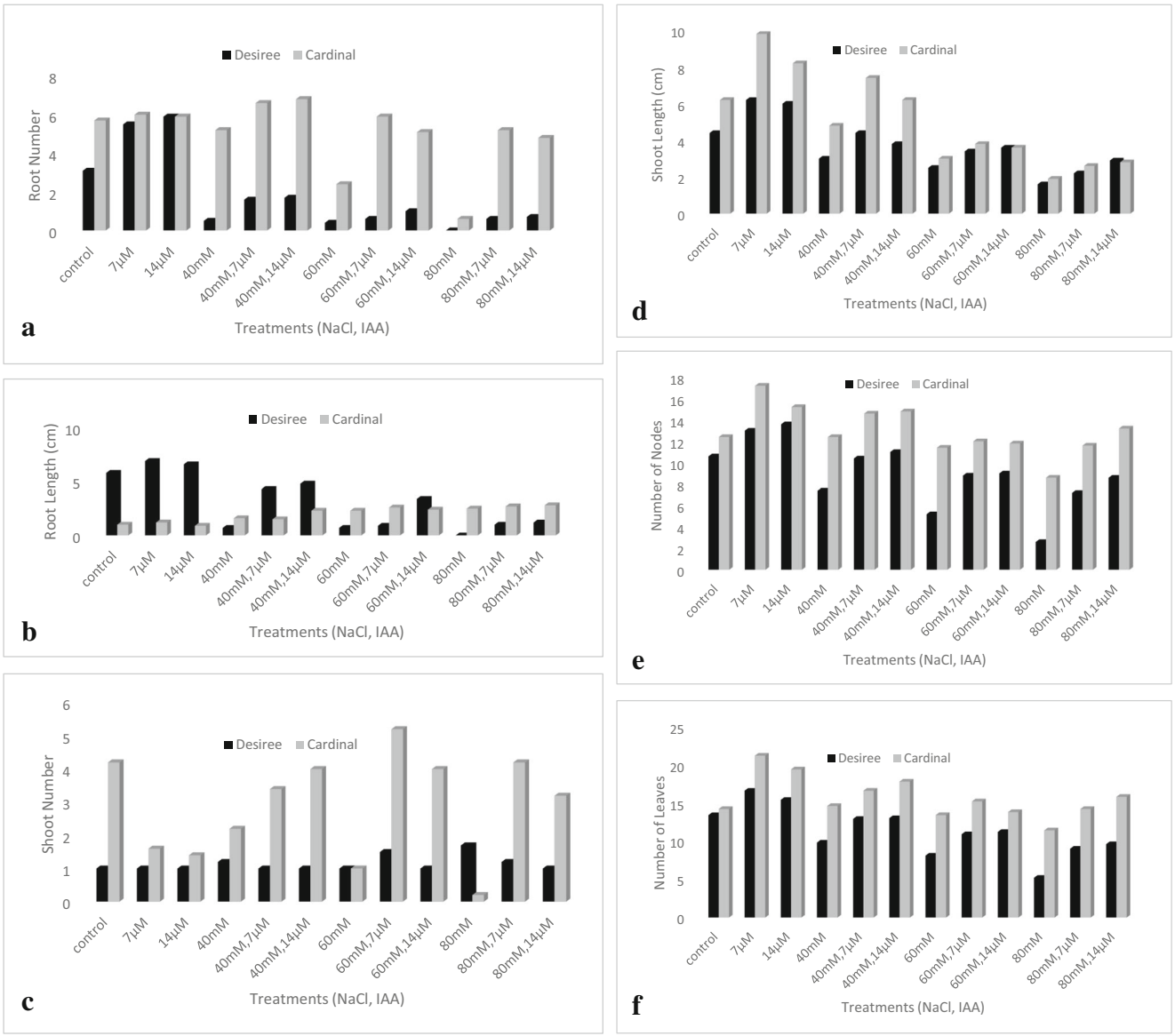

Figure 1. Comparative effects of indole-3-acetic acid (IAA: 0, 7, or 14 μM) and NaCl (0, 40, 60, or 80 mM) on morphological and biochemical parameters of *Solanum tuberosum* L.: (*a*) root number, (*b*) root length, (*c*) shoot number, (*d*) shoot length, (*e*) number of nodes, (*f*) number of leaves, (*g*) fresh weight, (*h*) protein, (*i*) Superoxide dismutase (SOD), and (*j*) Peroxidase (POD) in in vitro potato plants (cvs. Desiree and Cardinal).

(Scientech 5220). Other morphological parameters, which included number of leaves/nodes were determined at the same time. For calluses, the morphology, increase/decrease in fresh weight, and callus proliferation responses were recorded.

For biochemical analysis, 1 g of plant material was ground in liquid nitrogen using a mortar and pestle to obtain a fine powder. Two milliliters of phosphate buffer (0.1 M) containing 0.1 g polyvinypolypyrrolidone (PVP; Sigma-Aldrich) and Triton (0.01 mL; Sigma-Aldrich) were added to make a slurry, which was then centrifuged at 4°C for 30 min at 15,400×*g*. The supernatant collected was used for additional estimation as a crude enzyme extract.

For the estimation of total soluble protein, the Biuret method (Racusen and Johnstone 1961) was followed with minor modifications. Two test tubes (15 × 150 mm) were labeled as "control" and "experimental." In both tubes, 2 mL Biuret reagent was added. In the experimental tube, 0.2 mL crude enzyme extract was added, and in the control tube, 2 mL distilled water was added. Both tubes were vortexed and kept for 15 min at 25 ± 2°C to complete the reaction. The optical density (OD) was measured at 545 nm. The total soluble protein was calculated following a standard curve that was prepared by bovine serum albumin protein (Robinson 1979).

Quantitative estimation of the superoxide dismutase (SOD; E.C 1.15.1.1) activity was carried out using the method of Maral *et al.* (1977) with minor modifications. Briefly, to the 3-mL reaction mixture, which consisted of 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM nitroblue

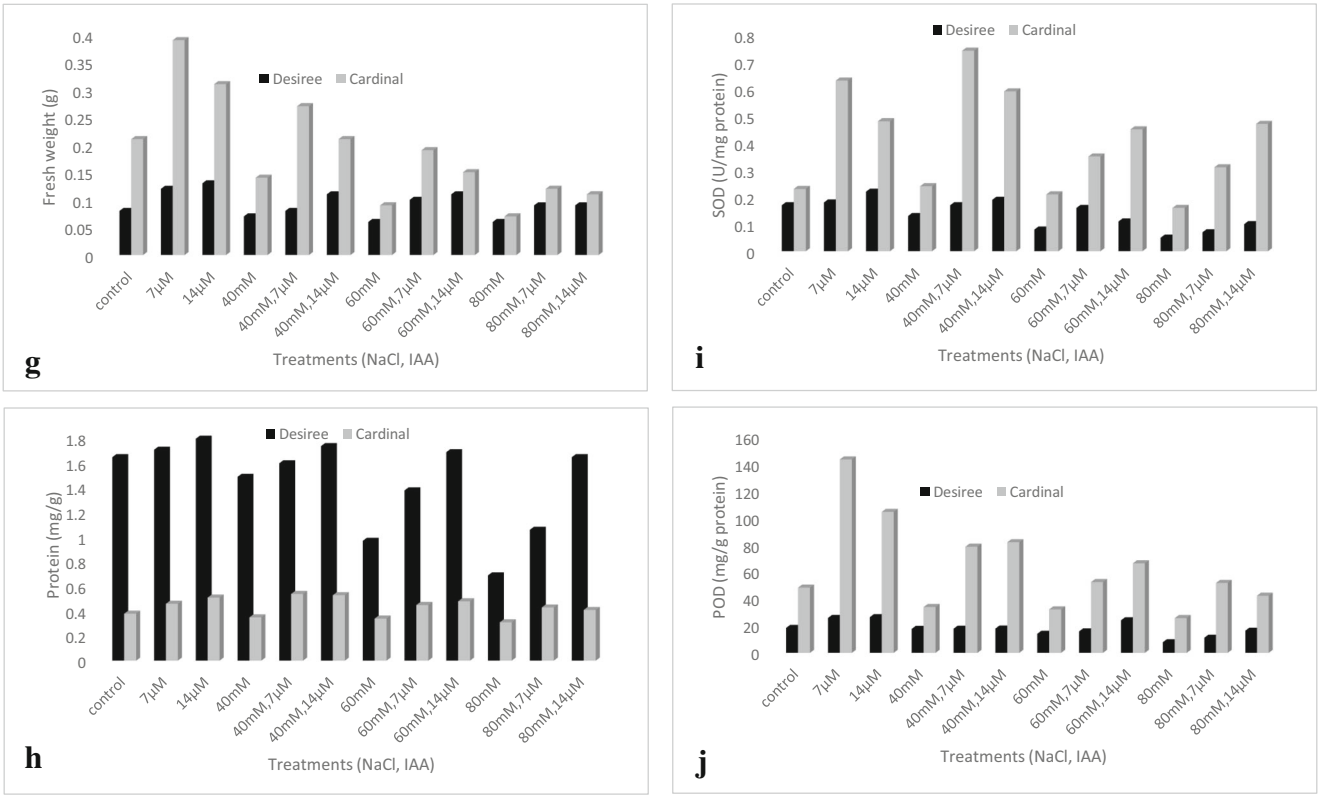

Figure 1. (continued)

tetrazolium, 0.1 mM ethylenediaminetetraacetate, and 2 µM riboflavin, 15 µL crude enzyme extract was added to the test tube labeled experimental, whereas distilled water (15 µL) replaced the crude enzyme in the control. Both samples were vortexed briefly following irradiation for 10 min with 40-W fluorescent cool-white light (40 µmol m^{-2} s^{-1} photon flux density). The absorbance was measured at 560 nm, and the SOD activity was calculated by the following formula:

$$\%\text{inhibition} = \frac{\text{Absorbance of control sample} - \text{Absorbance of experimental sample}}{\text{Absorbance of experimental sample}} \times 100$$

POD (EC 1.11.1.6) activity was measured using the Racusen and Foote (1965) method with some modifications. Two test tubes that were labeled control and experimental were used. To both tubes, 2.5 mL Tris-HCL buffer (0.1 M, pH 7.2) and 0.2 mL guaiacol (1%, *v/v*, purity 98%; Sigma-Aldrich, St Louis, MO) were added. In the experimental tube, 10 µL crude enzyme extract was added, while 10 µL distilled water was added in the control. Both samples were kept for 30 min at room temperature before the addition of H_2O_2 (0.3%, *v/v*; 0.2 mL). The absorbance was measured at 470 nm. The enzyme content was calculated as follows:

Peroxidase content (mg g^{-1} of tissue)$= \frac{A \times df}{EU \times Wt \times 1000.}$

where *A* is absorbance, df is the dilution factor, EU is the extract used, and Wt is fresh weight of the sample tissue.

Statistical analysis Data were analyzed statistically using analysis of variance (ANOVA). The dependent variables included root and shoot number and length, number of nodes/leaves, fresh weight of plants/calluses, callus morphology, proliferation response, protein, SOD, and POD. A full-factorial multivariate analysis was performed using SPSS 20.0.

Results

Effect of IAA and GA_3 on growth and biochemical parameters of potato under salt stress Nodal explants All of the tested growth parameters were negatively affected when nodal explants were grown on saline medium. However, the deleterious effects of salt stress were successfully alleviated with various concentrations of IAA and GA_3 (Figs. 1 and 2). In the cultivar Cardinal, a significant difference in shoot length was observed (Fig. 1). The reduction in

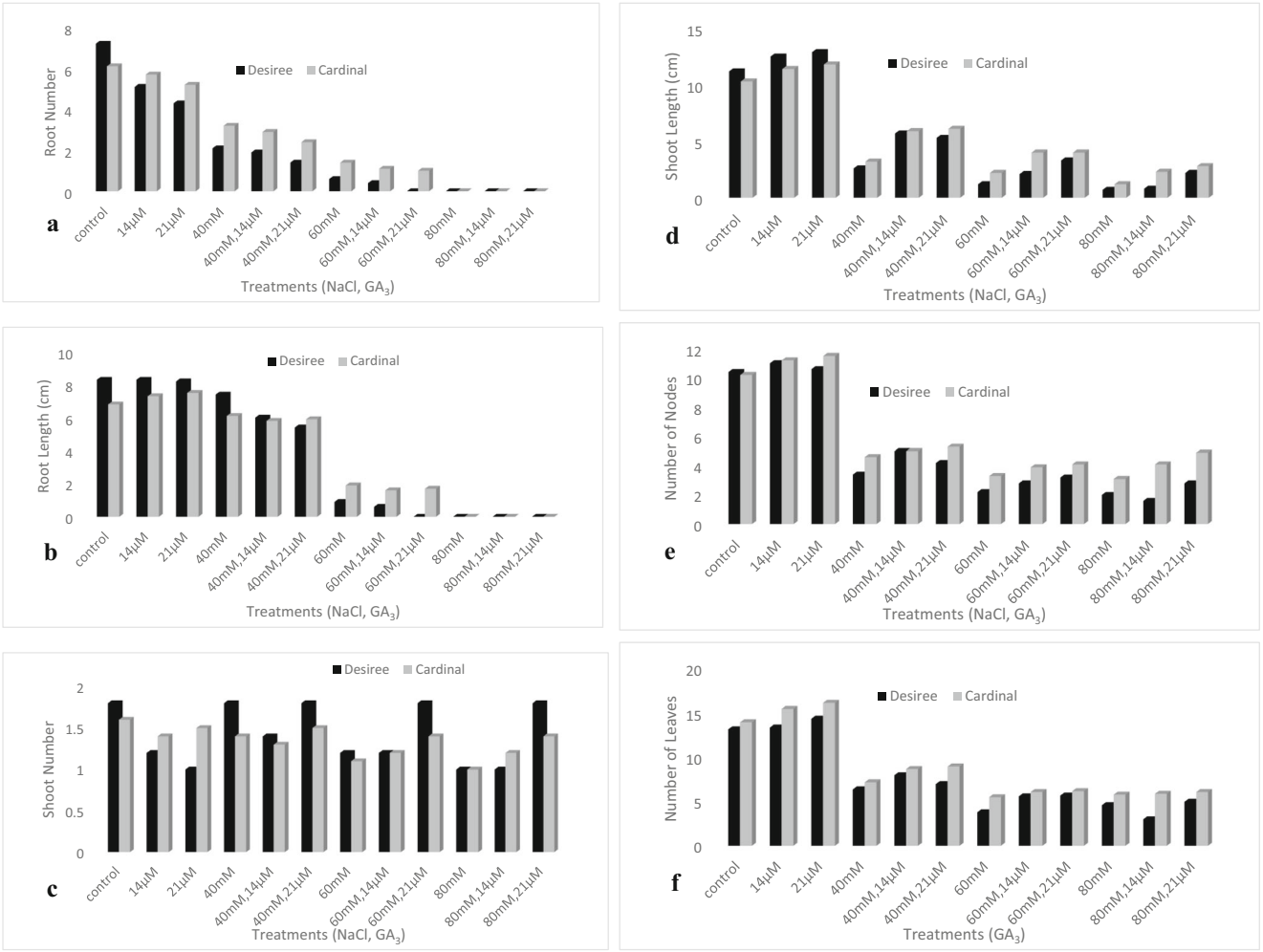

Figure 2. Comparative effects of gibberellic acid (GA_3; 0, 14, or 21 µM) and NaCl (0, 40, 60, or 80 mM) on morphological and biochemical parameters of *Solanum tuberosum* L.; (*a*) root number, (*b*) root length, (*c*) shoot number, (*d*) shoot length, (*e*) number of nodes, (*f*) number of leaves, (*g*) fresh weight, (*h*) protein, (*i*) Superoxide dismutase (SOD), and (*j*) Peroxidase (POD) in in vitro potato plants (cvs. Desiree and Cardinal).

growth at 40, 60, and 80 mM NaCl was successfully alleviated after treatment of IAA in both the cultivars (Fig. 1). In cv. Desiree, the root growth was completely inhibited at 80 mM NaCl, which was improved after IAA treatment (Fig. 1). Total protein content, SOD, and POD activities generally decreased with an increase in the NaCl concentration, which thereby increased with exogenous application of IAA (Fig. 1).

The data presented in Fig. 2 indicate that growth was reduced with NaCl alone, and the exogenous application of GA_3 alleviated these harmful effects in both of the tested cultivars. The number/length of roots was reduced with increasing salt concentrations and completely inhibited at 80 mM, which could not be improved by GA_3 application (Fig. 2). The long internodes were observed when nodal explants were supplemented with various GA_3 concentrations. Total protein content, SOD, and POD activities were reduced when the NaCl concentration was increased. However, an increase in the above-mentioned parameters was recorded with exogenous application of GA_3 (Fig. 2).

Callus cultures IAA affected the growth of calluses positively by increasing the fresh weights from 15.51 to 19.6% (7 µM) and 18.75% (14 µM; Table 1). The calluses were off white and friable. Under salt stress, the fresh weights decreased and the calluses were necrotic at 80 mM (Fig. 3). A similar response was observed in cv. Desiree (Table 2), in which the exogenous application of various concentrations of IAA improved the fresh weights of calluses, which could be observed from their morphological parameters because the dark brown/necrotic callus changed into a light brown color with exposure to IAA (7 or 14 µM; Table 2).

Exogenous application of GA_3 (14, 21 µM) also improved callus morphology and the fresh weights of both cultivars. A reduction of 8.16% in fresh weight of the calluses at 80 mM NaCl (cv. Cardinal) was increased to 3.92% (14 µM) and 2.08% (21 µM), respectively (Table 3). Likewise, an 11.53%

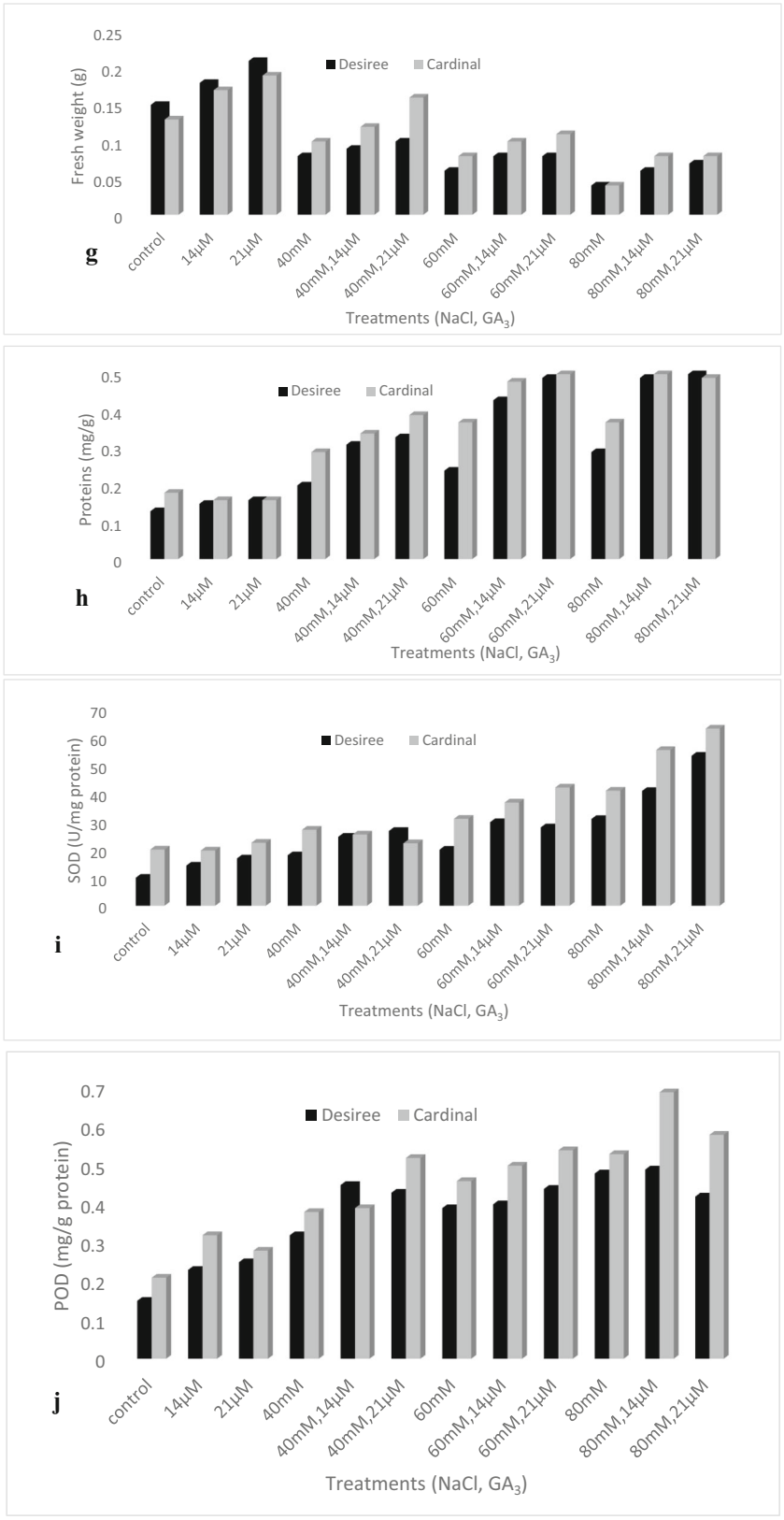

Figure 2. (continued)

Table 1. Effect of various concentrations of NaCl and IAA on the callus proliferation response in *Solanum tuberosum* L. cv. Cardinal

Treatments		Fresh weight at the time of treatment (g)	Fresh weight of callus after 30 d of treatment (g)	Callus morphology	Increase (+)/decrease (−) in fresh weight (%)
NaCl (mM)	IAA (μM)				
0	0	0.52 ± 0.02	0.54 ± 0.03	Off-white and friable	+ 3.84
	7	0.52 ± 0.03	0.56 ± 0.02	Friable and translucent	+ 7.69
	14	0.49 ± 0.02	0.56 ± 0.03	Off-white, friable and translucent	+ 14.28
40	0	0.51 ± 0.01	0.50 ± 0.02	Off-white with brown patches	− 1.96
	7	0.53 ± 0.02	0.55 ± 0.01	Whitish yellow, friable	+ 3.77
	14	0.52 ± 0.01	0.58 ± 0.03	Off-white, friable	+ 11.53
60	0	0.54 ± 0.02	0.48 ± 0.02	Yellow with brown patches, granular	− 11.11
	7	0.51 ± 0.01	0.55 ± 0.02	Yellow, granular	+ 7.40
	14	0.49 ± 0.02	0.53 ± 0.01	Yellow, granular	+ 8.16
80	0	0.53 ± 0.01	0.42 ± 0.03	Brownish yellow, necrotic	− 20.75
	7	0.52 ± 0.02	0.46 ± 0.02	Yellowish brown	− 11.53
	14	0.48 ± 0.03	0.43 ± 0.01	Yellowish brown granular	− 12.5

Results are means (± S.E.) from 30 replicate cultures. The increase or decrease in fresh weight is shown compared with the initial fresh weight in the respective treatment at day 30

decrease (60 mM NaCl) in cv. Desiree was reduced to 3.7% (14 μM) and 2.12% (21 μM; Table 4). In addition, the callus morphology was also positively affected by the exogenous application of GA_3 (Fig. 4).

Discussion

With exposure to salinity stress, a growth reduction in salt-sensitive plants is usually the first noticeable response (Parida and Das 2005). A decline in morphological parameters (root and shoot length, number of nodes/leaves, and fresh weight) was observed with a gradual increase in salt in the MS medium. The most drastic effect of NaCl stress was at the highest tested concentration (80 mM), in which the plantlets exhibited reduced shoot and root length but with an increased shoot number. These results are in agreement with the results of Ahmad *et al.* (2012) who reported a similar response of *in vitro*-grown potato under NaCl stress. Reduction of growth due to inadequate water uptake because of low osmotic potential is a common indicator of salt stress (Munns 2002; Borsani *et al.* 2003). Initially, salt stress decreases the water absorption capacity of the root system, which is followed by an increased water loss from the leaves. As a result of osmotic stress, physiological changes occur in plants, in which nutrient imbalance, membrane interruption, and reduced photosynthetic capacity have been observed (HanumanthaRao *et al.* 2016). Callus cultures also exhibited a reduction in the fresh weight of both cultivars with an increase in salt stress. These results are in agreement with a previous study conducted by Ochatt *et al.* (1999), in which a decrease in callus growth was observed at higher NaCl concentrations in *Solanum tuberosum* L. In another study, Liu and Van-Staden (1999) also reported a similar reduction in the fresh weight of calluses after 28 d, and callus growth was inhibited with a concentration of 100 mM salt. One reason for this salt-induced growth reduction could be the adverse effect of salt on roots, as it reduces uptake of water and nutrients. Hormonal imbalance is another probable reason, which leads to reduced growth in response to salinity. The repressive effect of salt stress on plant growth could also be attributed to the decline in endogenous levels of plant hormones (Hamayun *et al.* 2010).

IAA and GA_3 are phytohormones that promote cell expansion and elongation, vascular tissue development, maintain apical dominance, regulate phototropic and gravitropic behavior, and therefore ultimately enhance plant growth (Hamayun *et al.* 2010). In the present study, exogenous application of IAA and GA_3 enhanced callus/nodal explant growth and successfully counteracted the adverse effects of salt stress. The tested concentrations of both of the growth regulators significantly enhanced plant growth by increasing shoot and root length, the number of roots/node, and fresh weight in non-stressed and NaCl-stressed plants compared with the control. This growth enhancement could have helped the plants cope with stress by delaying the onset of the salinity tolerance threshold (Dalton *et al.* 2000). In the present study, a relatively lower concentration of IAA, such as 7 μM, or a slightly higher level of GA_3 (21 μM), were found to enhance growth in both

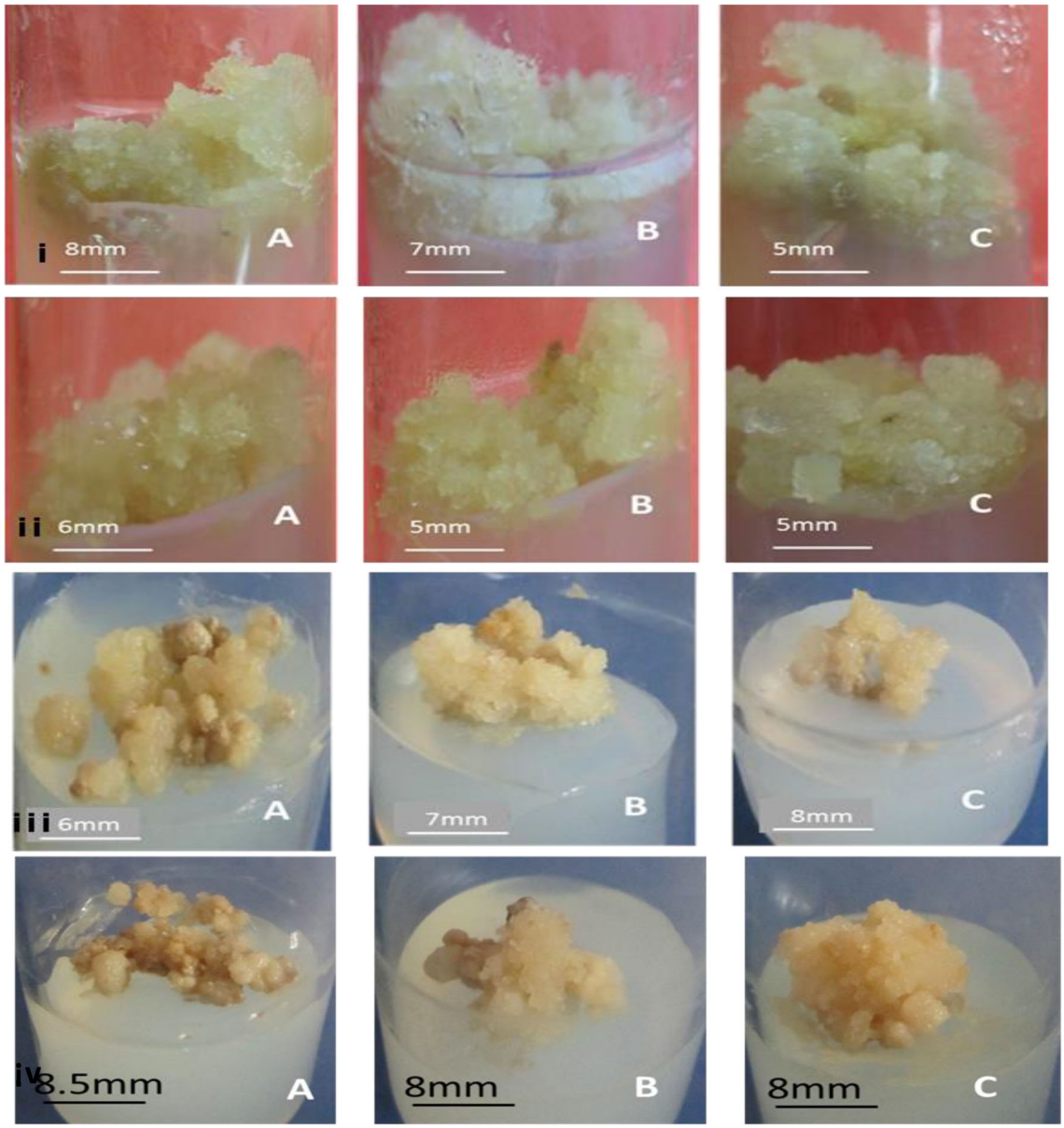

Figure 3. Off-white callus cultures of Solanum tuberosum L. cv. Cardinal: control (*A*); 7μM Indole-3-acetic acid (IAA) (*B*); and 14 μM IAA (*C*) with 0 mM NaCl (*i*), 40mM NaCl (*ii*), brownish yellow callus with 60 mM NaCl (*iii*), and yellowish brown calluses with 80 mM NaCl (*iv*).

normal and stress conditions. However, IAA influenced root growth positively, which is in agreement with several earlier studies, in which the role of IAA in the formation of lateral and adventitious roots was reported (Arteca 1996; Rahman *et al.* 2002). These results are also in agreement with the results of Chakrabarti and Mukherji (2003), Akbari *et al.* (2007), and Egamberdieva (2009) who described the positive role of IAA on seed germination and seedling growth under NaCl stress. Plant hormones act as a signal for an expression of numerous genes that contribute to salt tolerance (Shakirova *et al.* 2003). An immediate effect of salt stress is the reduction in the rate of leaf surface expansion, with additional growth reduction at higher salt concentrations (Wang and Nil 2000). Such a reduction in growth may be overcome by the exogenous application of certain plant growth regulators. Veselov *et al.* (2008) reported that exogenous IAA application not only stimulated growth but also alleviated the adverse effects of salt stress in maize. They attributed this response to an increase in the expression of *ZmEXPA1* expansin genes that promoted leaf cell extension.

Proteins are among the potential biochemical indicators of salinity tolerance. In this study, a progressive decrease in

Table 2. Effect of various concentrations of NaCl and IAA on the callus proliferation response in *Solanum tuberosum* L. cv. Desiree

Treatments		Fresh weight at the time of treatment (g)	Fresh weight of callus after 30 d of treatment (g)	Callus morphology	Increase (+)/decrease (−) in fresh weight (%)
NaCl (mM)	IAA (μM)				
0	0	0.49 ± 0.01	0.58 ± 0.02	Off-white, translucent, friable	+ 15.51
	7	0.51 ± 0.02	0.61 ± 0.03	Friable and translucent	+ 19.60
	14	0.48 ± 0.01	0.57 ± 0.02	Off-white, friable and translucent	+ 18.75
40	0	0.52 ± 0.02	0.46 ± 0.03	Off-white with brown patches, friable	− 11.53
	7	0.54 ± 0.03	0.58 ± 0.02	Yellow, friable	+ 7.40
	14	0.47 ± 0.02	0.52 ± 0.03	Yellow, friable	+ 10.63
60	0	0.53 ± 0.01	0.51 ± 0.01	Yellow with brown patches, granular	− 3.77
	7	0.54 ± 0.03	0.56 ± 0.02	Yellow, granular	+ 3.70
	14	0.49 ± 0.03	0.52 ± 0.03	Yellow, granular	+ 6.12
80	0	0.47 ± 0.02	0.35 ± 0.02	Brownish-yellow, necrotic	− 24.48
	7	0.52 ± 0.02	0.45 ± 0.01	Yellowish-brown,	− 13.46
	14	0.53 ± 0.03	0.46 ± 0.03	Yellowish brown-granular	− 13.20

Results are means (± S.E.) from 30 replicate cultures. The increase or decrease in fresh weight is shown compared with the initial fresh weight in the respective treatment at day 30

soluble protein content after exposure to NaCl was observed. These results are similar to the results of Wen *et al.* (2010), in which decreased levels of soluble protein content in potato were recorded under salt stress. The negative effect of salinization on protein content could be attributed to the osmotic effect (Yurekli *et al.* 2004) or due to a decreased availability of amino acids and denaturation of the enzymes involved in the amino acid and protein synthesis under saline conditions (Parida and Das 2005; Khalid 2017). Another possible reason could be the loss of potassium ions (K^+) under salt stress, as these are necessary for protein synthesis (Ayala-Astorga and Alcaraz-Meléndez 2010). It has been reported by Wang *et al.* (2003) that plants accumulate certain proteins that are likely protective in nature to survive under stressful conditions. Compared with the control used in this study (0 mM NaCl and 0 μM IAA in MS medium), the exogenous application of

Table 3. Effect of various concentrations of NaCl and GA_3 on the callus proliferation response in *Solanum tuberosum* L. cv. Cardinal

Treatments		Fresh weight at the time of treatment (g)	Fresh weight of callus after 30 d of treatment (g)	Callus morphology	Increase (+)/decrease (−) in fresh weight (%)
NaCl (mM)	GA_3 (μM)				
0	0	0.47 ± 0.02	0.53 ± 0.01	Yellow, friable	+ 12.76
	14	0.52 ± 0.02	0.61 ± 0.02	Whitish-yellow, friable	+ 17.30
	21	0.53 ± 0.03	0.61 ± 0.03	Friable, yellow	+ 15.09
40	0	0.52 ± 0.02	0.51 ± 0.01	Whitish-yellow with brown patches	− 1.92
	14	0.52 ± 0.03	0.55 ± 0.02	Yellow, granular	+ 5.76
	21	0.49 ± 0.02	0.52 ± 0.03	Yellow, granular	+ 6.12
60	0	0.53 ± 0.01	0.51 ± 0.01	Light brown with off-white and green patches	− 3.77
	14	0.52 ± 0.02	0.51 ± 0.02	Green with yellow patches	− 1.92
	21	0.48 ± 0.03	0.50 ± 0.03	Greenish-yellow	− 4.16
80	0	0.49 ± 0.01	0.45 ± 0.01	Black, necrotic	− 8.16
	14	0.51 ± 0.02	0.49 ± 0.02	Blackish-brown, necrotic	− 3.92
	21	0.48 ± 0.01	0.47 ± 0.01	Brown	− 2.08

Results are means (± SE) from 30 replicate cultures. The increase or decrease in fresh weight is shown compared with the initial fresh weight in the respective treatment at day 30

Table 4. Effect of various concentrations of NaCl and GA_3 on callus proliferation response in *Solanum tuberosum* L. cv. Desiree

Treatments		Fresh weight at the time of treatment (g)	Fresh weight of callus after 30 d of treatment (g)	Callus morphology	Increase (+)/decrease (-) in fresh weight (%)
NaCl (mM)	GA_3 (μM)				
0	0	0.51 ± 0.02	0.59 ± 0.01	Translucent, friable	+ 15.68
	14	0.53 ± 0.04	0.60 ± 0.02	Whitish-yellow, friable	+ 13.20
	21	0.52 ± 0.02	0.60 ± 0.03	Friable, yellow	+ 15.38
40	0	0.47 ± 0.03	0.46 ± 0.02	Whitish-yellow with brown patches	- 2.12
	14	0.52 ± 0.01	0.55 ± 0.04	Yellow, granular	+ 5.76
	21	0.53 ± 0.03	0.57 ± 0.03	Yellow, granular	+ 7.54
60	0	0.52 ± 0.04	0.46 ± 0.02	Light brown with off-white and green patches	- 11.53
	14	0.54 ± 0.03	0.52 ± 0.01	Green with yellow patches	- 3.70
	21	0.47 ± 0.01	0.46 ± 0.02	Greenish-yellow	- 2.12
80	0	0.54 ± 0.02	0.45 ± 0.05	Black, necrotic	- 16.66
	14	0.51 ± 0.02	0.49 ± 0.03	Blackish-brown, necrotic	- 3.92
	21	0.49 ± 0.01	0.46 ± 0.04	Brown	- 6.122

Results are means ± S.E. from 30 replicate cultures.

The increase or decrease in fresh weight is shown in comparison with the initial fresh weight in the respective treatment at day 30.

IAA increased the protein content in both the non-stressed and NaCl-stressed potato plants. This increased protein content could help plants maintain growth under stress conditions. These results are in agreement with the results of Agastian *et al.* (2000) and Fidalgo *et al.* (2004) who reported that stress-induced proteins play a major role in stress tolerance. High protein levels accumulate in plants under stress conditions and provide a storage form of nitrogen (Singh *et al.* 1987) and possibly play a role in osmotic adjustments (Ashraf and Harris 2004; Parida *et al.* 2004). In this study, increased protein content in response to IAA treatment could be due to a stimulating effect of auxin on the activation of K^+ uptake channels (Claussen *et al.* 1997), which might have enabled the plants to withstand the harmful effects of salt stress through osmotic adjustments or by balancing ionic homeostasis. This upregulation of proteins could be due to inclusion of salt stress proteins, as reported by Kim *et al.* (2004). Exogenous application of GA_3 also induced regulation of *salT* gene, which enhanced synthesis of proteins in response to salt stress (Wen *et al.* 2010).

Salt stress enhances the production of reactive oxygen species (ROS) and causes oxidative stress (Parida and Das 2005; Tanveer 2019). These ROS react with vital bio-molecules and cause pigment co-oxidation, lipid peroxidation, membrane disruption, protein denaturation, and DNA mutation (Molassiotis *et al.* 2006). In this study, the exogenous application of IAA alleviated the oxidative damage by increasing the superoxide dismutase (SOD) and peroxidase (POD) activities, in both the NaCl-stressed/non-stressed plants of potato compared with the control. Similar increases in the activity of SOD were reported by Rahnama and Ebrahimzadeh (2005) and Esfandiari *et al.* (2007) in salt-tolerant potato and wheat cultivars, respectively. In another study, Senthila *et al.* (2005) reported a similar increase in POD activity under salt stress in response to IAA. An increase in POD activity was also suggested to play a pivotal role in scavenging H_2O_2 in a salt-tolerant potato cultivar (Aghaei *et al.* 2009). The current studies are in agreement with results reported in earlier research (Meloni *et al.* 2001; Rahnama and Ebrahimzadeh 2005; Li 2009) who suggested that increased activities of antioxidant enzymes confer greater resistance in plants against stress-induced damage. SOD probably functions as the first line of defense against ROS, but its end product is the toxic H_2O_2. POD could provide a selective advantage for defense and play a role in scavenging H_2O_2. Higher POD activity decreases H_2O_2 levels in the cells and increases the stability of membranes and CO_2 fixation, as several enzymes of the Calvin cycle within the chloroplast are sensitive to H_2O_2 (Esfandiari *et al.* 2007). Similar results were observed in maize by Tuna *et al.* (2008). Manchandia *et al.* (1999) reported two- to four-fold increases in POD activity in cotton calluses with exposure to salt stress. However, in *Vigna radiata*, SOD and POD activities were reduced with GA_3 treatment under salt stress (Chakrabarti and Mukherji 2003), which indicated the complexity of biochemical events underlying salt tolerance and the various approaches diverse group of plants may have under stress conditions.

The results of this study demonstrate that IAA and GA_3 can alleviate the salt-induced damage under *in vitro* conditions in potato. These growth regulators likely act to

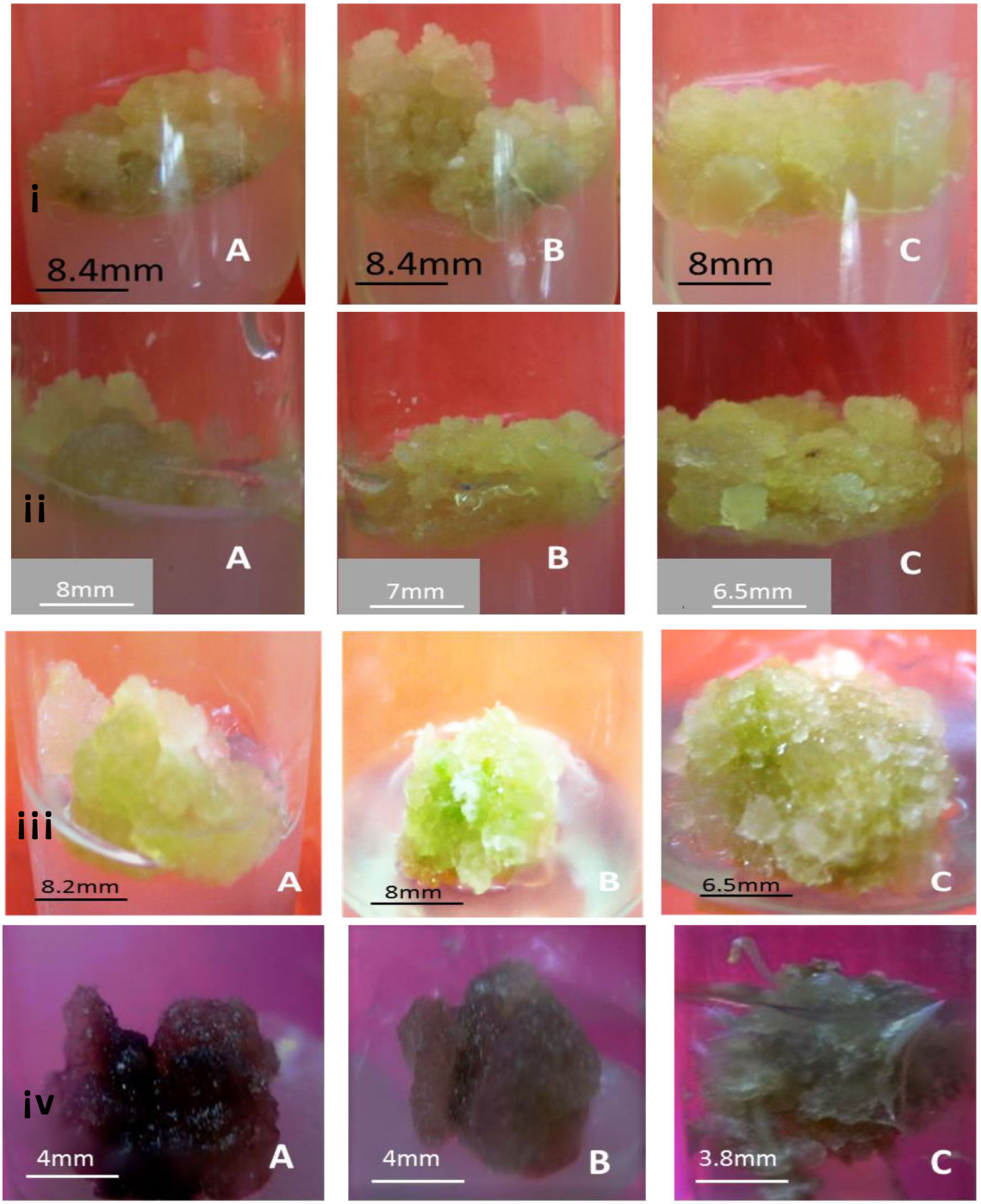

Figure 4. Off-white, yellow callus cultures of Solanum tuberosum L. cv. Desiree; control (*A*); 14 μM Gibberellic acid (GA3) (*B*); and 21 μM GA3 (*C*) with 0 mM NaCl (*i*), yellow calluses with 40 mM NaCl (*ii*), yellowish-green calluses with 60 mM NaCl (*iii*), and brown calluses with 80 mM NaCl (*iv*).

maintain the endogenous hormonal levels and/or increase the activities of the antioxidative enzymes, which promote growth even under stress. However, to harness potential benefits of this study and to determine the application for agronomic practices, these results need to be extended to future studies conducted in the greenhouse and in field conditions.

Acknowledgments We are grateful to the anonymous reviewers for their excellent reviews and feedback. We also thank the Copy Editor and the Editor in Chief for their contribution to enhance the outlook of this manuscript a great deal.

Funding information We thank Higher Education Commission Pakistan for providing research funds in the form of the Indigenous 5000 PhD Fellowship (106-1137-BM6-088) to Arifa Khalid.

References

Agastian P, Kingsley SJ, Vivekanandan M (2000) Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica 38:287–290

Aghaei K, Ehsanpour AA, Komatsu S (2009) Potato responds to salt stress by increased activity of antioxidant enzymes. J Integr Plant Biol 51:1095–1103

Ahmad P, Azooz, MM, Prasad MNV (Eds.). (2012) Ecophysiology and responses of plants under salt stress. Springer Science & Business Media

Akbari GA, Arab SM, Alikhani HA, Allakdadi I, Arzanesh MH (2007) Isolation and selection of indigenous Azospirillum spp. and the IAA of superior strains effects on wheat roots. W J Agri Sci 3:523–529

Al-Hakimi AMA, Hamada AM (2001) Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. Biol Plant 44:253–261

Amzallag GN, Lener HR, Poljakoff-Mayber A (1990) Exogenous ABA as a modulator of the response of Sorghum to high salinity. J Exp Bot 541:1529–1534

Anuradha S, Rao SSR (2007) The effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress. Plant Soil Environ 53:465–472

Arteca RN (1996) Plant growth substances. Chapman and Hall, New York, pp 286–287

Ashraf M, Fooland MR (2005) Pre sowing seed treatment—a shotgun approach to improve germination, plant growth and crop yield under saline and non-saline conditions. Adv Agron 88:223–271

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16

Ayala-Astorga GI, Alcaraz-Meléndez L (2010) Salinity effects on protein content, lipid peroxidation, pigments, and proline in Paulownia imperialis (Siebold & Zuccarini) and Paulownia fortunei (Seemann & Hemsley) grown in vitro. Electron J Biotechnol 13:13–14

Borsani O, Valpuesta V, Botella MA (2003) Developing salt tolerant plants in a new century: a molecular biology approach. Plant Cell Tissue Organ Cult 73:101–115

Chakrabarti N, Mukherji S (2003) Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. Biol Plant 46:63–66

Chauhan JS, Tomar YK, Singh NI, Ali S, Debrati (2009) Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. J Am Sci 5:79–78

Claussen M, Lüthen H, Blatt MR, Böttger M (1997) Auxin induced growth and its linkage to potassium channels. Planta 201:227–234

Dalton FN, Maggio A, Piccinni G (2000) Simulation of shoot chloride accumulation: separation of physical and biochemical processes governing plant salt tolerance. Plant Soil 219:1–11

Datta KS, Varma SK, Angrish R, Kumar B, Kumari P (1998) Alleviation of salt stress by plant growth regulators in *Triticum aestivum* L. Biol Plant 42:269–275

Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31: 861–864

Esfandiari E, Shekari F, Shekari F, Esfandiari M (2007) The effects of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedlings. Notulae Botanicae Horti Agrobotani Cluj-Napoca 35:58–56

FAO (2008) Hidden treasure. International year of potato, 2008. Food and agriculture organization of the United Nations. http://www.potato2008.org/en/index.html

Fidalgo F, Santos A, Santos I, Salema R (2004) Effect of long-term salt stress on antioxidant defense system, leaf water relations and chloroplast ultra-structure of potato plant. Ann Appl Biol 145:185–192

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Hamayun M, Khan SA, Khan AL, Shin JH, Ahmad B, Shin DH, Lee IJ (2010) Exogenous gibberellic acid reprograms soybean to higher growth and salt stress tolerance. J Agr Food Chem 58:7226–7232

HanumanthaRao B, Nair RM, Nayyar H (2016) Salinity and high temperature tolerance in mungbean [*Vigna radiata* (L.) Wilczek] from a physiological perspective. Frontiers. Plant Sci 7:957

Ilias I, Ouzounidou G, Giannakoula A, Papdopoulou P (2007) Effect of gibberellic acid and prohexadione-calcium on growth, chlorophyll fluorescence and quality of okra plant. Biol Plant 51:575–578

Karimi G, Ghorbanli M, Heidari RA, Assareh MH (2005) The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrate*. Biol Plant 49:301–334

Kaur H, Gupta N (2018) Ameliorative effect of proline and ascorbic acid on seed germination and vigour parameters of tomato (*Solanum tuberosum* L.) under salt stress. Int J Curr Microbiol App Sci 7: 3523–3532

Kaur S, Gupta AK, Kaur N (2000) Effects of GA_3, kinetin and indole acetic acid on carbohydrate metabolim in chickpea seedlings germinating under water stress. Plant Growth Regul 30:61–70

Khalid A (2017) Effect of exogenous application of IAA, BRs and GA_3 on growth, protein contents and antioxidative enzyme activities in *Solanum tuberosum* L. under salt stress. Doctoral dissertation, University of the Punjab, Lahore

Khalid A, Aftab F (2016) Effect of exogenous application of 24-epibrassinolide on growth, protein contents and antioxidant enzyme activities of Solanum tuberosum L. under salt stress. In Vitro Cell Dev Biol-Plant 52:81–91

Kim S, Kang JY, Cho DI, Park JH, Kim SY (2004) ABF2, an ABRE-binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance. Plant Physiol 40:75–87

Li Y (2009) Effects of NaCl stress on antioxidant enzymes of Glycine Soja sieb. Pak J Biol Sci 12:510–513

Liu T, Van-Staden J (1999) Selection and characterization of sodium chloridetolerant callus of *Glycine max* (L). Merrcb Acme 31:195–207

Manchandia AM, Banks SW, Gossett DR, Bellaire BA, Lucas MC, Millhollon EP (1999) The influence of α-amanitin on the NaCl-induced up-regulation of antioxidant enzyme activity in cotton callus tissue. Free Radic Res 30:429–438

Maral J, Puget K, Micheson AM (1977) Comparative study of superoxide dismutase, catalase, and glutathione peroxidase levels in erythrocytes of different animals. Biochem Biophys Res Commun 77: 1525–1535

Meloni DA, Oliva MA, Ruiz HA, Martinez CA (2001) Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. J Plant Nutr 24:599–612

Molassiotis AN, Sotiropoulos T, Tanou G, Kofidis G, Diamantidis G, Therios I (2006) Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitiol or sorbitol. Biol Plant 50:61–68

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250

Murashige T, Skoog F (1962) A revised medium for a rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Ochatt SJ, Marconi PL, Radice S, Arnozis PA, Caso OH (1999) *In vitro* recurrent selection of potato: production and characterization of salt-tolerant cell lines and plants. Plant Cell Tissue Organ Cult 55:1–8

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotox Environ Safe 60:324–349

Parida AK, Das AB, Mittra B, Mohanty P (2004) Salt-stress induced alterations in proteins profile and protease activity in the mangrove. *Bruguiera parviflora* Zeitschrift für Naturforschung, Z Naturforsch 59:408–414

Qasim A, Athar HR, Ahraf M (2006) Influence of exogenously applied brassinosteroids on the mineral nutrient status of two cultivars grown under saline conditions. Pak J Bot 38:1621–1632

Racusen D, Foote M (1965) Protein synthesis in dark grown bean leaves. Can J Bot 43:817–824

Racusen D, Johnstone DB (1961) Estimation of protein in cellular material. Nature 191:292–493

Rahman A, Hosokawa S, Oono Y, Amakawa T, Goto N, Tsurumi S (2002) Auxin and ethylene response interactions during Arabidopsis root hair development dissected by auxin influx modulators. Plant Physiol 130:1908–1917

Rahnama H, Ebrahimzadeh H (2005) The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol Plant 49:93–97

Robinson T (1979) The determination of proteins in plant extracts that contain polyphenols. Plant Sci Lett 15:211–216

Senthila A, Djanaguiraman M, Vijayalkashmi C (2005) Influence of seed treatment of growth regulators on some enzyme activities in groundnut under salinity. Agric Trop Subtrop 38:88–90

Shah SH, Ahmad I, Samiullah (2007) Responses of *Nigella sativa* to foliar application of gibberellic acid and kinetin. Biol Plant 51: 563–566

Shakirova FM, Sakhabutdinova AR, Bezukova MV, Fatkhutdinova RA, Fatkhutdinova DR (2003) Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci 164:317–322

Singh NK, Bracken CA, Hasegawa PM, Handa AK, Buckel S, Hermodson MA, Pfankoch F, Regnier FE, Bressan RA (1987) Characterization of osmotin. A thaumatin-like protein associated with osmotic adjustment in plant cells. Plant Physiol 85:529–536

Tal M (1983) Selection for stress tolerance. In: Evans DA, Sharp WR, Ammirato PV, Ymada Y (eds) Hand book of plant cell culture, vol 1. McMillan, London, pp 461–488

Tanveer M (2019) Role of 24-Epibrassinolide in inducing thermotolerance in plants. J Plant Growth Regul 38:945–955

Teixeira J, Pereira S (2007) High salinity and drought act on an organ-dependent manner on potato glutamine synthetase expression and accumulation. Environ Exp Bot 60:121–126

Tuna AL, Kaya C, Dikilitas M, Higgs D (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

Veselov DS, Sabirzhanova IB, Sabirzhanov BE, Chemeris AV (2008) Changes in expansin gene expression, IAA content, and extension growth of leaf cells in maize plants subjected to salinity. Russ. J. Plant Physiol 55:101–106

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: toward genetic engineering for stress tolerance. Planta 218:1–14

Wang Y, Nil N (2000) Changes in chlorophyll, ribolose bisphasphate arboxylaseoxygenase, glycine betaine contents, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. J Hortic Sci Biotechnol 75:623–627

Wen FP, Zhang ZH, Bai T, Xu Q, Pan YH (2010) Proteomics reveals the effects of gibberellic acid (GA_3) on salt-stressed rice (Oryza sativa L.) shoots. Plant Sci 178:170–175

Yurekli F, Porgali ZB, Turkan I (2004) Variation in abscisic acid, indole-3-acetic acid, gibberellic acid and zeatin concentrations in two bean species subjected to salt stress. Acta Biol Cracov 46:201–212

Zhu JK (2001) Overexpression of delta-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water and salt stress in transgenic rice. Trends Plant Sci 6:66–72