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PRE-SOWING APPLICATION OF ASCORBIC ACID AND SALICYLIC ACID TO SEED OF PUMPKIN AND SEEDLING RESPONSE TO SALT

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Abstract

The effects of seed soaking with salicylic acid or ascorbic acid on pumpkin seedlings growth under saline (10 dS m^{-1}) conditions were investigated. Seedlings fresh weight, protein contents, protease and nitrate reductase activities were significantly affected by 15 and 30 mg L^{-1} salicylic acid and 30 mg L^{-1} ascorbic acid priming treatments, under both normal and saline conditions. Priming reduced the severity of the salt stress, the amelioration was better due to 30 mg L^{-1} ascorbic acid or 30 mg L^{-1} salicylic acid treatments as these treatments showed best results on seedling growth, fresh and dry matter production under non-saline and saline environments. Application of seed priming with ascorbic acid and salicylic acid in pumpkin ameliorate the adverse effects of salt stress.

Introduction

Salinity levels can adversely affect agronomic and physiological attributes in crops (Cicek & Cakirlar, 2007; Al-Harbi *et al.,* 2008; Gómez-Pando *et al.,* 2010). Among many approaches/strategies used to combat salinity stress, exogenous application of plant growth regulators has received considerable attention (Afzal *et al.,* 2005; Dolatabadian *et al.,* 2008). Soaking seed before planting with growth regulators is beneficial in reducing negative effects of salinity on growth and physiological/biochemical responses of crops (Ashraf & Rauf, 2001; Afzal *et al.,* 2005). Priming typically affects germination time, leading to better growth and improved yield, especially in plants under stress (Halmer, 2004; Afzal *et al.,* 2005; Piri *et al.,* 2009).

Salicylic acid (SA) is a phenolic plant growth regulator having a role in regeneration of physiological processes in plants (Sakhabutinova *et al.,* 2003). The role of salicylic acid in seed germination (Cutt & Klessig, 1992), enzymatic activity (Dolatabadian *et al.,* 2008), photosynthetic rate (Khan *et al.,* 2003), uptake and transport of ions (Harper & Balke, 1981; Afzal *et al.,* 2005), and plant growth and yield (Hussein *et al.,* 2007) have been described. Inducing resistance against salinity (Shakirova & Bez-Rukova, 1997) and water stress (Senaratna *et al.,* 2000, Bezrukova *et al.,* 2001) in plants is a function of salicylic acid. Moreover, salicylic acid also reduced negative effects of salt stress by increasing levels of other plant growth regulators in plants (Sakhabutdinova *et al.,* 2003).

Ascorbic acid (AsA) is among the most abundant antioxidants found in plants. Exogenous application of ascorbic acid positively effects growth and physiological activities in *Triticum aestivum* L., (Amin *et al.,* 2007). Ascorbic acid alleviates adverse effects of salinity on plants by enhancing plant growth (Afzal *et al.,* 2005). Exogenous application of ascorbic acid induces activation of antioxidant enzyme system in canola (*Brassica napus* L.) resulting in reduction of detrimental effects of salinity (Dolatabadian *et al.,* 2008). However, little information is available regarding salt tolerance and response of pumpkin (*Cucurbita pepo* L.) in response to exogenously applied ascorbic and salicylic acids.

Considering the above mentioned facts about the effects of SA and AsA on plant growth and development, we hypothesized that if SA and AsA is applied exogenously, it might increase the salt tolerance ability of pumpkin. Hence, the study was carried out to find out the effects of pre-sowing application of salicylic and ascorbic acids on pumpkin seedlings under salt stress.

Materials and Methods

Pumpkin seed were obtained from the Vegetable Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Healthy seeds of similar size were used. Seed were soaked in solutions of 15 or 30 mg L^{-1} ascorbic acid or salicylic acid and for 12 hours in dark. Two treatments, control (distilled water) and 10 dS m^{-1} of NaCl (99.1%) salt (National Foods, Pakistan).

First the study was conducted in Petri plates lined with filter paper moistened with two salinity levels [0 (control) and 10 dS m^{-1}]. The experiment was arranged in a completely randomized design with six priming treatments. Seed soaking treatments included unsoaked seeds; priming in distilled water; 15 mg L^{-1} ascorbic acid; 30 mg L^{-1} ascorbic acid; 15 mg L^{-1} salicylic acid and 30 mg L^{-1} salicylic acid. There were three replications for each experimental unit. Mean maximum/minimum temperatures were 35.3/25.7°C. Seedling protease and nitrate reductase activity, total soluble proteins and fresh biomass/seedling were determined. Seedling protease activity was estimated according to the method of Ainouz *et al*., (1972). Seeds were ground in phosphate buffer (pH 7.0), centrifuged at 12000 rpm for 30 min at 4°C. The supernatant in test tubes was reacted with casein (1%). The solution then incubated in oven for one hour at 50°C, and then trichloro acetic acid (TCA) was added to terminate the reaction. Enzyme activity was estimated by protein analysis. Nitrate reductase activity was estimated according to the method of Sym (1984). Seedlings were extracted in 0.1*M* phosphate buffer (pH 7.0). The essay of the enzyme was with nitride analysis, the supernatant was treated with KNO_3 (0.2 M) and incubated for one hour at 32°C in dark. After one hour, incubated samples test tubes were treated with sulphanylamide and 1-naphthyl ethylenediamine dihydrochloride, incubated for 20 min and optical density was measured at 542 nm using a spectrophotometer (Hitachi, U1800, Tokyo, Japan).

In second phase, pumpkin seeds were grown in plastic glasses (15 x 8cm) filled with washed sand. The same six seed soaking treatments described earlier were applied. The experiment was arranged in a completely randomized design with six replications for each experimental unit. Hoagland's nutrient solution was added 4 days after sowing. Salinity was applied gradually after germination of plants. The study was conducted under maximum/minimum temperature of 36.3/27.0°C and mean photoperiod of 13-14 hours. Shoot and root fresh mass production and leaf chlorophyll was recorded four weeks after treatment. Chlorophyll content was measured according to the method of Arnon (1949). Fresh leaves were ground in 80% acetone and filtered using cheese cloth. Absorbance was measured at 645nm and 663nm.

Data were analyzed using Fisher's analysis of variance and treatment means compared by least significant difference (LSD).

Results

A 65% reduction in fresh shoot biomass and a 59% reduction in fresh root biomass occurred in plants treated with saline solutions compared to untreated plants. Hormonal priming of seeds with ascorbic acid and salicylic acid mitigated effects of salinity (Fig. 1a & b). The greatest fresh shoot biomass was for treatment with 15 and 30 mg L^{-1} SA without salt treatment. Plants treated with 15 mg L^{-1} AsA and 30 mg L^{-1} SA performed better under salt stress environment (Fig. 1a). Fresh root biomass, plants from seed treated with 15 and 30 mg L^{-1} SA had greater fresh root biomass when treated with or without salt (Fig. 1b). Treatment with salt resulted in a reduction in root and shoot dry matter. Priming seed with 15 mg L^{-1} SA and 15 mg L^{-1} AsA produced more dry matter than unsoaked and water soaked seeds (Fig. 1c & d). The most root dry matter with saline treatment was in plants from seed treated with 30 mg L^{-1} AsA. For non-saline controls, root/shoot length ratio was higher in plants from seeds treated with 30 mg L^{-1} AsA or 30 mg L^{-1} SA; 15 mg L^{-1} AsA treatment showed greater root/shoot length ratios when treated with salt (Fig. 2a). The fresh root/shoot matter ratio when plants were treated with salt was higher in plants from seed treated with 30 mg L^{-1} AsA (Fig. 2b). Priming with 30 mg L^{-1} AsA produced higher ratios of dry root/shoot matter under with and without saline treatment (Fig. 2c).

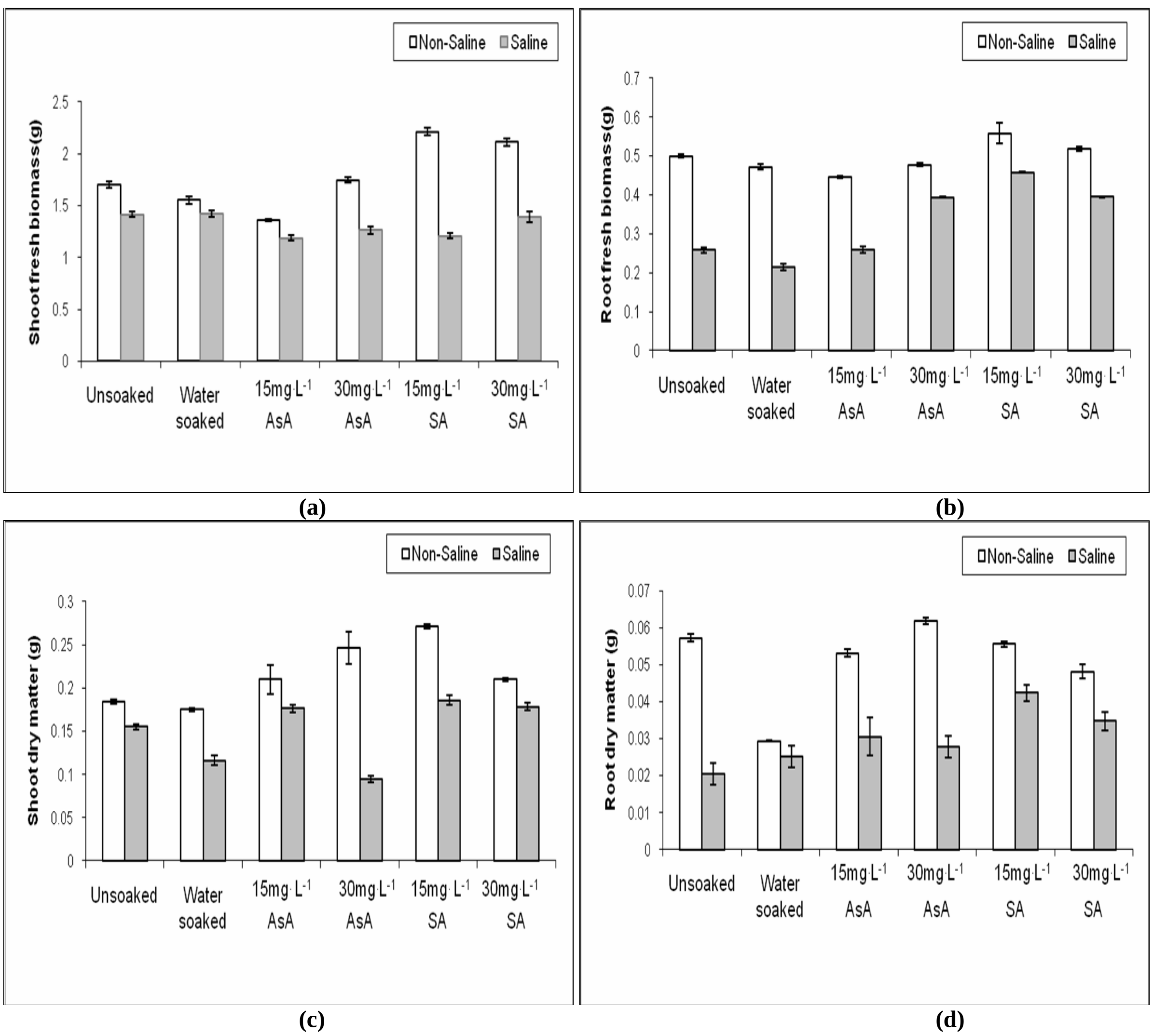

Fig. 1. Influence of pre-soaking seed with ascorbic acid and salicylic acid on a) shoot fresh biomass b) root fresh biomass c) shoot dry matter d) root dry matter of Pumpkin under normal and saline environment.

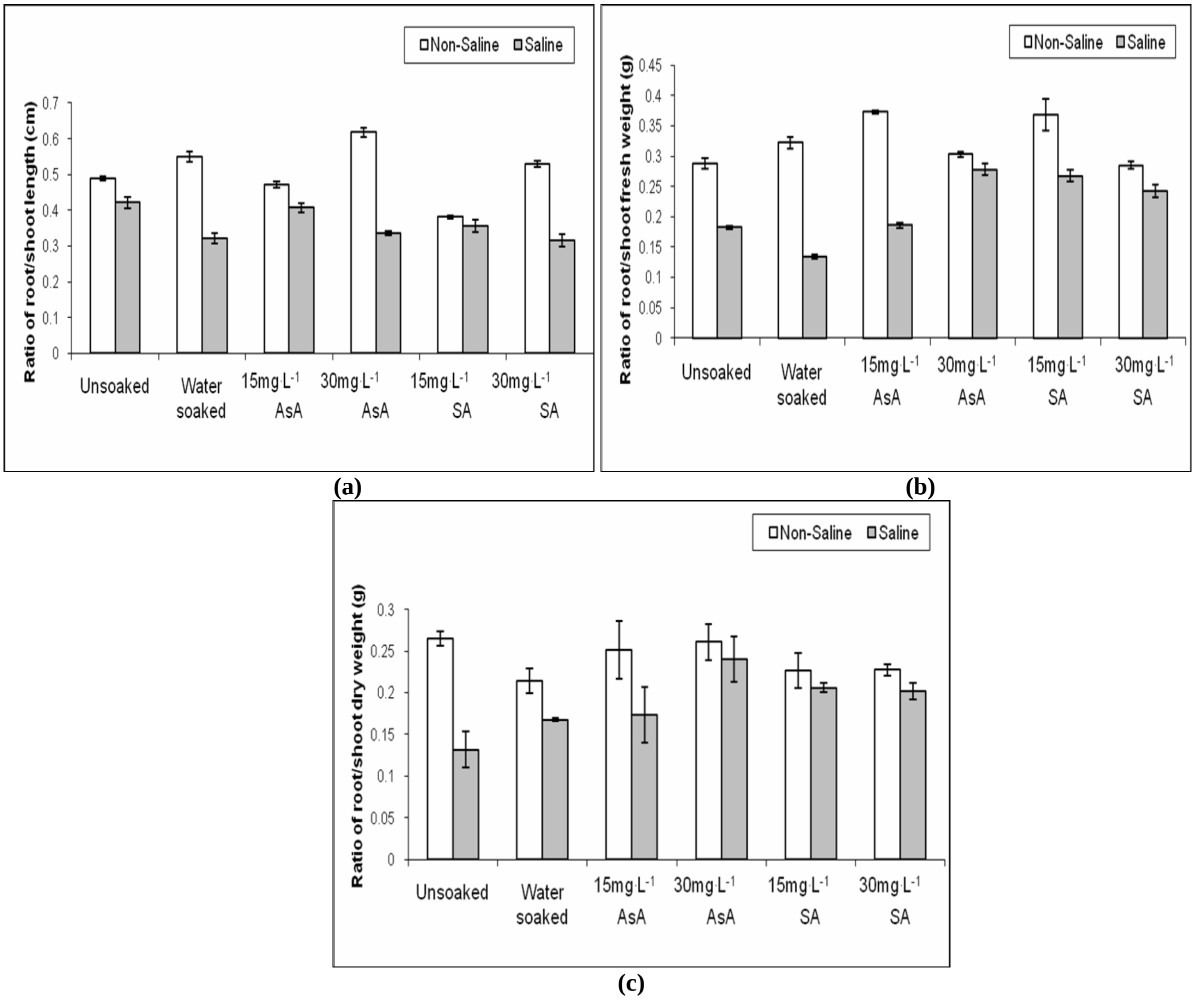

Fig. 2. Influence of pre-soaking seed with ascorbic acid and salicylic acid on the a) root/ shoot length ratio b) root/shoot fresh weight ratio c) root/shoot dry weight ratio of Pumpkin under normal and saline environment.

Saline treatment reduced leaf chlorophyll *a*, *b* and total chlorophyll contents. Salinity treated plants had 49, 53 and 56% less chlorophyll *a*, *b* and total chlorophyll, respectively, than untreated plants. Seed priming with AsA and SA reduced effects of salt on chlorophyll *a*, *b* and total chlorophyll. The most chlorophyll *a* was from plants raised from seed treated with 30 mg L^{-1} SA followed by plants from seeds treated with 15 mg L^{-1} SA and salt. The most chlorophyll *b* was in plants from seed treated with 15 mg L^{-1} SA but not treated with salt; 30 mg L^{-1} SA had higher values when seed were treated with salt (Fig. 3a, b). The most total chlorophyll was in plants from seed treated with 30 mg L^{-1} SA and salt (Fig. 3c).

Priming seed with 15 and 30 mg L^{-1} SA produced seed with the highest fresh weight. The most seedling fresh biomass, at five days after treatment, was in plants raised from seeds treated with 30 mg L^{-1} SA under non-saline environment and 15 mg L^{-1} AsA when treated with salt. Application of 10 dS m^{-1} decreased seedling fresh biomass (Fig. 4a).

Treating seed with 30 mg L^{-1} AsA or 15 mg L^{-1} SA produced the most nitrate reductase in leaves treated with salt. The most nitrate reduction, at five days after sowing, was in seedlings from seeds primed with 15 mg L^{-1} SA followed by plants from seed treated with 30 mg L^{-1} AsA (Fig. 4b).

Protease activity at sowing was higher in treated than untreated seed. The most protease activity was recorded in seeds treated with 15 mg L^{-1} AsA, followed by 15 mg L^{-1} SA treated seed in the presence of salt stress. Protease activity at 5 days after sowing in seedlings did not differ from that in plants from seed treated with AsA or SA (Fig. 4c).

Total soluble proteins, measured at sowing, were higher in treated than untreated seed. More total soluble protein was found in seeds treated with 15 mg L^{-1} SA than in other treatments. The highest seedling total soluble protein was at five days after soaking from plants from seed primed with 30 mg L^{-1} SA followed by plants from seed treated with 15 mg L^{-1} SA. Plants treated with 10 dS m^{-1} had 6% less total soluble protein than plants not treated with salt (Fig. 4d).

Discussion

Reduced plant growth under salt stress is a commonly occurring phenomenon (Iqbal & Ashraf, 2006; Raza *et al.*, 2007; Hosseini & Thengane, 2007). Pumpkin not treated with salt produced the most growth, and growth declined by addition of salt. Under high saline conditions water uptake by plants was reduced due to soil osmotic potential (Jamil *et al.*, 2007). Reduction in plant shoot and root dry matter is due to combined effects of osmotic and Cl^- and Na^+ ions (Hajer *et al.,* 2006). Presowing application of salicylic acid by soaking seed reduced adverse effects of salinity and improved fresh and dry weights of shoots and roots of salinity treated plants. Tari *et al*., (2002), Fariduddin *et al*., (2003), and Ahmet (2007) reported positive responses to exogenously applied salicylic acid in alleviating negative effects of salt in crops. Ascorbic acid also benefitted growth and may be due to the antioxidant activity of ascorbic acid protecting plants from damage due to abiotic stress (Beltagi, 2008).

Thylakoid membranes of the chloroplast become more sensitive under stress and the enveloped is destroyed causing leakage of chloroplast content. It has been reported that NaCl stress increases activity of the chlorophyll degrading enzyme cholorophyllase (Rao & Rao, 1981; Boughalleb *et al.,* 2008), resulting in instability of pigment protein complexes (Singh & Dubey, 1995), and reduction in total chlorophyll content. Hormonal priming of seeds with salicylic acid was effective in reducing negative effects of salt on chlorophyll content in pumpkin leaves. Tari *et al*., (2002), Czerpak *et al*., (2002), Fariduddin *et al*., (2003), and Singh & Usha, (2003) reported that exogenous supply of salicylic acid in low concentration enhanced the photosynthetic rate by improving leaf chl *a, b,* and carotenoids. Salicylic acid regulates physiological and biochemical processes in plants and can be used as a potential growth regulator to improve plant growth under saline conditions. Ascorbic acid also beneficially influenced damage reduction caused by salt. This may be due to salinity resulting in increased activity of reactive oxygen species (ROS) which may cause severe cellular damage. One proposed biochemical mode of ascorbate is to act as an antioxidant scavenging hydrogen peroxide (chloroplasts lack catalase) (Miyake & Asada, 1992; Beltagi, 2008).

An increasing trend in pumpkin seedlings protease activity due to salinity was observed in the present study. The enhanced protease activity in plants growing under saline environment has also been reported in some other species such as, *Bruguiera parviflora* (Parida *et al.,* 2004) and *Anabaena subcylindrica* (El-Naggar *et al.,* 2005). Pre-sowing seed soaking with each of 15 mg L^{-1}. AsA and SA further enhanced the protease activity within 24 hours of seed treatment, while, there was no significant improvement after 5days.

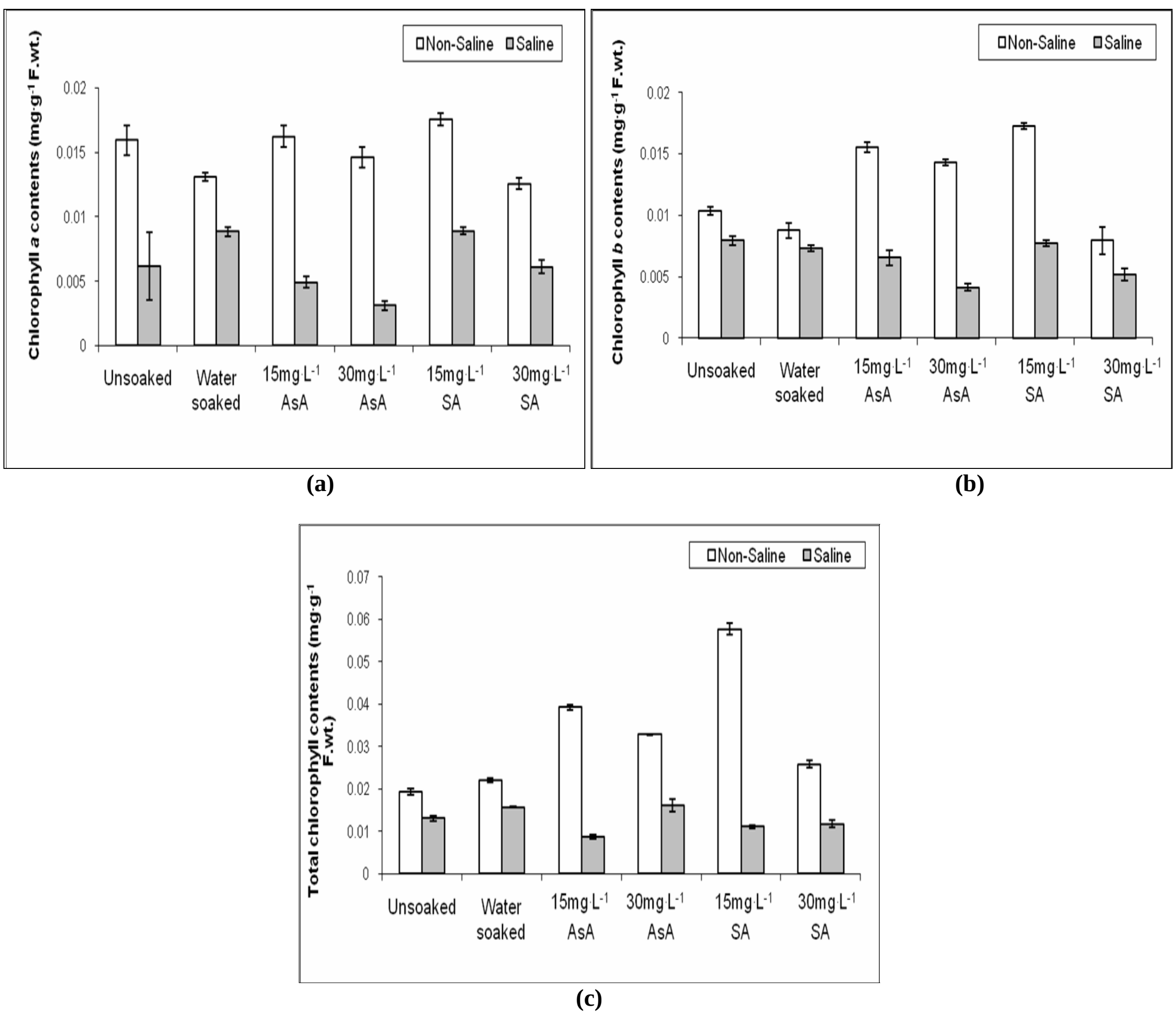

Fig. 3. Influence of pre-soaking seed with ascorbic acid and salicylic acid on a) chl '*a*' b) chl '*b*' c) total chlorophyll content of Pumpkin under normal and saline environment.

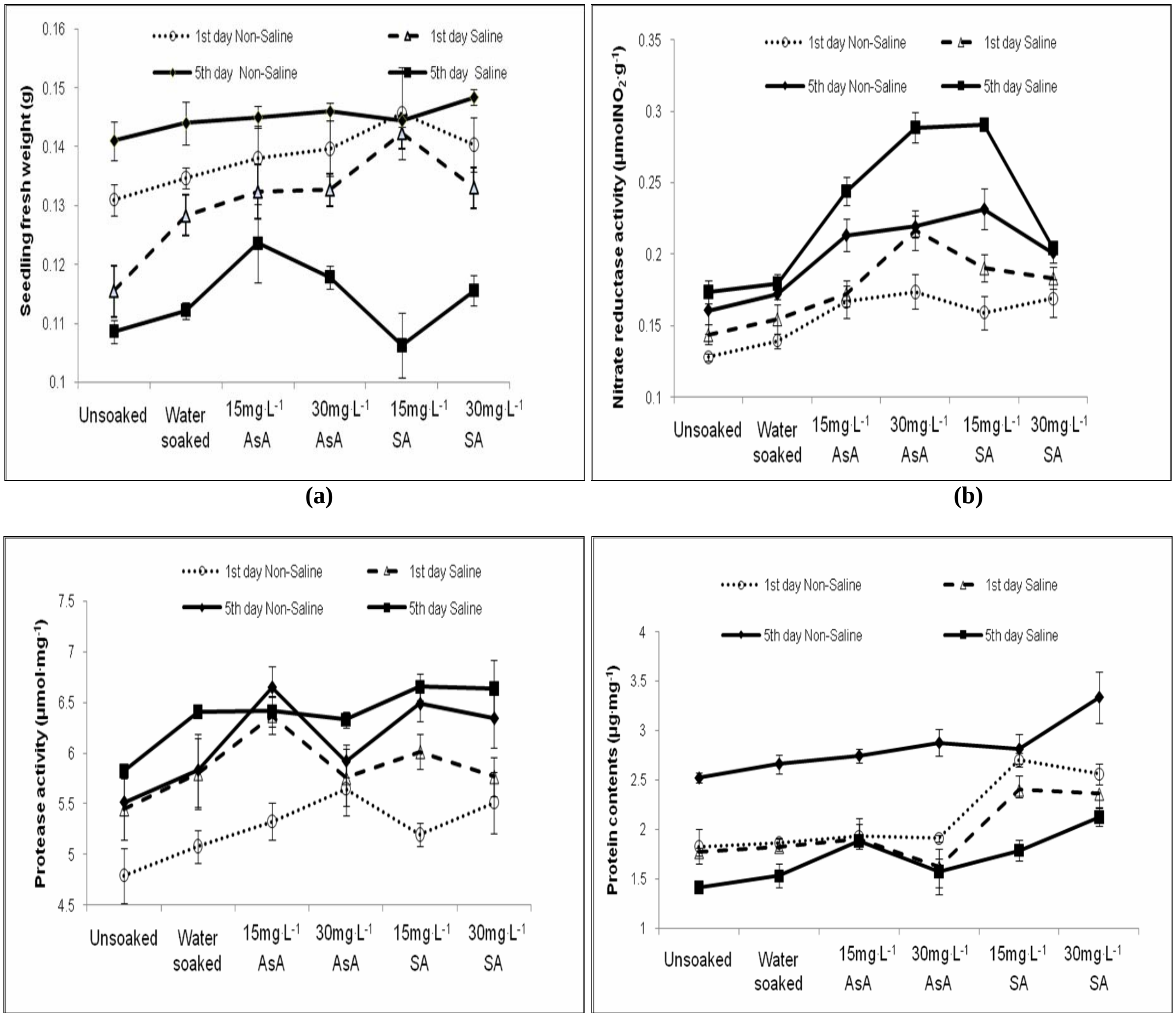

Fig. 4. Influence of pre-soaking seed with ascorbic acid and salicylic acid on a) fresh weight b) nitrate reductase activity c) protease activity d) protein content of Pumpkin seedling 24 hours after soaking under normal and saline environment.

Exposure of plants to salt levels of 10 dS m^{-1} resulted in a slight reduction in nitrate reductase activity. This may be due to steps in N metabolism being disturbed in the presence of salt, which is responsible for declines in plant growth rate (Meloni *et al.,* 2004). Priming seed with salicylic acid reduced adverse effects of salt and increased activity of nitrate reductase in pumpkin seed. Negative effects of salinity on pumpkin growth and biochemical responses can be reduced by use of plant growth regulators and hormonal priming with 30 mg L^{-1} salicylic acid or 30 mg L^{-1} ascorbic acid.

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