Ameliorative Effect of Proline and Ascorbic Acid on Seed Germination and Vigour Parameters of Tomato (\textit{Solanum lycopersicum} L.) Under Salt Stress

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A B S T R A C T

In this work, the influence of proline and ascorbic acid on physiological parameters in seeds subjected to different levels of salt stress was studied in tomato. The salinity stress declined seed germination and seed vigour parameters. Different treatments (hydration, different concentrations of proline 5mM, 10mM and ascorbic acid 1mM, 4mM) were given to seeds at 25mM, 50mM and 75mM NaCl concentrations. Seed germination was declined with the increasing level of salt stress. All the seed treatments increased the physiological parameters (percent germination, seedling length, seedling biomass, speed of germination, vigour index I and II) of seedlings as compared to control at different salinity levels. The results showed that proline 10mM and ascorbic acid 4mM were more effective than proline 5mM and ascorbic acid 1mM respectively.

Keywords
- Tomato
- Salt stress
- Seed treatments
- Proline
- Ascorbic acid
- Germination rate

Introduction

Tomato (\textit{Solanum lycopersicum} L.) is a major crop from family Solanaceae and is rich in minerals, vitamins, essential amino acids, sugars and dietary fibres that contribute to healthy and balanced diet (Palop \textit{et al.}, 2010).

Plants are exposed to several abiotic stresses during its growth and development. Salt-stress is one of the most prime hindrances in salt affected area of the world for crop production. At present, nearly 6.5% of whole area of the world and around 20% of the cultured land is affected by salinity (Billah \textit{et al.}, 2017). High levels of salt in soil causes imbalance in osmotic potential, ionic equilibrium and nutrient uptake (Nawaz \textit{et al.}, 2010).

Salinity stress reduce plant yield by affecting physiology as well as biochemistry of plant (Hemalatha \textit{et al.}, 2017). Excess amount of salt adversely affects plant growth and development and decreases yield and crop productivity (Manaa \textit{et al.}, 2011). In tomato, salinity affects adversely different growth stages (Zhang \textit{et al.}, 2017) and seed germination (Singh \textit{et al.}, 2012). Salinity reduces fresh weight and dry weight of seedlings (Mansour and Ali 2017).

Proline is proteinogenic amino acid which has a unique rigidity by conformation and is necessary for primary metabolism. Proline accumulates in response to drought and
salinity (Nahar et al., 2016). It has been reported that proline accumulation provides resistance to salinity stress (Nazarbeygi et al., 2011). Pre-sowing application of proline enhances germination parameters under abiotic stress conditions in radish (Shruti et al., 2015). Positive effects of seed treatment were reported for Tomato (Shalata and Neumann 2001).

Ascorbic acid (AsA) is one of the most abundant antioxidants found in plants. It is water-soluble and anti-oxidant molecule that acts as a primary substrate for detoxification of hydrogen peroxide (Akram et al., 2017). Ascorbic acid decreases adverse effects of salt stress on plant growth. Ascorbic acid play major role in stress by regulating complex sequence of biochemical reactions, induction of stress responsive protein synthesis, and the producing of various chemical defense compounds (Khan et al., 2011).

Seed treatment of ascorbic acid (vitamin C) in tomato increase resistance to salinity probably by decreasing the synthesis of active oxygen species (Sayed et al., 2016). Ascorbic acid is reported to improve the salinity tolerance in potato (Sajid and Aftab. 2009). Positive effects of ascorbic acid on growth are reported in wheat (Rafique et al., 2011).

Materials and Methods

Seeds of tomato genotypes (PVB-4 and Roma) were surface sterilized by 0.1 % solution of mercuric chloride followed by thorough washing by distilled water. Salinity stress was imposed by moistening the germination papers in petri dishes with solutions of different salinity concentrations of NaCl (Control, 25mM, 50mM, 75mM and 100mM NaCl). The petri dishes were placed in an incubator at 25°C and 60±15 % relative humidity for 14 days to record the effect of salinity on germination parameters. Three salinity levels were selected. The seeds were pre-treated for 2 hrs with different concentrations of proline (5mM amd 10mM) and ascorbic acid (1mM and 4mM). These treated seeds were subjected to salinity stress.

After fourteenth day, the seedlings were evaluated and the normal seedlings were counted and expressed in percentage (ISTA, 2011). At the time of germination count, five normal seedlings were selected at random from each replication and used for measuring seedling length. The values were expressed in centimeter. For calculating seedling dry weight, seedlings were dried in oven at 110˚C for 17 hours and expressed in milligrams. Vigour index of seeds were calculated as suggested by Abdul Baki’s Anderson (1973).

Vigour Index I = Germination (%) x Seedling length (cm)

Vigour Index II = Germination (%) x Seedling dry weight (g)

Statistical analysis

The statistical analysis was carried out by using SPSS-16.

Results and Discussion

Seed germination is an important stage that determines the crop production. Percent germination declines with increasing salinity levels. In present study, both the concentrations of proline and ascorbic acid increased the percent germination over control in PVB-4 and Roma at all salinity levels. But the affect of Proline 10mM and ascorbic acid 4mM was more as compared to proline 5mM and ascorbic acid 1mM respectively as showed in figure(1). Germination percentage declined with the increase in salinity levels (Hemalatha et al., 2017). Proline has an important role in increasing germination and it also increases resistance to various stresses (Kaur et al.,
2015). According to Talat (2013) foliar spray of proline promotes the salt tolerance and germination percentage, growth and chlorophyll contents in wheat. Pretreatment with ascorbic acid enhanced the germination in *Silybum marianum* (Ekmecki and Karaman 2012).

Similarly, it has been studied that seedling length was maximum at lower salinity level (25mM) and gradually decreased with higher salinity level in both the genotypes. It has been observed that pretreatment of proline as well as ascorbic acid enhanced seedling length at all salinity levels. As depicted in figure (2), the affect of ascorbic acid 4mM was maximum at all salinity levels in PVB-4 and Roma. Tomatoes inhabiting in saline environments lead to restricted root growth (Zhang *et al.*, 2016). Farooq *et al.*, (2011) reported that presoaking of seeds with different salts improved the seedling emergence, shoot and root length.

**Fig.1** Influence of seed treatments on percent germination of tomato (*Solanum lycopersicum* L.) under salt stress (a) Punjab Varkha Bahar-4 (b) Roma

![Graph showing the influence of seed treatments on percent germination of tomato under salt stress.](image-url)
Fig. 2 Influence of seed treatments on seedling length (cm) of tomato (*Solanum lycopersicum* L.) under salt stress (a) PVB-4 (b) Roma

(a)

![Graph showing seedling length under salt stress for PVB-4 with different treatments and NaCl concentrations](image)

(b)

![Graph showing seedling length under salt stress for Roma with different treatments and NaCl concentrations](image)
Fig. 3 Influence of seed treatments on fresh weight (mg) of tomato (*Solanum lycopersicum* L.) under salt stress (a) PVB-4 (b) Roma
**Fig. 4** Influence of seed treatments on dry weight (mg) of tomato (*Solanum lycopersicum* L.) under salt stress (a) PVB-4 (b) Roma

(a) PVB-4

(b) Roma
Fig. 5 Influence of seed treatments on seed vigour I of tomato (*Solanum lycopersicum* L.) under salt stress (a) PVB-4 (b) Roma
Fig. 6 Influence of seed treatments on seed vigour II of tomato (*Solanum lycopersicum* L.) under salt stress (a) PVB-4 (b) Roma
In the same way, seedling vigour and seedling biomass showed inverse relationship with salt concentrations. Both the concentrations of proline and ascorbic acid showed increased seedling fresh and dry weight. Proline 10mM and ascorbic acid 4mM showed prominent affect in seedling biomass during salt stress, as depicted in figure (3) and (4). Ismail (2014) reported that exogenously applied proline enhanced fresh and dry weight in wheat under salt stress. According to Clausen (2014), proline helps in increasing fresh weight in tomato under abiotic stress conditions.

Seed vigour is the sum total of those properties of seed which determine the level of activity and performance of seed during germination and seedling emergence. Vigour index declines with increasing salinity levels.

In present study, both the concentrations of proline and ascorbic acid increased the vigour index I and II over control in PVB-4 and Roma at all salinity levels. But the affect of Proline 10mM and ascorbic acid 4mM was more as compared to proline 5mM and ascorbic acid 1mM respectively as showed in figure (5) and (6).

Vigour index of seedling in maize was also significantly affected under different salt stresses (Janmohammadi et al., 2008). Chemicals like proline and ascorbic acid accelerates the metabolism occurring in treated seeds that leads to increase vigour index in sorghum seeds (Guan et al., 2014).

Hence, pre-seed treatments with ascorbic acid (4mM) and proline (10mM) can be used to mitigate the adverse effect of salt stress in tomato seeds.

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