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In vitro assessment of tomato (*Lycopersicon esculentum*) *and Cauliflower* (*Brassica oleracea*) seedlings growth and proline production under salt stress

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Abstract

Tomato and Cauliflower seedlings were grown *in-vitro* under salt concentrations (0, 2, 4, 8 and 10 dSm^{-1}) with objectives to investigate; (1) The effect of salinity on seedling growth and free proline production, (2) the correlation between seedling growth and proline contents, (3) comparative salt tolerance of both species. Different concentrations of salt showed considerable effect on percent (%) germination of seeds, length and biomass of shoot and root and also showed effect on percent water content of both plants seedlings. Germination rate in cauliflower was two times higher than tomato even at highest salt concentration (10 dSm^{-1}). Seedling growth of both species was less effected at low salt concentrations (2 and 4 dSm^{-1}) but at high concentrations (6 and 8 dSm^{-1}) the seedling growth of both species was significantly decreased. Particularly the tomato root was highly significantly reduced. The proline level linearly increased in both species with increasing salt concentrations up-to 4 dSm^{-1} and then declined. The cauliflower showed higher free proline level than tomato under all salt treatments. Overall, the cauliflower seedlings showed better growth response along with higher proline contents on comparison with tomato seedlings.

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Abbreviation: NaCl (Sodium Chloride), EC (Electrical Conductivity), MS (Murashig and Skoog), ANOVA (Analysis of Variance) LSD (Least Significant Differences).

Introduction

Salinity is a global problem for agriculture productivity. Soils having electrical conductivity (EC) more than or equal to 4 ds/m (40 mM NaCl) is considered as saline soils (Munns and Tester, 2008). Plants are sessile organisms and are directly exposed to environmental stresses such as salinity, drought, and low temperature. Salinity is a common consequence of irrigation in many parts of the world, especially in arid and semi-arid regions, particularly in areas where rainfall is insufficient to leach salts from root zone. Salinity is a significant factor in reducing crop productivity. Great effort has been devoted to overcome the deleterious effects of salinity on plants. Under salt stress, the plant increases the external osmotic pressure and as soon it increases, the shoot growth rate and new buds emergence significantly declines as well as the shoot dry weight is reduced (Munns and Termaat, 1986; Dadkhah, 2011). The shoot weight decrease in plant is an essential indicator of the salt tolerance (Jamil *et al.*, 1986).

Salinity is among the most common abiotic factors and about 20% of the agricultural lands are affected by increase in the concentration of salt (especially sodium salts), which leads to a reduction in the germination and growth rate, alteration in biochemical processes in plant metabolism (Martinez-Penalver *et al.*, 2012). Salt stress causes further osmotic and oxidative stress (Verslues *et al.*, 2006).

In plants a variety of protection mechanisms have developed to resist adverse environmental conditions, some of these mechanisms are the production of compatible metabolites (osmo-regulators) such as proline, the synthesis of stress proteins to protect the cell against oxidative and osmotic stresses, or decontamination procedures for exclusion and compartmentalization of sodium ions in the vacuoles (Yeo, 1998). The understanding of toxicity and plant responses to salts stress seems essential and various molecular and biochemical mechanisms in plants are involved to tolerate salt stress. Several amino acids, mainly proline accumulation under salt stress have been reported in plant tissues (Kemble *et al.*, 1954). Proline an amino acid is considered to be an essential compatible solute and a part of the adaptation mechanism to several stresses in plants (Ashton and Desh, 1993). The increased level of free Proline under stress was reported in rye grass (Kemble *et al.*, 1954) and then in many plants exposed to various stress conditions (Barnett and Naylor, 1966; Stewart and Lee, 1974). Proline is synthesized from glutamate and proline has been found to serve as a substrate for respiration (Britikov *et al.*, 1965) and as a source of nitrogen and other metabolites (Stewart and Lee, 1974). The accumulation of free proline may also contribute to the scavenging of abiotic stress induced active oxygen species by enhancing photochemical electron transport activities (Alia *et al.*, 1991).

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop in the world after potato. It is a valuable source of health promoting compounds such as the antioxidant lycopene which is a known anticancer agent and is used as functional food for cancer prevention. The cauliflower an important and low-calorie vegetable, is a rich source of vitamin C, beta carotene and flavonoids which give the cauliflower antioxidant proprieties and a potentially cancer preventative vegetable (Christopher, 1994). Both of tomato and cauliflower are important vegetables and a rich source of anticancer agents.

The present study was conducted to assess the comparative salt tolerance level of tomato and cauliflower seedlings based on germination, seedling growth (*in-vitro*) and free proline contents.

Materials and methods

Seeds of both tomato (*Lycopersicon esculentum*) and cauliflower (*Brassica oleracea*) were surface sterilized by immersing it in 70 % Ethanol (C_2H_5OH) (Sigma Aldrich) for five minutes and then properly washed with sterilized distilled water and placed in sterilized (autoclaved) Petri plates with the help of sterilized forceps in laminar air flow. Three replicate Petri plats were used for each treatment and control

and ten seeds were inoculated into each Petri plate containing filter-papers and cotton autoclaved prior to seed inoculation. For each plant total of fifteen petriplates were used and both plants were assessed in similar way.

Salt (NaCl) solution of different concentrations for treatments were prepared with addition of Murashig and Skoog (Murashige and Skoog, 1962) basal media and denoted as T1 (2 dSm^{-1}), T2 (4 dSm^{-1}), T3 (6 dSm^{-1}), T4 (8 dSm^{-1}) and T5 (10 dSm^{-1}). Control (C) was treated only with distilled water added with MS media. Six ml of each salt solution (treatments) and distilled water (control) was added to each Petri plate immediately after seed inoculation. Petri Plates were then placed for one week in growth cabinet (21 ^{o}C, dark, and 53% relative humidity) for germination. In order to prevent dehydration effect each day 1ml of sterilized distilled water was added to each Petri Plate along with MS media as a source of nutrients by using micropipette.

One week after seed inoculation, the percent (%) germination was recorded and five healthy seedlings were selected for growth (at 21 ^{o}C, 15 h photoperiod 210 µmol m^{-1} s^{-1} light intensity and 51 % relative humidity). After two weeks time the seedlings were removed from petriplates and fresh biomass was measured promptly.

Then shoot and root length was measured of each seedling with centimeter rule (Model: DK-436, Danking Enterprise Ltd., Taiwan) from joint to apices. The replicate seedlings (15 seedlings for each treatment and control) were divided in two groups. Seven replicate seedlings were dried in oven at 70 ^{o}C for 24 h and dry biomass was recorded. Other seven replicate seedlings were used for the proline analysis. Proline analysis was carried out using previously published method (Bates *et al.*, 1973) and proline contents were measured at 520 nm by spectrophotometer using standard curve. Data was subjected to analysis of variance (ANOVA) using Minitab 15 statistical software and mean values were compared by LSD for the significance of values. Microsoft Excel was used for correlation among parameters.

Results and discussion

Effect on Seed Germination

Seed germination data of tomato and cauliflower is presented in (Fig 1a and Fig 2a) respectively. All treatment significantly decreased germination rate except for cauliflower at T1 where the response was similar to control. Increased salt concentration linearly decreased germination and minimum germination was observed at highest concentration of salt for both plants. For both plants the highest germination was noted in control while the lowest was observed in T5 where salt concentration was highest (Fig 1a and Fig 2a). It shows that the rate of seed germination is decreased by increasing salt concentration. Germination stage is one of the most important phases in life cycle of plants and which is highly responsive to stress environment, such as salt, temperature, water, and drought (Al-Taisan, 2010; Brini *et al.*, 2009; Dash and Panda, 2001). Present study shows that germination percentage of tomato and cauliflowers was inhibited strongly particularly at highest levels of salt concentration. Similar results were observed in other plant species (Al-Taisan, 2010; Dkhil and Denden, 2010; Jamil *et al.,* 2005; 2006; 2007). It is assumed that extreme salinity might be toxic to the embryo of seed and then the germination is inhibited severely.

Effect of Salinity on Tomato and Cauliflower Shoots Length (cm)

Addition of saline water reduced the seedling growth of tomato and cauliflower as shown respectively in (Fig 1b, Fig 2b and Fig 2c). Seedling shoot length decreased significantly with increasing salt concentration (Fig 1b and Fig 2b). The highest shoot length was found in control while lowest shoot length was observed in T4 (8 dSm^{-1}) and T5 (10 dSm^{-1}) for both of tomato (Fig 1b) and Cauliflower (Fig 2b) plants. Present findings demonstrated that seedling shoot length of tomato and cauliflower during germination stage were inhibited severely especially at highest levels of salinity. Many scientists have

obtained similar results (Jamil *et al.*, 2005; 2006; 2007). Inhibition of seedling growth by salinity might be due to the inhibitory effect of ions (Brini *et al.*, 2009; Jamil *et al.*, 2007) or water absorption (Wener and Finkelstein, 1995). This might indicate that higher salt stress is not useful for seedling growth of tomato and cauliflower.

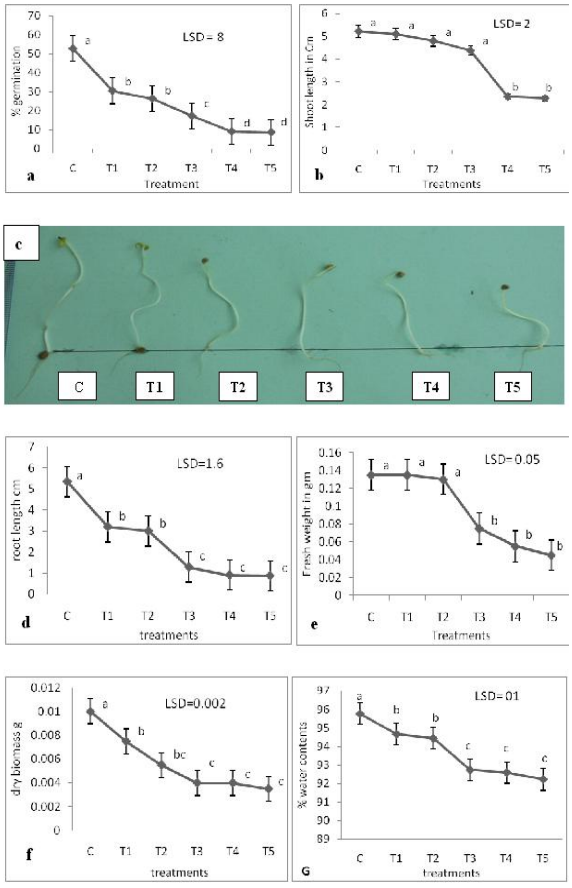

Fig. 1 (a-g). Effect of different treatment on (a) % germination, (b) shoot length, (c) seedling growth comparison, (d) root length, (e) fresh biomass, (f) dry biomass, (g) %water content of tomato. C (0 dSm^{-1}), T1 (2 dSm^{-1}), T2 (4 dSm^{-1}), T3 (6 dSm^{-1}), T4 (8 dSm^{-1}) and T5 (10 dSm^{-1}).

Effect of Salinity on Tomato and Cauliflower Root Length (cm)

The data on root length shows significant reduction on root length with increasing level of salt concentration as shown in (Fig 1d and Fig 2d) for tomato and cauliflower respectively. The highest root length was observed in control plant (without salt) while lowest was found in T4 (8 dSm^{-1}) and T5 (10 dSm^{-1}) as shown in (Fig 1d and Fig 2d). It shows that with increasing salt concentration, root length decreased. Seedling root length is an important parameter for salt stress because roots are mostly in direct contact with soil, and absorb solution from soil (Jamil *et al.*, 2006; 2007). It was found that seedling root length of tomato and cauliflower during germination stage were inhibited severely especially at highest levels of salinity. Similar results have been reported for other plants (Jamil *et al.*, 2005; 2006; 2007). Salt stress induces reactive oxygen species (ROS) production and leads to oxidative damages. These toxic oxygen species may react with macromolecules and lipid components of membranes causing damage through lipid peroxidation resulting in increased permeability of the membrane and shows adverse effect on plant (Singh, 2004).

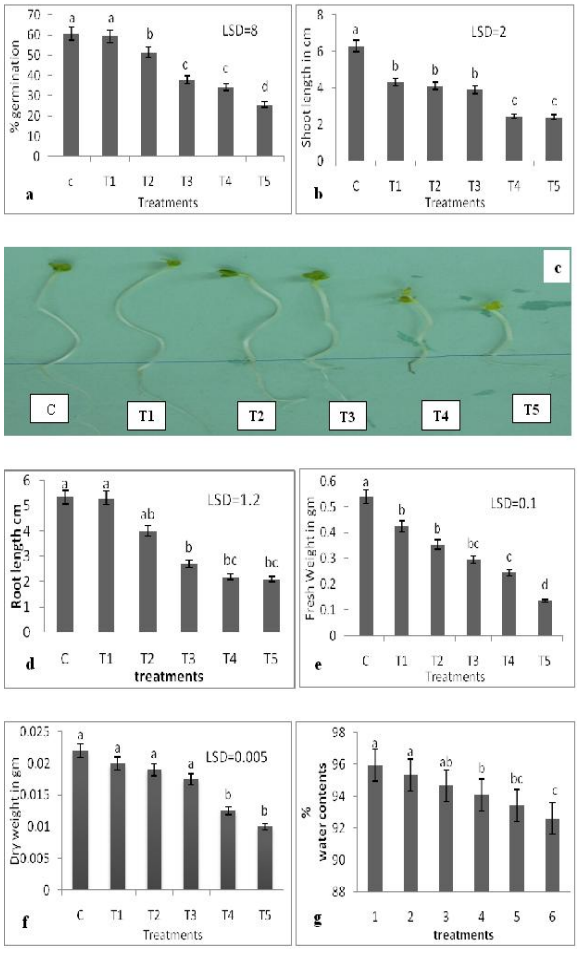

Fig. 2 (a-g). Effect of different treatment on (a) % germination, (b) shoot length, (c) seedling growth comparison, (d) root length, (e) fresh biomass, (f) dry biomass, (g) %water content of cauliflower. C (o dSm^{-1}), T1 (2 dSm^{-1}), T2 (4 dSm^{-1}), T3 (6 dSm^{-1}), T4 (8 dSm^{-1}) and T5 (10 dSm^{-1}).

Effect of Salt on Fresh and Dry Biomass of Tomato and Cauliflower

Salt concentrations significantly reduced fresh biomass of tomato (Fig 1e) and cauliflower (Fig 2e) seedlings. The maximum fresh biomass was found in control (without salt) while the lowest was found in T5 (highest salt concentration) for tomato and cauliflower as shown in (Fig 1e and Fig 2e) respectively. Fresh biomasses of both species were highly reduced by all treatments. The results in this investigation are similar to the findings of (Shannon and Grieve, 1999). Sodium chloride significantly reduces fresh biomass of plant (Saleem *et al.*, 2012). Dry biomass of both plants significantly decreased with increasing concentration of salt as shown in (Fig 1f and Fig 2f). The highest dry biomass was observed in control plant while minimum was noted in T5 for both plants (Fig 1f and Fig 2f). All treatments reduced the dry biomass of tomato and cauliflower species. This might be due to the toxic effects of Na^+ and Cl^- ions and the findings of (Shannon and Grieve, 1999) also support the present results.

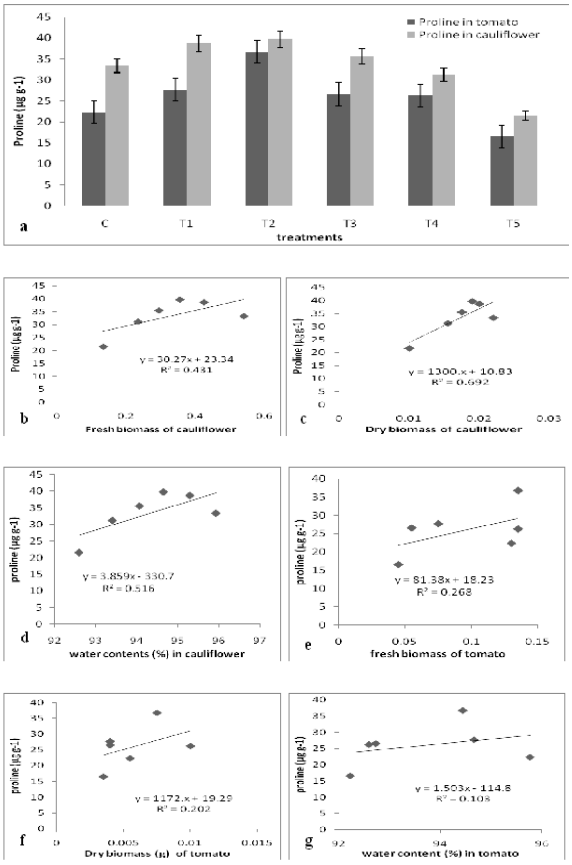

Fig. 3 (a-g). Proline content in cauiflower and tomato under different treatments (a). Correlation between proline and fresh biomass of cauliflower (b), prolin and dry biomass of Cauliflower (c), Proline and percent water content in cauliflower (d), prolin and fresh biomass of tomato (e), proline and dry biomass of tomoato (f), proline and percent water content in tomato (g). C (0 dSm^{-1}), T1 (2 dSm^{-1}), T2 (4 dSm^{-1}), T3 (6 dSm^{-1}), T4 (8 dSm^{-1}) and T5 (10 dSm^{-1}).

Percent (%) Water Content in Tomato and Cauliflower

Water content in seedling of both plants was significantly decreased with increasing concentration of salt shown in (Fig 1g and Fig 2g) respectively for tomato and cauliflower seedlings. There was a negative relationship between water content in different tissues and salt concentration. Maximum water content was found in control plants (without salt) while lowest water content was found in T5 where salt concentration was highest (Fig 1g and Fig 2g). Osmotic potential of tomato and cauliflower seedling might be reduced by salt treatments as already reported by previous researchers (Torrecillas *et al.*, 1994).

Proline and its Correlation with Growth Parameters under Salt Stress

Salinity stress had a significant effect on proline content. The highest proline content was found in T2 (4 dSm-1), while the lowest value was observed in T5 (10 dSm-1). Cauliflower seedling showed comparatively higher amount of free proline than tomato (Fig 3a). Accumulation of free proline in response to different environmental stresses seems to be wide-spread among plants (Banu *et al.,* 2009; Thippeswamy *et al.,* 2010). Proline can protect plants from stress through different mechanisms, including osmotic adjustment, detoxification of ROS, protection of membrane integrity, and stabilization of proteins/enzymes (Ozden *et al.*, 2009). Prolin content was higher in cauliflower than tomato (Fig 3a) and showed positive correlation with growth parameters in both plants species as shown in (Fig 3b to Fig 3g). Conclusively, the concentrations of salt treatments showed considerable effect on germination, early seedling growth, fresh and dry

biomass of tomato and cauliflower. The negative effect of salt on plants was increased with increasing concentration of salt. Overall the cauliflower seedling showed better growth response to salt stress as compared to tomato seedlings. It might be due to high concentration of proline content in cauliflower. The positive correlation between proline accumulation and salt stress suggests that proline could have some protective function in seedlings of cauliflower and tomato.

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