ROLE OF PROLINE TO INDUCE SALINITY TOLERANCE IN SUNFLOWER (*Helianthus annus* L.)

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Abstract

The potted experiment was conducted to determine the exogenous role of proline to induce salinity tolerance in sunflower (*Helianthus annus* L.). Salinity levels (0, 60 and 120 mmol) were created according to the saturation percentage of soil. Different levels (0, 30, 60 mmol) of proline were applied as a foliar spray on sunflower under saline and non saline conditions**.** Application of proline as a foliar spray ameliorated the toxic effects of salinity on growth, physiological and biochemical attributes of sunflower. Among different levels of proline, 60 mmol was found to be the most effective in ameliorating the toxic effects of salinity on sunflower.

Keywords: Proline, Salinity, Sunflower, Physiological and biochemical parameters.

Introduction

Salinity stress is one of the serious threats which affected the plant growth and production all around the world (Majeed et al., 2010; Bahantana and Lazarovitch, 2010). Total area of salt affected soil in the world is nearly 7% (Hussain et al., 2013). In Pakistan, 10 million ha area is affected by salinity, comprising 12.9 percent of country land (Hussain et al., 2013). Salinity is produced when low concentration of K^+ and Ca^{2+}content is present and concentration of NaCl and SO_4^{2-} contents increases (Dejampour et al., 2012). Therefore, in saline soil, sodium (Na^+) is a basic ion which plays a central role in toxicity because it is very rapidly absorbed and taken up by root cells of plants (Hasegawa, 2013). Physiological and biochemical changes as well as the process of growth, photosynthesis, respiration, translocation, ion uptake, nutrient metabolism and growth attributes are also affected by salt stress in plants (Schroeder et al., 2013,).

Salinity causes many adverse effects on plant developmental stages which are due to many factors such as low water potential of soil solution, nutritional imbalance, ions toxicity (salt stress) and hormonal imbalance (Miranda, 2011). Salinity causes osmotic stress and ionic toxicity due to the accumulation of sodium ion which enhanced the formation of reactive oxygen species (ROS), such as, superoxide (O^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($^1O_2$), which damage mitochondria, chloroplasts and cellular structure of the cell (Sharma et al., 2012; Malagoli et al., 2008). Therefore, ROS are scavenged by different antioxidant defense systems which consist of both enzymatic and non-enzymatic nature in plants (Ashraf, 2009). Salinity can also be ameliorated by the application of nitrogen and potassium (Khan et al., 2013). Osmolytes are neutral small molecules which provide protection to proteins and membranes of plant cells against toxic effects when excessive salinity levels are present. Osmoregulation is a defense mechanism which is adopted by plants to eliminate the toxic effects of salt, in which different osmotic regulators are used, such as, potassium, soluble sugar, proline and betaine (Hong-Bo et al., 2006). One of the best mechanisms for salinity tolerance is accumulation of proline into the plant cells. Proline plays a very important role in cell osmotic potential, stability of membrane and detoxification of toxic ions in plants under saline conditions (Ashraf and Foolad, 2007).

Oil seed crops are very valuable for human food and they have acquired third position among different crops, such as, cereals and legumes (Howard and Kinney, 2008). Sunflower (*Helianthus annuus* L.) has a great importance all over the world because of oil seeds but its production is decreasing in different areas where saline toxicity is rapidly increasing (Caterina et al., 2007). Thus the aim of present study was to investigate the role of exogenous application of proline on sunflower to induce the salinity tolerance.

Materials and Methods

The present study was conducted in the Department of Biological Sciences, University of Sargodha to determine the effect of foliar application of proline on sunflower under saline conditions. Different levels (0, 30, 60 mmol) of proline were applied as a foliar spray on sunflower under saline and non saline conditions**.** Different salinity levels (0, 60, 120 mmol) were adjusted according to the saturation percentage of the soil. The seeds of sunflower cultivar were obtained from Ayub Agricultural Research Institute, Faisalabad. Ten seeds were sown in each pot having 20 cm diameter and 24 cm depth containing 8 kg well

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mixed soil. After germination the plants were thinned to maintain six seedlings in each pot. The experiment was laid out in a complete randomised design with three replicates. The data for the following parameters was recorded.

Shoot and root length

Shoot and root length of three randomly selected plants from every pot was measured in cm and their mean was calculated.

Shoot and root fresh weight

Shoot and root of each plant were separated and fresh weight was determined separately with the help of a digital electrical balance.

Shoot and root dry weight

The plants were dried in an oven at 60°C for 24 hours and their dry weight was measured.

Chlorophyll contents

The chlorophyll a and b concentrations were determined by the method of Arnon (1949).

Determination of mineral elements in plant tissues

Sodium (Na^+) and potassium (K^+) in the leaves and roots were determined by the methods described by Allen et al. (1986).

Nitrate reductase activity (NRA)

Nitrate reductase activity was determined by the method as described by Sym (1984).

Protein: Protein content was determined by the method of Lowry et al. (1951).

Total amino acids

Total amino acids were determined as described by Hamilton and Van Slyke (1943).

Total sugars

Total sugars were estimated by the method of Yemm and Willis (1954).

Reducing sugar

Reducing sugar was determined by the method of Shahid and Tabbasum (2007).

Achene yield (g)

The achenes were collected from each treatment of plant and counted and 100 achenes were weighed by an electrical balance in grams and the average was calculated.

Statistical analysis

The data for all the traits was analysed by analysis of variance technique (Snedecor and Cochran, 1980). Differences for various characters were compared, using the least significant differences test at 0.05 level of probability.

Results and Discussion

Analysis of variance of the data of shoot and root length, shoot and root fresh and dry weight indicated that saline growth medium significantly reduced the shoot and root length, shoot and root fresh and dry weight of sunflower (Table 1). Application of proline as a foliar spray mitigated the effect of salinity and enhanced the above mentioned growth attributes of the plants (Table 2), among different concentration of proline, 30 mmol of proline was more effective on shoot length under non- saline conditions and 60 mmol of proline was more effective on shoot length and root length to reduce the effect of salinity (Table 2). Regarding root, shoot fresh and dry weight, 60 mmol of proline was more effective to reduce the effect of salinity and improve the growth of the sunflower plants (Table 2). Analysis variance of chlorophyll a, chlorophyll b and total chlorophyll indicated that salinity reduced the chlorophyll contents (Table 3). Application of proline as foliar spray increased the chlorophyll contents under saline and non-saline conditions. Among different concentration of proline, 60 mmol of proline was more effective to ameliorate the effect of salinity on chlorophyll a, chlorophyll b and total chlorophyll of sunflower under saline conditions (Table 4).

Analysis variance of shoot, root potassium and sodium indicated that salinity reduced the potassium contents in shoot and root of sunflower and increased the sodium contents in shoot and root of sunflower plants (Table 3). Application of proline as foliar spray increased the potassium contents and decreased the sodium contents in shoot, root of sunflower under saline conditions (Table 4).

Analysis of the variance of nitrate reductase activity (Table 5) indicated that salinity applied in root zone of sunflower plants significantly reduced the nitrate reductase of sunflower. Application of proline as a foliar spray significantly increased the nitrate reductase activity of the sunflower under saline and non-saline conditions (Table 6). However, among different concentrations (0 mmol, 30 mmol, 60 mmol) of proline applied as a foliar spray, 60 mmol of proline was more effective under saline conditions to ameliorate the salinity stress in sunflower plants (Table 6).

The same trend of results was also found in protein and total amino acid concentration in the experiment under study (Table 6). Regarding the amount of total sugar, reducing and non-reducing sugar concentration under saline and non-saline conditions, total sugar was observed to be greater under saline conditions by the application of 30 mmol proline as a foliar spray (Table 6), but in case of saline conditions, total sugar was observed to be greater by the application of 60 mmol of proline (Table 6). The same trend of non-reducing was observed under non-saline conditions (Table 6), but in case of saline conditions, 30 mmol of proline was more effective in increasing the non-reducing sugar content (Table 6). In case of reducing sugar, 60 mmol of proline was more effective to increase the reducing sugar under saline conditions (Table 6). Analysis variance of yield of sunflower indicated

that saline growth medium significantly reduced the achene yield of sunflower. Application of proline as a foliar enhanced the achene yield of sunflower under saline conditions (Table 6). Among different concentration of proline, 60 mmol of proline was found to be the most effective to ameliorate the effect of salinity (Table 6).

Table 1. Mean squares from analysis of variance (ANOVA) of the data for shoot length, root length, root fresh weight and shoot dry weight of the sunflower plants under saline condition.

Source	D.F	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g/ plant)	Root fresh weight (g/ plant)	Shoot dry weight (cm)	Root dry weight (cm)
Salinity	2	47.7159**	16.0370***	15.5926**	0.403414**	1.07444**	0.0286844***
Proline	2	1.4959*	6.2593*	4.9259*	0.031642	0.05152*	0.0006253*
Interaction	4	50.1259**	1.4815	3.0370	0.093896*	0.25340*	0.0051055*
Error	18	14.1907	3.2222	3.2963	0.071359	0.20857	0.0042669
Total	26						

Table 2. Influence of foliar application of proline on shoot length, root length, root fresh weight and shoot dry weight of the sunflower plants under saline and non-saline conditions.

Salinity levels (mmol)	0			60			120		
Proline (mmol)	0	30	60	0	30	60	0	30	60
Shoot length (cm)	35±4.3	37±3.3	36±2.8	24±3.1	27±3.3	29±2.6	20±2.9	23±3.1	25±3.4
Root length (cm)	6.0±0.9	5.5±0.4	5.6±0.7	4.5±0.2	5.5±0.3	5.6±0.5	3.5±0.2	4.5±0.3	5.5±0.4
Shoot fresh weight (g/ plant)	6.5±0.5	6.6±0.5	7.5±0.4	5.5±0.5	5.8±0.4	6.2±0.3	4.3±0.2	5.2±0.4	5.4±0.3
Root fresh weight (cm)	1.8±0.04	1.7±0.05	1.6±0.05	1.2±0.03	1.5±0.02	1.4±0.05	1.5±0.03	1.4±0.02	1.5±0.01
Shoot dry weight (cm)	2.3±0.08	2.5±0.09	2.6±0.02	1.8±0.03	2.2±0.04	2.4±0.03	1.2±0.03	2.4±0.02	2.5±0.01
Root dry weight (cm)	0.16±0.01	0.14±0.01	0.15±0.02	0.12±0.02	0.11±0.03	0.13±0.01	0.11±0.01	0.12±0.011	0.13±0.02

Table 3. Mean squares from analysis of variance (ANOVA) of the data for chlorophyll a, chlorophyll b, total chlorophyll, shoot, root potassium and shoot root sodium of the sunflower plants under saline conditions.

Source	D.F	Chlorophyl a (mg/ g f.wt)	Chlorophyl b (mg/ g f.wt)	Total chlorophyl a (mg/ g f.wt)	K^+ in shoot (mg/ g d.wt)	K^+ in root (mg/ g d.wt)	Na^+ in shoot (mg/ g d.wt)	Na^+ in root (mg/ g d.wt)
Salinity	2	0.0012247**	0.019917**	0.020981**	8.9084**	10.7201**	10.8226**	13.6712**
Proline	2	0.0132822*	0.159814*	0.106945*	12.8515*	11.1862*	2.4062*	36.4895*
Interaction	4	0.0151371ns	0.186925*	0.168341ns	2.4848*	5.2150ns	2.4444ns	7.5198ns
Error	18	0.0298406	0.110368	0.139284	4.9232	3.5856	3.8589	6.3022
Total	26							

Table 4. Influence of foliar application of proline on chlorophyll a, chlorophyll b, total chlorophyll, shoot, root potassium and shoot root sodium sugar of the sunflower plants under saline and non-saline conditions.

Salinity levels (mmol)	0			60			120		
Proline (mmol)	0	30	60	0	30	60	0	30	60
Chlorophyl a (mg/ g f.wt)	0.22±0.01	0.24±0.009	0.23±0.07	0.18±0.02	0.19±0.05	0.20±0.07	0.15±0.06	0.17±0.08	0.19±0.05
Chlorophyll b (mg/ g f.wt)	0.50±0.008	0.60±0.07	0.64±0.05	0.45±0.06	0.55±0.07	0.67±0.08	0.23±0.08	0.37±0.06	0.45±0.05
Total chlorophyll (mg/ g f.wt)	0.72±0.009	0.84±0.08	0.87±0.07	0.63±0.07	0.74±0.06	0.87±0.06	0.38±0.08	0.54±0.06	0.64±0.08
K^+ in shoot (mg/ g d.wt)	1.2±0.06	2.5±0.07	3.2±0.05	1.3±0.06	3.5±0.07	4.0±0.05	0.4±0.02	2.2±0.05	2.3±0.04
K^+ in root (mg/ g d.wt)	1.5±0.05	2.2±0.01	2.4±0.06	1.2±0.03	2.2±0.04	2.4±0.03	0.9±0.01	1.3±0.01	1.4±0.02
Na^+ in shoot (mg/ g d.wt)	0.5±0.004	0.4±0.002	0.3±0.002	5.0±0.007	4.5±0.005	5.3±0.004	7.3±0.006	6.5±0.004	5.7±0.006
Na^+ in root (mg/ g d.wt)	1.2±0.003	0.9±0.004	1.0±0.006	4.6±0.004	4.3±0.007	4.4±0.003	6.7±0.005	5.4±0.004	4.5±0.007

Table 5. Mean squares from analysis of variance (ANOVA) of the data for nitrate reductase activity, protein, total amino acid, total sugar, reducing sugar, non-reducing sugar and achene yield of the sunflower plants under saline conditions.

Source	D.F	Nitrate reductase activity	Protein	Total amino acid	Total sugar	Reducing sugar	Non-reducing sugar	Achene yield
Salinity	2	0.007*	1655.92**	36.525**	0.068***	0.111**	0.012**	0.248655**
Proline	2	0.051**	62.17ns	68.694**	0.0115**	0.015*	0.021*	0.093281*
Interaction	4	0.002ns	7.64*	24.239ns	0.011*	0.015ns	0.031*	0.137790*
Error	18	0.009	356.15	25.906	0.014	0.017	0.023	0.102076
Total	26							

Table 6. Influence of foliar application of proline on nitrate reductase activity, protein, total amino acid, total sugar, reducing sugar and non-reducing sugar of the sunflower plants under saline and non-saline conditions.

Salinity levels (mmol)	0			60			120		
Proline (mmol)	0	30	60	0	30	60	0	30	60
Nitrate reductase (µmol NO_2/g/f.wt/h)	7.1±1.2	7.16±1.4	7.25±0.9	7.1±1.2	7.12±1.3	7.22±1.4	5.15±1.2	5.90±0.8	6.30±0.7
Protein (µg/g f.wt)	50±2.3	75±2.4	73±2.4	60±2.3	45±2.5	42±2.6	45±3.1	50±3.2	47±2.5
Total amino acids (µg/g f.wt)	42±1.8	35±1.9	37±1.6	45±1.5	33±1.8	44±1.6	42±1.5	44±1.4	45±1.8
Total sugar (mg/g/d.wt)	3.75±0.6	3.87±0.7	3.65±0.6	3.85±0.8	3.73±0.4	3.70±0.3	3.8±0.6	3.90±0.4	3.87±0.4
Reducing sugar (mg/g/d.wt)	3.50±0.6	3.75±0.4	3.60±0.2	3.65±0.3	3.75±0.4	3.45±0.2	3.70±0.5	3.75±0.1	3.65±0.4
Non reducing sugar (mg/g. d.wt)	0.25±0.01	0.1±0.03	0.5±0.01	0.25±0.01	0.02±0.01	0.25±0.01	0.1±0.01	0.15±0.01	0.22±0.01
Achene yield/ plant (g)	3.4±0.03	4.2±0.04	5.4±0.05	3.5±0.05	4.6±0.06	5.2±0.04	2.1±0.03	4.6±0.03	5.4±0.02

Decreasing the crop losses due to various environmental factors is the major area of interest to meet the increasing demand of food as also reported by Shanker and Venkateswarlu, 2011. Plant used different physiological mechanisms for salinity tolerance (Gupta and Huan, 2014). Soil salinity can be ameliorated by the application of fertilisers, such as, nitrogen and potassium (Khan et al., 2013; Khan and Aziz, 2013) but the plants adopted different strategies to reduce the effect of salinity, such as, accumulation of different types of organic and inorganic osmolytes in the cytosol in response to salt stress which regulates the cellular processes, the same case is reported during current experiment (Chiraz et al., 2012). The production of osmolytes, such as, proline (Pro), is one of the best adaptive mechanisms in plants against salt stress conditions (Ashraf and Foolad, 2007) as it has been reported during current study. It has also been suggested that proline accumulation can serve as a selection criterion for most of the species against salinity tolerance (Ahmad et al., 2009). Application of proline as a foliar spray significantly increased the nitrate reductase activity of sunflower (Khan and Panda, 2008). Hoque et al. (2008) showed that proline improved salt tolerance in *Nicotiana tabacum* plants by increasing the activity of enzymes involved in the antioxidant defense system. Proline plays a vital role in salt tolerance by protecting the protein synthesis against harmful effect of salinity (Shi and Sheng, 2005). Total sugar observed from sunflower also involves in the reduction and alterations of biochemical, physiological, processes such as photosynthetic process and antioxidant defense system (Sundaram and Soumya, 2011). The results of the present study are supported by Nuran and Cakirlar (2002).

Conclusion

It may be concluded that the application of proline as a foliar spray significantly alleviated the adverse effects of salt stress on growth of sunflower by production of sugar in sunflower under saline conditions and maintained ion and water homeostasis, thereby, protecting photosynthetic machinery of sunflower against salt-induced oxidative stress.

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