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Full Length Research Article

Effect of proline stimulation on the growth and development of maize (*Zea mays* L.) seedlings

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Abstract

Proline biosynthesis and its osmoregulation in plants is a key phenomenon to alleviate various environmental stresses. While its role in growth and development of plant is still unclear. An experiment was conducted to determine the effect of proline in rooting medium on growth and development of maize seedlings. Three levels of proline (50, 75, and 100 ppm) were applied exogenously after establishment of seedlings and compared with control (0 ppm). The data for various morphological and biochemical parameters were recorded. Significant effects of proline were observed especially at higher dose i.e. 100 ppm which stimulated the root development and biosynthesis of total chlorophyll and carotenoid contents. Results concluded that proline impart positive effects on overall seedling establishment in maize which can help it to with stand under various biotic and a-biotic stress factors.

Keywords: Proline, Morphological and Biochemical parameters, Seedling Establishment

Introduction

Amino acids are the building blocks of protein which play an essential role in plant growth and development. A comprehensive research is an evidence to justify the positive correlation between plant stress and proline accumulation. Proline is extensively studied molecule in alleviating abiotic stress in plants. It helps the plants to tolerate various abiotic and biotic stresses. Different taxonomical studies suggested that many cereal crops i.e. maize with major portion containing starch are unable to produce sufficient level of proline that is necessary to withstand under stressful conditions (Slama *et al*., 2015). Thus in this situation, when proline is applied exogenously, it will promote production of proline endogenously, that directly contributed to plant stress tolerance (Ashraf and Foolad, 2007). Proline is found as an effective molecule in seed germination, enhance photosynthetic activity and promote lipid peroxidation which ultimately increases the productivity of the plant (Shaddad, 1990; Huang *et al*., 2009; Kahlaoui *et al*., 2013). These entire factors combine together to promote plant establishment under abiotic stress condition.

Other than acting as an effective amino acid during various stresses, proline under normal conditions act as a precursor of enzymes and proteins (Nanjo *et al*., 1999). It induces salt and drought stress tolerance in plants. Exogenous application of proline reduces the reactive oxidative species (ROS) in plants that can be produced in stressed conditions. As it adversely affects the cell membrane by lipid peroxidation due to its lipid bilayer structure. Thus, Proline plays its beneficial role by inducing ROS scavenging activity, and also prevents oxidation of lipids and save the cell from death.

The efficiency of proline depends upon its concentration. So, it is important to determine the optimum concentration of proline that applied exogenously which lead to improvement of plant tolerance to various abiotic stresses. The effectiveness of proline applied on seedling stage depends on the type of species, plant developmental stage, time of application and the concentration to be applied. Keeping in that view the potential of exogenous proline as a ROS scavenger, it may become a useful tool to counter the adverse effect of various biotic and a-biotic stresses. The present study was planned to assess the effectiveness of exogenous proline application on morphological and biochemical attributes of maize.

Materials and Methods

A maize variety “Neelum” was subjected to assess the effectiveness of proline on various growth attributes. A Pot experiment was conducted following randomized complete block design (RCBD) in the research area of Botanical Garden, University of Agriculture, and Faisalabad. Five seeds were sown in each pot, and proline having concentration i.e. 0, 50, 75 and 100 ppm was applied after 10 days of germination. After 30 days, three plants from each pot were subjected to study different growth parameters such as root fresh weight (RFW, g), root dry weight (RDW, g), shoot fresh weight (SFW, g), shoot dry weight (SDW, g), root length (RL, cm), shoot length (SL, cm), leaf weight (LW, g), leaf length (LL, cm), number of leaves (NL), leaf area (LA, cm^2), total chlorophyll contents (TChl, mg/g), chlorophyll A (ChlA, mg\g), chlorophyll B (ChlB, mg/g), Chlorophyll A/B ratio (A/B) and total carotenoids (Caro, mg/g). Leaf area was calculated with the following formula:

$$LA = Max.\,leaf\ length \text{ X } Max.\,leaf\ width \text{ X } C.F$$

The value of C.F. (Correction Factor) for leaf area is 0.68 which is standard in maize. For the determination of leaf chlorophyll contents, 0.1 gram of fresh leaf was put into Eppendorf tubes having acetone (80%). The Eppendorf tubes having leaf samples were then subjected to centrifugation at 10,000 rpm for five minutes and then placed over night at -40°C. Then supernatant was collected from the tubes and used for measuring the absorbance through spectrophotometer at 645, 663, and 480 nm. The formulae used for calculating the chlorophyll A, B, total chlorophyll and carotenoid contents are as following:

$$ChlA = [12.7\ (OD663) - 2.699\ (OD645)] \times \frac{V}{1000} \times \mathrm{W}$$

$$ChlB = [22.9\ (OD645) - 4.68\ (OD663)] \times \frac{V}{1000} \times \mathrm{W}$$

$$TChl = [20.2\ (OD645) + 8.02\ (OD663)] \times \frac{V}{1000} \times \mathrm{W}$$

$$Caro = \frac{[(OD480) + 0.114\ (OD663) - 0.638\ (\mathrm{OD645})]}{2500}$$

Here,
OD480 = Absorbance at 480 nm
OD645 = Absorbance at 645 nm
OD663 = Absorbance at 663 nm
V = Total volume made of acetone used in extract
W = gram weight of leaf tissue used

Results and Discussion

Significance differences of proline application were determined through analysis of variance (Table 1). Results of analysis of variance (ANOVA) presented hereby suggested that the application of proline brought significant changes in the studied parameters except RFW, LW, LL and NL. Differences in the responses by proline treatments as compared to control were determined through percent change analysis (Table 2, Figure 1). From the percent change analysis, it can be seen clearly that the application of proline causes increase in all of the parameters except NL and A/B ratio. Maximum change in most of the studied parameters especially chlorophyll contents and carotenoids contents was produced by 100 ppm of proline concentration. Increase in root length, fresh and dry weight with the application of proline is a positive contribution towards overcoming drought stress. This is why the induced proline concentration in drought tolerant genotypes is higher than that of drought sensitive genotypes. Similar effects of exogenous application of proline were observed by Ali *et al.* (2007) and Hayat *et al.* (2012). Findings of different researchers showed that increased uptake of proline by plants induces abiotic stress tolerance in plants, but it restricts the growth and development when the concentration increases from the optimum level. This showed harmful effects of very high concentration of proline in normal plant development (Nanjo *et al*., 2003; Nicolic *et al.*, 2008). Results also coincide with the findings of Guang *et al.*, (2013). With an increase in leaf area, increase in chlorophyll and carotenoid contents were observed which increases overall photosynthetic rate of the plant and thereby increasing productivity especially in stressed environments as also observed by Ebtihal *et al*. (2016). Due to increase in proline concentration, the level of chlorophyll *a* and *b* increases which indicates good physiological characters of maize seedling.

Principal component analysis (Figure 2) showed that first two principal components contributed most of the variation in studied parameters i.e., 55.2% and 42.7% respectively summing up 97.9% of the total variability. From the PCA biplot, it can be seen that most contribution in LL, LW and LA was from treatment no. 2 (50 ppm) while maximum contribution in ChlA, ChlB and Carotene was observed in the treatment no. 4 (100 ppm) of proline application. Hence it was found that application of proline increased plant responses, and it is suggested that application of proline at the rate of 100 ppm is helpful in efficient plant growth and development and inducing tolerance to various environmental stresses.

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Table 1: Analysis of Variance for studied parameters

Source	**DF**	**SL**	**RL**	**SFW**	**RFW**	**LW**	**LL**	**LA**	**NL**
Treatment	3	47.87**	311.39**	0.36**	0.48NS	0.02NS	28.43NS	136.56**	0.33NS
Error	8	0.62	6.85	0.02	0.15	0.03	8.36	9.85	0.08
Total	11								
Source	**DF**	**RDW**	**SDW**	**ChlA**	**ChlB**	**TChl**	**A/B**	**Caro**	
Treatment	3	0.001**	0.04**	1.65**	2.44**	7.31**	0.27*	1.002**	
Error	8	0.0008	0.0007	0.02	0.01	0.05	0.04	0.009	
Total	11								

Significance at 5% (*) and 1% (**). SL: Shoot length; RL: root length; SFW: shoot fresh weight; RFW: root fresh weight; LW: leaf weight; LL: leaf length; LA: leaf area; NL: number of leaves; RDW: root dry weight; SDW: shoot dry weight; ChlA: chlorophyll A contents; ChlB: chlorophyll B contents TChl: total chlorophyll contents; A/B: chlorophyll A-B ratio; Caro: carotenoid contents

Table 2: Percent change in response to treatments against control

Parameters	**50 ppm**	**75 ppm**	**100 ppm**
SL	22.04	16.35	8.33
RL	15.56	102.02	101.66
SFW	40.69	26.68	43.95
RFW	28.46	79.77	97.38
LW	14.28	7.14	4.76
LL	20.20	6.06	0.91
LA	35.82	30.79	19.32
NL	-11.76	-11.76	-11.76
RDW	22.22	0	12.96
SDW	23.46	-3.70	86.42
ChlaA	-9.64	153.89	247
ChlB	126.48	193.03	393.33
TChl	54..40	170.33	313.85
A/B	-62.85	-15.56	-31.56
Caro	13.96	68.51	81.02

SL: Shoot length; RL: root length; SFW: shoot fresh weight; RFW: root fresh weight; LW: leaf weight; LL: leaf length; LA: leaf area; NL: number of leaves; RDW: root dry weight; SDW: shoot dry weight; ChlA: chlorophyll A contents; ChlB: chlorophyll B contents TChl: total chlorophyll contents; A/B: chlorophyll A-B ratio; Caro: carotenoid contents

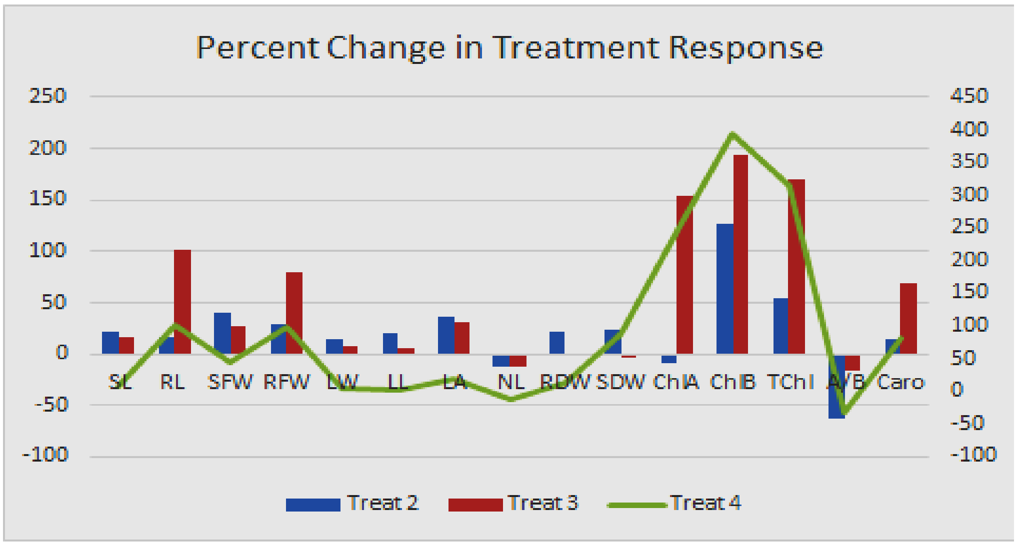

Figure 1: Percent change in studied parameters with proline application (Treat 2: 50ppm, Treat 3: 75ppm, Treat 4: 100 ppm)

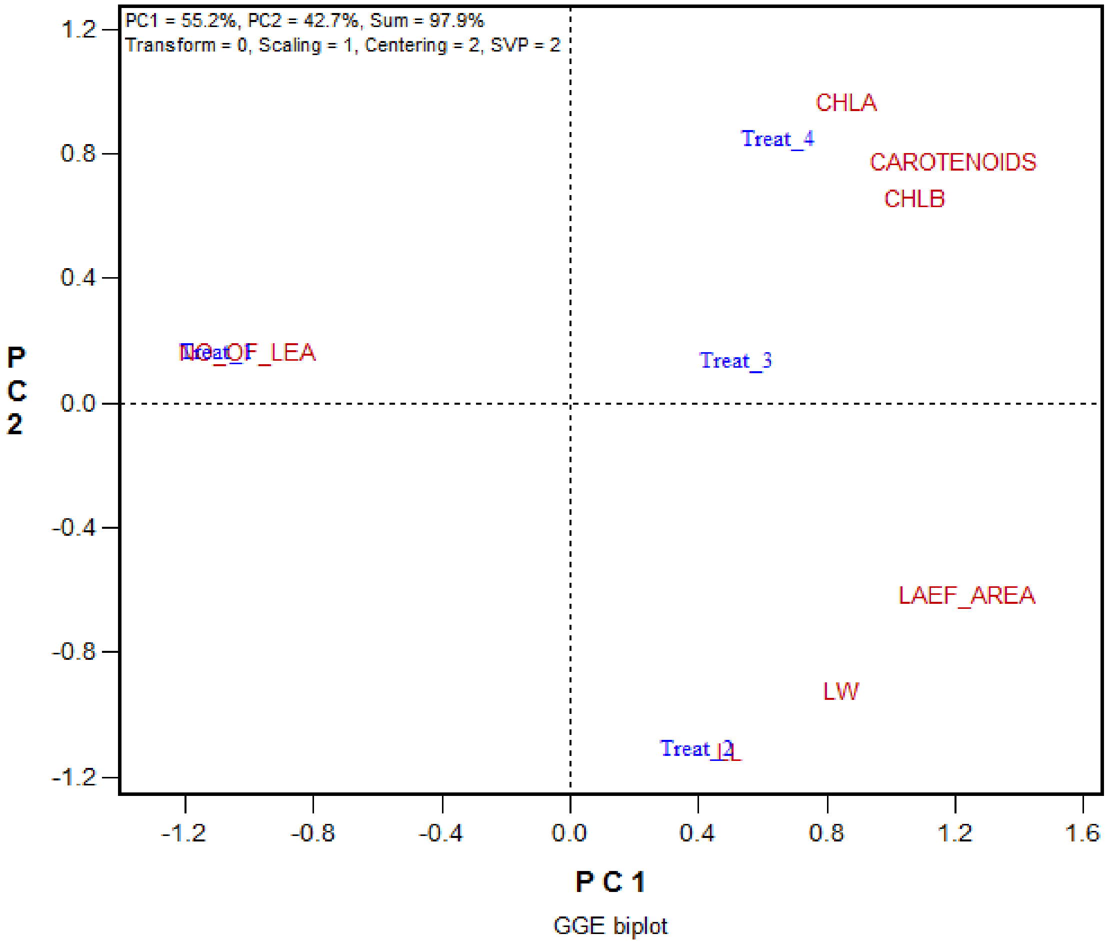

Figure 2: PCA Biplot of studied traits with applied proline treatments