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The Effect of Salt Stress on Proline Content in Maize (*Zea mays*) †

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Abstract: In this study, the effect of applied NaCl on the growth and proline concentration of maize varieties, i.e., (*Zea mays* L.), Syngenta 7720, Syngenta 6668, eco-91, Syngenta 7710 and Advanta Pac 751 vertex 751, was investigated. The experiment was conducted in laboratory conditions using a completely randomized design with three replications. The soil used for the experiment was salinized by applying NaCI (common salt) solution at the rates of 50 mM, 100 mM, 150 mM, 200 mM and 250 mM every three days for the duration of 12 days. We found that, according to the data we have collected on germination percentage and proline content, maize varieties (Syngenta 7720 and eco-91) had a significant increase in proline content and a decrease in plant growth as the concentration increased.

Keywords: maize (*Zea mays* L.); salinity; proline

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1. Introduction

Maize (*Zea mays* L.) plays an important role in human and animal nutrition in many developed and developing countries, [1]. It is a well-fit substitute crop for diversification of the rice-wheat system in Indo-Gangetic Plain (IGP). In India, the maize area has slowly expanded over the past few years, reaching 6.2 million ha (3.4% of the gross cropped area) in 1999/2000. In the country, it is grown under a wide range of climatic conditions, ranging from extreme semi-arid to sub-humid and humid. Traditionally, its growing area was more focused on Bihar, Madhya Pradesh, Rajasthan, and Uttar Pradesh, and to some extent in maize growing areas including Karnataka and Andhra Pradesh. It is also very popular in the low- and mid-hill areas of the western and northeastern regions [2].

Soil salinity is a key challenge in several regions of India, such as Gujarat, Uttar Pradesh, Maharashtra, West Bengal, Rajasthan and the coastal region of Odisha. Salt stress affects the growth and development of maize in several ways. However, the response of plants varies with the degree of stress at different crop growth stages. Short-term exposure of maize plants to salt stress influences plant growth to osmotic stress [3]. Salinity stress affects plant growth and productivity, water relation, photosynthesis and carbon assimilation, grain development, and filling. Salt resistance in some maize varieties is linked with higher potassium, low sodium and chloride fluxes, and cytoplasmic contents [4]. Photosynthesis is the most important process by which green plants convert solar energy into chemical energy in the form of organic compounds synthesized by the fixation of atmospheric carbon dioxide. Carbon fixation in maize is sensitive to salt stress [5]. Both stomatal and non-stomatal limitations and their combination are associated with salinity-induced reductions in maize photosynthesis [6]. Reduced stomatal conductance, impaired activities of carbon fixation enzymes, reduced photosynthetic pigments, and destruction of photosynthetic apparatus are among the key factors limiting carbon fixation capacity of maize plants under salt stress [5,6].

Proline is the most common endogenous osmolyte accumulated under various abiotic stresses including salinity [7,8]. When applied as an exogenous compound to crops, proline can improve salt tolerance [9]. For example, in salt-stressed maize, the foliar application of proline increased plant growth with a positive effect on yield characteristics [10]. The beneficial effects of exogenous proline application on salt stress tolerance have been the subject of several factors including crop growth stage and Na+/K+ ratio [11]. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious factors in plants exposed to salt stress has been widely reported [12,13]. Thus, the objective of this study was to see the effect of salinity on proline.

2. Materials and Methods

The seeds of five maize varieties (Syngenta 7720, Syngenta 6668, eco-seeds eco-91, Syngenta 7710, Advanta Pac 751 vertex 751) were sown in a germination tray on coco peat. Six levels of salinity solutions, viz., 0, 50, 100, 150, 200 and 250 mM NaCl were used to evaluate the resistance of maize varieties. The seeds were treated with 5 mL of NaCl solution every 3 days for a total 12 days. Proline accumulation was determined by using the method described by Bates et al. [14]. A 0.2 g fresh plant sample of leaves was homogenized with 3% sulfosalicylic acid. The homogenate was centrifuged at 3000 RPM for 20 min. The supernatant was treated with 200 µL acetic acid and 200 µL ninhydrin, then boiled for 1 h in a hot water boiling bath. After boiling for 1 h, the solution was kept in ice water. Then, absorbance at 520 nm was determined by UV-Visible using a spectrophotometer. Germination percentage, rate of germination, number of leaves, shoot length, leaf color, days of maturity, root length, number of roots, weight of seed at time of sowing, germination stress tolerance index (GSI), promptness index (PI), root length stress index (RLST), shoot length of stress index (SLSI), and proline content were measured.

3. Results

In this experiment, the effect of salinity on proline content in five maize varieties was determined (Table 1). We found that as the salt concentration decreased, the rate of germination and germination percentage increased, and also, as the concentration level increased, the proline contained in maize also increased. Among the five evaluated varieties, two varieties, i.e., Syngenta 7720 and eco-91 (Figure 1a,b), had higher yields, mostly due to their increased level of proline concentration.

$$\mu\text{moles per gram tissue} = [(\mu\text{g proline/mL}) \times \text{mL toluene})/115.5\ \mu\text{g}/\mu\text{mole}]/[(\text{g sample})/5]. \quad (1)$$

(**a**) Syngenta 7720 (**b**) eco-seeds eco-91

Figure 1. Varieties that have significant increase in proline.

Table 1. (Proline content).

	Proline Content in 520 nm Solution				
NaCl Concentration (mM)	**Advanta Pac 751 Vertex 751**	**Syngenta 7720**	**Eco-91**	**Syngenta 6668**	**Syngenta 7710**
0 mM	0.6720	0.0616	1.4941	0.2799	0.1709
50 mM	0.7527	0.2111	1.9645	0.2345	0.6480
100 mM	0.8096	0.2799	1.3844	0.1064	0.0888
150 mM	1.0901	0.5573	1.5434	0.6576	0.0201
200 mM	0.6152	0.5689	1.6250	0.3982	0.8162
250 mM	0.4762	0.8432	1.7653	0.3039	0.5191

4. Discussion

Proline accumulation in salt-stressed plants is a primary defense response to maintain osmatic pressure in a cell, which is reported in many crops [15]. Proline content increases after an increase in NaCl concentration. We reviewed the effect, resistance mechanisms and management of salt stress in maize. Consistent with our findings, in 2010, Kaya et al. [16] reported that proline experiences salt stress. At 400 mM NaCl, sweetcorn leaves accumulated more than 600 µmol g^{-1} proline. There were significant increases in proline according to Farooq et al. [3]. Foliar application of proline increased plant growth with a positive effect on yield characteristics in maize. Proline improves resistance against salinity through cell osmotic adjustment, membrane stabilization, and detoxification of injured ions in plants exposed to salt stress [13].

5. Conclusions

In this study, the effects of applied NaCl on the growth and proline concentration of maize varieties were investigated. We have concluded that there was a significant increase in growth and proline concentration in maize varieties. With our study, we can help farmers who are suffering from soil salinity. We can use the method of proline accumulation, as described by Bates et al., on different types of crops.

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