

THE EFFECT OF SALINITY STRESS ON GERMINATION CHARACTERISTICS AND CHANGES OF BIOCHEMICALLY OF SESAME SEEDS

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ABSTRACT. Objective of this study was to evaluate the effect of salinity stress on germination characteristics and biochemical changes of sesame seeds. Salinity stress at osmotic potentials of 0 (as control), -3, -6, -9 and -12 bar were adjusted using NaCl before the start of the experiment. Our results showed that, the effect of salinity stress for all traits was significant. By increases of salinity stress, germination percentage, germination, normal seedling percentage, seedling length and dry weight were reduced the ascorbate peroxidase and catalase activity, also proline content were at minimum at control and increased with increase in salinity stress, expressed by the osmotic potential.

Key words: Salinity stress; Germination characteristic; Ascorbate peroxidase; Catalase; Proline.

INTRODUCTION

Sesame (*Sesamum indicum* L.) cultivated in arid and semi-arid regions, is an annual oil seed crop cultivated for centuries, particularly in

the developing countries of Asia and Africa, for its high content of both excellent quality edible oil (42-54%) and protein (22- 25%).

Seed germination is an essential process in plant development to obtain optimal seedling numbers that results in higher seed yield. Germination and seedling growth declined with many abiotic factors such as salt and drought stress that are perhaps two of the most important grounded abiotic stress that limit number of seedling and seedling growth (Ashraf *et al.*, 1992; Almansouri *et al.*, 2001; Kaya *et al.*, 2006, Atak *et al.*, 2006; Ansari *et al.*, 2012; Ansari and Sharif-Zadeh, 2012; Ansari *et al.*, 2013).

Salinity stress delays the onset, reduces the rate and increases the dispersion of germination events, resulting in reduced plant growth and, finally, crop yield (Ashraf and Foolad, 2005). There for salinity has caused to

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significant decrease in germination characteristics in all studied species. The general response of many plants to increased salinity is to accumulate high concentrations of Na⁺ and Cl⁻ ions in their vacuoles or to distribute these ions to different parts of the plant to activate metabolic functions. In this way, the plants protect their cytoplasmic water potential (Munns, 2002). Certain biochemical strategies were used to enhance salt tolerance in plants, including the control of ion transfer from roots to leaves, the distribution of ions into cellular compartments, the synthesis of osmotic regulators, changes in photosynthesis and cell membranes, and the induction of antioxidative enzymes and certain plant hormones (Nakamura *et al.*, 2002). Enzymatic ROS-scavenging mechanisms in plants include production of superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), and glutathione reductase (GR) (Riley *et al.*, 1994; Skopelitis *et al.*, 2006). Stimulating of oxidative stress is the most important pathway applied by salinity for aquatic plants and may be applied for all of plants. Several studies have shown that salt tolerant species increase the activities of their antioxidant enzymes in response to salt treatment. Salinity has caused significant increase in SOD activity in all studied species. One way that plants adjust to high salt concentrations is by increasing their tissue osmotic potential. This results in the accumulation of inorganic and

organic solutes. The cellular response to short- and long-term salt stress by plants involves the synthesis and accumulation of osmoprotective compounds (Banu *et al.*, 2009; Yazici *et al.*, 2007), which are small nontoxic molecules. Osmotic active compounds increase the osmotic potential of the cell (Apse and Blumwald, 2002). Proline is the major compound that protects cells by stabilizing proteins and cellular membranes (Kumar *et al.*, 2003; Martinez *et al.*, 2003; Wang and Han, 2009; Çelik and Atak, 2011).

Therefore, the aim of study was to determine the effect of salinity stress on germination characteristics and biochemical changes of sesame seeds.

MATERIALS AND METHODS

Salinity stress at osmotic potentials of 0 (as control), -3, -6, -9 and -12 bar were adjusted using NaCl before the start of the experiment.

For the germination test, three replicates of 50 seeds were surface sterilized and imbibed on two layers of blotter paper in 9 cm diameter petri dishes at 25°C incubation, under different salinity stress. Germinated seeds were recorded every 24 h for 6 days. After test time expiration, some germination indexes were evaluated such as: germination percentage, germination Index, normal seedling percentage, seedling length and dry weight.

All extraction procedures were carried out at 4°C. The seed samples, weighting about 0.3 g, were homogenized with 3 ml of tris (pH 7.8), followed by centrifugation of 20000 g for 20 min. The

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supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically following H₂O₂ consumption at 240 nm (Chiu *et al.*, 1995). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Johnson and Cunningham (1972). The activities of APX and CAT were expressed per mg protein, and one unit represented 1 µmol of substrate undergoing reaction per mg protein per min.

Proline was measured as described by Bates *et al.* (1973). A quantity of 100 mg of frozen plant material was homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. A volume of 100 µl of the extract was reacted with 2 ml glacial acetic acid and 2 ml acid ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) for 1 h at 100 °C and the reaction was then terminated in an ice bath. The reaction mixture was extracted with 1 ml toluene. The chromophore-containing toluene was

warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20±100 mg.

All data were processed statistically by analysis of variance, using SAS Software. Data for germination and normal germination percentages were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS AND DISCUSSION

Analysis of variance showed that the effect of salinity levels, for all traits, was very light significant ($P < 0.01$) (*Table 1*). In agreement with the results, earlier reports (Patade *et al.*, 2011; Ansari *et al.*, 2012) have shown negative effect under the salinity stress on germination characteristics.

Table 1 - Variance analysis of studied traits in sesame seeds under salinity stress

S.O.V.	df	Germination percentage	Germination index	Normal seedling percentage	Seedling length	Dry weight
Treatment	4	2436.4*	336.26*	4082.26*	49.94*	0.01
Error	10	5.6	2.46	2.66	0.03	0.00003
CV%	-	3.84	8.04	3.14	3.08	6.97

* Indicate significant difference at 1% probability level.

Results showed that the highest germination percentage (*Fig. 1*), germination index (*Fig. 2*), normal seedling percentage (*Fig. 3*), seedling length (*Fig. 4*) and dry weight (*Fig. 5*) were attained from control conditions,

and with increasing of osmotic pressure this traits were reduced.

In agreement with the results, earlier reports (Ashraf and Foolad, 2005; Ashraf *et al.*, 1992; Almansouri *et al.*, 2001, Kaya *et al.*, 2006, Atak *et al.*, 2006; Ansari *et al.*, 2012; Ansari

and Shari-Zadeh, 2012; Ansari *et al.*, 2013) have shown negative effect under the stress conditions on germination percentage, germination index, normal seedling percentage, seedling length and dry weight. Also,

our results in this study suggested that salinity cause diminution in the seed characteristics as compared to the control conditions.

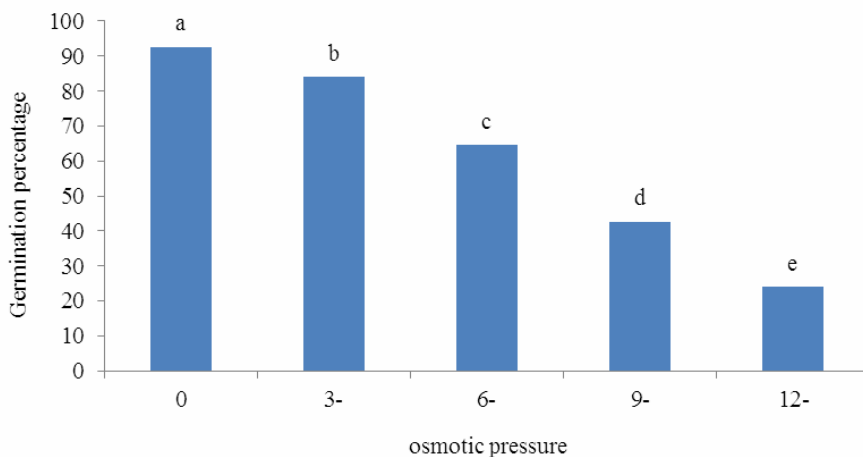


Figure 1 - The effect of salinity stress on germination percentage

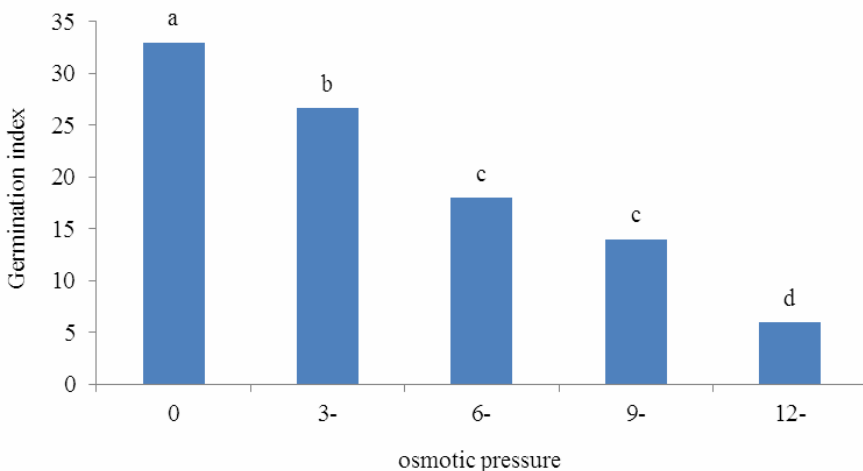


Figure 2 - The effect of salinity stress on germination index

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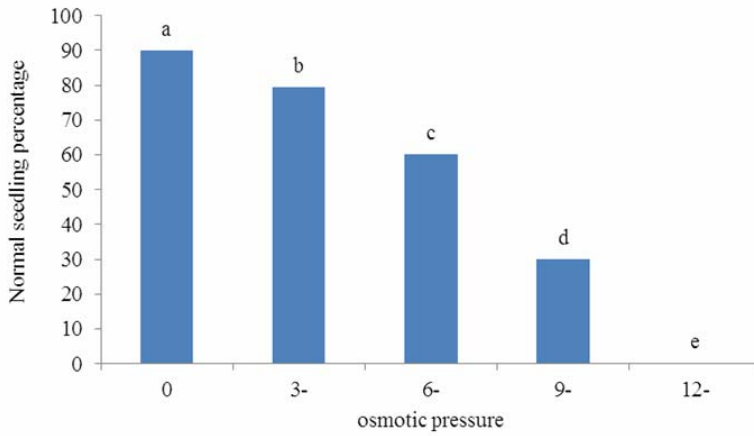


Figure 3 - The effect of salinity stress on normal seedling percentage

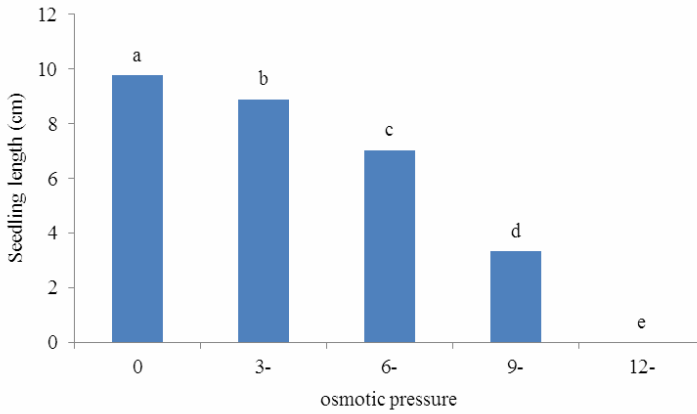


Figure 4 - The effect of salinity stress on seedling length

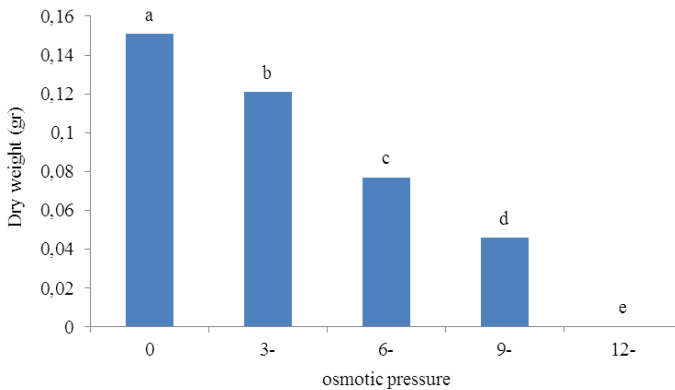


Figure 5 - The effect of salinity stress on dry weight

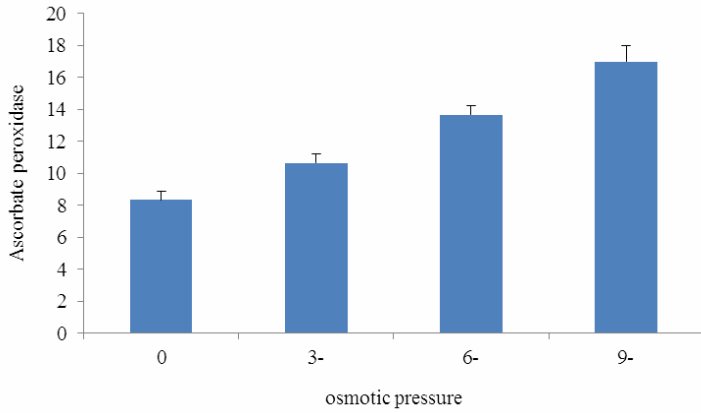


Figure 6 - The effect of salinity stress on ascorbate peroxidase activity

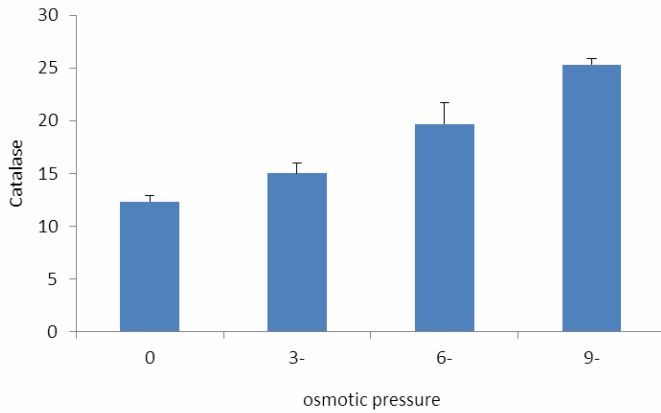


Figure 7 - The effect of salinity stress on catalase activity

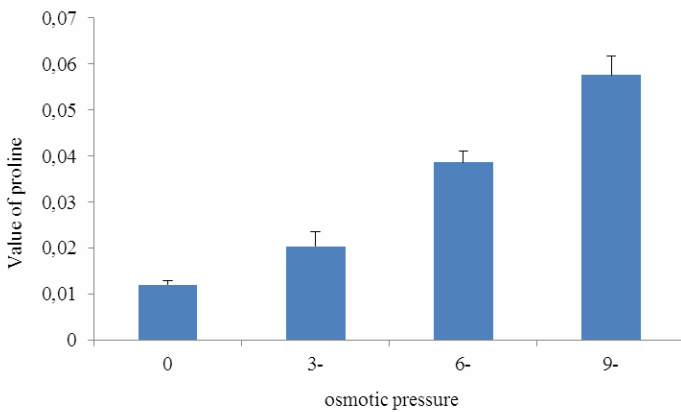


Figure 8 - The effect of salinity stress on value of proline

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The ascorbate peroxidase, catalase activity and proline content were lowest at control condition and increased by increase in salinity stress (Figs. 6, 7 and 8). In agreement with the results, earlier reports (Riley, 1994; Skopelitis *et al.*, 2006) have shown the ascorbate peroxidase and catalase activities was increased by increase in stress conditions. Proline accumulation stress is also supported by other researches in more crops (Kumar *et al.*, 2003; Martinez *et al.*, 2003; Wang and Han, 2009; Çelik and Atak, 2011). Also, a positive correlation between magnitude of free proline accumulation and stress tolerance has been suggested as an index for determining stress tolerance potential of cultivars. The major reason for increase in the proline concentration, during water stress, was due to lesser incorporation of continuously synthesized proline amino acid during proline synthesis (Çelik and Atak, 2011).

CONCLUSIONS

The effect of salinity stress for all traits was significant. By increases of salinity stress, germination percentage, germination index, normal seedling percentage, seedling length and dry weight were significantly reduced. The ascorbate peroxidase and catalase activities, also proline content were minimum at control conditions and increased by increase in salinity stress.

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