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RESEARCH ARTICLE

GROWTH PERFORMANCE OF *LENS CULINARIS* MEDIK UNDER NaCl INDUCED SALT STRESS

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ABSTRACT

The present study reports on the results of salinity stress induced by NaCl, which resulted in the physiological and biochemical changes during seedling development of lentil plants. Plants were subjected to various levels of salt stress of 0.1, 0.2 and 0.5 M, NaCl respectively. Salinity stress markedly affected the physiological and biochemical parameters. The effect of NaCl salinity on germination, seedling growth (root-shoot length) seedling survival, total chlorophyll, and proline content of lentil (*Lens culinaris* Medik.) were investigated. The addition of NaCl resulted in decrease of almost all the parameter taken in this study, but the proline content increased markedly. A slight reduction of germination, seedling growth, seedling survival and total chlorophyll was observed at lowest level of NaCl (01. M) and maximum reduction was observed in 0.5 M NaCl (critical salinity level).

INTRODUCTION

Salinity is the main environmental factor accountable for decreasing crop productivity in many areas of the world especially in arid and semi arid regions (De pascale and Berbieri 1997). On the other hand, not only the uncontrolled irrigation but also poor quality water may cause salinity. It has estimated that about one third of irrigated land has been affected by salinity. Salinity reduces growth and yield of the non halophytes plants by decreasing the availability of water to the roots due to the osmotic effect of external salts and by toxic effect of excessive salt accumulation with the plant (Turan *et al.*, 2007). Salinity is known to affect almost all aspects of plant growth, development and metabolism and it induces change in their morphology and anatomy. However, the type of physiological response and metabolic changes vary among plant species and as well as between different organs of the plant. Generally salinity is the presence of the successive concentration of soluble salts in the soil that suppress plant growth. The major cations contributing to salinity are Na^+, Ca^{+2}, Mg^{+2}, K^+ and anions are Cl^-, SO_4^{-2}, HCO^{-3} and NO_3^{-2}. It has been classified as "primary" or "secondary" salinity. Primary salinity, results from the accumulation of salts over long periods of time from two natural process first is the weathering of rocks containing soluble salts of various types and these are mainly chlorides of sodium calcium and magnesium and to a lesser extent sulphates and carbonates. Second is the deposition of oceanic salt carried inland by wind and rain. The salt composition of this deposited salt is that of sea water which is mainly sodium chloride. Naturally salt affected areas occur widely in arid and semiarid areas. They are often obvious as salt lakes but can also occur out of sight in the subsoil, where they are not associated with ground water or with rising water tables. This type of salinity is named "transient salinity" because it can made up and down the soil profile and in and out of the root zone, depending on the season (Manchanda and Garg 2008).
"Secondary salinity" results from human activities that change the hydrologic balance in the soil between water applied (irrigation and rainfall) and water used by crops (transpiration). Keeping all view the present investigation was carried out to screen the tolerance level of the lentil seedling towards different concentration of NaCl.

MATERIALS AND METHODS

An experiment was conducted under laboratory conditions (humidity 72 ± 5% and temperature 30 ± 2) in Agra, between November-July 2009 with lentil (*Lens cnlinaris* var. T-9) plants. The Evans and Nason (1953) nutrient medium containing 0.1, 0.2 and 0.5 M NaCl was used to create salinity. The control and NaCl treated seeds and seedlings were kept in the growth chamber and 14 days old seedling were used for various analysis.Seed germination was recorded daily upto 14 days after the start of experiment. A seed was considered germination when the radical emerged by 2mm in length (Mohammadi, 2009). In order screen lentil during seedling growth, 15 seedling (7 days old, in triplicate) were transferred to 100 ml plastic Jars containing growth nutrient medium (Evans and Nason 1953) as control or in the nutrient medium containing different concentration of NaCl. Following the measurement of initial length of seedling these were so placed that the lower half of each seedling was immersed in the

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Table 1. Effect of NaCl induced stress on the germination percentage and seedlings growth of lentil seeds.

Osmotic Stress	Concentration	Germination percentage	Root length (in cms)	Shoot length (in cms)
NaCl (M)	0 (Control)	99.66%	6.81 ± 0.07	10.75 ± 0.02
	0.1	97.33%	5.88 ± 0.16	9.06 ± 0.06
	0.2	93.66%	5.31 ± 0.08	7.09 ± 0.10
	0.5	24.33%	4.92 ± 0.04	5.12 ± 0.03

Data represent average percentage values of replicates having 100 seeds in each.

Table 2. Effect of NaCl induced tress on the seedling survival and total chlorophyll and carotenoids content of lentil.

Osmotic Stress	Concentration	Seedling survival Percentage	Total Chlorophyll (mgg^{-1}) of fresh wt.	Carotenoids (mg g^{-1}) of fresh wt.
NaCl (M)	0 (Control)	100%	4.98 ± 0.05	1.20 ± 0.04
	0.1	68.88%	4.18 ± 0.01	1.12 ± 0.02
	0.2	53.33%	3.66 ± 0.01	0.98 ± 0.06
	0.5	8.88%	3.18 ± 0.01	0.94 ± 0.01

Data represent average percentage values of 3 replicates having 15 seedling in each.
Values represent mean ± standard error.

solution. These were kept at room temperature. Fourteen days later the length of root and shoot were measured again with the help of a scale. Seedling survival were observed during the seedling growth period, the seedlings were daily observed and the number of dead seedlings were counted and discarded from the petri plates (Agnihotri 2002). Total chlorophyll of plant was extracted from the leaf (200 mg) with a mixture of acetone and ethyl alcohol (4:1) and absorption was measured in a UV-Vis double beam spectrophotometer (Systronics 2201) at 663 nm and 645 nm (Arnon 1949). For determination of proline concentration the sample were extracted and quantified in a UV-Vis double beam spectrophotometer (Systronics 2201) 520 nm as described by Bates *et al.*, (1973).

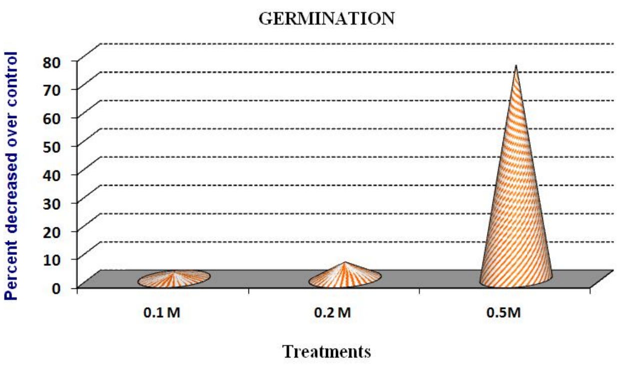
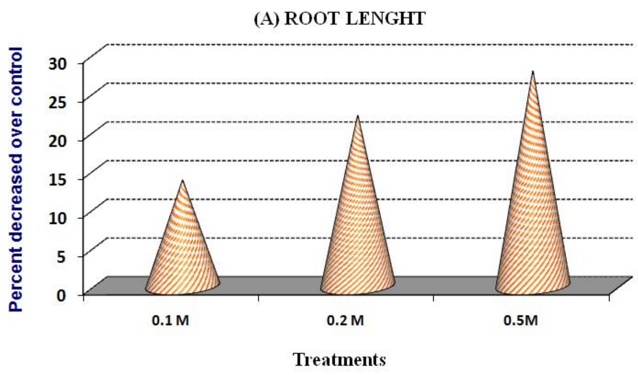


Fig. 1. Effect of NaCl induced stress on the germination of lentil seeds. Cones indicate reduction over control.

RESULTS AND DISCUSSION

Seed germination time was delayed with increasing the NaCl concentration. Therefore, germination process started at differently times in various NaCl concentrations. Significant differences in the seed germination percentage were observed at various salinity levels (0.1,0.2 and 0.5). Seed germination percentage decreased progressively with increase in the salinity level (Fig.1). The seeds showed best performance (99.6%) in control. The reduction in seed germination percentage was severe in seeds germinating at 0.5 M NaCl treatment (24.33% germination) and this salinity level was considered as critical for lentil seed germination. Increasing NaCl concentration gradually decreased the growth of lentil plants which was expressed as root and shoot length (Fig. 2). A slight reduction of root and shoot length was observed at the lowest level of NaCl as (15.7% in shoot and 13.6% in root) as compared to the control. In parallel to the increase of NaCl concentration, the effect of salinity was significant for lentil, i.e., growth reduction of plant at 0.5 M NaCl treatment was 52.3% in shoot and 27.7% in root) as compared to the control. Seedling survival was significantly affected when subjected to NaCl salinity (Fig. 3).



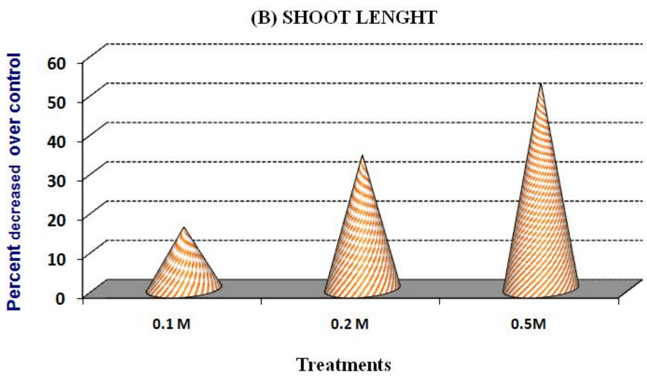


Fig. 2 (A,B) : Effect of NaCl induced stress on the root and shoot length of lentil plant. Cones indicate reduction over control.

Increase in the salinity level led to high reduction of seedling survival. The maximum reduction in seedling survival was observed when the concentration was increased from 0.2 – 0.5 M. Seedling survival decreased over the control by 31.1%, 46.6% and 91.1% under 0.1, 0.2 and 0.5 M NaCl, respectively. Total chlorophyll content of lentil plants were also affected significantly by salinity (Fig.4). The addition of NaCl in the medium decreased total chlorophyll concentration in plants. Total chlorophyll concentration decreased over the

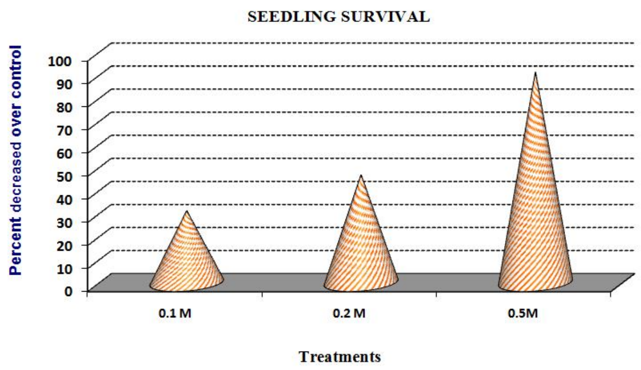


Fig. 3. Effect of NaCl induced stress on the seedling survival of lentil seedlings. Cones indicate reduction over control.

control by 16.0%, 26.5% and 36.1% under 0.1, 0.2 and 0.5 M NaCl, respectively. Application of salt stress caused a marked increase in the proline concentration. Proline concentration increased over the control by 34.8, 146.9 and 161.6% under 0.1, 0.2 and 0.5M NaCl respectively. *Lens culinaris* Medik is an important legume in the forming systems of the Mediterranean area because lentil seed is a source of high quality protein for human consumption and its straw is highly valued as animal folder. The crop is salt sensitive (Turan *et al*., 2007) like other leguminous crops. Upto a certain level of salinity, suppression of germination is brought about by osmotic effect (Narale *et al.*, 1969). In this study, at 0.2 and 0.5 M (critical level) a greater reduction in germination was observed, which may be due to the specific effect of salts in the nutrient medium, possibly as a result of toxic ions. Here "critical level" refers to the salinity level at which drastic inhibition in germination occurred. Elevated salinity shows down water uptake by seeds, thereby inhibiting their germination and root elongation. NaCl inhibited imbibition, germination and root elongation at a low osmotic potential. NaCl is likely to inhibit the activities of some enzymes that play critical role in seed germination (Katembe *et. al.*, 1998). Root length is an important trait against drought stress in plant varieties. In general, variety with longer root growth has resistant ability for drought (Leishman and Westolry 1994).

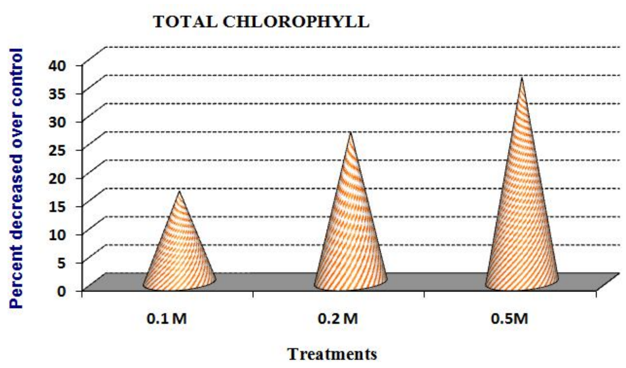


Fig. 4. Effect of NaCl induced stress on the chlorophyll content. Cones indicate reduction over control.

In the present study, decrease in the external osmotic potential caused a reduction in seedling growth of *Lens culinaris*. Although, the increasing concentrations induced salt stress leading to decrease in the root and shoot length, Similar results were reported by (Kaydam and Yagmur 2008). The total chlorophyll concentration of lentil leaves was reduced by increasing the level of NaCl. Similar results were reported for total leaf chlorophyll concentration of *Phaseolus vulgaris* (Yeo and Flower 1983) and wheat and bean (Turan *et al*., 2007). One of the most important mechanism exerted by higher plants under salt-stress conditions is the accumulation of compatible solutes such as proline. The salt treatments induced an increase in proline concentration in lentil plants. Proline accumulation under salinity may contribute to osmotic adjustment, protecting cell structure and function or may serve as metabolic or energetic reserve in plants (Harivandi *et al*., 1982). This study will be useful in screening the level of salinity which the lentil plant could tolerate as this region is particularly drought prompt area where cultivation of this crop is very common.

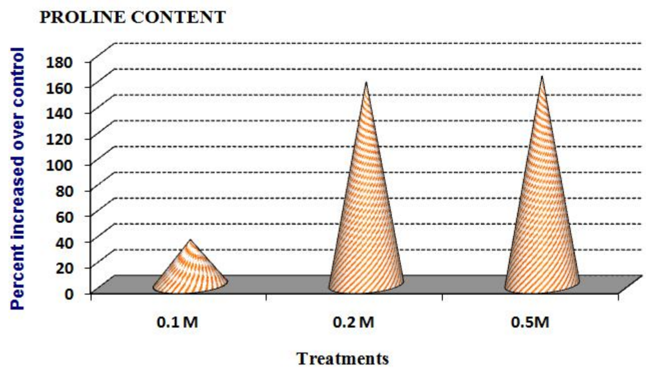


Fig. 5. Effect of NaCl induced stress on the proline content. Cones indicate reduction over control.

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