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Effect of salt stress on salt tolerant indices of morpho-physiological traits and yield attributes of lentil (*Lens culinaris* Medik.)

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Abstract

Salinity is considered in major abiotic stress creating constraints to plant growth and productivity. The knowledge of tolerant genotypes and their variability is an important requirement for success of any breeding programme. Therefore present study was attempted to screen salinity tolerance in ten lentil genotypes on the basis of their morphological, physiological and yield attributes. On the basis of experimental findings, the genotypes PDL1 were found to be highly tolerant and genotype PL07, PL04, IPL316, IPL406 was moderately tolerant whereas genotypes L4076, L4147, DPL62, DPL58 and K75 were found sensitive among studied genotypes under both salinity levels. However on the basis of yield attributes, the genotype PDL1 was found to be most tolerant among studied genotypes under both salinity levels. The genotype PDL1 will be very beneficial for the farmer belong to area which is salt affected to achieve higher pulse production.

Keywords: Salinity, lentil, yield attributes, leaf area

Introduction

Lentil (*Lens culinaris* Medikus) a self-pollinating, annual, cool season plant with an approximate genome size of 4 Gbp. Lentil belongs to the family *Leguminaceae* play a significant role in human and animal nutrition because it provides important dietary source of energy, protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds as well as diverse non-nutritional components such as protease inhibitors, tannins, α -galactoside oligosaccharides and phytic acid (Urbano *et al.*, 2007) [52]. Because of its leguminous nature lentil helps in soil fertility by providing high nitrogen fixation. This in turn allows it to be used under crop rotation with cereal crop which promote sustainable agriculture.

Instability of environment rises various stresses which affect growth and yield of plants. Soil salinity is one of the main abiotic stress in semi arid and arid area. Soil salinity is the abundance of salts of sodium chlorides, calcium and magnesium sulphates and bicarbonates in soil and water. (Hoang *et al.*, 2014) [19]. 830 million hectares (ha) of soil are salt-affected globally (Munns *et al.*, 2005) [38] and almost two million ha of land are not usable due to increase of salinity per year (Umali, 1993) [51]. The water balance of soil is disrupted naturally as well as due to interference of human that disturbs ecology of soil and leads to increase in soil salinity progressively. Agricultural productivity in India majorly affected by soil salinity. Out of the 6.73 million ha of salt-affected soil, 3.77 million ha. Are sodic soil and 2.96 million ha. are saline soil. It is estimated that globally large area under lentil production are affected by salinity or associated condition of sodicity. Salt tolerance is a multigenic trait both physiologically as well as genetically.

Conventional breeding approaches to improve varieties against salt stress had limited success rate (Flowers, 2004; Das *et al.*, 2015) [14, 9]. Salinity stress is regulated at every stage of development it is not covered under stage specific process and a single stage of plant development does not define the tolerance of all stage (Foolad, 2004) [16].

Lentil is a major source of pulse production in many countries and undergoes substantial reduction in growth and yield above a threshold ECe of 1.7 dSm⁻¹. It exhibits stunted or slow growth under saline conditions, with its yields falling to 50 % or even more of its true potential (Katerji *et al.* 2000) [18]. Soil salinization disturbs the texture of soil and hampers required air

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water balance for biological processes occurring in roots. This effect of salinity decreases crop yields.

Salt stress cause severe trouble to the plants morpho-physiological and biochemical traits such as poor photosynthetic rates (Hasegawa *et al.*, 2000; Munns, 2002; Ashraf and Shahbaz, 2003; Kao *et al.*, 2003) [20, 37, 23], reduction of plant height, leaf area, leaf area index and relative water contents. Toxicity of sodium ions disturbed stability of membranes (Marschner, 1986; Gupta *et al.*, 2002) [33, 18], ultimately sodium migrates to reproductive organs as well and reduces the growth and yield of plants (Munns, 2005) [38].

The first step of salt stress causes stomatal closure and a net decrease in photosynthesis as well as production of ROS. Water potential and osmotic potential of plants become more negative with increase in salinity, whereas turgor pressure increases with increasing salinity (Parida and Das, 2005) [40]. Protoplast starts shrinking as osmotic pressure of solution rises above the cells and then plasma membrane splits from the wall. Large gaps created between the plasma membrane and the wall may get filled with solution and allow an artificial apoplastic pathway for salts to move across the root. Moreover, salinity causes increase in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter in leaves of bean and cotton (Longstreth and Nobel, 1979) [30]. Salinity also reduces intercellular spaces in leaves and mitochondrial cristae and causes swelling of endoplasmic reticulum and mitochondria, and fragmentation of tonoplast and cytoplasm (Mitsuya *et al.*, 2000) [34].

The second step of the salt stress cause ion toxicity inside the plant cell and tissues where salts compartmentalize in vacuoles and excess salts starts hindering enzymatic processes (Munns, 2005) [38]. Increased treatments of NaCl induces increase in Na⁺ and Cl⁻ and decrease in Ca²⁺, K⁺ and Mg²⁺ leaves in several plants (Khan *et al.*, 1999; Khan *et al.*, 2000) [24, 25]. Physiological studies have indicated that during salt stress early effects (minutes to hours) on plant growth are due to water stress, whereas salt-specific effects only appear much later (days to weeks) (Denby and Gehring, 2005; Munns, 2002) [10, 37].

Plants adopted to live in saline environments have developed some additional biochemical and molecular strategies to cope with deleterious effects of ion accumulation in cytosol. Biochemical strategies include selective accumulation or exclusion of ions; control of ion uptake by roots and transport into leaves; and compartmentalization of ions (Munns, 2002) [37]. As a result of these alterations, the enzyme activity and metabolism are affected (De Lacerda *et al.*, 2003) [11]. Salinity build up the collection of NaCl in chloroplast of higher plants, affect photosynthesis and growth rate. In several plants salt stress inhibits PS-II (Kao *et al.*, 2003; Parida *et al.*, 2005) [23, 40] but some studies showed that salinity has no effect on PS-II (Brugnoli and Björkman, 1992; Morales *et al.*, 1992) [6]. The reduction of plant growth and dry matter accumulation under

saline conditions have been reported in several important grain legumes (Tejera *et al.*, 2006) [49].

In the present study we attempt to screen ten varieties of lentil for salt tolerance based on their morpho-physiological parameters and yield attributes.

Materials and methods

Plant material and experimental design

Ten genotypes of lentil (*Lens culinaris* L.) comprising of released varieties from different part of agro-climatic zone of India were selected for the present study (Table-1) and were procured from Indian Agriculture Research Institute (IARI), Pusa, New Delhi and CSUAT Kanpur. The experiment was conducted at experimental station, Department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology (SVPUA&T) Meerut, India, in two consecutive years 2016-2017 and 2017-2018. The study was conducted in Randomized Blocked Design (RBD) with three replicates of each variety in control (normal condition) and two treatments (saline condition). Experimental soil was sandy loam with initial pH 6.2 and EC_e (Electrical Conductivity of the extract of a saturated soil paste) is 1.39 dSm⁻¹. The salinity was created at standardized level of which affects the growth parameters of lentil. The level of salinity was created at two levels EC_e 4 dSm⁻¹ and 6 dSm⁻¹ and a control using normal soil (1.39 dSm⁻¹) by adding desired amount of NaCl, Na₂SO₄, CaCl₂.2H₂O (1: 2 :1 ratio) and mixed thoroughly in the irrigation water and irrigated the pot at pre flowering stage (30 DAS). Twenty seeds were sown in each pot and thinning was done after 20 days leaving five plants in each pot. Same fertilizer and irrigation was given to each pot. All the parameters pertaining to salinity tolerance were observed after 65 days after sowing (DAS) of plant.

Meteorological observations

During the study period meteorological data was recorded at automatic weather station of Indian Institute of Farming System Research (IIFSR), Modipuram, Meerut, India.

The meteorological data (2016-17) indicated that weekly minimum and maximum temperature during the crop season ranged from 5.6^{0c} to 24.3^{0c} and 18.5^{0c} to 34.5^{0c} respectively. Total rainfall received was 86.5 mm during the entire crop season and average relative humidity of morning and evening was found to be varying from 97.7% to 65.9% and total 141.3 sunshine hrs. Received during the entire crop season (Fig. 3.1). However, meteorological data (2017-18) indicated that weekly minimum and maximum temperature during the crop season ranged from 6.1^{0c} to 19.5^{0c} and 19.7^{0c} to 33.3^{0c} respectively. Total rainfall received was 13.8 mm during the entire crop season and average relative humidity was found to be very from 97.7 to 26.6 % and 148.4 hrs total sunshine received during the entire crop season, (Figure 3.2) calculated as standard meteorological week (SMW) during experiment conducted from October 2016 to march 2017 and October 2017 to march 2018 (Figure 1.1,1.2).

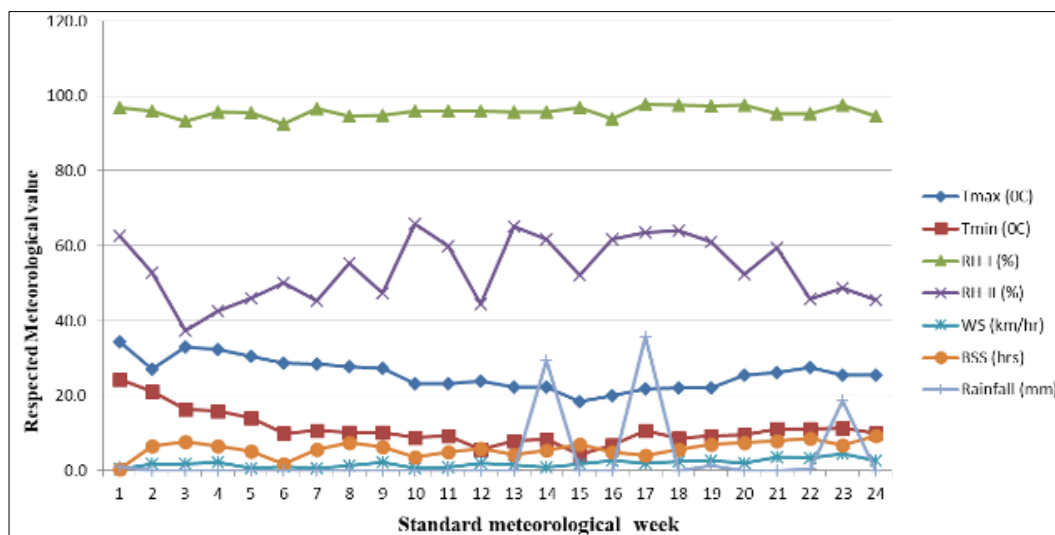


Fig 1.1: Standard meteorological weather (SMW) during October 2016 to March 2017, Line graphs indicate Weekly SMW.

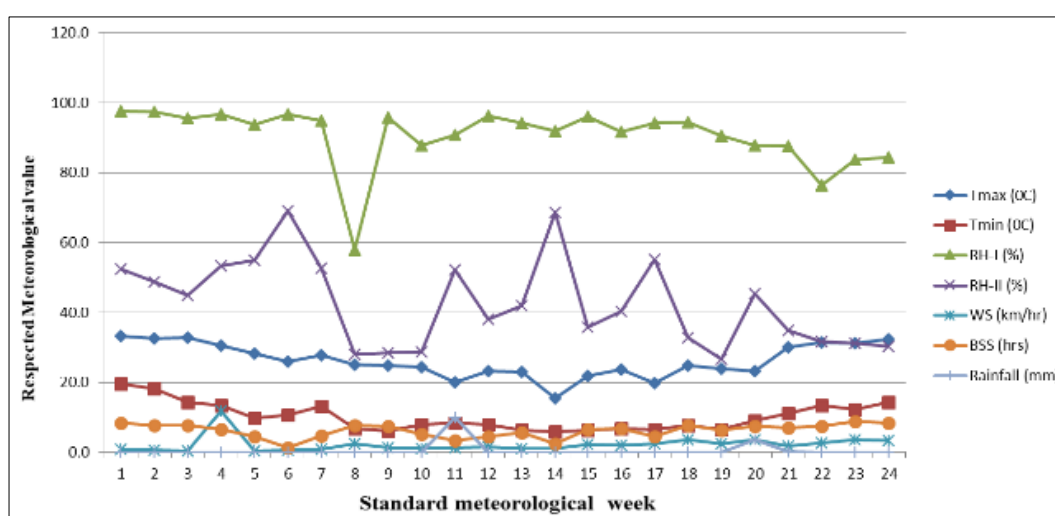


Fig 1.2: Standard meteorological weather (SMW) during October 2017 to March 2018, Line graphs indicate Weekly SMW.

Measurement of Parameters

The genotypes were observed for morphological, physiological and biochemical traits at growth phase (75 days after sowing). The investigated traits were plant height (PH), leaf area (A) Leaf area index (LAI), relative water content (RWC), membrane stability index (MSI), Total chlorophyll content (SPAD), potassium ion accumulation (K^+), sodium ion accumulation (Na^+), pod plant⁻¹, seed plant⁻¹, yield plant⁻¹ and 1000 seed weight.

Estimation of Plant height

Plant height was measured from the base of the plant to the top fully opened leaf of the main shoot at growth stage. Measurements were taken from the main shoot in each treatment tagged earlier and average height of the single plant was calculated and expressed in cm.

Estimation of Leaf area plant⁻¹

Leaf area plant⁻¹ was calculated by taking as per the method given by Mondal *et al.* 2013 [35]

Estimation of Leaf area index

Leaf area index was calculated according to Sestak *et al.*, 1971 [35].

$$\text{Leaf area index} = \frac{\text{Leaf area}}{\text{Land area}}$$

Estimation of Relative Water Content

The relative water content in recently matured leaves was determined using the method suggested by Brass and Weatherly (1962) [5]. Completely extended leaves were removed and fresh weight of leaf (leaf discs) was recorded. Three leaves sample were collected randomly in each treatment and weighed accurately up-to fourth decimal on an electrically operated single pan analytical balance. This was considered as the fresh weight. The weighed leaf discs were allowed to float on distilled water in a petri dish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight. Subsequently these leaf discs were dried in an oven at 70 °C for 48 hours, the dry weight was recorded and designated as dry weight. The RWC was calculated by the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Estimation of Membrane stability index

Membrane stability index (MSI) estimated according to Sairam (1994) [34]. Two sets of leaf tissues (0.1 g) were placed in 10 ml of double-distilled water. One set was kept at 40 °C for 30 min and its conductivity recorded using a conductivity bridge (C1). The second set was kept in a boiling water bath

(100 °C) for 10 min and its conductivity also recorded (C2). The membrane stability index was calculated as:

$$MSI = [1 - (C1/C2)] \times 100$$

Estimation of chlorophyll content

Total Chlorophyll content was measured using Chlorophyll meter SPAD-502 Plus (KONICA MINOLTA). The SPAD meter was calibrated by pressing the measuring head closed without inserting leaf and then measurement was taken for each control and treatment. The third fully expanded leaf from the top selected and measuring head of chlorophyll meter was pressed and noted. The measurement was taken three times and averaged. The concept of SPAD meter was given by Sudhakar *et al.*, (2010) [46].

Estimation of percent Sodium (Na⁺) and Potassium (K⁺)

Plant samples were analyzed for total K and Na (%). For potassium and sodium analysis, plant samples were analyzed through wet digested in di-acid mixture. K and Na (%) was determined by Flame Photometer (Jackson, 1973) [21]. For estimation of potassium and sodium percentage in plant samples di-acid digestion method was used. The mixture of concentrated HClO₄ and HNO₃ was used in the ratio of (1: 2). 0.5 g of dried fine powdered leaves sample was transferred to a 200 ml of test tube containing 10 ml of di-acid mixture. The test tube was kept on hot plate and heated at 180-200 °C for 8hr for complete digestion until the dense white fumes are evolved. Further the mixture was cooled, and the volume was adjusted to 100 ml by adding doubled distilled water in volumetric flask and filtered through Whatman filter paper. Finally, the filtrate was used for analysis as per the method described by Jackson, (1973) [21].

Estimation of Potassium (%)

Known quantity of plant digest in 100 ml volumetric flask has the dilution factor of 200 (0.5 ml made up to 100 ml gives 200 times dilution factor). The flame photometer reading was adjusted to zero with 0 ppm and with 20, 40, 60, 80, 100 ppm KCl solution (K standard solution). Diluted solutions of plant digest were fed into the flame photometer and their readings were recorded. Concentration of K in diluted plant digest was estimated by referring to a standard curve of K concentrations. Percentage of K was calculated by using the formula.

$$K (\%) = \frac{\text{ppm conc of K}}{10^6} \times \frac{\text{volume of digest}}{\text{wt of sample (gm)}} \times \text{Dilution factor} \times 100$$

Estimation of Sodium (%)

Known quantity of plant digest in 100 ml volumetric flask has the dilution factor of 200 (0.5 ml made up to 100 ml gives 200 times dilution factor). The flame photometer reading was adjusted to zero with 0 ppm and with 20, 40, 60, 80, 100 ppm NaCl solution. Flame photometer reading for each diluted solution of plant digest was recorded. Concentration of Na in diluted plant digest was estimated by referring to standard curve. Percentage of Na was calculated by using the formula.

$$Na(\%) = \frac{\text{ppm conc of Na}}{10^6} \times \frac{\text{volume of digest}}{\text{wt of sample (gm)}} \times \text{Dilution factor} \times 100$$

Number of pods per plant

Pods of each replication were counted and then average number of pods for each plant was counted

Number of seeds per plant

The total number of seeds produced by each plant were harvested separately and carefully counted.

Seed yield per plant

The seeds were separated from pods manually and then sun dried and weighted.

1000-seed weight

Thousand clean sun dried seeds were counted from the seed stock obtained from the sample plants and weighted by using electronic balance.

Salt Tolerant Trait Indices (STTI)

Salt tolerant trait indices (STTI) for each of the studied trait were calculated according to the formula of Ali *et al.*, (2007) [2].

$$STI = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under control condition}} \times 100$$

Results and Discussion

The morpho-physiological traits and yield attributes are exploited under this study to collect the valuable informations. These informations are valuable for production of commercial cultivars and salt tolerant varieties for semi arid and arid area which is salt affected. The experimental results of this study showed there is greater difference in performance of varieties and can be useful in future for production of salt tolerant varieties.

Morphological parameters

Plant height, leaf area and leaf area index are considered important parameters for growth under salinity stress. The results indicated a wide range of salt tolerance index (STI) for plant height ranged from 68.51 (IPL406) to 89.25 (PDL-1) with a mean of 78.34 in salinity level S-1 and 63.61 (IPL-406) to 80.40 (PDL-1) with a mean of 72.14 in salinity level S-2 in 2016-17(Table-2) while in 2017-18 ranged from 76.20 (L-4076) to 91.69 (PDL-1) with a mean of 82.24 in salinity stage S-1 and 56.77 (DPL62) to 81.60 (PDL-1) with a mean of 70.82 in salinity level S-2(Table-2).

The salt tolerance index (STI) revealed a wide range of leaf area plant⁻¹ ranged from 53.48 (DPL62) to 72.89 (PL04) with a mean of 61.83 in salinity level S-1 and 48.78 (DPL62) to 59.72 (PL04) with a mean of 52.90 in salinity level S-2 in 2016-17(Table-2) while in 2017-18 ranged from 55.55 (DPL62) to 74.27 (PL04) with a mean of 62.69 in salinity level 1 (S-1) and 47.95 (DPL62) to 58.28 (PL04) with a mean of 53.02 in salinity level 2 (S-2) (Table-2).

The salt tolerance index (STI) revealed a wide range of leaf area index ranged from 59.14 (DPL62) to 78.16 (PL04) with a mean of 65.96 in salinity level S-1 and 52.61 (DPL62) to 64.67 (PL04) with a mean of 58.10 in salinity level S-2 in 2016-17(Table-2) while in 2017-18 ranged from 57.87 (DPL62) to 81.78 (PL04) with a mean of 67.18 in salinity level 1 (S-1) and 55.03 (DPL-62) to 66.38 (PL04) with a mean of 58.78 in salinity level 2 (S-2) (Table-2).

The results showed reduction in plant height, leaf area, and leaf area index in salt sensitive cultivars whereas salt tolerant breeding line and cultivars showed less reduction in comparison to salt sensitive varieties. The genotypes PL04 and PDL1 were found the most tolerant among studied genotypes whereas PL07, IPL316 and IPL406 were moderately tolerant and L4076, L4147, DPL62, DPL58 and K75 were found to be salt sensitive. The results found in this study were supported

by the findings of Singh *et al.* (2017)^[31] and Kumawat *et al.*, (2017)^[19]. The wide range of salt tolerance indices (STI) obtained here for different traits indicate that genotypes had broad genetic base for these characters. In breeding programs, where many genotypes will be evaluated, relative salt tolerance indices (RSTI) can be exploited for these parameters to provide a ranking of the genotypes for salt tolerance.

Physiological parameters

Lentil genotypes in this study were evaluated for physiological characters *viz.*, relative water content, membrane stability index, total chlorophyll content using SPAD meter, percent sodium ion and potassium ion. All the genotypes were evaluated for physiological features under non-saline and saline conditions during 2016-17 and 2017-18.

Relative water content

In stress conditions, physiological changes are the initial response after morphological changes against stress. When plants sense stress in the environment, some physiological changes occur. The salt tolerance index (STI) revealed a wide range of relative water content ranged from 77.70 (L-4076) to 95.79 (PDL-1) with a mean of 86.39 in salinity level (S1) and 67.90 (K-75) to 88.93 (PDL-1) with a mean of 76.95 in salinity stage S-2 in 2016-17(Table-3) while in 2017-18 ranged from 77.97 (DPL-62) to 94.56 (PDL-1) with a mean of 85.01 in salinity level 1 (S-1) and 66.19 (K-75) to 86.41 (PDL-1) with a mean of 76.62 in salinity level 2 (S-2) (Table-3). The study of this parameters can produce valuable information regarding the plant tolerance ability (Munns *et al.*, 2002; Apel and Hirt 2004; Singh *et al.*, 2017)^[37, 3, 45]. There is strong coordination between various physiological response of lentil to salt stress and their tolerance mechanism such as relative water content (Sidari *et al.*, 2008)^[44] and membrane stability index (Jamil *et al.*, 2012)^[22]. Relative water content (RWC) has been reported as an important indicator salinity stress. As the salinity increases relative water content decreases (Monem *et al.*, 2008)^[36] and affects the balance between water supply to leaves and transpiration rate (Farquhar *et al.*, 1989)^[8]. High amount of RWC in leaf denotes that the plant can improve its inner aquatic relation under stress condition (Chakherchaman *et al.*, 2009)^[6]. Higher relative water content in tolerant genotypes influence the ability of plant to recover from salt stress, consequently maintaining the yield (Lilley *et al.*, 1996)^[21].

Membrane stability index

Many aspects of plant growth are affected by salt stress; one of them is membrane stability index (MSI). Under salt stress, the cell membrane of lentils get damaged which leads to deleterious effect of salts, consequently decreasing growth and yield. The salt tolerance index (STI) revealed a wide range of membrane stability index percent ranged from 64.82 (DPL-62) to 90.01 (PDL-1) with a mean of 76.69 in salinity level 1 (S-1) and 46.99 (DPL-62) to 80.36 (PDL-1) with a mean of 64.48 in salinity level 2 (S-2) in 2016-17 (Table-3) while in 2017-18 ranged from 64.93 (L-4076) to 90.29 (PDL-1) with a mean of 76.94 in salinity level 1 (S-1) and 48.35 (L-4076) to 82.85 (PDL-1) with a mean of 65.22 in salinity level 2 (S-2) (Table-3). Higher membrane stability index represents the tolerance capability against salinity stress (Lee *et al.*, 2013)^[20].

Total chlorophyll content using SPAD meter

The effect of salt stress on chlorophyll content shows decreased chlorophyll content /salt stress (Lee *et al.*, 2013)

^[20]. When plants are grown under salt stress condition it leads to reduced growth and productivity. Photosynthetic activity is severely impaired due to decreased chlorophyll content, since chlorophyll content correlates directly with the growth and development of plant (Acosta-Motos *et al.*, 2017)^[1]. The salt tolerance index (STI) revealed a wide range of total chlorophyll content (SPAD value) ranged from 51.10 (L-4076) to 95.24 (PDL-1) with a mean of 64.08 in salinity level 1(S-1) and 38.85 (L-4076) to 86.21 (PDL-1) with a mean of 48.67 in salinity level 2 (S-2) in 2016-17(Table-3). While in 2017-18 ranged from 53.56 (K75) to 93.38 (PDL-1) with a mean of 67.02 in salinity level 1 (S-1) and 48.37 (K-75) to 85.97 (PDL-1) with a mean of 57.63 in salinity level 2 (S-2) (Table-3). The decrease in chlorophyll content suggested substantial damage to the photosynthetic mechanism (Pareek *et al.*, 1997; Lee *et al.*, 2013)^[31, 20]. Our results showed that the salt treatment significantly decreased the chlorophyll content.

Sodium and Potassium

The effect of ions correlates with salt tolerance mechanism in crop plants. The concentration of sodium ion leads to salt tolerance mechanism in plants which limits the uptake or transport of ions from the root to areal parts (Lopez *et al.*, 1999)^[23]. The higher salt concentration leads hampers salt regulation mechanisms. Salt sensitive genotypes gets rapidly saturated with salt ions at lower level of salinity as compared to salt tolerant genotypes. Due to this rapid saturation plants start showing deleterious effect of sodium (Singh *et al.*, 2017)^[31]. The salt tolerance index (STI) revealed a wide range of sodium percent ranged from 472.00 (PDL-1) to 654.55 (L4076) with a mean of 584.34 in salinity at level S-1 and 528.00 (PDL-1) to 845.00 (L4147) with a mean of 730.88 in salinity level S-2 in 2016-17(Table-3). While in 2017-18 ranged from 504.35 (PDL-1) to 657.14 (L4076) with a mean of 610.90 in salinity level 1 (S-1) and 600.00 (PDL-1) to 845.45 (DPL-62) with a mean of 764.74 in salinity stage 2 (S-2). (Table-3) In the present investigation, at higher concentration sodium uptake increases in salt sensitive genotypes as compared to tolerant genotypes. The genotype which accumulated higher amounts of sodium was L4076, L4147, K75, DPL62, DPL58 whereas PDL1 showed the least uptake. Hence, we can say that tolerant genotypes have better mechanism to avoid salt accumulation and bypass detrimental effect of sodium on plant metabolism. This observation is in accordance to those observed in other species (Marshner 1986; Femandes *et al.*, 1994)^[25, 9].

The salt tolerance mechanism can be correlated with selective uptake of potassium over sodium (Wenxue *et al.*, 2003)^[41]. The salt tolerance index (STI) revealed a wide range of potassium percent ranged from 35.07 (DPL-62) to 69.44 (PDL-1) with a mean of 53.42 in salinity at stage S-1 and 20.15 (DPL-62) to 51.06 (PDL-1) with a mean of 37.32 in salinity stage S-2 in 2016-17 (Table-3). While in 2017-18 ranged from 37.34 (L-4076) to 70.01 (PDL-1) with a mean of 54.01 in salinity stage 1 (S-1) and 20.18 (DPL-62) to 50.08 (PDL-1) with a mean of 36.99 in salinity stage 2 (S-2) (Table-3). The salt tolerance mechanism revealed by the selective uptake of potassium and not sodium was considered one of the important physiological mechanism. The deficiency of potassium enhances sodium toxicity (Flower *et al.*, 1983). In present study, it was also found that potassium content decreased with increasing sodium concentration, it indicates antagonistic nature between the absorption of sodium and potassium. In tolerant genotype potassium concentration

decreased marginally with less increase of sodium as compared to sensitive genotypes. This suggests that tolerant genotypes have better regulation of potassium concentration with proper maintenance of sodium concentration. Thus, tolerant genotype can maintain relatively higher potassium level. The higher potassium concentration is related with better growth and higher seed yield.

The wide range of relative salt tolerance indices found in this study for different traits would be fruitful in breeding programs for genotype ranking for salt tolerance. Based on these physiological parameters, PDL1, PL04, PL07, IPL316 showed higher relative salt tolerance indices whereas L4076, L4147, K75, DPL62 showed lower range of relative salt tolerance indices and DPL58, IPL406 had moderate range of relative salt tolerance indices. Therefore, on the basis of relative salt tolerance indices, genotypes can be classified as tolerant, moderately tolerant and sensitive for salinity. Our results are in agreement with Sidariet *et al.*, (2008) [36]; Monem *et al.*, (2008) [28] for relative water content and Monem *et al.*, (2008) [36]; regarding chlorophyll content. Similar findings were reported for sodium and potassium relationship by Turan *et al.*, (2007) [40]; Ashraf *et al.*, (1993) [3]; and Singh *et al.* (2017) [31].

Yield attributes

Salinity stress disturbs the metabolic activity of plants (Dua 1998) [7] and the uptake of sodium by plant ultimately leads to translocation of sodium in reproductive organs that disturbs pod per plant, seed per plant and seed yield per plant statistics which ultimately affects the seed weight of lentil. Tolerant genotypes have ability to check the uptake of sodium which leads to lower level of sodium translocation to the reproductive organs hence resulting in greater number of pods, seeds, and seed yield per plant and 1000 seed weight is better in comparison to those of sensitive genotypes which have poorer sodium uptake regulation. Higher sodium levels cause necrosis of leaves which affect the life of leaflets ultimately resulting in yield reduction (Tester and Davenport 2003) [39].

Pod Plant⁻¹

The salt tolerance index (STI) revealed a wide range of Pod/Plant ranged from 32.00 (L-4147) to 63.59 (PDL-1) with a mean of 41.81 in salinity level S-1 and 22.00 (K-75) to 48.68 (PDL-1) with a mean of 30.65 in salinity level S-2 in 2016-17 (Table-4). While in 2017-18 ranged from 32.65 (L-4147) to 64.44 (PDL-1) with a mean of 42.68 in salinity level 1 (S-1) and 22.42 (K-75) to 48.68 (PDL-1) with a mean of 31.34 in salinity level 2 (S-2) (Table-4).

No. of seed Plant⁻¹

The salt tolerance index (STI) revealed a wide range of No. of seed/Plant ranged from 35.00 (K-75) to 71.86 (PDL-1) with a mean of 47.92 in salinity level S-1 and 24.00 (K-75) to 50.72 (PDL-1) with a mean of 33.27 in salinity level S-2 in 2016-17 (Table-4). While in 2017-18 ranged from 34.62 (K-75) to 74.52 (PDL-1) with a mean of 48.53 in salinity level 1 (S-1) and 23.68 (K-75) to 52.54 (PDL-1) with a mean of 33.63 in salinity level 2 (S-2) (Table-4).

Seed yield Plant⁻¹

The salt tolerance index (STI) revealed a wide range of seed yield/Plant ranged from 32.69 (L-4147) to 67.59 (PDL-1) with a mean of 42.26 in salinity level S-1 and 22.00 (K-75) to 67.59 (PDL-1) with a mean of 33.17 in salinity level S-2 in 2016-17 (Table-4). While in 2017-18 ranged from 32.19 (L-4147) to 69.93 (PDL-1) with a mean of 42.17 in salinity level 1 (S-1) and 20.00 (K-75) to 69.93 (PDL-1) with a mean of 32.05 in salinity level 2 (S-2) (Table-4).

1000 seed weight

The salt tolerance index (STI) revealed a wide range of 1000 seed weight ranged from 36.18 (K-75) to 84.65 (PDL-1) with a mean of 54.41 in salinity level S-1 and 26.38 (DPL-62) to 83.28 (PDL-1) with a mean of 44.03 in salinity level S-2 in 2016-17 (Table-4). While in 2017-18 ranged from 36.04 (K-75) to 85.53 (PDL-1) with a mean of 44.08 in salinity level 1 (S-1) and 26.25 (DPL-62) to 84.13 (PDL-1) with a mean of 44.08 in salinity level 2 (S-2) (Table-4).

The relative salt tolerance indices in this study suggest that sodium concentrations have negative impact on yield and yield related attributes. Based on yield attributes PDL1 were found most tolerant genotypes. The results of present study are in the agreement with study of Ashraf *et al.*, (1993) [3], Gadala *et al.*, (2007) [13], Mondal *et al.*, (2013) [35], Tesfaye *et al.* (2014) [38], Singh *et al.*, (2017) [31]. The above study is useful in assessment of salt tolerant genotype of lentil. Our study showing similarity with other researchers and their Finding in different plant species like chickpea, faba bean.

Group ranking and score based on Salt tolerance index (STI)

All the ten studied lentil genotypes were ranked according to Salinity tolerant index (STI) and two salinity levels S1 and S2 and positioned of group rank are shown in table (Table-5, 6, 7). The Performance of PDL-1 in STI scores and ranking was more superior in all the studied traits with moderate ranking of PL07 and PL04.

Table 1: Lentil genotypes used in this study

Sr. No.	Genotypes	Source
1	L4076	IARI Pusa
2	L4147	IARI Pusa
3	PL04	IARI Pusa
4	PL07	IARI Pusa
5	PDL1	IARI Pusa
6	DPL62	IARI Pusa
7	DPL58	IARI Pusa
8	IPL406	IARI Pusa
9	K75	CSAU Kanpur
10	IPL316	IARI Pusa

Table 2: Relative salt tolerance indices at Salinity Level S-1 & Salinity Level S-2 of following traits studied for year 2016-17 (I) & 2017-18 (II) in Lentils

Genotypes	Plant height (cm)		Plant height (cm)		Leaf Area Plant ⁻¹ (cm ²)		Leaf Area Plant ⁻¹ (cm ²)		Leaf Area Index		Leaf Area Index	
	I		II		I		II		I		II	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
L4076	74.95	69.78	76.20	68.74	62.44	53.89	63.41	54.95	65.20	58.80	63.24	56.72
L4147	75.32	71.33	77.28	70.88	58.44	52.94	59.54	51.67	63.56	59.11	66.19	58.20
PL04	81.47	74.72	84.52	73.41	72.89	59.72	74.27	58.28	78.16	64.67	81.78	66.38
PL07	83.16	77.2	85.91	75.15	67.25	52.03	66.23	53.54	70.17	58.35	73.51	60.84
PDL1	89.25	80.44	91.69	81.62	68.69	54.54	69.07	55.20	71.76	59.50	75.09	62.09
DPL62	71.87	66.15	76.91	56.77	53.48	48.78	55.55	47.95	59.14	52.61	57.87	55.03
DPL58	82.25	72.27	83.31	72.06	57.29	48.90	58.32	49.71	61.60	54.40	59.88	55.53
IPL406	68.51	63.61	78.37	61.02	65.76	58.02	67.28	57.54	68.28	62.83	70.38	60.64
K75	72.56	67.75	79.28	71.70	54.46	49.99	56.34	50.55	60.51	55.80	60.97	56.89
IPL316	84.11	78.24	88.94	76.9	57.63	50.19	56.94	49.87	61.25	55.00	62.90	55.48

S1- Salinity (EC_{iw}= 4.00 dSm⁻¹), S2- Salinity (EC_{iw}= 6.00 dSm⁻¹).**Table 3:** Relative salt tolerance indices at Salinity Level S-1 & Salinity Level S-2 of following traits studied for year 2016-17 (I) & 2017-18 (II) in Lentils

Genotypes	RWC (%)		RWC (%)		MSI (%)		MSI (%)		Total Chlorophyll Content (µg/cm ²)		Total Chlorophyll Content (µg/cm ²)		Na ⁺ (%)		Na ⁺ (%)		K ⁺ (%)		K ⁺ (%)	
	I		II		I		II		I		II		I		II		I		II	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
L4076	77.70	74.61	79.15	73.08	64.94	47.79	64.93	48.35	51.10	38.85	58.79	50.75	654.45	813.64	657.14	842.86	38.32	25.06	37.34	24.46
L4147	83.27	75.27	80.18	75.20	65.74	48.54	66.30	50.32	52.63	40.00	60.24	51.29	645.01	845.00	625.00	835.00	40.16	28.35	39.67	27.83
PL04	89.92	79.63	88.68	79.65	84.85	76.92	85.55	77.12	69.35	48.30	66.66	56.17	610.09	765.02	621.05	736.16	65.43	44.64	64.64	43.38
PL07	91.56	80.44	91.70	82.54	85.73	77.79	86.79	78.29	70.88	50.00	74.90	63.19	571.43	714.29	621.00	700.00	66.94	48.68	65.41	47.60
PDL1	95.79	88.93	94.56	86.41	90.01	80.36	90.29	82.85	95.24	86.21	93.38	85.97	472.06	528.00	504.35	600.00	69.44	51.06	70.01	50.08
DPL62	82.22	70.65	77.97	72.26	64.82	46.99	66.17	49.84	53.76	41.19	57.61	49.14	625.01	812.50	631.82	845.45	35.07	20.15	42.59	20.18
DPL58	90.45	78.12	86.05	78.38	83.79	73.75	83.94	73.39	68.49	49.09	67.94	55.54	613.63	772.73	628.57	804.76	63.04	43.65	63.03	43.36
IPL406	81.40	72.71	81.34	70.84	71.43	58.82	70.99	58.19	51.55	40.73	61.47	54.66	550.02	718.18	626.32	789.47	47.41	31.85	47.53	33.6
K75	78.41	67.90	79.97	66.19	67.24	55.56	66.10	54.80	55.55	39.15	53.56	48.37	604.76	771.43	633.33	790.48	41.19	30.24	43.74	31.01
IPL316	93.15	81.27	90.49	81.63	88.36	78.26	88.34	79.10	72.27	53.19	75.65	61.27	496.02	568.00	571.43	676.19	67.22	49.54	66.14	48.42

S1- Salinity (EC_{iw}= 4.00 dSm⁻¹), S2- Salinity (EC_{iw}= 6.00 dSm⁻¹).**Table 4:** Relative salt tolerance indices at Salinity Level S-1 & Salinity Level S-2 of following traits studied for year 2016-17 (I) & 2017-18 (II) in Lentils

Genotypes	Pod Plant ⁻¹		Pod Plant ⁻¹		No. of Seed Plant ⁻¹		No. of Seed Plant ⁻¹		Seed yield Plant ⁻¹ (g)		Seed yield Plant ⁻¹ (g)				1000 Seed weight (g)		1000 Seed weight (g)	
	I		II		I		II		I		II				I		I	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
L4076	36.82	24.55	37.23	25.04	40.95	28.67	40.86	28.53	36.86	24.58	36.69	23.19	17.26	11.28	43.78	28.45		
L4147	32.00	26.23	32.65	26.39	35.72	26.54	36.00	26.68	32.69	32.69	32.19	32.19	11.86	9.35	53.23	41.77		
PL04	47.63	35.72	49.16	37.07	47.68	36.98	47.29	36.62	46.68	35.01	46.93	33.12	18.02	13.32	74.19	54.56		
PL07	45.69	32.78	47.00	33.91	52.73	38.80	56.05	41.18	45.81	32.86	47.57	32.54	17.76	12.15	66.13	45.01		
PDL1	63.59	48.68	64.44	48.68	71.86	50.72	74.52	52.54	67.59	67.59	69.93	69.93	20.47	20.14	85.53	84.13		
DPL62	34.00	25.00	34.68	25.65	48.00	27.00	47.19	26.46	34.00	25.00	33.84	24.13	12.34	8.78	37.12	26.25		
DPL58	32.46	23.61	33.55	24.58	47.25	26.70	49.16	27.69	33.88	24.64	33.20	23.21	13.47	10.60	41.59	32.62		
IPL406	44.00	33.00	45.32	34.15	46.91	34.47	47.98	35.20	42.13	31.60	42.07	30.58	14.99	12.45	55.54	46.00		
K75	36.00	22.00	36.29	22.42	35.00	24.00	34.62	23.68	36.00	22.00	35.20	20.00	11.16	10.31	36.04	33.26		
IPL316	46.00	35.00	46.56	35.60	53.13	38.83	51.69	37.71	47.02	35.78	44.17	31.67	16.05	14.69	53.42	48.81		

S1- Salinity (EC_{iw}= 4.00 dSm⁻¹), S2- Salinity (EC_{iw}= 6.00 dSm⁻¹).**Table 5:** Ranking of genotypes by using average STI Scores of various Morphological and physiological characters (2016-17)

S. No.	Genotype	Based on PH, LAP ⁻¹ , LAI				Based on RWC, MSI and SPAD, Na ⁺ , K ⁺			
		S-1		S-2		S-1		S-2	
		GR	TR	GR	TR	GR	TR	GR	TR
1	L4076	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
2	L4147	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
3	PL04	1	Tolerant	2	Moderate	1	Tolerant	1	Tolerant
4	PL07	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant
5	PDL1	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant
6	DPL62	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
7	DPL58	2	Moderate	2	Moderate	2	Moderate	2	Moderate
8	IPL406	3	Susceptible	3	Susceptible	2	Moderate	2	Moderate
9	K-75	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
10	IPL316	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant

GR-Genotype rank; TR-Tolerance rank

Table 6: Ranking of genotypes by using average STI Scores of various Morphological and physiological characters (2017-18)

S. No.	Genotype	Based on PH, LAP ¹ , LAI				Based on RWC, MSI, Chlorophyll (SPAD), Na ⁺ , K ⁺			
		S-1		S-2					
		GR	TR			GR	TR	GR	TR
1	L4076	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
2	L4147	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
3	PL04	1	Tolerant	2	Moderate	1	Tolerant	1	Tolerant
4	PL07	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant
5	PDL1	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant
6	DPL62	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
7	DPL58	2	Moderate	2	Moderate	2	Moderate	2	Moderate
8	IPL406	3	Susceptible	3	Susceptible	2	Moderate	2	Moderate
9	K-75	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
10	IPL316	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant

GR-Genotype rank; TR-Tolerance rank

Table 7: Ranking of genotypes by using average STI Scores of various Yield Attributes (2016-17) and (2017-18)

S. No.	Genotype	Based on Yield attributes Pod plant ¹ , seed plant ¹ , yield plant ¹ and 1000 seed weight (2016-17)				Based on Yield attributes Pod plant ¹ , seed plant ¹ , yield plant ¹ and 1000 seed weight (2017-18)			
		GR	TR	GR	TR	GR	TR	GR	TR
1	L4076	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
2	L4147	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
3	PL04	2	Moderate	2	Moderate	2	Moderate	2	Moderate
4	PL07	2	Moderate	2	Moderate	2	Moderate	2	Moderate
5	PDL1	1	Tolerant	1	Tolerant	1	Tolerant	1	Tolerant
6	DPL62	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
7	DPL58	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
8	IPL406	2	Moderate	2	Moderate	2	Moderate	2	Moderate
9	K-75	3	Susceptible	3	Susceptible	3	Susceptible	3	Susceptible
10	IPL316	2	Moderate	2	Moderate	2	Moderate	2	Moderate

GR-Genotype rank; TR-Tolerance rank

Conclusion and Recommendations

On the basis of above findings, following useful conclusions, both having fundamental and applied values, may be drawn. The increased in salinity stress leads to disturbance in various studied characters such as morphological traits like (Plant height, leaf area palnt¹, leaf area index), Physiological parameters like (RWC, MSI, Total Chlorophyll contents, Na⁺ and K⁺ contents and yield attributes. On the basis of these studied parameters PDL1 were found most tolerant genotypes whereas PL04, PL07, IPL406 and IPL316 were found moderately tolerant genotypes although L4076, L4147, DPL62 and DPL58 were more susceptible genotypes. The overall performance of PDL1 under salt stress was found superior than among studied genotypes. The genotype PDL1 will be very beneficial for the farmer belong to area which is salt affected up to 6 dSm⁻¹ to achieve higher pulse production. These attributes can be used for screening tool for salinity tolerance in other cultivars as well.

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