International Journal of Economic Plants

https://ojs.pphouse.org/index.php/IJEP

Crossref

IJEP May 2025, 12(3): 01-08

Article IJEP5439

Full Research

Stress Management

Doi: HTTPS://DOI.ORG/10.23910/2/2025.5439

Effect of Salt Stress on Germination and Early Seedling Growth in Lentil

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Article History

Received on 08th May, 2024
Received in revised form on 15th April, 2025
Accepted in final form on 08th May, 2025
Published on 24th May, 2025

Abstract

The present study was conducted during December, 2018–February, 2019, to assess the genetic variation and association of the characters concerning germination and seedling parameters with salt tolerance index and subsequently evaluate the five varieties of lentil as per their response against varied level of salinity during early seedling stage at laboratory condition. Salinity poses a significant challenge to crop productivity worldwide, affecting various physiological and metabolic pathways in crops like lentils, particularly in arid and semi-arid regions where lentils are commonly cultivated. Seeds of five varieties of lentil were grown at four different levels of salinity (0, 50, 100 and 150 mM NaCl) in completely randomized block design (CRD) with factorial combination. The germination percentage, shoot length, root length, shoot: root ratio, seedling fresh weight, seedling dry weight and seedling vigour index were studied and data were recorded. Results revealed that most of the studied traits reflected significant genetic variation. High heritability coupled with high genetic advance was detected for most of the traits and confirmed the importance of selection for improving the traits. Salinity levels drastically reduced seedling vigour and other parameters with increasing salt concentration. The degree of diminution varied with different varieties. Among the five varieties, Ranjan was detected as salt tolerant followed by Moitree and ILL-6002 was identified as most sensitive to salinity. Ranjan can be considered as a valuable genetic resource for lentil breeding programme under salt stress situation.

Keywords: Genetic variation, germination percentage, lentil, salinity, seedling vigour

1. Introduction

Salinity represents a prominent global issue, with over 800 million ha of land currently affected by high salt levels (Shahid et al., 2018). In saline soils sufficient water is their but available water is scarce due to presence of excess concentration of soluble salts which possess oxidative damage in crops, thereby restricting water absorption (Muscolo et al., 2020; Panuccio et al., 2022). However, different authors defined salinity in various ways. The most widely accepted definition of a saline soil is one that has an electrical conductivity in the saturation extract (ECe) exceeding 4 mmhos cm^{-1} (4 dS m^{-1}) at 25°C. Food and Agriculture Organization (FAO)/ The United Nations Educational, Scientific and Cultural Organization (UNESCO) reported soils with ECe(s) exceeding 15 mmhos cm^{-1} as strongly saline soils (Abiala et al., 2018; Nadeem et al., 2019). Salinity alters the physiological processes inside plant systems by causing a rise in turgor pressure and a lack of water availability, which impairs cell membrane integrity, interrupts enzyme activity, produces salt toxicity, and eventually reduces photosynthesis (Banerjee et al., 2019; Singh et al., 2020). Legumes are usually sensitive to salinity. Soil salinity particularly disturbs the symbiotic interaction between legumes and rhizobia (Farooq et al., 2017; Tlahig et al., 2021) and also affects plant development and dry matter accumulation when grown in saline environments (Tejera et al., 2006; Jha et al., 2019). Thus, salinity exerts significant and varied impacts on overall growth, yield, potential as well as the quality and composition of the food legume (Bouzroud et al., 2023).

Lentil (*Lens culinaris* Medik.), a significant cool–season food legume, holds the rank of the fourth most crucial legume crop globally. Serving as an economical source of dietary protein, lentil play a vital role in providing nutrition to the economically backward segments of developing nations (Chatterjee et al., 2023). The lentil grain is abundant in starch, proteins, dietary fibers, and possesses a high concentration of both macro and micronutrients (Bhattacharya et al., 2022). Like other pulses, lentil is also sensitive to salinity which significantly reduces growth and yield of this crop if grown in more than 1.7 Ds m^{-1} ECe soil (Katerji et al., 2001). Lentil exhibits its sensitivity at early stage of crop growth by reduced germination percentage, seedling vigour, shoot and root length, seedling dry and fresh weights and ultimately loss of plant life (Tesfaye et al., 2014). Hence, the screening parameters that are extensively employed to delineate the salt tolerant genotype include germination and seedling growth profiling under saline environment because of high heritability of the traits even though the trait is of quantitative in nature as well as extreme sensitivity of the seedling stage as compared to the established plants (Arshi et al., 2002). Several workers conducted experiments to study the effects of the salinity on lentil for plant growth and early seedling parameters (Arslan et al., 2016; Hossain et al., 2018; Foti et al., 2019; Pandey and Sengar, 2020; Yasir et al., 2021).

As the global population continues to rise and cultivable land diminishes, the pursuit of resilient cultivars capable of thriving in saline environments emerges as the most comprehensive strategy to address this challenge. Given the varying impact of salinity on different stages of plant growth and considering the diversity among cultivars, our research hypothesis was that different lentil cultivars in agricultural fields may exhibit distinct responses to salinity. So, keeping pace with the backdrop, the present study has been contemplated to examine the variability regarding the effect of salt stress on germination and early seedling growth among the lentil varieties and to recommend the tolerant variety in future lentil breeding programme.

2. Materials and Methods

2.1. Plant materials

The present study was conducted under the laboratory condition at the Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, West Bengal (741 252), during December, 2018 to February, 2019. The experiment was conducted with 5 cultivars of lentil *viz*., Ranjan, Moitree, KLS-218, HUL-57 and ILL-6002. Among these 5 cultivars, 4 were microsperma (small seeded) and Pilosa type and ILL-6002 was from Mediterranean region and macrosperma type (bold seeded).

2.2. Treatment and experimental details

To enumerate the effect of salinity on germination and seedlings growth, seeds of the tested lentil varieties were sown in glass petri plates of 9 cm diameter. The petri plates were first washed by chromic acid and rinsed in tap water for thrice. After that petri dishes were kept for drying in hot air oven for 4 hrs followed by autoclaving for sterilization at 121°C and 15 PSI. A total of fifty disinfected seeds of five lentil varieties *viz*., Ranjan (V_1), Moitree (V_2), KLS-218 (V_3), HUL-57 (V_4) and ILL-6002 (V_5) were placed on sterilized Whatman filter papers in petri plates at 20±2°C temperature inside BOD incubator. Initially, the lentil seeds were surface sterilized by treating with sodium hypochloride (NaOCl) @ 12% for 10 minutes followed by washing with distilled water thrice. Five salinity levels *viz*., control (0.0 mM), 50 mM, 100 mM and 150 mM NaCl concentrations were prepared by dissolving 0, 0.7305 g, 1.461 g, 2.191 g NaCl in 250 ml of double distilled water, and henceforth designated as T_0, T_1, T_2 and T_3, respectively. Whatman filter paper was moistened with 3 ml of test solutions daily to maintain the condition for germination. The experiment was conducted following completely randomized design (CRD) in a factorial combination maintaining three replications for each concentration.

2.3. Recording of observation and data analysis

Five seedlings were randomly selected from each petri plates to record the data on shoot length (cm), root length (cm), shoot to root length ratio, shoot fresh weight (mg), root fresh weight (mg), shoot and root dry weight (mg). The data on root and shoot length was recorded after 15 days of sowing. Observation on root and shoot dry weight were recorded after drying in hot air oven for 48 hours at 65°C. Germination % was recorded on 7 days after sowing as per the protocol of Anonymous (1996) with the following formulae:

Germination % (GP)=(No. of seeds germinated/No. of seeds sown)×100

Subsequently, seed vigour was calculated with the following formulae:

Seed Vigour=GP×seedling length

Finally, salt tolerance Index (STI) was calculated comparing the root and shoot dry weight of five seedlings observed in salt concentration with the dry weight obtained in control condition.

Salt tolerance index (STI)=(Total dry weight at T_x/Total dry weight at T_0)×100

The data on germination % was transformed to arcsine before statistical analysis. Mean values of all the parameters were used for statistical analysis. The Analysis of Variance (ANOVA) was accomplished for determining the total variances among the lentil varieties for different treatments, and their significance was assessed using the ‘F’ test. Difference between the treatment mean was tested by Duncan’s multiple range tests (DMRT) at 5% probability level. Different genetic parameters like phenotypic variance (σ^2p), genotypic variance (σ^2g), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability in broad sense (h^2bs) were estimated using the earlier method (Ghosh et al., 2022; Meher et al., 2024). Correlation coefficient was measured by the formula suggested by Johnson et al. (1955). The SPAR (Statistical Package for Agricultural Research) tool for Windows version 2.0 was used to conduct the statistical analysis (Ahuja et al., 2008).

3. Results and Discussion

3.1. Studies on genetic variation

The analysis of variance over different salinity levels (Table 1) indicated that the salinity levels (Treatment), genotypes

Table 1: The ANOVA over different salinity levels for various traits

Sources of variation	DF	Mean square								
		Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot dry wt (mg)	Root dry Wt (mg)	Seedling vigour	Salt tolerance index	Germination %
Treatment	3	77.12**	170.52**	4321.03**	594.53**	24.45**	21.64**	814641.49**	16625.31**	2047.74**
Geno	4	12.96**	11.87**	384.56**	210.55**	6.48**	16.97**	158373.68**	1384.62**	714.87**
Treat.:Geno	12	1.74**	7.41**	81.93**	78.71**	2.07**	2.80**	11936.72**	511.01**	43.34**

*: represent significant at (p=0.05); **: (p=0.01) level of significance, respectively

and salinity levels×genotypes interaction exhibited significant mean sum of squares difference for all the seedling parameters**.** This finding confirmed that the lentil varieties responded differently to various salinity levels for all the traits and have inherent variability. The findings reflected there was significant genetic variability within the varieties for salt tolerance, despite the relatively modest range of genetic material studied (Kumawat et al., 2018; Foti et al., 2019).

The performance of lentil cultivars at varying salt concentrations was represented by a box plot (Figure 1). It was detected that shoot length, shoot dry weight, shoot and root fresh weight represented consistent performance whereas for rest of the traits incongruous response was observed.

In the present study, presence of ample genetic variability was also confirmed with different genetic parameters (Table 2). In most of the traits, the difference between PCV and GCV was significant and reflected the environmental influence except root fresh and dry weight as well as germination %. The highest PCV and GCV was observed in case of root dry weight (83.47; 83.02) followed by root fresh weight (39.44; 38.67) and both these parameters were lowest for germination % (14.76; 14.71). Moderate to high heritability coupled with moderate to high GA as % over mean was recorded for most of the studied traits. This finding validated that most of the genetic variation was due to additive component. Therefore, simple section would be advantageous for improvement of these traits. Similar findings were also reported in earlier studies (Kayıs and Ceyhan, 2015; Kumawat et al., 2018).

3.2. Influence of salinity on germination and other seedling growth parameters

In the present study, it was depicted that salt stress altered germination percentage negatively with increase in salinity levels. Amid the five varieties tested, the germination percentage was significantly different in various salt concentrations. The highest germination percentage was observed in control (T_0), followed by 50 mM salt (T_1), and

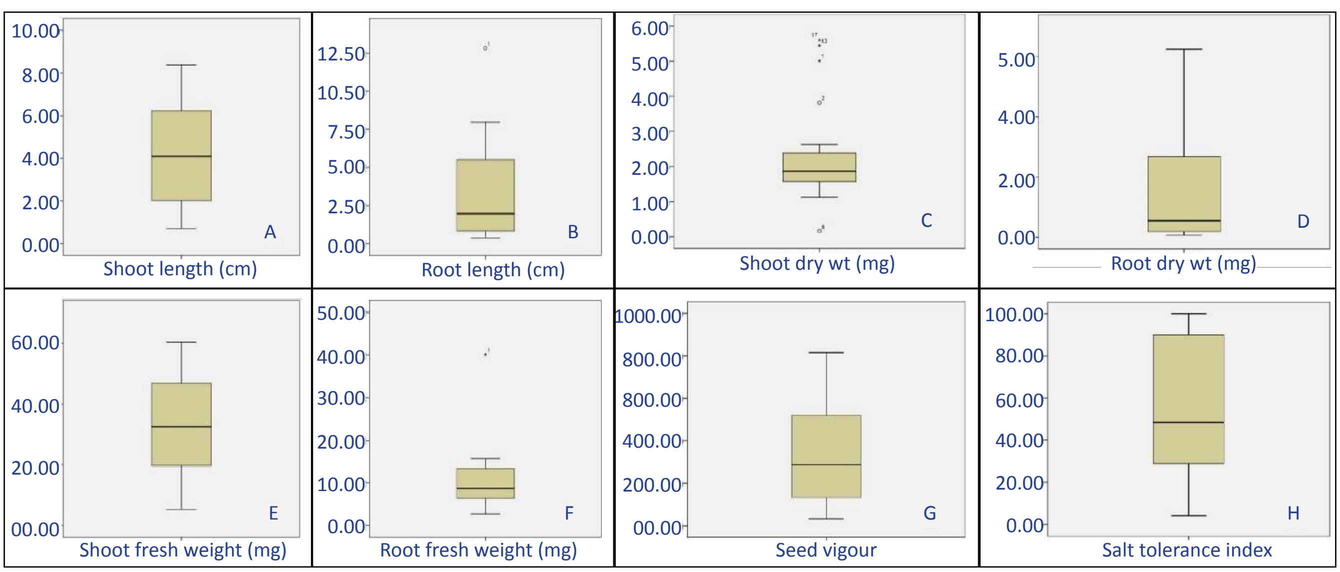

Figure 1: Box plot view representing the performance of lentil varieties at various levels of salt concentration; A: Distribution pattern of shoot length; B: Distribution pattern of root length; C: Distribution pattern of shoot dry weight; D: Distribution pattern of root dry weight; E: Distribution pattern of shoot fresh weight; F: Distribution pattern of root fresh weight; G: Distribution pattern of seed vigour; H: Distribution pattern of salt tolerance Index

Table 2: Genetic parameters of 5 lentil varieties at different levels of salt concentration

	Grand mean	PCV	GCV	Genetic advance	Genetic advance as % of mean	Heritability (%)
Shoot length (cm)	4.17	26.33	24.19	1.91	45.82	84.40
Root length (cm)	3.40	31.35	28.13	1.77	52.13	80.50
Shoot fresh weight (mg)	33.54	20.64	14.65	7.18	21.41	50.30
Root fresh weight (mg)	10.76	39.44	38.67	8.40	78.06	96.10
Shoot dry wt (mg)	2.33	34.93	29.70	1.21	52.00	72.30
Root dry wt (mg)	1.43	83.47	83.02	0.43	30.08	98.90
Germination %	71.98	14.76	14.71	21.72	30.17	99.20
Seedling vigour	334.23	35.55	33.77	220.89	66.09	90.30
Salt tolerance index	55.83	21.84	17.80	16.68	29.88	66.40

was lowest in 150 mm (T_3) salt concentration (Table 3). The values ranging from 80% to 63% in Ranjan (V_1), 73% to 45% in Moitree (V_2), 70% to 40% in KLS-218 (V_3), 72% to 42% in HUL-57 (V_4) and 68% to 37% for ILL-6002 (V_5). The reduction in germination % was highest in ILL-6002 followed by HUL-57 and KLS-218 whereas, Ranjan and Moitree exhibited congruous response at varied salt concentration (Figure 2). Determining the germination % under salt stress is the easiest selection criteria to delineate cultivars for their sensitivity to salt stress. Salt tolerance is a measure of a seed's ability to survive the impacts of excessive salt concentrations in the medium during germination. Excessive salt decreases the germination medium's water potential, creating physiological drought, thus slowing down or totally inhibiting germination. Earlier reports suggested the high heritable nature of this trait thus validated the usefulness of this trait as a good selection index (Mann et al., 2019; Banerjee et al., 2019; Singh et al., 2020).

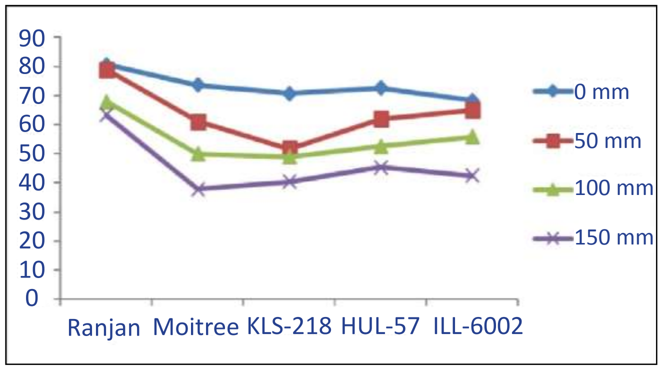

Figure 2: Effect of different salt concentrations on germination % of 5 lentil varieties

Root length is the main determinant of a plant's response to salinity (Khodarahmpour, 2011). The result revealed that both root and shoot length were consistently decreased with raising salt levels among different varieties and seedling root lengths were more severely affected compared to shoot length in saline conditions (Table 3). Ranjan recorded highest root length (12.83 cm) at varied salt concentration ranging from 12.83 cm to 0.83 cm followed by Moitree and lowest root length was observed in IIL-6002 (0.37 cm to 5.3 cm). Regarding shoot length also at differential level of salt concentration, Ranjan depicted the highest shoot length (8.37 cm) among all the varieties tested. It was affirmed by earlier workers that salt has a negative effect on shoot and root elongation as because cell wall becomes rigid due to excessive accumulation of salts that ultimately affects cell wall elasticity and turgor pressure thus reduces cell expansion and elongation ultimately leads to reduced root and shoot growth (Tesfaye et al., 2014; Foti et al., 2019; Rao et al., 2023). The present result was in full agreement with the earlier reports in lentil (Asghar et al., 2009; Tesfaye et al., 2014; Hossain et al., 2018).

Shoot and root fresh weights are the important parameters for judging the impact of abiotic stresses in any species. It was observed that shoot and root fresh weight exhibited variable response under different salt concentrations. Among all the tested varieties, Ranjan (V_1) exhibited highest shoot and root fresh weight and lowest was observed in ILL-6002 (V_5). The average shoot fresh weight of Ranjan was 41.1 mg whereas the average root fresh weight was 42.12 mg. Strikingly, varieties like Ranjan and Moitree exhibited maximum values of seedling shoot and root length along with higher shoot and root fresh weight even at alleviated salinity level compared to the other varieties that exhibited sensitivity to salinity and recorded least values for seedling shoot and root length along with meagre values for shoot and root fresh weight. Earlier studies in lentil and *Phaseolus* species also supported our results (Jeannette et al., 2002; Foti et al., 2019; Yasir et al., 2021).

Shoot and root dry weights followed similar trend as that of shoot and root fresh weights. Both root and shoot dry weights were linearly decreased with increasing salt concentration (Table 3). Ranjan (V_1) recovered maximum shoot and root dry weights and ILL–6002 (V_5) recovered minimum weights in different treatments. The reduction in shoot dry weight was more at all the treatments than the root dry weight. Stress caused by salt highly inhibited the root elongation causes

Table 3: Comparison of various levels of salt concentration in 5 varieties of lentil

Salt Conc.	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot dry wt (mg)	Root dry wt (mg)	Seedling vigour	Germination %	Salt tolerance index
Variety: V_1									
T_0	8.37^{a}	12.83^{a}	60.3^{a}	68.08^{a}	5.6^{a}	6.25^{a}	815.07^{a}	80.64^{a}	100^{a}
T_1	6.67^{b}	5.73^{a}	46.5^{ab}	44.8^{b}	3.82^{b}	3.64^{b}	642.00^{b}	78.98^{a}	80.29^{b}
T_2	4.83^{c}	2.17^{c}	36.93^{b}	38.57^{c}	2.16^{c}	2.62^{c}	414.67^{c}	68.06^{b}	61.47^{bc}
T_3	2.03^{d}	0.83^{c}	20.67^{c}	17.03^{c}	1.13^{c}	0.48^{d}	162.47^{d}	63.44^{c}	54.75^{c}
Variety: V_2									
T_0	7.67^{a}	7.97^{a}	57.67^{a}	55.77^{a}	5.8^{a}	3.81^{a}	697.37^{a}	73.65^{a}	100^{a}
T_1	6.73^{a}	4.57^{b}	42.5^{ab}	40.63^{a}	2.1^{b}	2.74^{b}	524.47^{b}	65.16^{b}	78.15^{b}
T_2	3.4^{b}	1.77^{b}	29.33^{bc}	28.13^{b}	1.85^{b}	0.7^{c}	213.87^{c}	55.76^{c}	42.26^{c}
T_3	2.57^{b}	0.67^{b}	18.67^{c}	15.84^{b}	1.69^{b}	0.17^{b}	129.83^{c}	45.38^{d}	27.47^{b}
Variety: V_3									
T_0	5.77^{a}	6.67^{a}	50.07^{a}	44.2^{a}	2.62^{a}	1.19^{a}	513.50^{a}	70.67^{a}	100^{a}
T_1	4.1^{b}	2.33^{b}	35.53^{b}	25.37^{ab}	1.88^{b}	0.37^{a}	321.93^{ab}	61.12^{b}	42.16^{b}
T_2	2^{c}	1.00^{b}	$23.9b^{c}$	11.33^{b}	1.63^{b}	0.19^{c}	133.80^{c}	49.99^{c}	30.42^{b}
T_3	0.9^{c}	0.57^{c}	17.07^{c}	7.33^{c}	1.47^{b}	0.14^{c}	37.73^{c}	40.39^{d}	14.58^{d}
Variety: V_4									
T_0	7.13^{a}	7.9^{a}	56.07^{a}	50.87^{a}	5.01^{a}	3.07^{a}	615.77^{a}	72.56^{a}	100^{a}
T_1	4.2^{a}	2.9^{b}	37.13^{b}	29.39^{b}	2.05^{a}	1.68^{b}	334.80^{b}	62.03^{b}	67.3^{b}
T_2	3.63^{a}	1.31^{bc}	25.5^{bc}	13.00^{b}	1.66^{a}	0.22^{b}	213.07^{b}	52.54^{c}	33.0^{c}
T_3	1.7^{b}	0.63^{b}	17.53^{c}	8.3^{b}	1.51^{a}	0.15^{a}	64.10^{c}	42.51^{d}	19.03^{c}
Variety: V_5									
T_0	4.9^{a}	5.3^{a}	46.77^{a}	32.37^{a}	1.89^{a}	0.61^{ab}	451.07^{a}	68.32^{a}	100^{a}
T_1	4.07^{b}	1.5^{b}	24.23^{b}	18.14^{b}	1.72^{a}	0.29^{a}	252.87^{b}	51.75^{b}	39.97^{b}
T_2	1.97^{c}	0.83^{b}	13.07^{b}	8.21^{bc}	1.12^{ab}	0.19^{a}	114.07^{c}	49.02^{c}	21.47^{c}
T_3	0.7^{c}	0.37^{b}	10.23^{b}	5.8c	0.16^{b}	0.07^{c}	32.07^{c}	37.86^{d}	4.17^{d}
Grand mean	4.17	3.39	33.53	10.76	2.33	1.43	334.23	55.82	59.49

lower water uptake and toxicity which ultimately reduced growth of seedlings in salt treatments compared to control condition. This gradual decrease in shoot and root dry weights were also reported by Hamdy et al. (2002) and Tesfaye et al. (2014) in lentil and Kandil et al. (2012) in seedlings of mungbean

Seedling vigour is a key indicator to represent the amount of damage caused by particular stress and the crop's ability to cope with the specific stress (Copeland and McDonald, 2012). Under various salinity level, seedling vigour decreased with the increment of salt concentration (Table 3). High magnitude of seedling vigour was observed in Ranjan (V_1) followed by Moitree (V_2) and it was lowest in ILL-6002 (V_5). Several Earlier reports were in accordance with the present finding which implied that seedling vigour decreased under salinity and this trait exhibited acute sensitivity to salt stress (Khodarahmpour, 2011; Keshtiban et al., 2015; Kumawat et al., 2018).

The highest reduction in salt tolerance was detected in ILL–6002 followed by KLS–218 and HUL–57. Therefore, STI was used as a basis for ranking of the varieties against salinity tolerance. At T_0 condition, there was no significant difference between the varieties concerning STI. Ranjan being the best variety having maximum STI and less affected by salinity stress compared to other varieties considered in the study; followed by Moitree. HUL–57 revealed moderately tolerant response to salinity whereas, KLS–218 and ILL–6002 were the sensitive varieties.

In the present study, all the lentil varieties exhibited significant differences for various seedling parameters studied and indicated broad range of values at various salt concentration except in control condition. Almost all the characteristics mean values were influenced negatively by the salinity gradient due to toxicity effects on early seedling stages which influences ion uptake and decreased water availability (Kayıs and Ceyhan, 2015; Ouerghi et al., 2016; Kumawat et al., 2018).

3.3. Correlation study

Correlation study indicated different degree of association among the traits. STI exerted positive and significant correlation with most of the early seedling growth parameters, such as root and shoot length, root fresh and dry weight seed vigour and germination % (Table 4). Therefore, these characters should be considered as key traits during selection of salt tolerant lentil varieties. Since the number of varieties considered in the study were meagre in number, therefore, highly significant correlation was recorded for most of the seedling parameters. Similar findings were also mentioned in previous reports in lentil (Kayıs and Ceyhan, 2015; Kumawat et al., 2018).

Table 4: Correlation matrix among the variables at different level of salt concentrations in 5 varieties of lentil

	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot dry Wt (mg)	Root dry wt (mg)	Seed vigour	Germination %
Root length(cm)	0.390							
Shoot fresh weight (mg)	0.901**	0.438						
Root fresh weight (mg)	0.43	0.895**	0.357					
Shoot dry wt (mg)	0.757**	0.349	0.779**	0.293				
Root dry wt (mg)	0.40	0.752**	0.29	0.796**	0.400			
Seed vigour	0.984**	0.866**	0.429	0.394	0.389	0.434		
Germination %	0.846**	0.756**	0.809**	0.722**	0.699**	0.757**	0.903**	
Salt tolerance index	0.768**	0.812**	0.455	0.812**	0.440	0.731**	0.783**	0.710**

4. Conclusion

Significant genetic variation was observed for most of the studied traits at various levels of salt concentration. Correlation study revealed significant and positive association between shoot and root length, root fresh and dry weight, germination % and seed vigour with STI. Based on STI, Ranjan was detected as the least effected variety whereas, ILL-6002 was the highly sensitive one. The identified salt tolerant cultivar would be recommend for cultivation in the saline zone of the country.

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