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EFFECT OF SALINITY STRESS ON GERMINATION OF FIVE TUNISIAN LENTIL (*LENS CULINARIS L.*) GENOTYPES.

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Abstract

Salinity is one of the major stresses especially in arid and semi-arid regions, which severely limites crop production. It is a significant problem affecting agriculture worldwide and is predicted to become a larger problem in the coming decades. This study was conducted to assess the effect of different salinity level (0, 50, 150, 250 mMol of NaCl) on lentil seed germination efficiency (germination, seedling shoot length, seedling root length, seedling fresh shoot weight and seedling fresh root weight). Five Tunisian genotypes of lentil (*Lens culinaris* M) namely: Kef, Siliana, Nefza, Ncir, and Local oueslatia were investigated. Results showed that there were significant differences among the different NaCl solution for all evaluated traits. Indeed, the experiment showed that the concentrations of salt have a negative impact on the germination and growth of lentil. As a result when the concentration of salt increases, the germination, length of root and shoot and fresh weight of root and shoot decreases. At 250 mM salt stress level, seed germination percentage of all genotypes was notably reduced compared with non-stress condition (0.0 mMol). Moreover, the seeds were not germinated by the 250 mM salinity level for kef genotype. From the results of this present investigation, it can be concluded that seeds of Kef and Ncir genotypes were susceptible to higher concentrations of salt solutions in germination stage. However, Siliana, Local oueslatia and Nefza genotypes can be considered as tolerant to salt stress compared to the other ones. These genotypes could be used for further analysis and for hybridization in the breeding program for enhancing lentil cultivation in newly reclaimed soils.

Keywords: Lentil (*Lens culinaris* L.), salinity, germination, Tunisian genotypes

Introduction

Lentil (*Lens culinaris* L.) is an important legume in the farming systems of the Mediterranean area, because it is a source of high quality protein in human diet and animal consumption (Katerji et al. 2001). It offers most practical means of solving the protein malnutrition. Lentil is characterized by its ability to enter into a symbiotic relationship with the bactrium *Rhizobium leguminosarum* in the fixation of atmospheric nitrogen. It helps in reducing the amount of added nitrogenous fertilizer to the plants.

Agricultural productivity in arid and semi-arid regions of the world is very low due many complex factors such as accumulation of salts in soils (Munns 2002). Total area under salinity is about 953 million ha covering about 8 percent of the land surface in the world (Singh 2009). It has estimated that about one third of irrigated land has been affected by salinity (Stockle, 2001).

In Tunisia, the crop is commonly grown under either semi-arid and rainfed condition during winter on soils that conserve moisture from the preceding monsoon. Total production of lentil in Tunisia was 1687 tons from an area of 3750 acres with an average yield of 4.4 qx ha-1 (Fao, 2013).

Seed germination is one of the most important events in the life cycle of higher plants. It is the sum of all physiological processes occurring inside the seed which starts with the imbibitions in water and ends with emergence of embryonic root. When a viable seed is soaked, water is taken up, that initiates respiration, protein synthesis and other metabolic activities. Almansouri et al. (2001) reported that soil salinity affected plants at seedling stage much higher than other plant growth stage because seed germination usually occurs in the uppermost soil layers which accumulate soluble salts as a result of evaporation and capillary rise of water. There is not always a positive correlation between salt tolerance at germination and during the later stages of growth. Some of the crop species are very tolerant to salts during later stages of growth are quite sensitive during germination and vice versa (Berstein and Hayward 1958). Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on plant species (Ungar 1996). Soil salinity may influence the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or the toxic effects of Na+ and Cl^- ions on the germinating seeds (Khajeh et al. 2003). Under salt stress, there is a decrease in water uptake during imbibitions. Furthermore, salt stress may cause excessive uptake of ions (Murillo et al. 2002).

Turan et al. 2007 showed that 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 in the plant. 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 according to plant species as well as plant organs.

The present study was initiated to investigate the influence of salinity on the germination and early seedling growth of some Tunisians lentil genotypes.

Materials and Methods

The study was conducted under laboratory condition at room temperature and based on morphological variation among lentil genotypes in order to assess the salt tolerance in terms of seed germination and seedling growth. Seeds of lentil were sterilized in sodium hypochloride (12%) for 15min then washed three times with distilled water. Three replicates of 20 seeds of each genotype were germinated in sterilized petri-dishes on filter papers (Whatman N^o 1).These seeds were subjected to 50, 150 and 250 mM concentrations of NaCl and distilled water. The filter paper was kept moist by adding the respective salt solutions. The germination was recorded at 24 h interval for 15 days. The radicle emergence (≥2mm) was taken as the criterion for germination. After 15 days germination, the length of root and shoot were measured with the help of a scale.

The measured parameters included:

*Germination percentage: Seven days after seeds were put into the Petri dishes, germinated seeds were counted, and the germination percentage calculated. The germination percentage calculated as:

Germination percentage = Number of germinated seeds / number of total seed X 100.

*Seedling Shoot Length (cm): Fifteen days after 15 days of sowing, shoot lengths were measured in centimeters.

*Seedling Root Length (cm): Fifteen days after 15 days of sowing, root lengths were measured in centimeters.

*Seedling Fresh Shoot Weight (g): was measured after 15 days of sowing by weighting the mass of shoots using sensitive balance.

*Seedling Fresh Root Weight (g): was measured after 15 days of sowing by weighting the mass of roots using sensitive balance.

Data analysis

For all investigated parameters, analysis of variance was performed using the statistica software package. Significant differences among the mean values were compared by LSD test ($P < 0.05$).

Results and Discussion

Influence of Salinity on Germination

No significant difference between genotypes was observed under control condition (salinity level of 0.0 mMol). Results showed that untreated seeds (0.0 mMol) germinated readily. Germination process present a sigmoid curve, with a first stage characterized by a low germination rate, which lasted until 24 h after seeding; an intermediate stage between 24 to 48 h with a maximum germination rate, and a final stage of a static character in which the germination velocity increases minimally (Figure1). These results agree with those presented by Abd El-Monem et al 2008, in case of lentil, who showed that germination process can be considered in terms of three sequential steps: (i) seed imbibition, (ii) radical initiation and (iii) radicle emergence.

Seeds of Siliana genotype were the most rapid to germinate, presenting in the first stage a rate of 6.6 germinated seeds per day, reaching 33% germination at 24 h after seeding. This speed germination is in contrast to that presented by the local oueslatia. The latter presented only 1.7 seed per day reaching close to 8.3% of germinated seeds at the end of the same time (Figure 1). In this stage Kef, Ncir and Nefza genotypes presented intermediate values of 3.7, 2.3 and 3.7 seed day-1, respectively.

In the second stage of germination kinetics (between 24 and 72 h), the differentials among the germination rates observed in the first stage tended to be higher. However, owing to the marked differences presented in the first stage, the percentages of germination between Local Oueslatia and Ncir were higher significantly different ($P \leq 0.01$) at the end of the second stage (72 h). Indeed, at the end of this stage the Local Oueslatia genotype reached 100% germination, while Ncir reached only 80%. Maximum germination (98.3 to 100%) was reached at 48 h (Siliana), 72 h (kef, Ncir and Nefza) and at 96h (local oueslatia).

Results of analysis of variance showed that percentage of germination was significantly affected by salt stress and genotypes ($P \leq 0.01$), where increasing in salinity stress reduced percentage of germination (Tab. 1).

Table2 show that germination percentage of lentil genotypes decreased when salt stress level was increased. Indeed, level salinity of 150 mM NaCl had different effect on germination percentage of lentil genotypes, where Ncir genotype was the most susceptible and Siliana genotype was the most tolerant to salt stress. Very low germination occurred by 250 mM NaCl.

Inhibition of germination due to salinity has been also reported by Buchade and Karadge 2014 who studied the seed germination of five legume crops: *Dolichos biflorus*, *Lens esculenta* Moench. *Phaseolus aureus*, *P. aconitifolius* and *Trigonella foenum graecum*.

As it is shown in figure1, the increasing salinity caused a delay and a gradual decrease in germination. These results were in agreement with those reported by Almansouri et al. 2001 explaining that salt and osmotic stresses are responsible of both inhibition or delayed seed germination and seedling establishment. Strong reduction was observed mainly at the highest level of salt concentration compared to control. Nevertheless, genotypes responded differently to different salinity levels. Indeed, seed germination percentage of Siliana, Kef, Nefza and Local oueslatia genotypes were not significantly influenced by 50 mMol of salt stress level, whereas under this level seed germination percentage was significantly decreased for Ncir genotype. However, at 150 mM NaCl, germination percentage was significantly reduced in Kef (40%) and Ncir (43.3%). At the same salinity level (150 mM NaCl) genotypes Siliana, local oueslatia and Nefza germinated more than the other ones (Ncir and kef), exhibiting a fair degree of salt tolerance. Decrease and delay in germination in saline medium has also been reported by Guo et al., 2010; Fethi et al., 2011).

At 250 mM salt stress level, seed germination percentages of all genotypes were notably reduced compared with non-stress condition (0.0 mMol). Moreover, the seeds were not germinated by the 250 mM salinity level for kef genotype. From the results of this present investigation, it can be concluded that seeds of Kef and Ncir genotypes were susceptible to higher concentrations of salt solutions in germination stage. However, siliana, local oueslatia and Nefza genotypes can be considered as tolerant to salt stress compared to the other ones. Gulzar et al., 2001, showed that salinity inhibits germination of seeds in one of two ways: (1) preventing germination without loss of viability at higher salinities; and (2) delaying germination of seeds at salinities that cause some stress to seeds but do not prevent germination.

Influence of Salinity on Seedling Shoot and Root traits

The study of morphological traits showed that salinity caused a significant reduction on almost traits measured at the highest NaCl concentration.

Seedling shoot length

The analysis of variance for seedling shoot length data showed a highly significant variation in seedling shoot length ($p < 0.001$) among genotype, salinity level and their interaction (Table 1).

A slight reduction of shoot length was observed at 50mMol of NaCl as 15.2% in shoot as compared to the control. In parallel to the increase of NaCl concentration, the effect of salinity was significant for shoot lentil, i.e., growth reduction of plant at NaCl treatment was 76.8% and 93.3% respectively at 150 mM and 250 mM as compared to the control. Seedling survival was significantly affected when subjected to NaCl salinity (Fig. 2). This result showed that increment of NaCl treatments resulted a significant reduction in shoot growth. The results of this study show conformity with research result of Kagan et al. 2010 and Saratale et al. 2010 who reported that salinity inhibits elongation of shoot in lentil and increasing NaCl treatment results significant reduction of shoot growth.

Azene et al. 2014 reported that the reduction of shoot length was probably due to the excessive accumulation of salts in the cell wall elasticity, thus, secondary cell appears sooner and cell wall becomes rigid as a consequence the turgid pressure efficiency in cell enlargement decreases that result in short shoot growth.

Seedling root length

The analysis of variance (ANOVA) for seedling root length indicates that there was highly significant variation in seedling root length among lentil genotypes, salinity levels and their interaction ($p < 0.001$) (Table 1).

A slight reduction of shoot length was observed at the lowest level of NaCl (50 Mm) as 17.1% in shoot as compared to the control. In parallel to the increase of NaCl concentration, the effect of salinity was significant for shoot lentil, i.e., growth reduction of plant at NaCl treatment was 72.8% and 89.9% respectively at 150 mM and 250 mM as compared to the control. Seedling survival was significantly affected when subjected to NaCl salinity (Fig. 3).

At 50 mM salt concentration, Nefza genotype attained the longest root length than other accession while, salinity adversely reduced root growth in local oueslatia genotype which showed the shortest root length. Furthermore, at 150 Mm and 250 mM salt concentration, the longest root length was recorded in Nefza genotype, whereas the shortest root length was observed in siliana and kef genotypes. The result elucidate that the increment of NaCl concentration caused the reduction of seedling root length of lentil accession. The result is in full agreement with those found by Ashrafand and Waheed (1990) for lentil, Arshi et al. (2003) for senna plant and Duzdemiro et al (2009) for pea.

Munns (2002) showed that salinity stress cause a multitude of physiological problems in plant processes. It causes a significant reduction in germination percentage, germination rate, shoot and root length, root and shoot weight, and dry root and shoot weight, and seed yield which lead to the death of the entire plant (Gupta and Minhas 1993 and Jamil et al. 2006).

In the current investigation, 50 mMol of salinity has a more pronounced effect on root length with respect to shoot length, as roots are directly exposed to salt solution. The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake for overall osmotic adjustments of the plant body under high salt stress condition as reported by Majid Abdoli et al. 2013.

Fresh weight of shoot and root

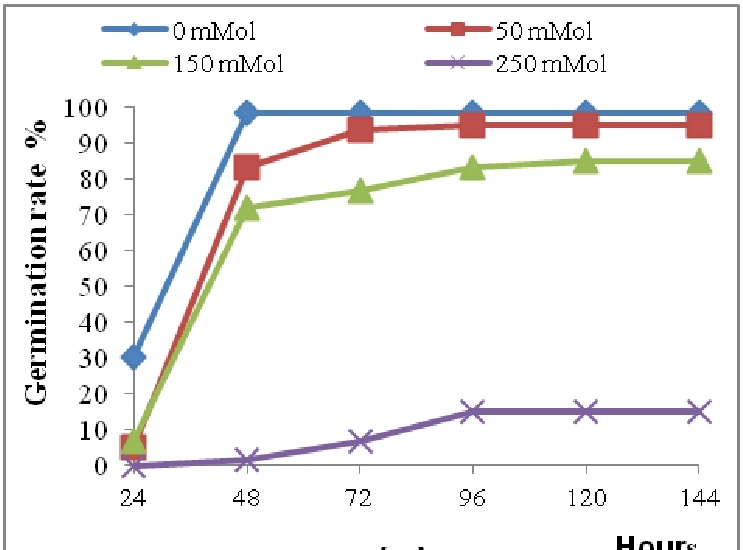
Salinity had significant effect on shoot and root fresh weight ($P \leq 0.01$), where increasing in salt concentration reduced these traits (Tab.1). Differences in fresh weight of shoot and root were significant in different genotypes under different salinity level ($P \leq 0.01$). Siliana genotype had the highest value of fresh weight of shoot and root under control (0.0 mM) treatment. Regarding fresh weight of shoot and root, the effect of salt stress was pronounced from 50mM Nacl concentration onwards. It was completely inhibitory from 250 mM onwards for kef genotype (fig 4 and Fig.5). The result was in line with previous research findings of Stoeva and Kaymakanova (2008) who reported a rapid decrease in seedling fresh shoot weight of leguminous plants under saline environment. This reduction may be due to limited supply of metabolites to young growing tissues, because metabolic production takes place within in the leaves and is significantly perturbed at high salt stress, either due to the low water uptake or toxic effect of NaCl concentration (Hussain et al. 2009; Munns 2002 ; Taffouo et al. 2009). Salinity reduced the growth of root in all genotypes, but the degree of reduction of seedling fresh root weight for Siliana, Ncir and Nefza genotypes was less than the other accessions. Consequently, those genotypes had the maximum value of seedling fresh root weight even at higher salinity level than the other accessions. On the other hand, salinity highly inhibited the root elongation of kef and local oueslatia. These genotypes had minimum value of the seedling fresh root weight. The result justified that salinity reduced seedling fresh root weight of lentil accessions. The findings of this study are in line with those of Jeannette et al. 2002 and Kagan et al. 2010 who reported salinity increment significantly reduced fresh root weight in phaseolus species and Lentil, respectively.

Table1. Mean square values and significance of NaCl treatment, genotype and their interaction effect on germination % (24h, 48h and 72h), Seedling Shoot Length, Seedling Root Length, Seedling Fresh Shoot Weight and Seedling Fresh Root Weight.

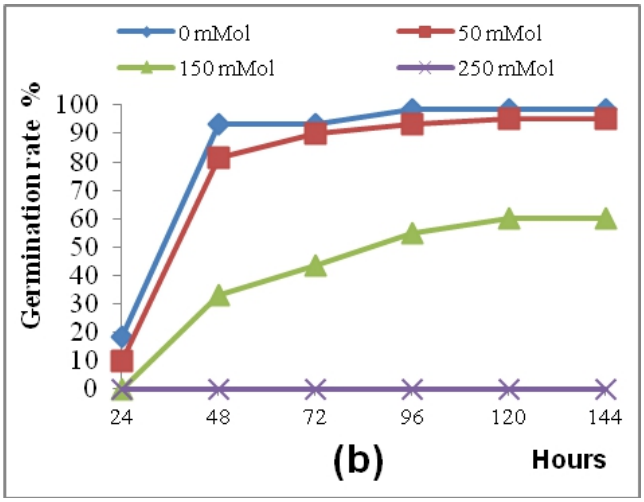
	Source of variation		
	Genotype	**NaCl**	**Interaction genotype* NaCl Treatment**
Germination % (24h)	44.16	904.86**	70.83**
Germination % (48h)	1250.62**	21657.08**	409.51**
Germination % (72h)	1138.54**	22142.63**	276.31**
Seedling Shoot Length	22.9	220.17**	5.75
Seedling Root Length	9.95**	88.64**	2.38**
Seedling Fresh Shoot Weight	0.003	0.61**	0.002
Seedling Fresh Root Weight	0.00015	0.016**	0.00014

Table2. The effect of salinity levels on Seed Germination percentage of different lentil genotypes. Values with the same letter are not significantly different using LSD tests at 5%.

	0 mM NaCl	**50 mM NaCl**	**150 mM NaCl**	**250 mM NaCl**
Siliana	98.33j	95ij	85ghi	15bc
Kef	98.33j	95ij	60de	0a
Ncir	100j	70ef	56.66d	6.66ab
Nefza	100j	90hij	76.66fg	21.66c
Loc. Oues.	100j	96.66j	80fgh	16.66bc
Mean	99.33	89.33	71.66	11.99



(a)


0 mMol
50 mMol
150 mMol
250 mMol
Germination rate %
100
90
80
70
60
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40
30
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10
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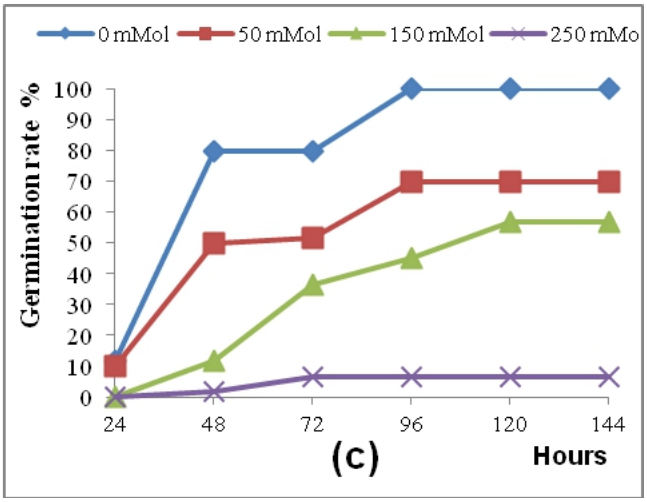
24
48
72
96
120
144
(b)
Hours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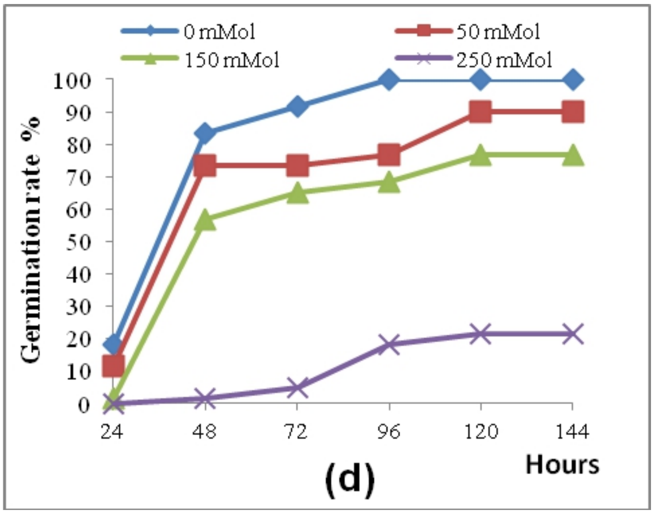
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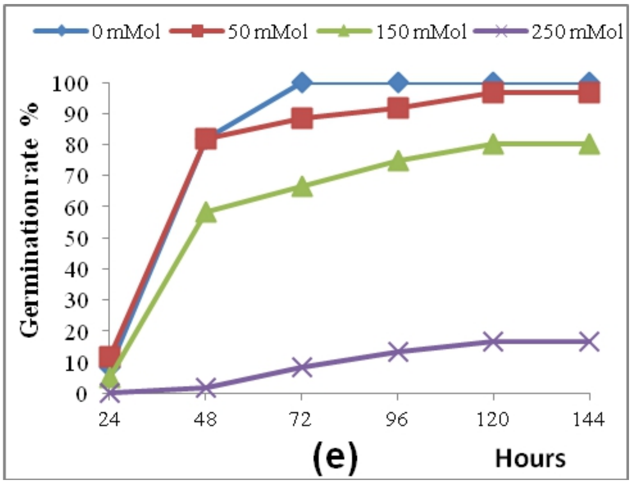

Figure 1: Germination rate in control (0mMol NaCl) and in salt stress conditions (50, 150 and 250 Mmol NaCl) of five lentil varieties studied (a: Siliana, b: kef, c: Ncir, d: Nefza, e: Loc. Oueslatia).

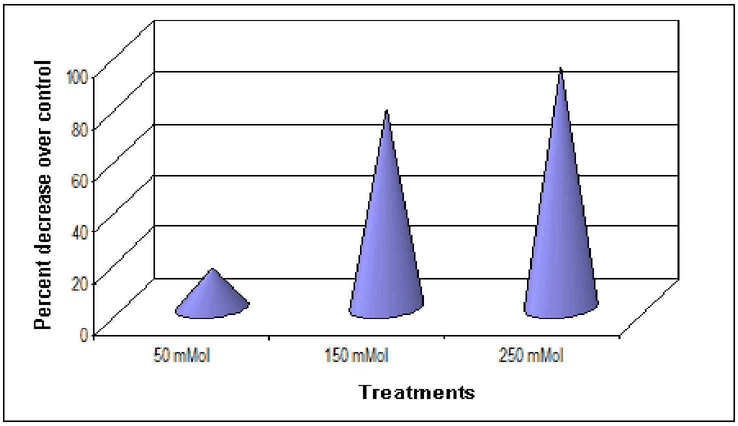

Figure 2: Effect of different levels of NaCl on shoot length.

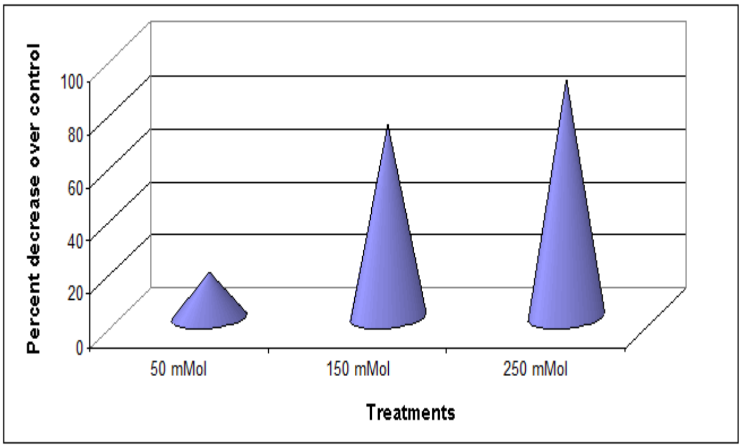

Figure 3: Effect of different levels of NaCl on root length.

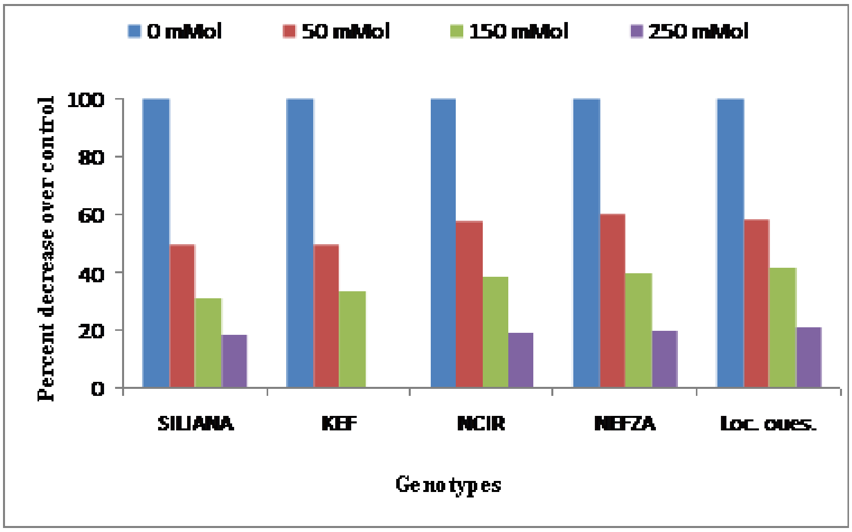

Figure 4: Effect of different levels of NaCl on root fresh weight.

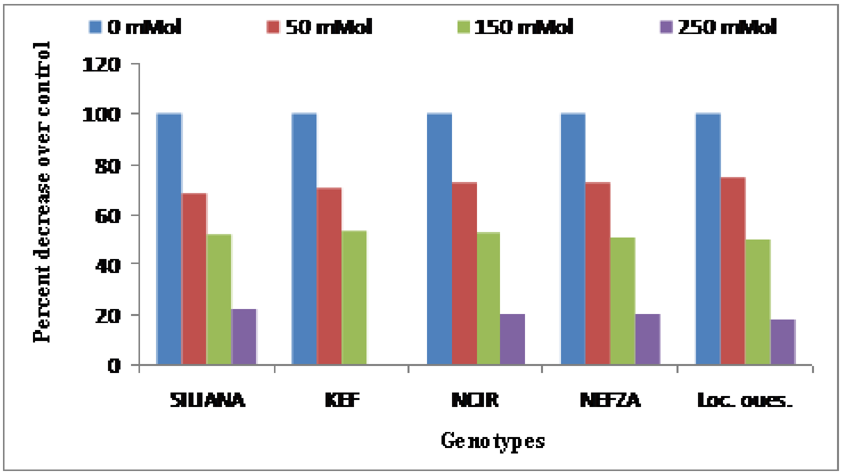

Figure 5: Effect of different levels of NaCl on shoot fresh weight.

Conclusion

This study demonstrated that lentil seed germination varied according to the change in NaCl level. Therefore NaCl has direct harmful effects on lentil seed germination.

In general, it can be concluded that under control condition (no salt stress) all five genotypes of lentil had good growth. But they showed different response to higher levels of salinity (150 mMol). However, salinity reduced all germination properties of lentil cultivars, especially seed germination. These results indicate that genetic variation exists among lentil genotypes in terms of germination percentage under salt stress condition. Under sever salt stress, Siliana, Local oueslatia and Nefza genotypes were the most tolerant genotypes which can be suggested for cultivation under salt stress condition. The germination tolerance of plant species to salinity under laboratory conditions does not necessarily correlate with their response to salinity under Field conditions.

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