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Research Paper

Effect of salt stress on the germination of chickpea (*Cicer arietinum* L.)

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Abstract

In order to understand the mechanisms involved in plant tolerance to salinity, experiments are carried out on ten genotypes of chickpeas (*Cicer arietinum* L.), to compare their germination behavior at different concentrations of NaCl (0 mM, 68 mM and 170 mM), the measurements carried out have concerned the earliness, kinetics, the daily and final germination rate and the length of the radical. For this last parameter, the results were analyzed statistically to select the most tolerant varieties. Results suggest that the final rate of germination of most of the genotypes studied is not affected by salinity, whereas for the parameter length of the radical the effect of salinity is significant.

Keywords: chickpea (*Cicer arietinum* L.), genotypes, salt stress, NaCl, tolerance.

Introduction

Abiotic stress is a major factor around the world in limiting plant growth and productivity[1,2]. Exposure of plants to a stressful environment during various developmental stages appears to induce various physiological and developmental changes[3]. Salinization of water and soil is considered a major limiting factor in agricultural production in several Mediterranean countries, including Algeria. In order to enhance saline areas and/or areas with only brackish water resources, it is essential to select genotypes capable of developing and producing in these areas. From an agronomic point of view, the work on stress should aim to provide solutions to better define technical routes, facilitate varietal selection processes (better target the desired phenotypes), identify relevant genetic traits and make their binding possible in genotypes with good agronomic performance[4].Chickpea is one of the most important grain legumes traditionally cultivated in deprived areas and saline soils[5]. This work proposes an analysis of the effects of salinity at increasing concentrations of NaCl on the germination of ten genotypes of chickpea "*Cicer arietinum* L.", by studying the precocity, the final, the kinetics of the germination and the length of the radicle.

Material and methods

The study area

Experiments were carried out at the National Institute of Agronomic Research, a western research unit of Sidi bel abbés.

Plant material and experimental device

Vegetal material

Ten (10) genotypes of chickpea were used in this experimentation, whose origins are different, introduced from INRA France, ICARDA, and ICRISAT, and the locals are recovered from the farmers and their multiplication took place at the INRAA of Sidi Bel Abbes, are used in this e. (Table 1)

Table 1: The origin of the genotypes studied in this experiment

Origins	ICARDA	ICRISAT	INRAA FRANCE	LOCAL
Genotypes	ILC 482	MARRON	INRA 199	ZOUAOUI
	ILC32-79	(ICC 4107)		BENAZZOUZ
	ILC 607			REGUIG
	ILC 603			BELKHADEM

ICARDA: the international center of agricultural research in the dry areas
ICRISAT: international crops research institute for the semi-aride tropics.
INRAA: the national institute for agricultural research of Algeria

Preparing of watering solutions and seeds for germination

Concentrations of watering solutions are as follows: 0 mM, 68 mM and 170 mM are prepared with distilled water and NaCl.

A germination test was conducted under different concentrations of sodium chloride. The 10 seeds are disinfected with 8% sodium hypochlorite and then rinsed with distilled water, then germinated in 10 cm diameter Petri dishes, between two layers of filter paper, it is humidified with 15 ml of solution.

Figure 1: the experimental germination device

The parameters studied

Precociousness of germination: It is expressed by the rate of the first seeds germinated corresponding to the time interval between the seedling and the beginning of the emergence of the radicle through the membrane[6].

Final germination rate: It is expressed by the ratio of seed germination to total seed number.

Germination kinetics: It most often represents the evolution of cumulative germination percentages over time. This kinetics is based on the cumulative germination rates, that is to say, the variation in germination rates with time expressed in days under all test conditions[7].

The length of the radical: Measurements of the length of the radicle are made on the last day of germination using millimeter paper.

Figure 2: The effect of Salinity on the length of the radical.

Statistical processing
Results obtained on three repetitions are statistically analyzed using the 5% NEWMAN KEULS test to assess the effect of salinity on the genotypes of chickpea.

Results and discussion

The precociousness of germination
The rate of seeds germinated after 24 h is shown in the figure below.

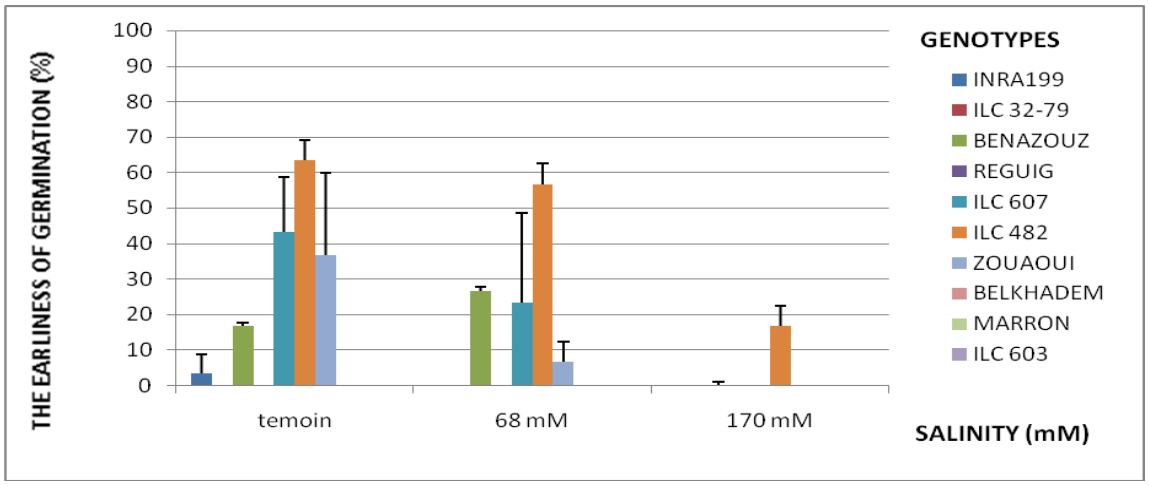


Figure 3: Effect of salinity on the earliness germination of chickpea seeds

After 24 hours the ILC 482 genotype is the earliest under the three treatments, the decrease is clear and is explained by the effect of salinity, for the genotypes: ILC 607 and ZOUAOUI rates are lower, and only 3.33% of seeds germinated in the absence of salt for the INRA199 genotype.

For other BELKHADEM, MARRON, ILC 603, REGUIG and ILC 32-79, no seed germinated after 24 hours, this lack of germination recorded shows that there is a difference in germination power.

The final rate of germination: The final rate is recorded after the stability of the number of seeds germinated.

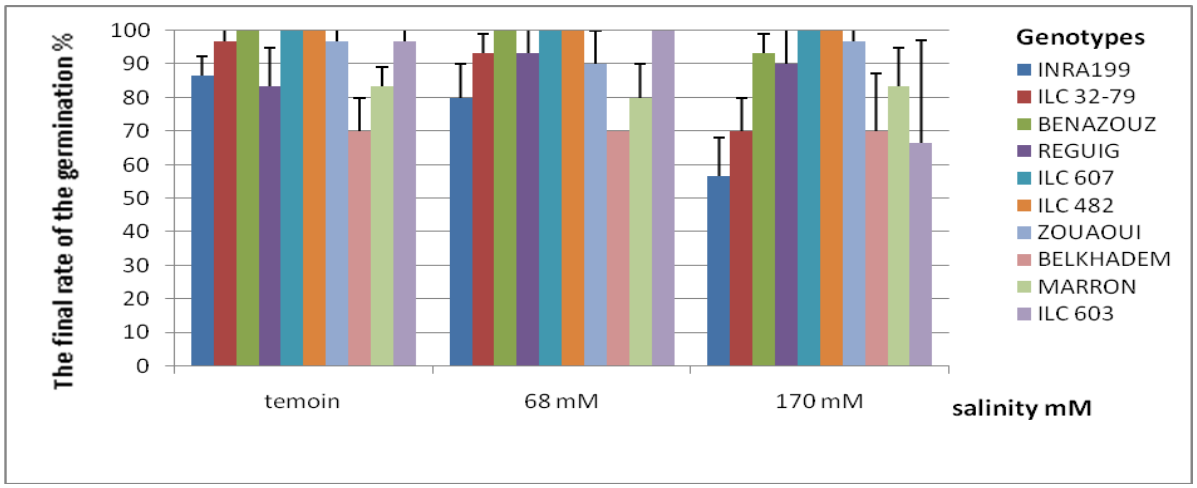


Figure 4: Effect of salinity on final rate of germination of chickpea seeds

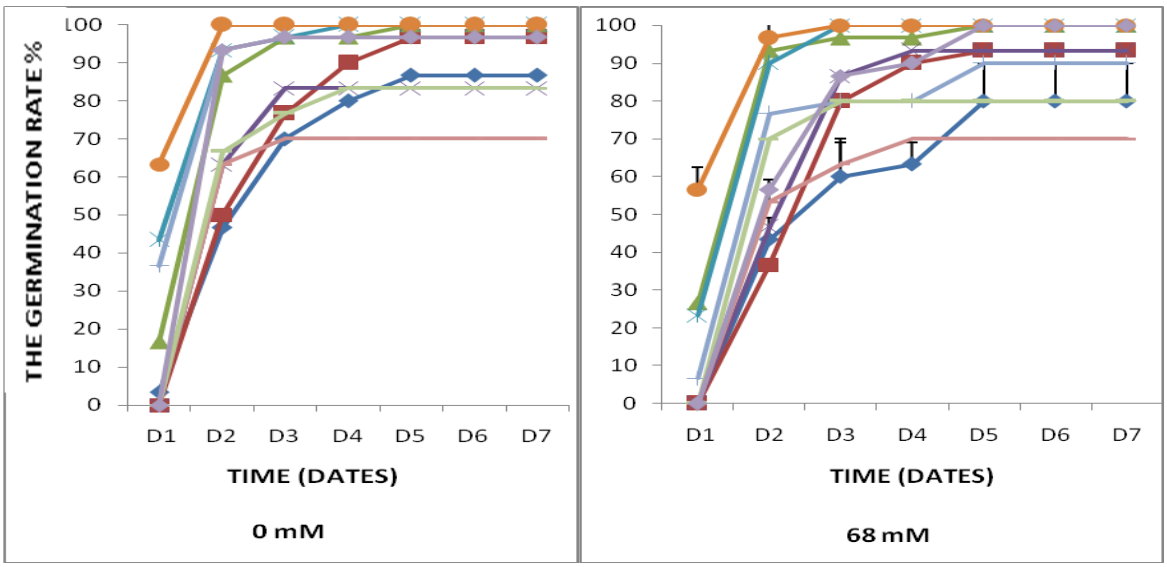
After seven days of germination, the BENAZOUZ genotype reached 100 % under the 03 salt treatments, the genotypes INRA 199 and ILC 603 had the lowest rate, the values were 56 and 66% respectively and these genotypes have low germinative power under the concentration 170 mM of NaCl.

For the genotypes BELKHADEM, ILC607 and ILC482 the rate remains the same under the three concentrations, located between 70 and 100%, respectively. For the genotypes, REGUIG and MARRON, the values are between 80 and 96 to 93% under the concentration 170 mM of NaCl treatment, which can be explained by the fact that salinity has no effect on the final rate of these genotypes.

The germination kinetics

The effect of salinity on germination kinetics is shown in the figure 5.

Figure 5 shows that germination duration is different from genotype to genotype. Differences are observed within the same genotype under different levels of salt stress. Germination curves distinguish three phases. A latency phase, needed to appear the first germinations, during which the germination rate remains low. The duration of this phase varies with NaCl concentration, it is short, in the order of 24 hours in the control plants and those cultivated in 68 mM of NaCl but, it becomes more or less long, especially in the plants subjected to the treatment 170 mM of NaCl, for which this phase can go up to 36 hours.



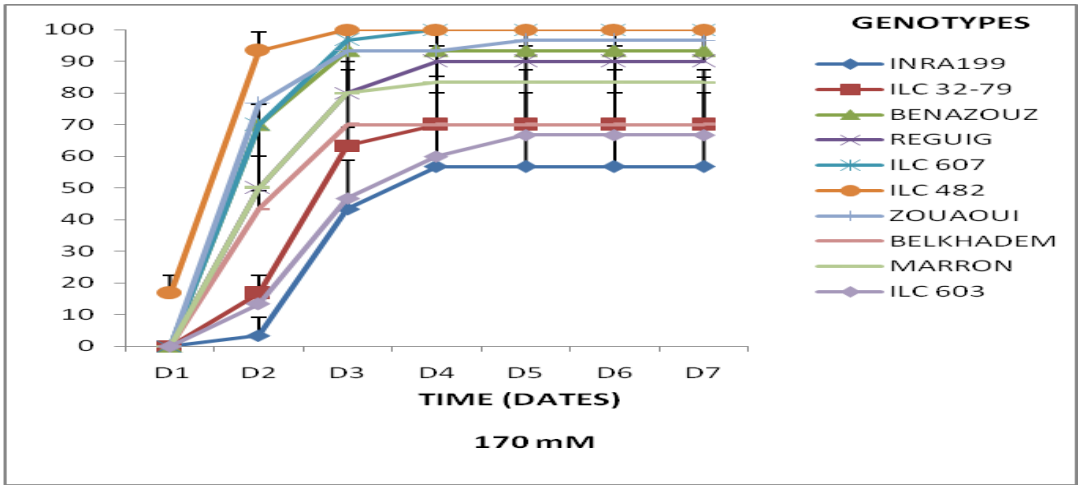


Figure 5: effect of salinity on germination kinetics

For the last day after germination only the genotypes INRAA199, BENAZOUZ, ILC 482, and ZOUAOUI germinated. The BELKHADEM genotype presents the same duration of this phase for the three treatments, we deduce that the salinity has no effect on the germination kinetics of this genotype. It can be seen that under the 170 concentration, the latency phase is longer, only the ILC 482 genotype germinated after 24 hours, and its final rate is not affected by the salinity with the ILC 607 genotype, while for the others genotypes there was a decrease in the final rate of the germinated seeds with respect to salinity.

The length of the radical

The length of the radical was measured on the last day of the experiment and the results are shown in Table 2.

Table 2: Analysis of variance for the parameter

	The length of the radicle		
genotypes/ treatments	**witness**	**68 mM.l^{-1}**	**170 mM.l^{-1}**
ILC482	6,033	5,436	1,416
ILC 607	5,0966	4,52	1,263
BENAZOUZ	5,1633	3,773	1,183
ZOUAOUI	3,9766	2,176	1,05
MARRON	3,0366	1,116	0,433
ILC603	2,9933	1,61	0,383
REGUIG	1,9233	1,186	1,253
INRAA199	3,3333	1,64	0,483
ILC 32-79	3,81	1,22	0,586
BELKHADEM	1,79	0,716	0,693
O.A		2.32	
C.V		25.0%	
S.D		0.58	
Genotype effect		HS	
Salt stress effect		HS	
Interaction f1*2		HS	

O.A: Overall average, C.V: Coefficient of variation. S.D: Standard of deviation, mM.l^{-1} : millimolar per liter.

The results of the variance analysis for the radical length parameter are highly significant (Table 2).

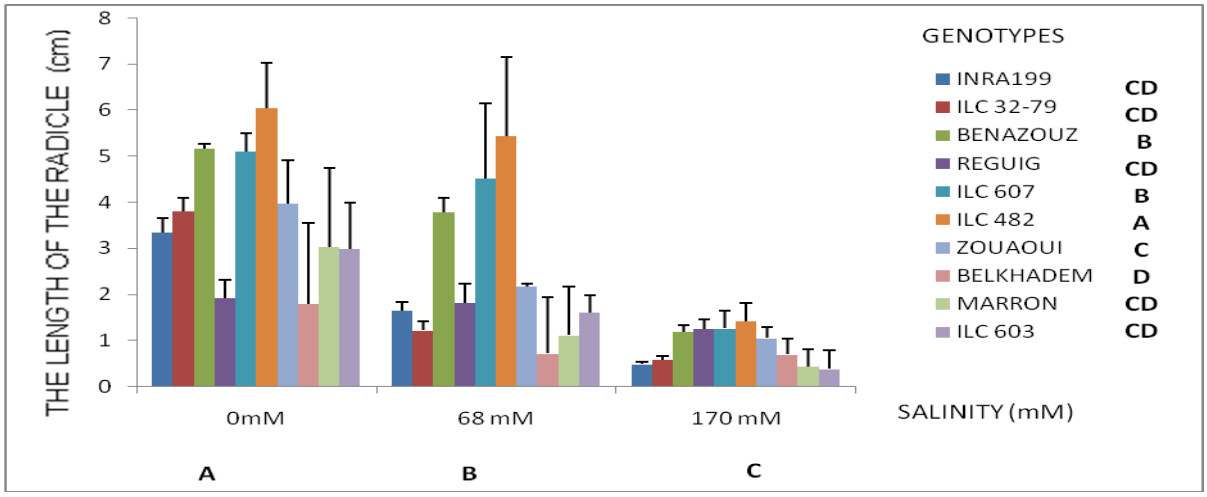


Figure 6: Effect of salinity on the length of the radicle of chickpea genotypes

According to the figure, the different concentrations belong to the 03 different homogeneous groups, which means that the length of the radical decreases by increasing the concentration of NaCl in the irrigation solution.

Whereas for the genotype factor we can distinguish that the genotypes are divided into five homogeneous groups. The ILC 482 genotype has the highest length of the radicle, followed by the two ILC607 and BENAZOUZ genotypes which belong to the same homogeneous group, the ZOUAOUI genotypes belongs to the third homogeneous group with an average of 2.29 cm. The difference between the five genotypes MARRON, ILC603, REGUIG, INRAA199 and ILC 32- 79 is not significant. The BELKHADEM genotype belongs to the last homogeneous group whose length of the radicle is the smallest (1.07cm).

Statistical analysis of the Radicle Length parameter shows that the most tolerant genotypes can be selected during the germination stage are: ILC 482, ILC 607, BENAZOUZ and ZOUAOUI respectively.

On germination, the results obtained have demonstrated the effect of salinity by reducing the number of first seeds germinated, this can be explained by the fact that salinity affects all germination processes, following the decline of water potential around seeds, which makes water inaccessible to the latter, for rehydration and the resumption of active life of the embryo[8].

Germination started rapidly in the absence of salt, but was slower under 170 mM, so the delay before first seed (latency phase) appeared increased with NaCl concentration. This delay in seed germination of all genotypes with increasing NaCl concentration is explained by the time it takes for the seed to put in place mechanisms to adjust its internal osmotic pressure[9] demonstrated that NaCl has adverse effects on water transport in radicle cells. In addition, NaCl affects the permeability of the plasma membrane by increasing the influx of external ions and the efflux of cytosol solutions[10]. According to the work of Levigneron et al. 1995 and Levingneron et al. 1995 germination of potato tubers may be delayed by 3 to 7 days depending on the degree of salinity of the soil[11,12]. The authors reported that seed sensitivity during germination is mainly due to the effect of salinity on reserve mobilization[7,13,14]. The slowdown in the mobilization of reserves is due either to the delay in activation or synthesis of hydrolases or to the inhibition of the transfer of products from hydrolysis from endosperm to embryo.

For the final rate of germination, the results showed the genotypic response of germination to saline stress. It is noted that in medium containing 68 mM sodium chloride, germination capacity for most genotypes is not affected and the recorded values are close to the values obtained in control plants. For the BELKHADEM genotype the final rate under the three treatments is 70% which can be explained by the fact that its germination power is low.

The final rate for seven genotypes (BELKHADEM, ILC607, ILC482, MARRON, BENAZOUZ, REGUIG and ZOUAOUI) is not affected by salinity. Our results are consistent with Duar 1992[15], which showed

that the final germination rate of the chickpea genotypes studied had not decreased with increased salinity. For the other three genotypes ILC32-79, ILC603 and INRAA199, the final rate is affected, this decrease in the final rate with an increase in saline concentration is either an increase in external osmotic pressure, which affects the absorption of water by seeds and/or the accumulation of K+ and Na+ ions in the embryo[16]. The results also indicate that some ecotypes of *Trifolium isthmocarpum*, *Trifolium cherleri* and Lotus. *Ornithopodioide* germinated at the highest concentrations of NaCl with a 50% reduction in germination rate from their controls[17]. According to Taffouo et al. 2008, germination rate may be used as an early criterion for selecting salinity-tolerant legumes[18].

The effect of salinity on the growth of the radicle is significant, for the genotype LC482 its final rate of germination is not affected by salinity, but the length of the radicle is affected as in all other genotypes, this parameter suggests that all genotypes germinate better in the absence of salinity. According to Gomes et al. 1983 the emergence of the radicle would be controlled by the osmolarity of the medium during germination, while the subsequent growth of the seedling would be limited by mobilization and transport of the reserves to the embryonic axis[19]. Such a situation may be explained by the fact that salt stress significantly affects the growth of the root system[20]. In line with the results of[21], which found that in the sunflower genotypes studied, salt significantly reduced root length and volume with a greater reduction in root volume. Later for Arous 2009, in the bean (*Phaseolus vulgaris* L), the results showed large variations in length across the different saline treatments; generally the highest length values were found at the control lot level[22]. Results for this latter parameter show that chickpea is a plant susceptible to the action of NaCl at germination at salt concentrations of 170 mM, which are interesting indicators for further elucidating the relationship of salt stress and seed behavior of all 10 genotypes of chickpea.

Conclusion

Based on our results, we can conclude from our experiment that:

- The results of our study of the different parameters reveal intraspecific variability in salinity tolerance among the different varieties studied.
- Salinity does not influence the final germination rate for most genotypes, but it has a much greater influence on its duration and the length of the radicle.
- It is interesting to move towards a selection of the varieties of tolerant or salinity resistant from the germination stage.
- This study allowed for the classification of tolerant and sensitive genotypes for different traits and the assessment of genetic variability.

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