

Evaluation of Algerian Cowpea genotypes for salt tolerance at germination stage

¹Fahima NABI, ¹Assia Chaker-Haddadj, ¹Sihem Tellah, ²Abdelkader Ghalem, ¹Ghania Ounane, ¹Naima Ghalmi, ³Réda Djebbar and ¹Sidi Mohamed Ounane

¹Laboratoire "Amélioration intégrative des Productions Végétales (AIPV), Département de production végétale. École Nationale Supérieure Agronomique (ENSA), Avenue Hassan Badi, 16004, El Harrach, Alger Algérie.

²Département de chimie, Université Saad Dahleb, Rue de Soumaa Soumaa, Blida, Algérie.

³Laboratoire de Biologie et Physiologie des Organismes, faculté des Sciences Biologiques. Université des Sciences et Technologies Houari Boumediene (USTHB). BP 32. El Alia. 16025 Bab Ezzouar Alger (Algérie)

Address For Correspondence:

Fahima NABI, Laboratoire "Amélioration intégrative des Productions Végétales (AIPV), Département de production végétale. École Nationale Supérieure Agronomique (ENSA), Avenue Hassan Badi, 16004, El Harrach, Alger Algérie.
Phone : +213 (0) 556 39 05 01; E-mail: fahimanabi@yahoo.com

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ABSTRACT

The lack of information about the effects of salinity on germination of Algerian cowpea prompted us to study the salt tolerance levels of five populations (EK1, TZ7, A17, B23 and IS13) of cowpea *Vigna unguiculata* (L.) Walp. The objective was to determine genotypic differences among Cowpea accessions in terms of salt stress. In this study, Daily and final germination percentage, as well as salt tolerance index and speed of germination of the seeds of 5 cowpea landraces were determined under various salt (NaCl) stresses with electrical conductivities of T0 (control, distilled water), T1 (4 dS m⁻¹), T2 (8 dS m⁻¹), T3 (12 dS m⁻¹), T4 (16 dS m⁻¹), T5 (20 dS m⁻¹) for 8 days under controlled conditions. Statistical tests were performed on the various parameters studied to highlight the relationship between the salt stress and the germination of cowpea. Germination percentage, final germination percentage and speed of germination decreased significantly with increased NaCl concentration (from EC 12 dS m⁻¹). Genotypic responses were significant for all germination traits, which were all generally retarded by salt stress. Salt total and salt ranking tolerance indices grouped the cowpea landraces into tolerant (EK1, TZ7 and B23) and susceptible ones (A17 and IS13). Final germination percentage of EK1, TZ7 and B23 has been reduced significantly from 16 dS m⁻¹ (TZ7) and 20 dS m⁻¹ (EK1 and B23). Based on the results of the experiment, The tolerant genotypes can be considered for inclusion in Cowpea breeding programme and in future genetic studies for salt tolerance.

KEYWORDS: Salinity, landraces, Maghreb, *Vigna unguiculata*, germination

INTRODUCTION

Salinity is one of the major abiotic stresses for crop production in many parts of the world [2] including the south of Algeria. These regions are predominantly arid to semiarid and are affected by salinity due to low rainfall, high evapotranspiration and saline groundwater. With the persistent drought in these regions, the surface water availability for irrigation has declined and groundwater is increasingly used by farmers for irrigation. Djili et al. [12] indicated that the salinity, which was initially lower than 2 dS/m in surface horizons, increased to more than 12 dS/m after five irrigation, they showed that secondary salinisation is a factor of soil degradation, substantially reducing crop yields. Due to the salinization and reduction of available water resources, growers in the south of Algeria facing great challenges to even grow crops. Therefore, identifying alternative crops that are salt tolerant is imperative for sustaining some economic viability for growers in this region of Algeria.

The accumulation of soluble salts in soils leads to an increase in osmotic pressure of the soil solution, which may limit the absorption of water by the seeds or by the plant roots. Salt damage to plants is attributed to the reduction in water availability, toxicity or specific ions, and nutritional imbalance caused by such ions [16]. However, the magnitude of the effect of salinity varied with the plant species, type and level of salinity. There are interspecies, intraspecies and intercultivar variations, and even individual lines differ at different ontogenetic stages to salt tolerance, which provides scope for selection of genotypes for salt tolerance [35].

Earlier studies of many crop, including *Vigna unguiculata*, suggests that salt tolerance is a developmentally regulated, stage specific phenomenon such that tolerance at one stage of plant development may not be correlated with tolerance at other developmental stages [28;15]. Therefore, specific stages throughout the ontogeny of the plant, such as germination and emergence, seedling survival and growth, and vegetative and reproductive growth, should be evaluated separately during the assessment of germplasm for salt tolerance. Such assessments may facilitate development of cultivars with salt tolerance characteristics throughout the ontogeny of the plant [2]. Screening and selection for any characters are desired at the earliest developmental stage possible [31].

Germination of seeds, one of the most critical phases of plant life [35], is greatly influenced by salinity [43]. Salinity may cause significant reductions in the rate and final percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields [16]. Ashraf and Foolad [2] proved that genetic information regarding seed germination and related traits could help improve seedling emergence in saline soils through breeding programs. Rapid, uniform, and complete germination is a prerequisite for successful transplant production and stand establishment in vegetable crops [16].

Cowpea (*Vigna unguiculata* L. Walp.) is a species widely grown for different purposes, for example as a fodder crop for livestock, as a green vegetable and as a dry bean [39]. The cultivated area of cowpeas worldwide was estimated as being 14 million ha in 2000 [36] with the most production being in Africa in the Sudanian Savanna zone and the Sahelian zone. Most of the areas where cowpeas are grown are semi-arid [23]. Cowpea, is reputed to be the most drought and heat resistant crop in semi-arid Africa [21] and tolerate low soil fertility due to their high rate of nitrogen fixation [23]. However, production is constrained by susceptibility to biotic and abiotic stresses and the absence of improved cultivars [40]. According to the divisions for classifying crop tolerance to salinity, cowpea is a moderately sensitive crop [27] but exhibits greater salt tolerance during later stages of plant growth [28]. Therefore, cowpea can play an important role in agricultural development, particularly in the Algerian Sahara where the drought and salinity frequently limit crop production. The effect of salinity on the germination, vegetative growth, or yield of cowpea has been studied [39;28;30,31;20;40]. However, there are no data about the salinity tolerance of current cowpea accessions from Algeria. For this reason, the main aim of the present study is to provide the first information on the tolerance of cowpea during germination stage to salinity changes and to establish their possible range of salinity tolerance and information about the promising and diverse genotypes which may help to execute further research on breeding programs for genetic improvement of cowpea for saline environment. Therefore, the present study was undertaken to evaluate the effect of salinity on seed germination in 5 genotypes of Algerian cowpea and further to assess their genotypic variation.

MATERIAL AND METHODS

1. Plant materials :

Seeds of 5 cowpea landraces were collected from several areas across Algeria during years 2003, 2004, and 2005 [19]. The seeds were conserved *ex situ* (seeds dried and stored at -20°C and multiplied in a field collection) at the "Institut Technique des Cultures Maraichères" (ITCM Staouali, Algiers). Based on the locations in which they were collected, the cowpea landraces were assigned the following names: EK1, TZ7, A17, IS13 and B23 native respectively of El Kala, Tizi Ouzou, Adrar, In Salah and Bechar. The selection of cultivated accessions was based on contrasting seed size, colour and origin. Some agromorphological features of cowpea landraces, as well as their geographical and climatic distribution within the Algerian territory, are reported in table 1. Kabylia is a region in the North of Algeria, part of the Tell Atlas located at the edge of the Mediterranean Sea. Its climate is sub-humid with a temperate winter and rainfall >700 mm/year. Local soils, which are composed of clay-sand to marl, are often degraded on the slopes. El Kala is located in the extreme Northeast of Algeria in a triangle of coastal plains and hills bounded by the Mediterranean Sea and the Tunisian border. The climate of El Kala is humid to sub-humid with mild winters with annual average precipitation of 910–1,300 mm. Several soil types are present: marshes, boggy soil, dunes, and wet meadows that become dry in the summer. The oases of the Sahara are characterized by a hyper-arid climate with cold winters and very hot, dry summers. Seasonal variation may be huge, from over 50°C during summer days, to temperatures below freezing during winter nights. Daily variation is also very important. Not only does the Sahara receive very little rain, but precipitation events are also extremely irregular, ranging from 1 to 180 mm/year with a high degree of evapotranspiration. Local soils are mainly comprised of sand.

2. Methods:

2.1. Germination experiments:

Seeds were surface sterilized in 5% sodium hypochlorite solution for 5 min, subsequently washed with distilled water and air-dried before being used in the germination experiments to avoid fungus attack. Petri dishes (9 cm diameter) containing two disks of Whatman No. 1 filter papers were prepared. Three replications of 10 seeds of the landraces EK1, TZ7, A17, IS13 and B23 of cowpea (*Vigna unguiculata* L. Walp), were submitted to germination test in germinator at $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and $80\% \pm 1\%$ of relative humidity, according to a totally randomized design.

These seeds germinated in "germitest" papers imbibed in distilled water (H_2O) or in sodium chloride (NaCl) solutions in a proportion of 2,5 times the weight of the paper [10]. The NaCl concentrations were 0, 4, 8, 12, 16 and 20 dS m^{-1} , which electrical conductivity (EC, dS m^{-1}) was measured with a conductivimeter. Petri dishes were tightly sealed using parafilm to prevent evaporation; thus minimizing changes in concentration of salt solutions. seeds were considered to be germinated with the emergence of the radical [9].

Germination parameters calculation:

The final germination percentage (FGP) was calculated by using the following equation:

$$\text{FGP} = N_g/N_t \times 100 \quad (1)$$

N_g is the number of the germinated seeds on the last day of counting (day 8) and N_t is the total seeds in each treatment.

The speed of germination (SG, without unit), was calculated using the equation below, as described by Maguire (29), and Shahba *et al.* (34), respectively.

$$\text{SG} = \sum i [g_i - g_{(i-1)} / i] \quad (2)$$

g is the total germination percentage on an incubation day i , minus the total germination percentage on the previous day $g_{(i-1)}$, and divided by the incubation day i .

The Salt tolerance index (STI) status of each genotype was determined as a ratio of germination response (mean value) under salt stress to control condition [18] (3)

2.2. Statistical analyses :

The experiments were carried out in factorial completely randomized design (CRD). Factor-1: Genotypes having 5 levels and Factor 2: Salinity having 6 levels with three replications where each replication consisted of 10 samples. The statistical analyses of germination data were done using IBM statistics SPSS 21 software for Windows, for analyse of variance, and means were separated by least significant difference (LSD) using SPSS statistical software. The averages followed by the same alphabetical letter are not significantly different with the threshold from 5%.

Results:

The anova for final germinal poucentage, speed of germination and salt tolerance index showed that all factors (salinity and genotype) and all interactions of factors were significant at $P < 0.05$ (Table 3).

Five cowpea landraces showed different germination percentages (Figure 1, Table 2) and speed of germination (Figure 2) in both non-treated control and treatments with salinity. For non-treated control, the germination percentages were as follows: 100% for the landraces EK1, TZ7 and B23, which is achieved the third day and the fourth day for A17. For the population IS13 the final germination pourcentage (81, 47%) is attained the seventh day (Figure 1). IS13 showed a significant lower ($P < 0.05$) final germination pourcentage at non-treated control than the other landraces, while no significant differences were observed among the other three landraces. The speed of germination for non-treated control showed a similar trend with germination percentages, as shown in Figure 2. The speed of germination (mean and SD) were as follows: B23 ($0,46 \pm 0,01$) > TZ7 ($0,46 \pm 0,03$) > EK1 ($0,44 \pm 0,02$) > A17 ($0,33 \pm 0,03$) > IS13 ($0,28 \pm 0,02$). IS13 and A17 showed a significant lower ($P < 0.05$) speed of germination than the other landraces while no significant differences were observed among the other three landraces.

For the salinity treatments (Figure 1), seed germination showed three effects of increasing salinity in the germination medium. However, the magnitude of these effects varied among landraces At salinities 4, 8 and 12 and dS/m , there was a delay in germination (Figure 1) but no significant reduction in final germination percentage. However, both 12 and 16 and 20 dS/m^{-1} significantly ($P < 0.05$) delay and reduced final germination percentage (Table 2). Germination was completely inhibited in the présence of higher salt concentrations.

The first effect of salt stress is noted for EC of 4, 8, 12, 16 dS/m^{-1} in EK1 and B23, EC of 4, 8 and 12 dS/m^{-1} in TZ7, EC of 4 and 8 dS/m^{-1} in A17, EC of 4 dS/m^{-1} in IS13 respectively.

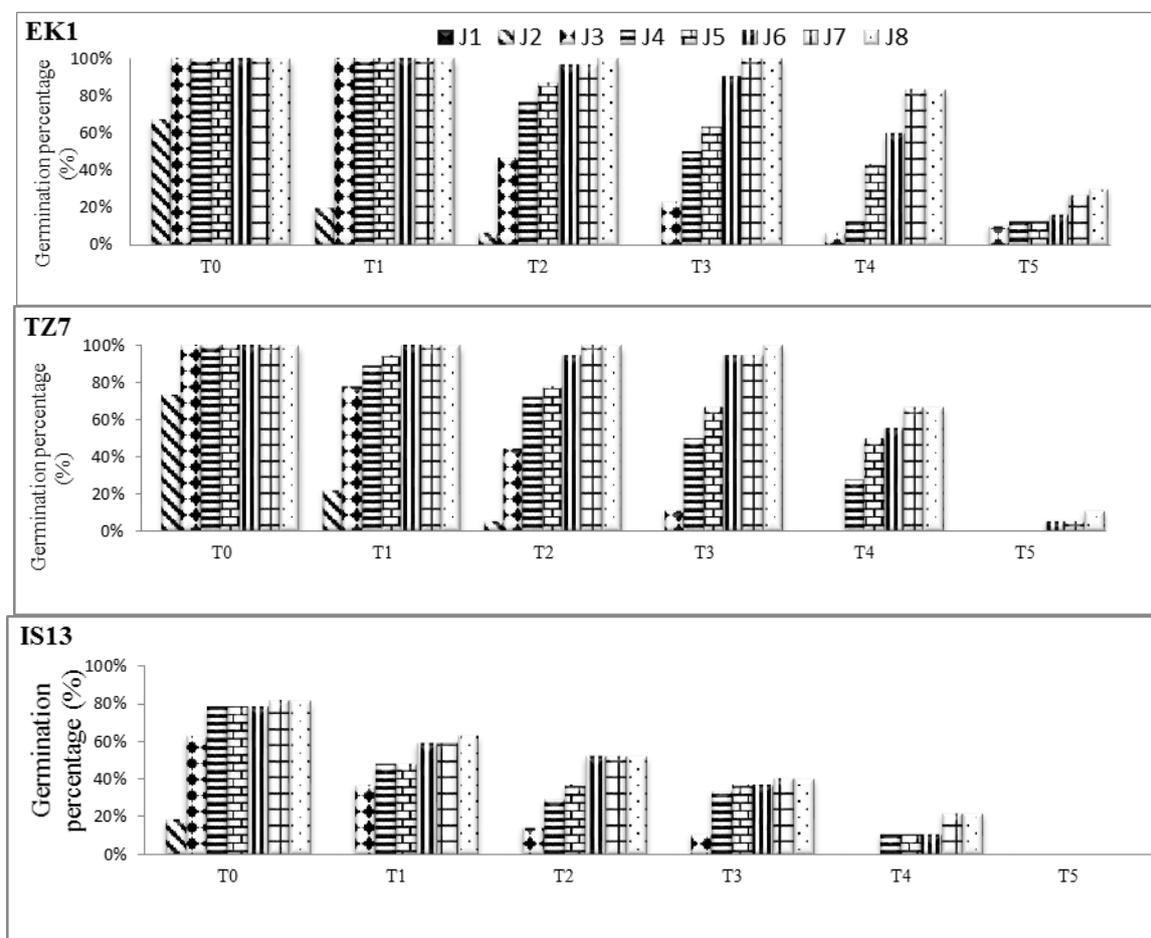
The second effect is showed in EC of 20 dS/m^{-1} in EK1 and B23, EC of 16 dS/m^{-1} in TZ7, EC of 12, 16 and 20 dS/m^{-1} in A17 and EC of 4, 8, 12, 16 and 20 dS/m^{-1} in IS13 respectively. The delay in germination was very obvious for IS13 and A17, with the first seeds only germinating respectively on day 3 and 5 at 12 dS/m^{-1} .

In most of the genotypes completion of germination at higher salt concentrations (16 dS/m^{-1}) was also delayed to 3 day (EK1,) and 4 day (TZ7, IS13 and B23) and 7 day (A17) from the start of incubation.

Germination percentages declined sharply with 12, 16 and 20 dS/m^{-1} treatments (Table 2). At these NaCl concentrations, differences among the genotypes were significantly different. TZ7, EK1 and B23 had final germination percentages relatively high with the 16 dS/m^{-1} treatment (83,33%, 66,66% and 83,33), while IS13 and A17 had lower germination percentages with the same NaCl treatment (22,22% and 5,56%). The germination percentages of EK1 and B23 were significantly ($P < 0.05$) reduced with the 20 dS/m^{-1} and TZ7 with the 16 dS/m^{-1} . IS13 showed a significant lower ($P < 0.05$) final germination percentage at all level of salt stress than the other landraces.

These results showed that 12 and 16 dS/m^{-1} treatments can be used effectively to identify sensitive and highly resistant genotypes respectively (Table 2). The third effect of salt stress was observed for EC of 20 dS/m^{-1} in all landraces, germination of A17 and IS13 was completely inhibited at this salt concentrations. Responses of cowpea speed of germination to salinity showed a similar trend with germination percentages, as shown in Figure 2.

Concerning the salt tolerance index of genotypes (Table 2). The salt tolerance index varied between 0,39 and 1 with T3, 0,06 and 0,83 with T4, 0 and 0,30 with T5. EK1 (1), TZ7 (1) and B23 (0,83) were the best performing genotypes with the T3 treatment; The tolerance indices of genotypes with lower performances than EK1, TZ7 and B23, were above 0,50 (IS13) and 0,39 (A17) with the T3 treatment. EK1 (0,83), TZ7 (0,67) and B23 (0,83) were the best performing genotypes with the T4 treatment; the other genotypes IS13 and A17 did not perform well their salt tolerance indices were respectively 0,06 and 0,27 with the T4 treatment.



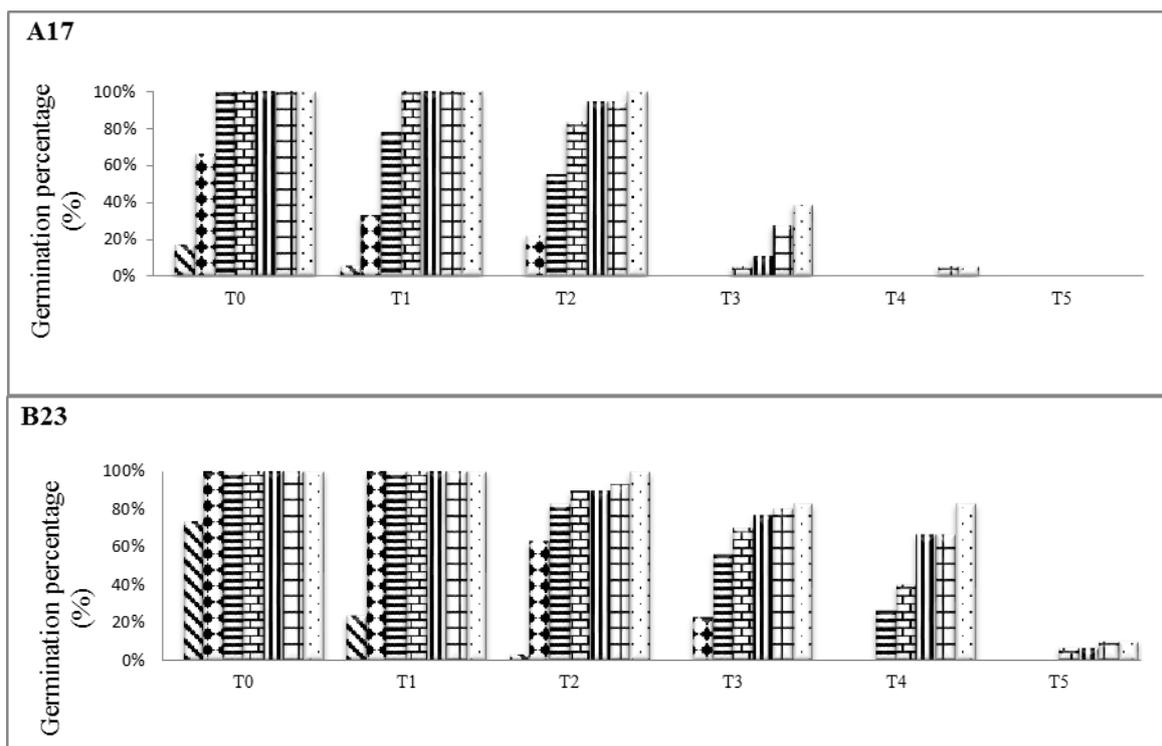
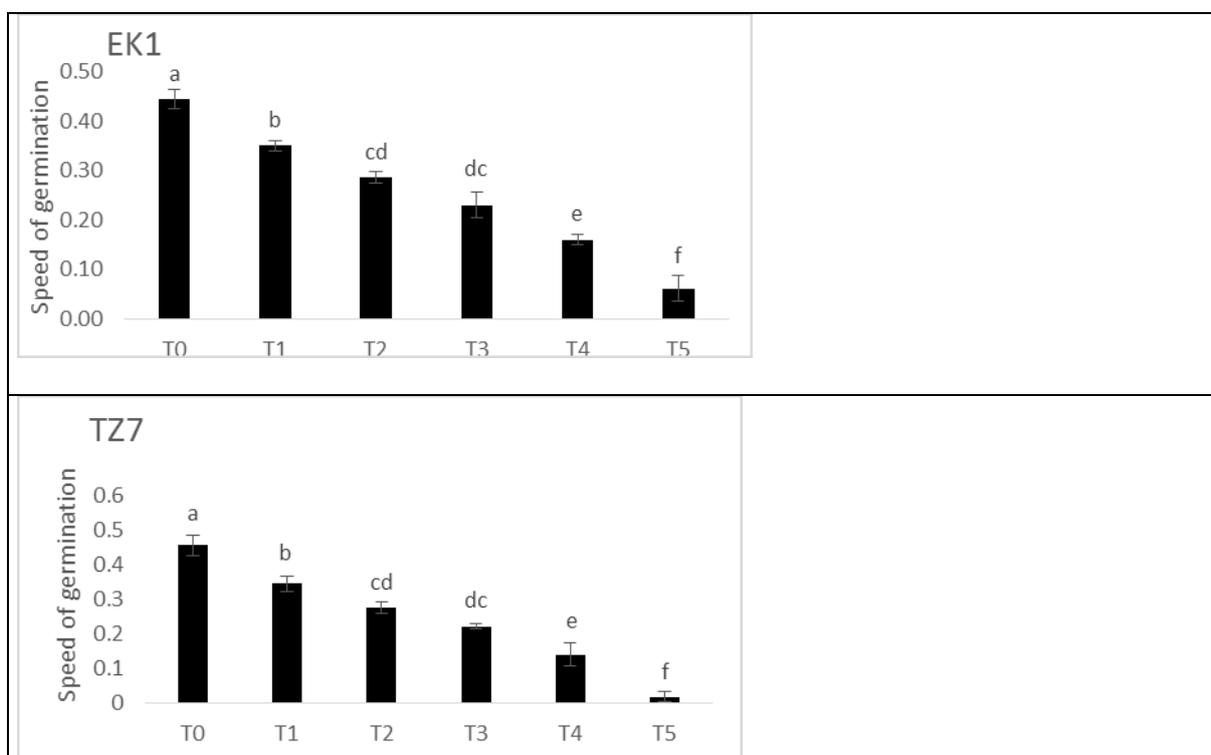


Fig. 1: Salt stress effects on germination of cowpea seeds for 8 days. Values represent the mean of three replicates. T0 : control, T1 : EC= 4 dS m⁻¹, T2 ; EC= 8 dS m⁻¹, T3 :EC = 12 dS m⁻¹ et T4 :EC = 16 dS m⁻¹, T5:EC = 20 dS m⁻¹ (DAY)



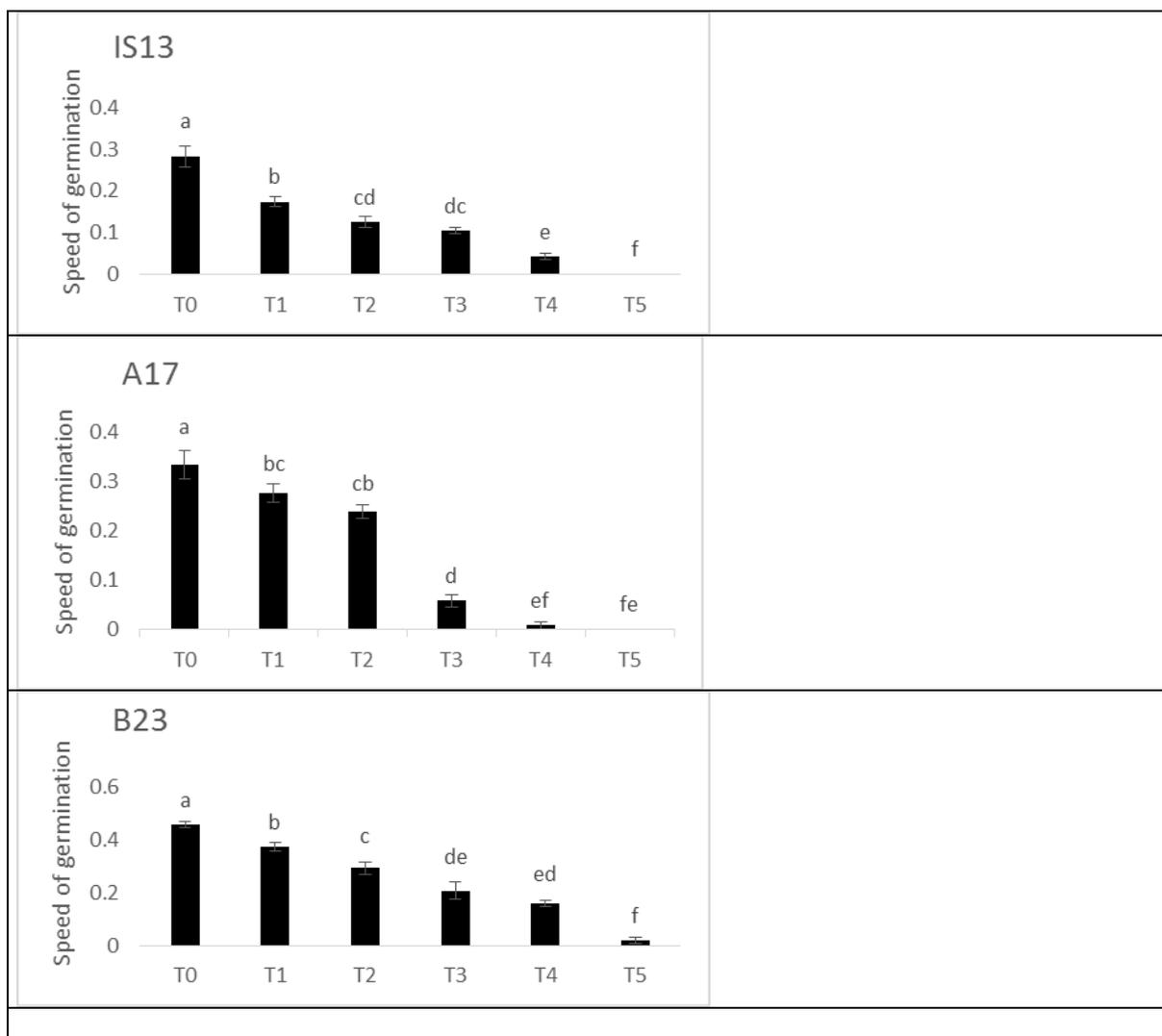


Fig. 2: Speed of germination of 5 cowpea landraces under different salinity. Values represent the mean of three replicates and error bars represent standard deviation. Columns containing same letters indicate no significant difference among treatments; T0 : control, T1: EC= 4 dS m⁻¹, T2 ; EC= 8 dS m⁻¹, T3 :EC = 12 dS m⁻¹ et T4 :EC = 16 dS m⁻¹, T5:EC = 20 dS m⁻¹

Table 1: Some Morphological characters of Algerian cowpea landraces, and their geographical location.

Accession	Geographical origin	Longitude	Latitude	Altitude (m)	Seed colour	Eye colour	Seed weight (g 100 seed)
EK1	Tonga Ouest, El Kala	08°31E	36° 53N	2.2	white	brown	23,43
TZ7	TiziOuzou, Kabylie	03°95E	36°54N	344	white	black	29,20
IS13	In Salah, Sahara	02°30 E	27°14 N	265	black	Absent	7,28
A17	Bouda, Adrar, sahara	00°11W	27°49N	279	cream	Absent	8,66
B23	El Ouata, Bechar, sahara	01°83W	29°86N	419	cream	Absent	9,87

Table 2: Final germination percentage (PGP) and salt tolerance index (STI) of 5 cowpea landraces grown with different NaCl treatments (for 8 days).

Genotypes	Salt concentration (dSm ⁻¹)										
	T0	T1		T2		T3		T4		T5	
	FGP (%)	FGP (%)	STI	FGP (%)	STI	FGP (%)	STI	FGP (%)	STI	FGP (%)	STI
EK1	100±00aA	100±00aA	1,00Aa	100±00aA	1,00Aa	100±00aA	1,00Aa	83,33±6,67aA	0,83aA	30,00±15,28aB	0,30aB
TZ7	100±00aA	100±00aA	1,00Aa	100±00aA	1,00Aa	100±00aA	1,00Aa	66,66±16,67aB	0,67Ba	11,11±11,11aC	0,11Ca
IS13	81,47±7,41bA	62,96±3,70bB	0,77bA	51,85±7,41bB	0,64ABb	40,74±3,70bB	0,50Bb	22,22±6,41bC	0,27Bb	0±0bD	0,00Cab
A17	100±00aA	100±00aA	1,00Aa	100±00aA	1,00Aa	38,89±11,11bB	0,39Bb	5,56±5,56bC	0,06Cb	0±0bC	0,00CbC
B23	100±00aA	100±00aA	1,00Aa	100±00aA	1,00Aa	83,33±8,82aA	0,83Aa	83,33±8,82aA	0,83Aa	10,00±5,77aB	0,10Ba

Note: Within each column, the same lower case letter indicates no significant difference among genotypes. Within each line the same capital letters indicates no significant difference among treatments ($P < 0.05$). Les valeurs sont représentées par les moyennes \pm ESM. T0 : control, T1: EC= 4 dS m⁻¹, T2 ; EC= 8 dS m⁻¹, T3 :EC = 12 dS m⁻¹ et T4 :EC = 16 dS m⁻¹, T5:EC = 20 dS m⁻¹

Table 3: Effects of NaCl on the final germination percentage, speed of germination of cowpea seeds and salt tolerance index: summary of anova results

Source of variation	df	Final germination percentage(%)			Speed of germination			Salt tolerance index		
		Mean square	F value	P level	Mean square	F value	P level	Mean square	F value	P level
Salinity	5	16.354,077	131,236	0,000***	2808,961	281,204	0,000***	1,854	116,931	0,000***
Genotype	4	5.730,005	45,981	0,000***	696,914	69,768	0,000***	,454	28,619	0,000***
SalinityXGenotype	20	761,361	6,110	0,000***	36,868	3,691	0,000***	7,521	4,742	0,000***
Error	60	124,616			9,989			1,586		
Total	90									

*** Significant at $P < 0,0001$ **Discussion:**

Acceptable growth of plants in arid and semi-arid lands which are under exposure of salinity stress is related to the ability of seeds for best germination under unfavorable conditions [33]. Thus, screening of genotypes at the early stages may be an important criterion for selecting salt tolerant genotypes, thus saving considerable time. Germination potential of seeds in saline environments could be correlated with the tolerance at later growth stages [2]. However, salt tolerance at early growth stages is not always correlated with that in the following growth stages [5]. In the present investigation, we focused on evaluation of the potential tolerance of cowpea genotypes traditionally grown in different climatic regions of the Algerian Maghreb to salt stress at germination stage.

The results showed that the seed germination significantly delayed under salinity condition in all the genotypes, maximum seed germination occurred in distilled water. The susceptible genotypes required more number of days for germination as compared to those of tolerant genotypes. Increase in salinity levels significantly reduced the germination percentage and speed of germination of the 5 cowpea landraces with varying response.

Several investigations of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development [26].

The delay in germination of cowpea observed in the higher salinity treatments had been reported in Mungbean [33], Tomato [24] and *Origanum* [26]. Kaveh *et al.* [24] indicate that ascending salt concentrations not only prevent the seed germination, but also extend the germination time by delaying the start of germination.

Generally, low salt concentration delays germination by inducing a state of dormancy, and not appreciably reduce the final percentage germination [4]. Meanwhile, high salt concentration inhibits the seed germination and decreases the germination percentage and increases germination time (1 ; 35).

Although external salinity of 4, 8 and 12 dS/m^{-1} NaCl delayed cowpea seed germination, the final percent germination after 8 days was not affected in some genotypes, such as TZ7, EK1 and B23. However, at 16 and 20 dS/m^{-1} there was both a delay in germination and in the final germination percentage (Figure 1, Table2).

Cowpea seeds germination was significantly reduced when electrical conductivity in sand culture exceeded 12 dS/m^{-1} [39], decreasing 30% when exposed to 15.6 dS/m^{-1} [30], decreased when treated with NaCl 100 mol m^{-3} , the equivalent to 10.2 dS/m^{-1} [10]. Similar results were obtained in our study because germination of A17, TZ7 seeds decreased significantly when treated respectively with NaCl 12 and 16 dS/m^{-1} . However, These values are lower compared to those obtained in our study because germination percentage of EK1 and B23 decreased significantly when treated with NaCl 20 dS/m^{-1} . In the present study, all landraces were not able to germinate rapidly under the highest salt stress level germination (20 dS/m^{-1}), germination was completely inhibited in some genotypes (IS13 and A17) at this salt concentrations. This is due to the fact that an increase in salinity causes an excessive negative osmotic potential [38]. Similar results were observed for *Vigna unguiculata* (32 ; 38). Bernstein [7] indicate that this concentration is higher than what is observed in most saline agricultural soils (75-150 mM).

It has been reported by several authors that salt stress can affect seed germination by decreasing the ease with which the seeds take up water (osmotic stress) [25], because the activity and events normally associated with germination are delayed and/or proceed at a reduced rate [41]. Salinity can also affect germination by facilitating the uptake of toxic ions (ion toxicity), which can cause changes to certain enzymatic or hormonal activities of the seed [11].

The first effect of salinity is similar to that shown for *Vigna unguiculata* [39] and may be considered to be purely an osmotic effect as germination was merely delayed. Reduced final germination at higher concentrations of NaCl 16 and 20 dS/m may be an osmotic or toxic effect or a combination of both [39]. Physiological studies to distinguish between the two effects are limited but evidence suggests that low water potential of the germination medium is a major limiting factor [8]. In the context of this discussion, the term salt tolerance during seed germination was used only to refer to situations where the seed germinated rapidly under salt stress conditions. No distinction was made between osmotic and ionic effects of the salinity stress.

Germination failure under saline conditions was also ascribed to oxidative stress induced by salinity [42].

Heavy seeds germinated in greater numbers and more quickly than light seeds [37]. Our results suggest a relationship between salinity tolerance and the seed weight. Seeds of EK1, TZ7 and B23 were heavier than

seeds of IS13 and A17 (Table 1), and it may be one of the reasons why germination for EK1, TZ7 and B23 seeds were greater than for IS13 and A17 seeds at all NaCl concentrations.

On the basis of results of germination percentage and salt tolerance index, our investigation demonstrated genetic variation in seed germination responses to salinity among five cowpea landraces. This study indicated that EK1, TZ7 and B23 had superior germination performance at low, moderate and high levels of salt stress. These landraces are identified as the most tolerant, being able to germinate rapidly under both control (nonstress) and salt stress conditions. These landraces ranked well in final germination percentage, speed of germination and salt tolerance index. Foolad *et al.* [16] indicate that germination processes that facilitate rapid germination under salt and nonstress conditions possibly were controlled by similar genetic and physiological mechanisms. Conversely, the other landraces (IS13 and A17) germinated rapidly under control conditions but germinated poorly at the highest salt stress level, thus exhibiting low salt tolerance index. Thus, in these landraces, the physiological processes required for germination were sensitive to stress [6]. These landraces may be deficient in genetic elements required for coping with salinity [14].

There was a high level of variation in germination percentage under salinity. The differences in germination among the landraces were greater in the presence of salinity in comparison to the absence of salinity. These differences can be attributed to genotypic variation in germination response being expressed to a greater degree under stress than under non-stress conditions [41]. Certain genes may be stress-inducible and expressed only under salt-stress conditions [14].

In conclusion, the results of this study demonstrate that salt tolerance during germination exists within Algerian cowpea landraces. Knowing the difference in salt tolerance between species and genotypes of special importance to ecological and economic aspects, allowing proper agricultural production in regions that are facing this problem. Cultivar EK1, TZ7 and B23 appears to be more tolerant to salt stress than cultivar A17 and IS13. EK1, TZ7 and B23, represent a genetic resource for improvement of salt tolerance of cowpea. The desirable traits governing salt tolerance can be introduced in to the susceptible or recurrent genotypes through breeding for the genetic improvement of cowpea for saline soils in near future. Further studies are recommended 1) screen more salt tolerant Cowpea genotypes, which are suitable to grow in Algerian Sahara soils ; 2) assess whether these genotypes characterized as tolerant based on their responses to salt stress in germination stage, maintain their degree of salt tolerance till crop maturity.

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