



Research article

Influence of salinity on germination and early seedling root growth traits of alfalfa (*Medicago sativa* L.) landraces collected in Southern Algerian oases

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Article Info

Article history:

Received 22 April 2021

Revised 4 November 2021

Accepted 8 November 2021

Available online 20 December 2021

Keywords:

Alfalfa,
Diversity,
Salt tolerance,
Seeds,
Seedling root

Abstract

The perennial forage *Medicago sativa* L. (alfalfa) is a very important leguminous forage crop. Screening of alfalfa landraces to salt tolerance through the seed germination and early seedling root growth stages is crucial for their evaluation. Ten local landraces of alfalfa from Algerian oases and one commercial cultivar (Giulia) were evaluated for germination and early seedling root growth under increasing NaCl salt concentrations (0 mM, 85.6 mM, 171.1 mM, 256.7 mM, 342.2 mM). Five traits were studied: precocity of germination (PG), final germination rate (FGR), mean germination time (MGT), radicle length (RL) and the IC₅₀ which represents the osmotic potential that inhibits 50% of viable seeds. The results showed that there were significant differences among alfalfa cultivars under non-stress and salt stress for all the studied traits, suggesting high diversity among the cultivars. Hassi Laabid had the highest FGR (mean ± SD; 74.53 ± 32.88), whereas Megganine had the lowest FGR (63.20 ± 29.07). For MGT, Megganine had the highest value (3.49 ± 0.94) while Hassi Laabid had the lowest value (2.29 ± 1.15). Giulia had the highest reduction while Guemar had the lowest reduction under increasing NaCl salt concentration. The cultivars with the greatest IC₅₀ values were Janet (-0.77 MPa), Hassi Laabid (-0.74 MPa), Temacine (-0.73 MPa) and In Salah (-0.69 MPa), indicating that they required the greatest osmotic potentials to limit their germination and would potentially be more tolerant to the effect of NaCl during germination and seedling establishment. Correlation coefficients between all possible combinations were estimated and the results indicated that PG, FGR, MGT and RL had significant negative or positive correlations with each other.

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<https://doi.org/10.34044/j.anres.2021.55.6.08>

Introduction

Lucerne or alfalfa, *Medicago sativa* L., often called “The Queen of the Forages”, is a perennial, herbaceous, forage legume cultivated under a wide range of climatic conditions, from oases in North Africa to Siberia, making it the most important leguminous forage crop and the most harvested worldwide (Nordkvist and Åman, 1986). Alfalfa is appreciated for its highly digestible energy and protein production that makes it an extremely valuable feed (Lacefield et al., 2009). In addition, alfalfa is characterized by its relevant agronomic faculty to restore the structure and fertility of the soil (Russi et al., 1997). Furthermore, alfalfa is particularly efficient at improving water infiltration, increasing soil organic matter contents and reducing diseases and weeds (Magdoff and Weil, 2004; Lacefield et al., 2009).

In different parts of the world, especially in dry Mediterranean areas, alfalfa is often confronted with saline stress, where salinization of soils and persistent irrigation result in salinized land (Rozema and Flowers, 2008). The salinity problem is especially dangerous in combination with a lack of water for land irrigation and it has been estimated that at least 20% of all irrigated lands are salt-affected (Pitman and Läuchli, 2002). Furthermore, the frequency and severity of drought events accelerate and spread the processes of salt mobilization and accumulation and these conditions will inevitably threaten the persistence of alfalfa because alfalfa is only moderately salt tolerant (Maas and Hoffman, 1977).

In the Algerian Sahara, alfalfa is by far the main cultivated forage species (Chaabena et al., 2012). For centuries, the farmers in this region have been able to preserve and select local landraces which, according to Trabut and Mares (1906), had impressive yields that no other fodder plant could produce under the same conditions. In a comparative trial between local and introduced alfalfa cultivars in southern Algeria, Bouabboub-Mossab (2001) found that local cultivars were significantly better with an average production of dry matter yield greater than 30 t/ha/yr compared to 20 t/ha/yr for introduced populations with 16 cutting cycles annually against 11 to 12 cuts in the introduced cultivars. In the same context, recent reports have pointed to possible interest in north-African landraces as valuable, salt-tolerant, parent germplasm (Pecetti et al., 2013).

Under soil salinity conditions, every aspect of plant growth can be compromised. Smith (1993) pointed out that germination, seedling growth, and reproduction are stages when alfalfa plants are most sensitive to salinity. In glycophytic

species, germination and seedling development may be among the most salinity sensitive stages (Chang, 1961). Glycophytic seeds and seedlings tend to be negatively affected by osmotic stress as well as ionic toxicity, usually resulting in reduced and delayed germination and seedling growth (Peng et al., 2008; Farissi et al., 2011; Cordeiro et al., 2014; Petrović et al., 2016). Understanding plant responses at the germination and seedling stages is particularly important for elucidating the mechanisms of salt tolerance, sensitivity and survival in plants (Peng et al., 2008).

Many studies have highlighted the significant genetic diversity that exists in alfalfa using different traits (Herrmann et al., 2018; Ahmed et al., 2019; Benabderrahim et al., 2020), and although these different studies have shown that alfalfa is characterized by high inter-population variability, few studies have been reported on the genetic diversity of alfalfa at the germination and seedling growth stages and even less under salt stress. Thus, the current study aimed to assess variability in a set of alfalfa landraces collected from Algerian oases for laboratory germination and seedling establishment in the presence and absence of salt (NaCl). The NAAIC procedure was used, which defined a standard test for assessing cultivar salt tolerance from seed germination under saline conditions (Rumbaugh, 1991). The final objective was to identify genotypes likely to possess adaptations to saline conditions and to select the genotypes most tolerant to salinity.

Materials and Methods

The experiment was carried out during March 2016 at the Plant Physiology Laboratory of the Agronomy Department of the University of Mohamed Boudiaf in M'sila (Algeria). In this experiment, 11 populations were used (Table 1). One population was a commercial cultivar (Giulia) widely used by farmers, while 10 were local cultivars obtained from a local alfalfa producer that originated from different oases in the South Algerian Sahara (Table 1). These populations have been cultivated for many decades and are still widely used by farmers in these traditional agro-ecosystems (Chaabena et al., 2012). All these cultivars have been previously evaluated over a period of 3 yr based on several biometric parameters (Chaabena et al., 2012). In this experiment, cultivars were evaluated for laboratory germination and seedling establishment under increasing levels of salt concentration. Cultivars and salt concentrations were considered as factors and were assessed in a completely randomized design in three replications.

Table 1 Name, label, origin, germplasm type and characteristics of 11 alfalfa cultivars studied

Cultivar	Label	Origin site	Type*	Altitude (m)	Latitude	Longitude
Megganine	P1	Touggourt	Lan	65	33°09'	6°05'
In Salah	P2	In Salah	Lan	275	27°11'	2°28'
Janet	P3	Janet	Lan	1037	24°33'	9°29'
Temacine	P4	Temacine	Lan	78	33°00'	6°00'
Hassi Laabid	P5	El Meniaa	Lan	398	30°37'	2°52'
Nezla	P6	Touggourt	Lan	67	33°05'	6°04'
Touijine	P7	Jamaa	Lan	21	33°32'	6°02'
Tamentit	P8	Sidi Youcef	Lan	242	27°45'	0°15'
Guemar	P9	Guemar	Lan	61	33°29'	6°47'
Chott	P10	Ouargla	Lan	132	31°57'	5°22'
Giulia	P11	Italy	Com	-	-	-

Com = commercial variety; Lan = farm landrace

The experiment was conducted following a modified version of the North American Alfalfa Improvement Conference (NAAIC) protocol, “Salt Tolerance of Germinating Alfalfa Seeds” (Rumbaugh and Pendery, 1990). Seeds were germinated in sterilized Petri dishes containing Whatman No. 2 filter paper moistened with either distilled water (control), or 85.6 mM, 171.1 mM, 256.7 mM or 342.2 mM sodium chloride (NaCl) solution. Each dish contained 4.5 mL of the appropriate salt solution or distilled water that was applied only once at the initiation of the trial. Three replicates of 25 seeds each were used for each treatment. The plates were placed in the dark in an incubator, the temperature was maintained at 25°C and seeds were considered germinated when the radicle had emerged (Ranal and De Santana, 2006). Germinated seeds were counted daily until the end of the germination period of 7 d. To maintain adequate moisture, 3 mL of the original salt solution was added to each Petri dish on the third day. Traits observed were: precocity of germination (PG), final germination rate (FGR), mean germination time (MGT), radicle length (RL) and the osmotic potential that inhibited 50% of viable seeds (IC_{50}).

PG was defined as the rate of seed germination from the first day (Equation 1):

$$PG (\%) = (n / N_t) \times 100 \quad (1)$$

where n is the number of germinated seeds on the first day and N_t is the total number of seeds sown for germination.

FGR was defined as the rate of seeds germinated on the seventh day (Equation 2):

$$FGR (\%) = (N_7 / N_t) \times 100 \quad (2)$$

where N_7 is the number of total seeds germinated at the end of trial and N_t is the total number of seeds sown for germination.

The numbers of seeds germinated with salty treatments were adjusted by dividing the germination in each Petri dish by the germination of the same cultivar in the same replicate at the control (0.0 mM NaCl) treatment level and multiplying by 100 to convert to a percentage (Carlson et al., 1983).

MGT was calculated according to the formula of Ellis and Roberts (1980), as shown in Equation 3:

$$MGT = \sum n \cdot D / \sum n \quad (3)$$

where N is the number of seeds newly germinated at time D , D is the days from the beginning of the germination test and $\sum n$ is the number of final germinants.

RL was measured after seven days of growth.

The mean FGR values were used to develop an IC_{50} value which was used to describe the osmotic potential required to inhibit germination of 50% of viable seeds, as described by Rumbaugh (1991). This was accomplished by converting salt concentrations to reflect osmotic potential measured in megapascals (MPa) according to the NAAIC procedure (Rumbaugh, 1991). Then, the corresponding osmotic potential values were graphed on the x-axis with the average germination percentage on the y-axis for each cultivar, with linear regression modelling used to fit a trendline from which the slope equation was calculated. The IC_{50} value was then calculated using the slope equation, according to Equation 4:

$$IC_{50} = (0.5 - b) / a. \quad (4)$$

where b is the y-intercept and a is the slope.

Data analysis

For statistical analysis, values for PG, FGR, MGT and RL for each cultivar under different salt concentrations were calculated and subjected to two-way analysis of variance (ANOVA) using the R software (R Core Team, 2020). Prior to ANOVA, the arcsine square root transformations of FGR and PG were performed to stabilize the treatment variances (Scott et al., 1984). Fisher's least significant difference (LSD) method calculated using the R software was used to detect significant differences among means. Correlation (Pearson's method) and hierarchical cluster analysis (Ward's method) were performed using the 'factoextra' package in the R software. All tests were considered significant at $p = 0.05$.

Results

Precocity of germination

There was a highly significant effect of NaCl on PG and a highly significant difference between cultivars (Table 2). In addition, the interaction between the two studied factors was highly significant. The evolution of early seed germination from the 11 cultivars, according to different concentrations of NaCl, is shown in Fig. 1, Table 3 and Table S1. Both the figure and tables have been provided for the interaction to best present the information. Germination precocity was retarded under increasing salinity concentration compared to the control. Under the control treatment, seeds started germination after one day with values varying between $40.00 \pm 11.78\%$ and $91.00 \pm 3.46\%$ (Table S1). Whatever the cultivar, the differences in germination began to decrease as the salt concentration increased until there was no more germination (342.2 mM; Table 3, Table S1). The cultivar Giulia (P11) had a later early germination rate in all types of medium,

including the control. However, the cultivar Hassi Laabid (P5) had the highest rate ($p < 0.05$), as shown in Fig. 1 and Table 3. The comparison between cultivars showed that Giulia (P11) had a high reduction rate compared to Guemar (P9), which had a low reduction rate (Table S1).

Final germination rate

The results of ANOVA for the final germination rates showed that there were significant differences between cultivars and between salinity stress levels; however, no interaction effects were found (Table 2). Comparisons of cultivar means showed that all cultivars had significantly higher final germination percentages than the commercial cultivar Giulia (P11), as shown in Table 3. Seed from Giulia (P11) produced only $49.20 \pm 37.03\%$ germination, whereas Hassi Laabid (P5), Janet (P3), Temacine (P4) and In Salah (P2) had germination rates of $74.53 \pm 32.88\%$, $73.66 \pm 28.75\%$, $73.26 \pm 28.73\%$ and $72.40 \pm 31.30\%$, respectively (Table 3).

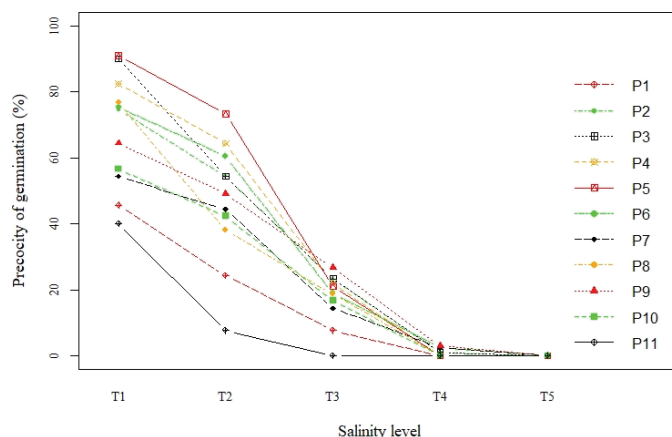


Fig. 1 Interaction effects of NaCl concentration (T1= 0.00 mM, T2 = 85.6 mM, T3 = 171.1 mM, T4 = 256.7 mM, T5 = 342.2 mM) and cultivars (P1–P11, names as indicated in Table 1) on precocity of germination of alfalfa seedlings

Table 2 Results of 2-way analysis of variance, showing effects of salinity levels, cultivars, and salinity levels $\times$ cultivars interaction for germination indices and radicle length

Dependent trait	Cultivars			Salinity levels			Cultivars $\times$ Salinity level		
	DF	F	p-value	DF	F	p-value	DF	F	p-value
Precocity of germination	10	0.179	< 0.001	4	6.178	< 0.001	40	0.039	< 0.001
Final germination rate	10	0.150	< 0.001	4	5.054	< 0.001	40	0.033	0.148
Mean germination time	10	1.883	< 0.001	4	27.652	< 0.001	40	0.054	0.147
Radicle length	10	39.300	< 0.001	4	704.000	< 0.001	40	13.400	0.0024

Table 3 Effects of cultivars and NaCl concentrations on precocity of germination (PG), final germination rate (FGR), mean germination time (MGT) and radicle length (RL)

Cultivar	Traits			
	PG (%)	FGR (%)	MGT (days)	RL (mm)
P1	15.53±18.98 ^e	63.20±29.07 ^c	3.49±0.94 ^a	7.00±4.14 ^b
P2	30.66±31.40 ^{abc}	72.40±31.30 ^{ab}	2.58±1.26 ^{bc}	9.53±6.50 ^a
P3	33.73±36.19 ^{ab}	73.66±28.75 ^{ab}	2.41±1.13 ^{bc}	11.21±7.82 ^a
P4	33.73±36.02 ^{ab}	73.26±28.73 ^{ab}	2.45±1.13 ^{bc}	9.70±5.80 ^a
P5	37.06±39.80 ^a	74.53±32.88 ^a	2.29±1.15 ^c	9.41±6.77 ^a
P6	31.40±33.07 ^{abc}	68.80±29.81 ^{abc}	2.54±1.29 ^{bc}	7.48±3.99 ^b
P7	23.06±23.59 ^d	69.93±32.22 ^{abc}	2.22±0.88 ^c	6.98±3.64 ^b
P8	26.66±30.69 ^{cd}	67.40±33.42 ^{abc}	2.32±0.89 ^c	6.65±3.95 ^b
P9	28.60±26.36 ^{bc}	66.86±30.76 ^{bc}	2.44±1.19 ^{bc}	6.78±3.45 ^b
P10	23.13±24.94 ^d	63.26±31.70 ^c	2.42±0.71 ^{bc}	6.51±3.82 ^b
P11	9.53±16.77 ^f	49.20±37.03 ^d	2.84±1.17 ^b	6.59±5.00 ^b
NaCl Concentration	PG (%)	FGR (%)	MGT (days)	RL (mm)
T1 (0.00 mM)	68.27±18.80 ^a	91.81±7.26 ^a	1.50±0.38 ^c	14.03±4.36 ^a
T2 (85.6 mM)	46.57±20.67 ^b	90.87±8.29 ^a	1.91±0.76 ^d	11.41±3.26 ^b
T3 (171.1 mM)	17.51±9.38 ^c	81.24±12.94 ^b	2.40±0.67 ^c	5.54±1.71 ^c
T4 (256.7 mM)	0.80±1.91 ^d	57.45±20.50 ^c	3.18±0.69 ^b	2.61±0.42 ^d
T5 (342.2 mM)	0.00±0.00 ^d	16.12±11.07 ^d	3.75±1.07 ^a	6.53±4.60 ^c

Cultivars are defined in Table 1. T1–T5 = treatments with different levels of salinity shown in parentheses.

Mean ± SD in a column superscripted with different lowercase letters indicate significant ($p < 0.05$) differences among means within each main effect.

FGR significantly decreased with increasing salinity (Table 3). Comparison between FGR under the control treatment and for the salt media showed that until T2 (85.6 mM) there was only a 10.5% reduction; however, at T3 (171.1 mM) and T4 (256.7 mM), there were reductions of 34.36% and 75.69%, respectively (Table 3).

Mean germination time

The results obtained for MGT showed highly significant differences between cultivars (Table 2). In addition, there were significant differences between salinity stress levels (Table 2). The maximum mean germination time was obtained at 342.2 mM. The cultivars Touijine (2.22 ± 0.88 d), Hassi Laabid (2.29 ± 1.15 d) and Tamentit (2.32 ± 0.89 d) had the lowest mean germination times despite the increasing salinity of the medium, while the cultivar Megganine (P1) had the highest mean germination time (3.49 ± 0.94 d), as shown in Table 3.

Radicle length

The ANOVA analysis showed there was a highly significant effect of NaCl on RL and a highly significant difference between cultivars (Table 2). In addition, the interaction between the two factors was significant. The comparison between cultivars according to different concentrations of NaCl is shown in Fig. 2, Table 3 and Table S1. In the control treatment, the highest value of radicle length was observed in cultivars Janet (P3; 21.44 ± 1.72 mm) and In Salah (P2; 18.83 ± 2.92 mm), while the shortest were the commercial cultivar Giulia (P11; 10.53 ± 0.97 mm) and Guemar (P9; 10.11 ± 0.30 mm), respectively (Fig. 2, Table S1). After application of salt stress, radicle elongation was greatly inhibited in salinities over 85.6 mM of NaCl, and the radicle length declined to below half that of the control (Table 3). Except for P11 (Giulia), which was substantially suppressed at 342.2 mM of NaCl, the salinity level that most affected the radicle length was 256.7 mM, with a reduction of over 80% (Fig. 2, Table 3, Table S1). Despite the salinity level among the tested alfalfa cultivars, the cultivars with the longest radicles were In Salah (P2), Janet (P3), Temacine (P4) and Hassi Laabid (P5), as shown in Table S1.

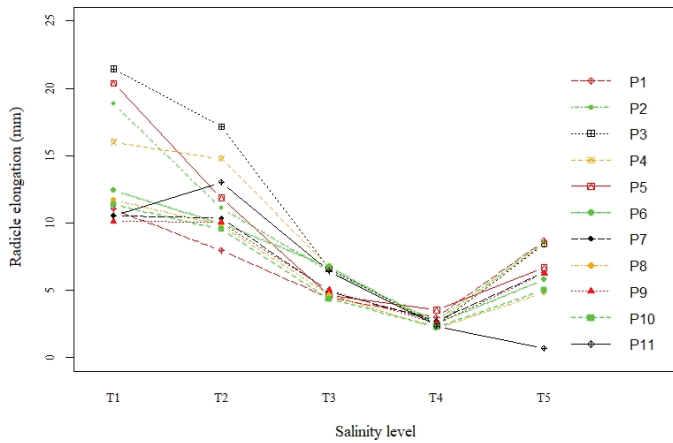


Fig. 2 Interaction effect of NaCl concentration (T1 = 0.00 mM, T2 = 85.6 mM, T3 = 171.1 mM, T4 = 256.7 mM, T5 = 342.2 mM) and cultivars on radicle elongation of alfalfa seedlings (P1–P11, names as indicated in Table 1)

IC₅₀ values

The IC_{50} values that provided a clear indicator of the osmotic potential that inhibited 50% of viable seeds (Table 4). The cultivar with the greatest IC_{50} value was Janet (P3) at -0.77 MPa. Of the 11 cultivars, 8 had IC_{50} values greater than -0.5 MPa, indicating that they required a high osmotic potential to limit their germination and would potentially better tolerate the effect of NaCl during germination (Table 4). On the other hand, the lowest performance was exhibited by the commercial population Giulia (P11) and the land race Touijine (P7), with values of -0.38 MPa and -0.25 MPa respectively (Table 4).

Correlation

There were negative significant correlations between precocity of germination (PG) and mean germination time (MGT) under the control treatment and salinity levels of 85.6 mM and 171.1 mM (Table 5). There were also negative significant correlations between MGT and the final germination rate (FGR) at salinity levels of 85.6 mM and 171.1 mM (Table 5). In contrast, there were positive significant correlations between PG and FGR under the control treatment and the salinity level of 171.1 mM. Furthermore, radical length (RL) was positively correlated with FGR under the control treatment and at the salinity level of 342.2 mM (Table 5).

Classification of alfalfa cultivars

Hierarchical cluster analysis indicated the cultivars could be arranged into three groups (Fig. 3). The first cluster included four cultivars that originated from In-Salah (P2), Janet (P3), Temacine (P4) and Hassi Laabid (P5). The cultivars in this group had on average the best results for precocity of germination, final germination rate and radicle length as well as lower mean germination times. The second cluster was made up of five cultivars: Nezla (P6), Touijine (P7), Tamentit (P8) Guemar (P9) and Chott (P10). This group was distinguishable by intermediate values for all the studied traits. The third cluster contained the cultivar Megganine (P1) and the commercial cultivar Giulia (P11). These last two had the lowest mean values for precocity of germination, final germination rate and radicle length and had higher mean germination times.

Table 4 Osmotic potential for 11 alfalfa cultivars

Cultivar	Label	IC_{50} osmotic potential		Linear regression line equation	<i>p</i> -value
		(-Mpa)	%		
Janet	P3	-0.77	1.70	$y = 0.73x + 1.06$	0.026
Hassi Laabid	P5	-0.74	1.64	$y = 0.82x + 1.10$	0.047
Temacine	P4	-0.73	1.62	$y = 0.73x + 1.03$	0.026
In Salah	P2	-0.69	1.54	$y = 0.80x + 1.05$	0.034
Nezla	P6	-0.68	1.51	$y = 0.75x + 1.01$	0.062
Tamentit	P8	-0.61	1.36	$y = 0.85x + 1.01$	0.034
Guemar	P9	-0.59	1.32	$y = 0.79x + 0.96$	0.019
Megganine	P1	-0.55	1.23	$y = 0.75x + 0.91$	0.007
Chott	P10	-0.52	1.17	$y = 0.82x + 0.93$	0.006
Giulia	P11	-0.38	0.86	$y = 0.97x + 0.86$	0.002
Touijine	P7	-0.25	0.87	$y = 0.74x + 0.95$	0.040

IC_{50} = osmotic potential that inhibits 50% of viable seeds

Table 5 Correlations between measured traits under control treatment and salinity conditions

Traits	T1 (Control)				T2 (85.6 mM)			
	PG	FGR	MGT	RL	PG	FGR	MGT	RL
PG	1.00				1.00			
FGR	0.89***	1.00			0.58	1.00		
MGT	-0.72*	-0.44	1.00		-0.79**	-0.64*	1.00	
RL	0.80	0.85**	-0.60	1.00	0.26	0.11	-0.45	1.00
Traits	T3 (171.1 mM)				T4 (256.7 mM)			
	PG	FGR	MGT	RL	PG	FGR	MGT	RL
PG	1.00				1.00			
FGR	0.87***	1.00			0.30	1.00		
MGT	-0.76**	-0.76**	1.00		-0.54	-0.46	1.00	
RL	0.10	0.005	-0.04	1.00	-0.076	0.43	-0.27	1.00
Traits	T5 (342.2 mM)							
	PG	FGR	MGT	RL				
PG	1.00							
FGR	-	1.00						
MGT	-	0.57	1.00					
RL	-	0.83**	0.59	1.00				

*, **, *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

Discussion

Salt tolerance is critical in the germination and establishment stages of plant development (Smith, 1993). The current results showed clearly that in the germination stage, there were significant differences among the studied alfalfa cultivars regarding their ability to germinate under saline or non-saline conditions, suggesting the existence of high diversity among the cultivars and inhibitory effects of salt stress on seed germination traits. Genetic diversity in *Medicago sativa* has been well documented, but most studies have focused on post-emergence traits, such as morphological traits (Julier et al., 2007; Herrmann et al., 2018) and biomass production (Maamouri et al., 2017; Benabderrahim et al., 2020). However, few studies have reported the diversity in germination under salt stress (Rumbaugh and Pendery, 1990; Benabderrahim et al., 2011) and in the early seedling establishment stage (Soltani et al., 2012). According to Meyer and Allen (1999), seed germination depends on several associated factors such as genotype and the germination environment. The seed behavior of several species under different salt concentrations showed that they reached maximum germination in non-saline conditions and are very sensitive to high salinity in the germination and seedling establishment stages (Carlson et al., 1983; Khalil et al., 2011; El Naim et al., 2012).

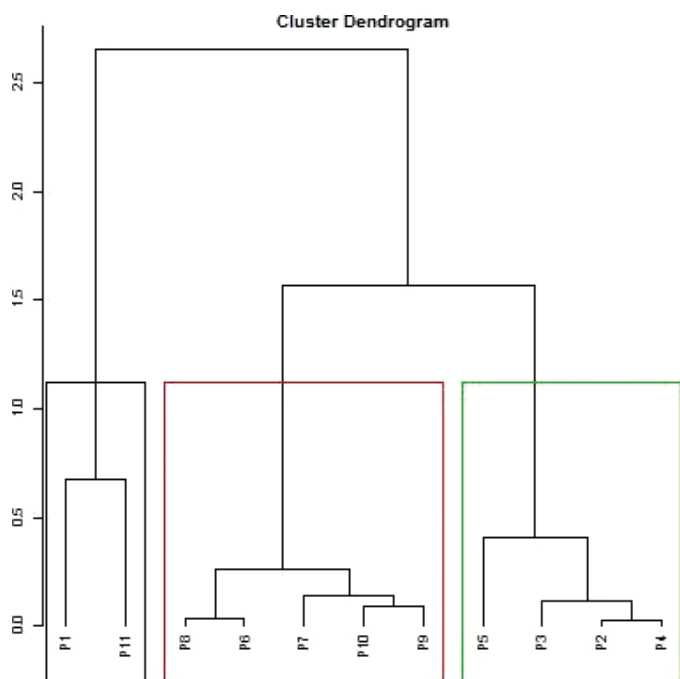


Fig. 3 Cluster analysis classifications of 11 cultivars determined based on Euclidian distance on four measured traits (precocity of germination, final germination rate, mean germination time, radicle length), where P1–P11 are cultivar names as indicated in Table 1, green box indicates salt-tolerant cultivars, black box indicates sensitive cultivars and red box indicates intermediate cultivars

In the current study, there was a considerable reduction in germination at high salinity levels. This decrease can be attributed to the ability of seeds to tolerate the decreased osmotic potential of the germination media or an ability to cope with the toxic effects of NaCl, or a combination of both effects (Allen et al., 1985). According to Redmann (1974), the osmotic effect of NaCl on the germination of alfalfa was more important than the toxic effect. In the same context, Allen et al. (1986) observed that there was a significant increase in the Na^+ and Cl^- ions in seeds of two alfalfa cultivars (Mesa-Sirsa and AZST, 1982) as the NaCl concentration of the germination media increased. The entry into the seed of Na^+ and Cl^- ions in high concentrations could potentially be toxic and affect embryo viability and therefore, germinability (Esechie, 1993).

In the current study, the precocity of germination and final germination rate traits were negatively affected by increasing NaCl salt levels. These results agreed with those reported by Pujol et al. (2000) and Cokkizgin (2012) on common beans, who observed that an increase in salinity induced both a delay in the initiation of the germination process and a reduction in the final percentage of germinating seeds. This was also confirmed by the correlation analysis in the current study, which showed a positive and highly significant correlation between precocity of germination and final germination rate. In other similar studies, Farissi et al. (2011) with *Medicago sativa* and Yakoubi et al. (2019) with Okra (*Abelmoschus esculentus* L.), found that tolerant genotypes had earlier and higher germination.

The increased saline concentration of the medium had a significant effect on the mean germination times among ecotypes, so that when seeds were exposed to different salinity levels, they took longer to germinate, which decreased the water potential (Verslues et al., 2006). This was also shown in the results of other research (Alom et al., 2016; Arias et al., 2018). According to Miyamoto et al. (1985), a faster germination rate would allow emerging seedlings to avoid prolonged contact with the surface layer of saline soil. The cultivar Megganine (P1) in contrast to all the other cultivars, had the highest mean germination time (3.49 d), exceeding the time reported by Soltani et al. (2012) in a similar experiment (2.9 d). In the current study, a negative phenotypic correlation was found between the mean germination time and final germination rate. This was also reported in the results of Cokkizgin (2012) with common bean and by Fuller et al. (2012) with wheat.

The comparison between cultivars at different salinity levels indicated that radicle length was always higher in

non-saline water than at any NaCl concentration. The radicle growth of the 11 varieties declined with increasing salinity and the critical concentration for radicle growth was 256 mM NaCl. Similar results were reported by Soltani et al. (2012) for 20 alfalfa cultivar and four levels of salinity stress, where at a concentration of 225 mM NaCl, only the beginning of primary root protrusion was noticeable. However, at 350 mM, all the landraces except for the commercial cultivar Giulia, showed improvement in radicle length, which was unexpected. Similar results were found by Shen et al. (2003) with *Suaeda salsa*. According to Galvan-Ampudia and Testerink (2011), in highly saline environments, early root development has a positive impact on the water absorption capacity, which in turn buffers against saline-induced osmotic stress. A significant positive correlation was found between radicle length and both precocity of germination and final germination rate traits. Some authors have also observed a correlation between seed and seedling salinity tolerance (Donohue et al., 2010; Cordeiro et al., 2014; Nunes et al., 2019), which indicates that rapid screens for germination or early radicle-related growth traits in a saline environment may offer a simple means of identifying genotypes likely to possess adaptations to saline conditions (Cordeiro et al., 2014).

According to the NAAIC procedure that defined a standard test for assessing cultivar salt tolerance from seed germination under saline conditions (Rumbaugh, 1991), cultivars with an IC_{50} value greater than approximately 1.6 % NaCl should be considered as salt-tolerant, while those with an IC_{50} value lower than approximately 1.45 % NaCl would be as salt susceptible. In the current investigation, the land races Janet (P3), Hassi Laabid (P5) and Temacine (P4) exceeded this threshold value for stress tolerance, while In Salah (P2) and Nezla (P6) had intermediate values. All other populations had lower IC_{50} values, indicating susceptibility. In a similar experiment that measured the salt resistance of 761 alfalfa plants from different centers of diversity during germination, Rumbaugh and Pendery (1990) found that populations from more arid African centers were better able to tolerate NaCl during germination, while the mean IC_{50} value reported by Rumbaugh and Pendery (1990) was approximately -0.73 MPa that was in the vicinity of the mean IC_{50} value measured in the current study (-0.62 MPa). In other similar research, Pecetti et al. (2013) described the cultivar Tamentit (P8) as being a tolerant population ($\text{IC}_{50} = 1.78\%$), whereas in the current study, Tamantit had an IC_{50} value of 1.36%. According to Smith and Dobrenz (1987), salt stress affected the germination percentage in aged seed differently to fresh seed. In the current study,

this difference could be explained by advanced seed age as well as the storage conditions. Finally, based on the results from the cluster analysis, it can be concluded that the ecotypes of the first cluster (Janet, In Salah, Temacine, and Hassi Laabid) were superior to the others in terms of salt tolerance at the germination stage and at seedling establishment. The cultivar Megganine and the commercial cultivar Giulia were the most sensitive, while the cultivars Nezla, Touijine, Tamentit, Guemar and Chott had intermediate values. However, given the variation among rankings at different salt concentrations, it is premature to determine the most salt-tolerant cultivar based on these data alone. Germination is only one stage of growth in a plant, and the ability of the plant to tolerate saline conditions throughout its life is also critical. Thus, it is vital to further explore the behavior of these cultivars under salt stress before it will be possible to select for increased resistance or sensitivity of salt stress. However, all cultivars should be used in large field trials to confirm their salt tolerance and stability in different multilocal trials.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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