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Comparative Salt Tolerance Study of Some Moroccan Alfalfa Varieties during Germination and Seedling Emergence Stages

Laila Aberchane[1], Khadijatou Taoufiq[1], Khouzaima Elbiari[2], Mustapha Faghire[1]

[1]Laboratory of Vegetal Biotechnology, Department of Biology, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco
[2]Department of Biology, Faculty of Sciences, Abdelmalek Essadi University, Tétouan, Morocco
Email: laberchane@gmail.com

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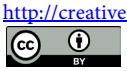


Abstract

The aim of this study was to evaluate and compare the response of seed germination and seedling emergence of three Moroccan alfalfa cultivars (Ifrane, Marocaine and Demnate) and an introduced variety (Siriver) under different NaCl concentrations, in order to explore possibilities of selecting salt-tolerant genotypes. Seeds were kept under salt stress at 7 treatment levels: 0, 50, 100, 150, 200, 250, and 300 mM of NaCl. Seed germination, seedling length, fresh weight, germination speed and other parameters were recorded. The results showed that all germination parameters were significantly reduced for all genotypes as NaCl concentration increased. However, significant interspecific variation for salt tolerance was observed. Indeed, at the highest salt stress level (250 mM NaCl), the Ifrane and Marocaine varieties had higher germination rates (73% and 64% respectively), whereas the Demnate and Siriver varieties had a low germination rate (25% and 13% respectively). Growth parameters such as height and biomass of young seedlings were reduced under salt treatment. The intraspecific variability of lucerne with respect to salinity is significant. However, both in the presence and in the absence of salt, the local germplasm (Ifrane, Demnate and Marocaine) showed the best germination behavior. In fact, the Ifrane genotype was the most tolerant and it exhibited a particular adaptability to the saline environment, at least at these two stages of the life cycle, and could therefore be an interesting asset for the establishment of these plants in irrigated perimeters in arid zones faced with salinity.

Subject Areas

Agricultural Science

Keywords

Salt Stress, Seed Germination, Seedling Stage, Salt Tolerance, Intraspecific

Variability

1. Introduction

The phenomenon of salinisation poses a real threat to global food security, not only reducing the production of many crops, but also reducing the area under cultivation and the quality of crops [1], contributing to increasing poverty and food insecurity around the world [2]. According to FAO [3], salinisation has already affected more than 800 million hectares worldwide, and more than 10% of arable land has become desertified or salinised [4] [5]. On an annual basis, 0.3 to 1.5 million ha of arable land are lost due to salinization, and 20 - 40 million ha are severely affected by salinity [6]. Furthermore, it is estimated that more than 50% of arable land will be affected by salinity by 2050, and global economic losses due to salinity stress are estimated to exceed tens of billions of US dollars per year [7].

In Morocco, more than 5% of the territory is already affected by salinity to varying degrees [8]. Indeed, more prolonged and severe droughts have led farmers to rely on low quality water sources and to use unsustainable irrigation and fertilisation practices that increase soil salinisation [9]. In particular, they correspond to arid and semi-arid regions where 80% of the water available for irrigation has a salinity of 5 g/L or more [10]. Most of these high-salinity areas are devoted to the cultivation of alfalfa (*Medicago sativa* L.), which is the most common and important forage crop, with more than 106,000 ha (22.8%) of the total area devoted to forage crops [11]. The cultivation of alfalfa strongly contributes to the socio-economic development of local families [12]. The age of lucerne in Morocco, combined with the pedoclimatic diversity of the areas of its distribution, has led to the appearance of genetically diverse ecotypes [13]. Thus, these alfalfa ecotypes have acquired a certain adaptation in parallel with the worsening salinity problem in these regions, which gives them a certain tolerance to salinity. Alfalfa is moderately tolerant to salinity, but there is a large variation among the cultivars of this forage crop compared to the center of diversity [4].

Salinity stress severely reduces plant growth and development by causing various morphological, physiological, biochemical, and metabolic changes [2]. It causes water unavailability, nutrient deficiency, and ion imbalance at all stages of plant development, including seed germination, emergence, growth and productivity. However, the germination and seedling stages are the most crucial for plant establishment. The salinity exerts depressive effects on the germination capacity of the seeds and it depends on the concentration of NaCl and varies among the plant species. This effect results in a delay or complete inhibition of germination especially at high salt concentrations [14]. Indeed, salinity affects the germination of seeds causing an external osmotic potential that prevents wa-

ter uptake (osmotic effect), ion disequilibrium (ionic effect) on the germinating seeds, and generating secondary effects due to the generation of reactive oxygen species (ROS), and causes membrane lipid peroxydation (oxidative stress) affecting the uniformity of plant density with a negative effect on yield [15] [16]. To overcome the salt stress effect, plants have evolved various mechanisms that help them to adapt to the osmotic, ionic and oxidative stress caused by high salinity [14].

The best economic approach to maximize the agricultural productivity of lucerne in these saline-affected areas is to use the potential reservoir of these rustic resources in the selection of salt-tolerant Lucerne genotypes at the germination and emergence seedling stages capable of maintaining a reasonable yield in saline-affected soils. Precocious germination under salinity stress can be considered as a tolerance criterion. Indeed, the selection of varieties for rapid and uniform germination under salinity stress conditions can contribute to early seedling establishment. In this context, the aim of the present study was to evaluate the effects of salinity on seed germination and seedling emergence parameters of alfalfa varieties, in terms of morphological and physiological response; and to analyze the sensitivity and salt stress tolerance of alfalfa varieties in order to select the tolerant ecotypes.

2. Materiels and Methods

2.1. Plant Materiel and Germination Experiments

The plant material used in this work consists of three Moroccan alfalfa cultivars: the Marocaine cultivar originating from the Tata region, the Ifrane cultivar originating from the Guelmime region, both of which are located in the arid and semi-arid regions of the Anti-Atlas. The Demnate cultivar originates from the High-Atlas mountains, in addition to an introduced variety, Siriver. The seeds were surface disinfected with 6% sodium hypochlorite solution for 5 minutes and thoroughly washed 7 to 8 times with distilled water to remove traces of sodium hypochlorite solution. The study was conducted in a completely randomised design with three replicates of each treatment and40 seeds per Petri dish were used to test the germination of the four lucerne varieties. The seeds were placed in Petri dishes containing two layers of filter paper soaked with 0 (distilled water), 50, 100, 150, 200, 250, and 300 mmol·L^{-1} NaCl. Germination was carried out in an incubator in constant darkness at 25˚C.

The seeds were used in a factorial design combining two factors in a randomized complete block design. The two factors were variety with 4 levels and salinity with 7 levels. Three replications with 40 seeds per box were used for the germination test with three replications for each treatment and for each cultivar. Germination was monitored daily for 8 days, counting germinated seeds every 24 hours; radicle extension is the event that ends germination and marks the start of seedling growth [17]. Seeds are considered germinated when the radicle has extended at least 2 mm beyond the seed coat [18].

2.2. Germination Variables Computation

Several germination parameters were calculated to characterize salt tolerance, including the germination rate (GR), final germination percentage (FGP), mean germination time (MGT) [19], and germination index (GI) [20].

The germination rate was calculated by using the following formula:

$$\mathrm{GR} = \sum \frac{n_i}{s} \times 100$$

The final germination percentage formula is:

$$\mathrm{FGP} = \frac{n_f}{s} \times 100$$

The mean germination time is a measure of the rate and time-spread of germination (lower values indicating faster germination) [19]. It was estimated as:

$$\mathrm{MGT} = \sum \frac{n_i t_i}{n_i}$$

The germination index (GI) described by Maguire [20] is a measure of seedling vigor and includes not only germination but also emergence characteristics. High values obtained using this expression mean higher seedling vigour. The value of GI was higher when the seeds germinated earlier. GI was calculated by using the following formula:

$$\mathrm{GI} = \left[\frac{n_1}{1} + \frac{n_2}{2}\right] + \cdots + \left[\frac{n_x}{x}\right]$$

where, n_i represents the number of newly germinated seeds on day i; n_f represents the number of germinated seeds at the end of the experiment; S represents total number of seeds in an experimental unit (germinated/viable/non-germinated); t_i is the number of days from the start of the experiment to day i; n is the number of germinated seeds at each day after sowing and 1, 2, … and x is the corresponding day of germination.

2.3. Kinetics of Germination

The kinetics of germination is expressed by the number of seeds germinated at 1, 2, 3, ..., 7 days after sowing. It is a parameter that makes it possible to better understand the ecological significance of the germination behavior of the studied cultivars, as well as the series of events that begin with the stage of water absorption by the seed and end with the elongation of the embryonic axis and the emergence of the radicle.

2.4. Physiological Analysis

We determined the physiological responses of two varieties contrasting in their responses to salinity according to the results of germination tests; Ifrane, being the most tolerant and Siriver, the less tolerant. After exposure to 0 or 200 mM NaCl for 7 days, the seeds developed young seedlings with radicals, hypocotyls and cotyledons. The length and the fresh weight of the young seedlings devel-

oped were measured.

2.5. Statistical Analysis

All measurements were subjected to a two-way analysis of variance (ANOVA II) with cultivars and salinity treatments as factors, followed by a Student-Newman-Keuls test. A difference was considered statistically significant when $p < 0.05$. All statistical analyses were performed on Excel.

3. Results

3.1. Effect of Salinity on Seed Germination

For the 4 cultivars, the two-way ANOVA revealed a highly significant main effect of both cultivar and salinity regarding final germination percentage and germination rate index ($p < 0.001$) (Table 1, Table 2). However, the existence of a significant interaction between these two effects ($F = 12$ for (FGP) and $F = 4$ for (GRI)) indicated that the populations studied did not respond similarly to the effect of salt at a given concentration of NaCl.

The effects of salt on the final germination percentage (FGP) of alfalfa were shown in Table 3. For all the cultivars studied, Ifrane, Marocaine, Demnate, and Siriver, the highest FGP occurred in the control with 100%, 99%, 96% and 93% respectively. However, increased salinity reduced the final germination percentage of alfalfa seeds compared to the corresponding controls (Table 3). The reduction was more pronounced with increasing NaCl concentrations. For each concentration of NaCl, a significant variation in both seed germination potential and seedling vigor was observed among the four cultivars studied. In addition, all cultivars showed an increase in Mean Germination Time (MGT), indicating that seeds take more time to germinate with increasing salinity (Table 4).

Comparing the mean values of the different genotypes (Table 3), the latter can be divided into two groups with different responses to sodium chloride: The first, composed of the Ifrane and the Marocaine genotypes, showed the highest germination rate of 81% and 75% respectively, while the second group consisted of the Demnate and Siriver genotypes, which had the lowest germination rates of 55% and 50% respectively.

At the control treatment (0 mM) and the lowest stress treatment (50 mM), all the cultivars formed a homogeneous group which showed no significant difference with the control for FGP. At this salinity level, the seeds germinated rapidly and no significant change in germination speed was noticed (MGT not different from the control and between cultivars) (Table 4).

The means of FGP at 100 mM and 150 mM NaCl showed no significant differences, 89% and 84% respectively. However, the results showed considerable variation in the response of seed germination to salinity among the cultivars studied at these two salt concentrations. Thus, at 100 mM NaCl, two groups were distinguished, the first was formed by the cultivar Ifrane, which showed a very high percentage of germination (FGP = 98%) and a high germination index

Table 1. Two-way analysis of variance (ANOVA) for final germination percentage (FGP) of the 4 cultivars at different NaCl concentrations.

Source of variation	df	MC	F
Cultivars (var.)	3	386,234	105,679***
Salinity (salt)	6	1905,194	521,290***
Var. X salt	18	43,882	12,006***
Error	83		

Df: Degree of freedom, MC: Mean of square, F: Ratio of variance, ***Significant at $P = 0.001$.

Table 2. Two-way ANOVA for germination rate index (GRI) of the 4 cultivars at different NaCl concentrations.

Source of variation	df	MC	F
Cultivars (var.)	3	877,162	60,926***
Salinity (salt.)	6	4552,278	316,196***
Var. X salt	18	69,314	4,814***
Error	195		

Df: Degree of freedom, MC: Mean of square, F: Ratio of variance, ***Significant at $P = 0.001$.

Table 3. Final Germination Percentage of different genotypes of lucerne under different NaCl concentration.

Cultivars	NaCl concentrations (mM)							Average
	0	50	100	150	200	250	300	
Ifrane	99a	98a	98a	96a	78b	73b	23c	81 (A)
Marocaine	100a	99a	93b	88c	68d	64d	14e	75 (AB)
Demnate	93a	91a	83ab	80b	53c	25d	13d	55 (BC)
Siriver	96a	95ab	85b	70c	41d	13e	2f	50 (C)
Average	97 (A)	96 (A)	89 (B)	84 (B)	60 (C)	44 (D)	13 (E)	

In the same row (a, b, c, d, e, f), significant differences (test Student, $P < 0.05$) if the letters are different. The averages followed by the same letter (A, B, C, D) are not different at the 5% threshold (test Student).

Table 4. Mean Germination Time (MGT) of different cultivars in various NaCl concentrations.

cultivars	NaCl concentrations (mM)						
	0	**50**	**100**	**150**	**200**	**250**	**300**
Ifrane	1.01^{a}	1.15^{a}	1.16^{a}	1.25^{a}	1.56^{b}	1.64^{b}	2.04^{c}
Demnate	1.05^{a}	1.09^{a}	1.32^{b}	1.58^{b}	1.73^{c}	1.77^{d}	2.50^{e}
Marocaine	1.05^{a}	1.19^{a}	1.41^{b}	1.47^{b}	1.98^{c}	2.08^{c}	2.50^{d}
Siriver	1.15^{a}	1.35^{a}	1.50^{b}	1.85^{b}	2.16^{c}	2.13^{d}	3.50^{e}

Values followed by the same letters in a row are not significantly different at $p < 0.05$, (test student).

(Figure 1) (GI = 94.78), and the second formed by the cultivars Marocaine, Demnate and Siriver, with no differences for FGP (93%, 83% and 85% of FGP respectively) and for GI (82.49, 76.5 and 78.57 respectively). For the MGT, there were no significant differences between the four cultivars studied. At 150 mM NaCl, the values of FGP and MGT recorded for each cultivar were significantly different and the means showed no difference with those recorded at 100 mM NaCl. Thus, the Ifrane cultivar showed earlier seed germination MGT (1.25), highest FGP (96%) and the highest seedling vigor (GI = 92.04), whereas the Siriver variety showed the highest MGT (1.84), lowest FGP (70%) and lowest seedling vigor (GI = 51.76).

At 200 mM of NaCl, the results showed considerable variation in the response of seed germination to salinity among the cultivars studied. The Ifrane cultivar showed a high germination percentage of 78% and an earlier seed germination (MGT = 1.56), followed by the Marocaine cultivar with 68% germination percentage and MGT < 2 days. The germination percentage of the Demnate cultivar showed a significant reduction at this salinity level, 53%, the MGT value registered were also under 2 days (1.73). However, the Siriver variety seemed to be severely affected by this concentration, it showed the lowest final germination percentage (FGP = 41%) and the seed germination took longer (MGT = 2.16).

At 250 mM NaCl, the genotypes studied can be divided into two groups according to their germination response to this salinity level. The first group includes the cultivars Ifrane and Marocaine, which showed the highest FGP (73% and 64% respectively), with no difference from those registered at 200 mM NaCl. The second group includes the cultivars Demnate and Siriver, whose germination was strongly affectedby this concentration in the same way, showing the lowest FGP (25% and 13% respectively), with a significant difference with those registered at 200 mM. The MGT values registered indicate that the Ifrane cultivar showed the earliest seed germination (MGT < 2 days) at different salinity levels, while for the Siriver cultivar, germination takes a lot of time to take place (MGT > 2 days); the Marocaine and Demnate cultivars occupied an intermediate position between the tolerant cultivar (Ifrane) and the most sensitive (Siriver). The germination index at this concentration showed a high seedling vigour for the Ifrane and the Marocaine cultivars (GI = 55.19 and GI = 42.43 respectively), whereas the Demnate and the Siriver cultivars showed a lower seedling vigour (GI = 21.84 and GI = 8.71 respectively).

The high salt dose (300 mM of NaCl) caused a significant reduction in seed germination and delayed its initiation in all cultivars. The cultivar Siriver was the most affected by this concentration, almost no germination took place, with a germination percentage not exceeding 2%, and the time to germination gradually lengthened (MGT > 3 days).

The effect of NaCl on the germination behavior of alfalfa results in an increase in the latency time and a decrease in the germination rate (Figure 2). The study of the germination kinetics shows that an increasing salt concentration causes a delay

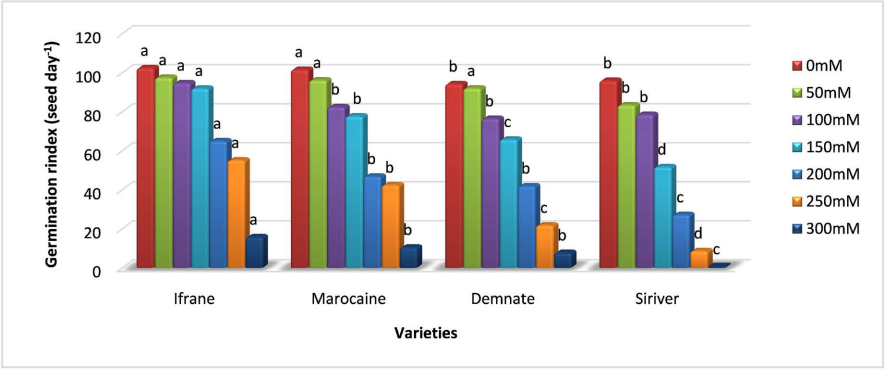


Figure 1. Germination index of different alfalfa varieties in various NaCl concentrations, at each concentration NaCl, means of populations having the same letter are not significantly different ($p < 0.05$) (test student).

in the germination. These results show that the Ifrane cultivar is the most tolerant to salt stress, whereas the Siriver cultivar is the most susceptible at the germination stage. Both cultivars with contrasting salinity responses were used for further characterization.

3.2. Changes in Fresh Weight and Growth of Two Alfalfa Seedlings

Plant biomass and yield data are used as an agronomic index to define stress tolerance. We analyzed the fresh weight and length of seedlings of two alfalfa genotypes under salt stress (200 mM of NaCl) for 7 days after imbibition. Our results showed that alfalfa growth (fresh weight and length of seedlings) is negatively affected by increasing salinity. Under normal conditions, the Siriver variety had lower fresh weight and seedling length than Ifrane ecotype (**Figure 3(a)** and **Figure 3(b)**). However, the fresh weight and length of seedlings of both cultivars were significantly inhibited by 200 mM NaCl ($P < 0.01$). The reduction was greater in Siriver (262 mg fresh weight and 3.9 mm seedling length) than in Ifrane (314 mg fresh weight and 7.8 mm seedling length) with a significant difference ($P < 0.01$), indicating that the latter is more tolerant to salt stress than the former.

4. Discussion

4.1. Effect of Salinity on Germination

Soil salinity is one of the major plant stressors, especially in arid and semi-arid regions and can severely limit crop production [21]. The results obtained in this study showed that the salt treatment produced depressive effects at the developmental stages studied. Monitoring of the germination process revealed that salt stress was responsible for both a delay in seed germination and a reduction in seedling establishment in the four alfalfa genotypes studied. This suggests that seed germination takes longer under salt stress. However, a significant difference was noted between the populations studied in their reactions to salt. The Ifrane cultivar had the highest germination percentage and the lowest average germination

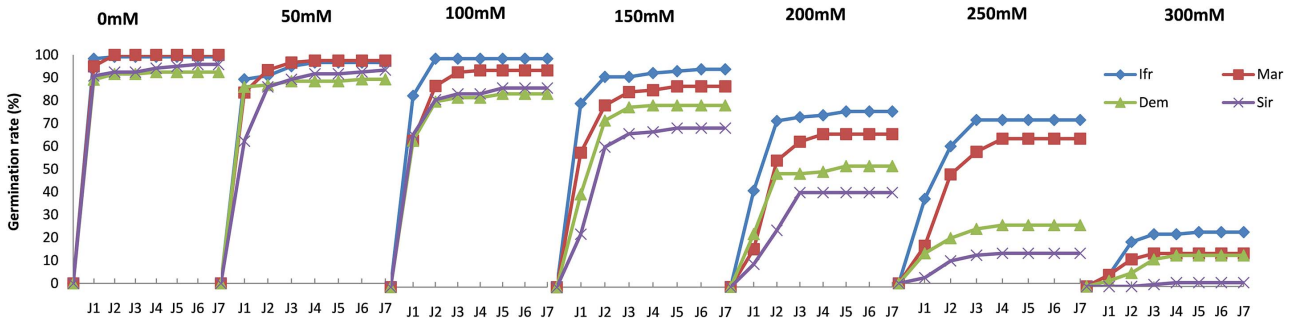
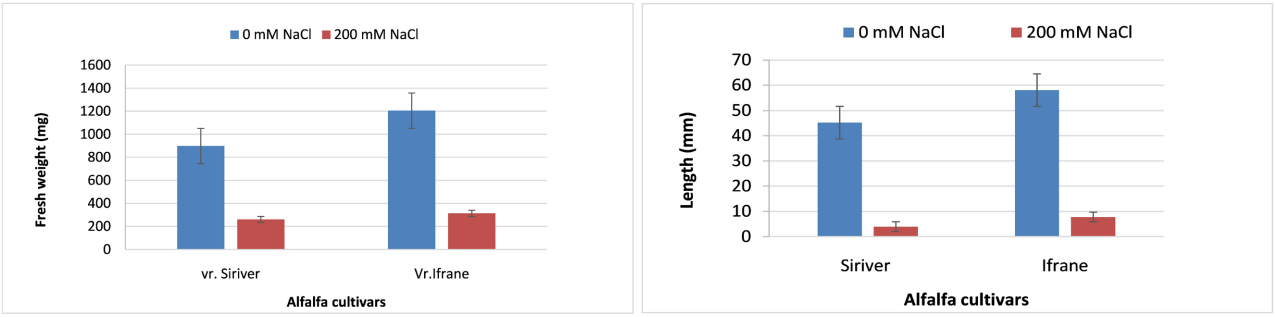


Figure 2. Kinetics of seed germination of four alfalfa genotypes, Ifrane, Marocaine, Demnate and Siriver, during germination for 7 days, and under different NaCl concentrations.



(a) (b)

Figure 3. Changes in fresh weight and length of stress-tolerant and sensitive alfalfa cultivars under 200 mM NaCl treatment for 8 days. Fresh weight of young seedlings (a); Length of young seedlings (b). **indicates that mean values were significantly different between the treatments and the control $p < 0.01$. Bars are standard error.

time, while the Siriver variety had the lowest germination percentage and the highest average germination time. On the other hand, the Marocaine and Demnate ecotypes showed an intermediate position, with the Marocaine cultivar clearly superior. Thus, the Moroccan alfalfa genotypes used in this study proved to be more tolerant than the variety (Siriver) introduced, testifying to the richness of the local material in terms of tolerance to salinity, and to its usefulness and effectiveness in maximizing the agricultural productivity of alfalfa in areas affected by salinity. The effect of salinity on alfalfa seed germination and seedling establishment has been studied by several researchers, who have shown a wide variation in salt tolerance between alfalfa varieties at different stages of development [12] [22] [23] [24].

The reduction in seed germination affected by salt stress may be due to the direct effect of sodium chloride on embryo growth [25]. Salinity affects seed germination and reduces it by decreasing the rate of water absorption by osmotic effect and increasing the rate of ion efflux by modifying hormonal and enzymatic activities due to the toxic effects of sodium and chloride ions [12]. The emergence of the radicle is mainly hindered by a reduction in the water potential gradient between the seed and the external environment [14]. Water uptake is necessary to complete an endogenous physiological process during seed germination. Salinity also disrupts the hormonal balance of plants [26] and reduces

the use of seed reserves [27].

4.2. Effect of Geographical Origin of Populations on Salt Stress to Lerance

A significant variation in germination potential revealed by ANOVA was observed between Moroccan alfalfa populations with respect to the different NaCl concentrations used. This variation is partly a function of their geographical origin. Indeed, the Ifrane and Marocaine cultivars from arid and semi-arid regions (Anti-Atlas), which are representative of the "African" type, are the most tolerant to salinity and showed the highest FGP and strong seedling vigour for high NaCl concentrations. The Demnate cultivar from the High-Atlas mountains, which is related to the 'Falcata' type, and the Siriver variety were sensitive to salt stress. The results obtained indicate a certain correlation between the origin of the cultivars and resistance to salinity. This result can be explained by the great diversity of traditional oasis and mountain agro-ecosystems and by the adaptation of populations to the pedoclimatic conditions of their ecosystem of origin, such as drought, cold and salinity.

Similar results have been reported in several studies [15] [28]. Genetic material from Indian and African arid areas excelled in resistance to NaCl during germination, while genetic material related to the European origins 'Falcata' was the least resistant [29].

4.3. Effect of Salinity on Seedling Growth

A reduction in seedling growth resulting from an increase in salinity level has been reported in alfalfa and certain other species [12] [22] [23] [30] [31]. The results obtained in our study showed that salt treatment negatively affects the growth of young seedlings by reducing fresh weight and seedling height, with significant differences between ecotypes. The reduction in fresh weight and seedling length was greater for the Siriver variety than for the Ifrane cultivar. Seedling growth is one of the most important indices of salt tolerance, as shown by various studies [24] [30] [32]. This confirms the concept that plant biomass data are proposed as agronomic parameters to define stress tolerance. The results obtained show the tolerance of the Ifrane ecotype and the sensitivity of the Siriver variety at the germination and young seedling stages.

5. Conclusions

The results of the present study showed that the salt treatment produced depressive effects at different stages of development in the four alfalfa genotypes. At the germination stage, the effect of salinity was manifested by a reduction in seed germination capacity and an increase in mean germination time. At the young seedling stage, we noted that the parameters taken into consideration were significantly affected. However, important differences in the germination behavior were observed between the Moroccan cultivars and the Siriver variety. In fact,

germination was more affected in the introduced variety (Siriver) than in the Moroccan cultivars (Ifrane, Marocaine and Demnate).

The results obtained confirm that the local alfalfa germplasm is genetically diverse and we noted a clear and significant variation between the different Moroccan populations considered in terms of their reactions under salt stress. Such variation could be a determining factor in the selection of populations that are tolerant to this stress. Thus, the cultivars Ifrane and Marocaine originated from Oasis areas show particular adaptability to the saline environment, at least at this stage of the life cycle, and could therefore be an interesting asset for the establishment of these plants in irrigated perimeters in arid zones faced with salinity, for improving lucerne productivity in this saline areas and thus make a major contribution to the socio economic development of local families. However, the choice of this genotype must be made on the basis of a large number of traits (morphological, biochemical and physiological) that control the complex genetic determinants of salinity tolerance.

Conflicts of Interest

The authors declare no conflicts of interest.

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