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Effect of salt stress on *Pistacia atlantica* rootstock seedlings in nursery conditions

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Abstract. One year old seedlings of 9 genotypes (mother trees) were separated in three classes: C1, C2 and C3. Classes were ranged decreasingly from the most to the least salt tolerant. Salt stress was induced by adding sodium chloride (NaCl) in the irrigation water. Three salt concentrations were tested (0, 100 and 200 mM). Growth parameters and mineral contents were recorded after 60 days of culture. Results showed no significant variation for all tested seedling classes between the control and stressed plants for stem length and whole plant fresh weight. The C1 and C3 plant classes reduced leaf number at 100 mM and 200 mM NaCl respectively. For all classes, salt treatments induced greater sodium increase in leaves and stems than in roots in comparison with control conditions. Potassium nutrition was similar in control and NaCl treated plants for all seedlings indicating the salt tolerance of this specie. C1 seedlings accumulated larger amount of chlorides in leaves and stems than in roots. C2 and C3 seedlings had an opposite behaviour.

Keywords. *Pistacia atlantica* – Rootstock – Salt tolerance – Growth – Minerals.

Effet de la salinité sur des plants issus de semis de* Pistacia atlantica *en conditions de pépinière

Résumé. *Des plants d'un an issus du semis des graines de 9 génotypes de* P. atlantica *ont été séparés en trois classes : C1, C2 et C3. La classification a été établie par ordre décroissant suivant leur degré de tolérance à la salinité. Le stress salin a été imposé par l'addition de chlorure de sodium (NaCl) dans l'eau d'irrigation. Trois concentrations de sel ont été testées (0, 100 et 200 mM). La croissance et les teneurs en sodium (Na^+), chlorures (Cl^-) et potassium (K^+) ont été déterminées après 60 jours de culture. Les résultats n'ont montré aucune différence significative entre le témoin et les plants stressés chez les trois classes pour la longueur de la tige et le poids frais de la plante. Chez les plants C1 et C3, le nombre de feuilles a diminué respectivement à 100 et 200 mM NaCl. La salinité a induit une augmentation plus importante de la concentration en sodium dans les feuilles et les tiges comparée à celle des racines. La nutrition potassique était comparable au témoin chez toutes les plantes ce qui montre la tolérance à la salinité de cette espèce. Les plantes de classe C1 ont accumulé plus de chlorures dans les feuilles et les tiges que dans les racines alors que C2 et C3 ont adopté un comportement inverse.*

Mots-clés. Pistacia atlantica *– Porte-greffe – Tolérance à la salinité – Croissance – Minéraux.*

I – Introduction

Pistachio (*Pistacia vera* L.) nut tree is usually propagated by grafting on seedlings of many *Pistacia* species. Rootstocks can play an important role in salt tolerance of grafted plants. In Tunisia, a wild endangered specie *P. atlantica* exists as isolated aged trees in arid and semi arid areas. Despite its adaptation to hostile environment such as salinity (Ferguson *et al*., 2005), drought and nematode tolerance and its good performance as rootstock compared to *P. vera*, it is, nowadays, rarely used as rootstock for pistachio varieties, probably because of seeds lack and variable germination capacity. Heterozygocy and dioecy of *Pistacia* species induce a great variability in sexual propagated progenies that is needed in breeding programs. It was reported that salt tolerance can vary among seedlings of one species but it also differed according to seed source (Dochinser and Townsend, 1979; Khasa *et al.*, 2002) and developmental stage

(Dasgan *et al.*, 2002). In order to set up a breeding program for early selection of salt tolerant genotypes to be used as rootstock, the first step was to develop an efficient method to select mother trees susceptible to give high rates of vigorous salt tolerant seedlings based on seed performance at germination stage. For this purpose, it was thought essential to answer the question whether a high rate seed germination under salt stress of a mother tree could be an efficient indicator of its progenies salt tolerance at seedling stage. The aim of this study was to compare growth and nutrition under salt stress of three *P. atlantica* genotype classes that showed different salt tolerance performances at germination stage.

II – Materials and methods

Mature seeds of *P. atlantica* mother trees (genotypes) were collected from the Tunisian centre west Sidi Bouzid site. Seed germination capacity was studied at different salt concentrations. Genotypes were then, classified into three classes C1, C2 and C3 from the highest to the lowest salt tolerant according to their germination capacity at 180 mM NaCl. One year seedlings of similar vigour, obtained from these mother trees, were chosen without previous selection for salt tolerance and potted in 2 litres plastic bags filled with equal parts mixture of compost, garden soil and manure and irrigated twice a week with tap water supplemented with 0, 100 and 200 mM of sodium chloride (NaCl) for 60 days. A gravel layer was settled up below the plastic pots to ensure water draining. Axillary shoots of all seedlings were removed keeping only the main stem. A plastic film was covering the plants to protect them from rain water. Seedlings (33 to 45) per treatment were arranged in a randomized complete block design with 3 replications.

At the end of the culture period, the principal shoot length was measured and leaves and axillary shoots were counted. Leaves, stems, roots and whole plant fresh weights were determined. These plant parts were then dried at 70°C for at least 72 h until weight stabilisation. Dried material was grinded and dry ashed at 400°C before mineralization with nitric acid 1N. The sodium (Na^+) and Potassium (K^+) contents were quantified by flame emission photometry, chlorides (Cl^-) were analysed by titration with silver nitrate ($AgNO_3$) in the presence of potassium bichromate following Mohr colorimetric modified method (Mathieu and Pieltain, 2003). Data were subjected to one-way ANOVA analyses using SPSS software. Differences were determined by Duncan test at $p \leq 0.05$.

III – Results

1. Stem length and leaf and axillary shoot numbers

Table 1 shows the sodium chloride effect on *P. atlantica* stem length and leaf and axillary shoot numbers after 60 days of culture. Salinity affected seedling growth differently depending on the parameter considered and the genotype class. Indeed, NaCl did not affect significantly the shoot length of all seedlings. However, leaf number showed more variation according to seedling classes. Indeed, leaf number decreased significantly at 100 mM NaCl for C1 seedlings and at 200 mM for C3 plants while no significant variation was recorded for C2 seedlings. Axillary shoot formation decreased with salt stress for all plants (4.35 to 35.53%) but this reduction was significant only for C3 seedlings at 200 mM NaCl. When compared to control conditions (Fig. 1), the seedlings belonging to the three classes behaved differently in the presence of salt stress: C1 plants showed a great reduction of leaf number (41.4 and 61.7% at 100 and 200 mM NaCl respectively) but were capable to maintain the growth of principal shoot and the formation of axillary shoots to a level similar to control conditions (respectively 12.6 and 4.4% reduction at 200 mM) at the two NaCl concentrations. The C2 seedlings recorded 15 to 23% of growth reduction for all parameters studied at 100 mM NaCl but at 200 mM NaCl, only leaf number continue its decrease while the two other parameters did not vary at all. For the C3 *P. atlantica* class, a decrease of 14 to 31% was observed for stem length and leaf and axillary shoot numbers at 100 mM of salt added to irrigation water. At the highest salt treatment, leaf and axillary shoot number decreased again up to 38 and 36% respectively but stem length was maintained at the level reached at 100 mM NaCl.

Table 1. Sodium chloride effect on stem length and leaf and axillary shoots numbers of the three classes of *P. atlantica* seedlings

Seedling class	NaCl (mM)	Principal stem length (cm)	Principal stem leaf number	Axillary shoot number
C1	0	$28.6^{a} \pm 1.46$	$13.3^{a} \pm 9.13$	$4.6^{b} \pm 3.06$
	100	$25.6^{a} \pm 0.86$	$7.8^{bc} \pm 9.51$	$4.4^{b} \pm 3.76$
	200	$25^{a} \pm 1.01$	$5.1^{c} \pm 10.13$	$4.4^{b} \pm 3.81$
C2	0	$26.5^{a} \pm 1.33$	$8.9^{bc} \pm 9.45$	$5.9^{ab} \pm 4.06$
	100	$24.2^{a} \pm 0.53$	$6.9^{c} \pm 6.96$	$4.7^{b} \pm 4.29$
	200	$24.2^{a} \pm 0.42$	$4.7^{c} \pm 6.87$	$4.7^{b} \pm 3.24$
C3	0	$25.5^{a} \pm 1.17$	$11.3^{ab} \pm 6.91$	$7.6^{a} \pm 5.07$
	100	$24.5^{a} \pm 0.4$	$7.8^{bc} \pm 5.34$	$6^{ab} \pm 4.95$
	200	$24.6^{a} \pm 0.43$	$7^{c} \pm 7.12$	$4.9^{b} \pm 4.62$

Columns having the same letter are not significantly different at $p \leq 0.05$.

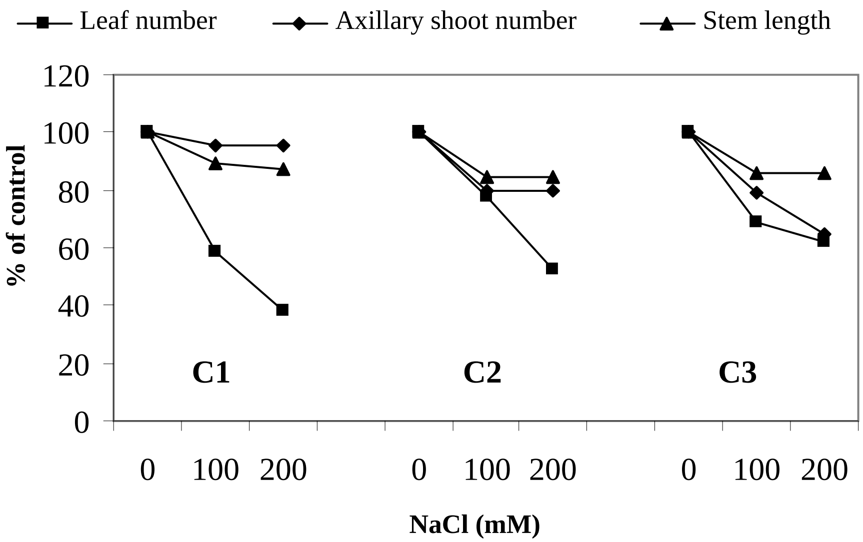

Fig. 1. Sodium chloride effect on *P. atlantica* seedling growth expressed in percent of control.

2. Fresh weight

The fresh weights of the whole plants were measured after 60 days of culture under salt stress conditions. No significant variation with salinity was noticed for all seedlings. However, when each part of the plant was considered alone, leaves appeared as the most affected organ by NaCl treatment at both applied concentrations while stems and roots fresh weights have not been greatly affected by salt (Table 2). Indeed, fresh leaf biomass decreased significantly at 100 mM for C1 and C3 plants and at 200 mM for C2 ones.

Table 2. Sodium chloride effect on *P. atlantica* seedling fresh weight

Seedling class	NaCl (mM)	Leaves	Stems	Roots	Whole plant
C1	0	1.48[a] ± 1	2.17[a] ± 1.23	1.33[a] ± 0.66	5.09[a] ± 2.65
	100	0.67[bc] ± 0.7	2.06[a] ± 1.37	1.19[a] ± 0.71	3.96[abc] ± 2.52
	200	0.57[bc] ± 0.65	1.96[a] ± 1.29	1.35[a] ± 0.84	3.91[abc] ± 2.53
C2	0	0.96[b] ± 0.92	1.73[a] ± 1.01	1.14[a] ± 0.61	3.87[abc] ± 2.13
	100	0.61[bc] ± 0.71	1.87[a] ± 1.21	1.14[a] ± 0.76	3.66[bc] ± 2.38
	200	0.34[c] ± 0.42	1.79[a] ± 1.20	1.04[a] ± 0.66	3.2[c] ± 2.10
C3	0	1.33[a] ± 0.61	2.17[a] ± 1.01	1.22[a] ± 0.58	4.76[ab] ± 2
	100	0.89[b] ± 1.22	2.12[a] ± 1.09	1.28[a] ± 0.71	4.35[abc] ± 2.66
	200	0.63[bc] ± 0.68	2.01[a] ± 1.08	1.13[a] ± 0.74	3.84[abc] ± 2.23

Columns having the same letter are not significantly different at $p \leq 0.05$.

Compared to control plant fresh weight, the reduction caused by salt stress did not exceed 23.5% (Fig. 2). All plants showed a great decrease in leaf fresh weight while the highest level (64%) was reached for C2 at 200 mM NaCl. Otherwise, the stem and root decrease did not surpass 10.5% (C1 at 200 mM NaCl).

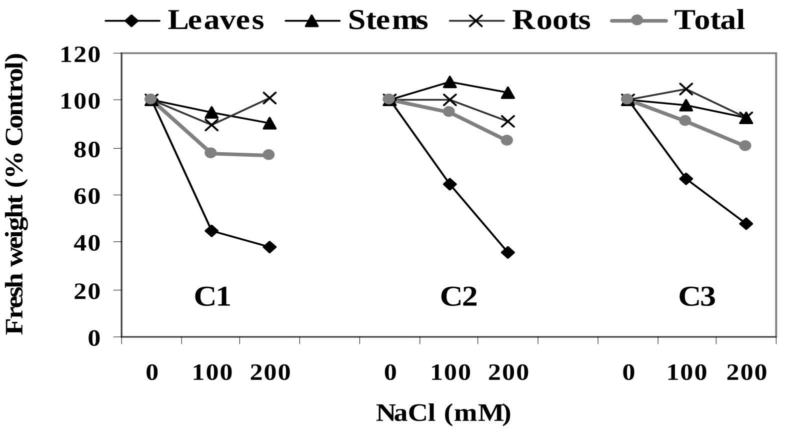

Fig. 2. Effect of sodium chloride on leaves, stems and roots fresh weight (% of control).

3. Mineral contents

The three groups of plants showed nearly similar pathway in accumulating Na^+ ions under salt stress conditions tested (Tables 3, 4 and 5). For all seedlings Na^+ content in roots was higher than in leaves and stems. However, the comparison with control conditions showed that for all seedlings, Na^+ concentration increased more in shoots than in roots. This increase was slightly higher in shoots (leaves + stems) of the C1 plants at 200 mM NaCl (3.2 fold) than in the C2 and C3 ones (3.06 and 2.25 fold respectively). Inversely, the Na^+ concentration raise was more important in the C3 roots (1.27 and1.34 fold at 100 and 200 mM NaCl respectively) than in the C2 (1.25 and 1.24 fold at 100 and 200 mM NaCl respectively) and C1 ones (1.17 and 1.18 respectively at 100 and 200 mM NaCl). Chloride uptake showed important variations according to the tolerance class and the organ considered. This variation was more pronounced at 200 mM NaCl. When compared to the control, Cl leaf content reached a level 11 fold higher than control for C1 group and did not exceed 2.6 and 4.5 fold of control for C2 and C3 seedlings respectively at 200 mM NaCl. On other hand, potassium nutrition of all plants seems not to be negatively affected by NaCl in the irrigation water.

Table 3. Sodium chloride effect on sodium (Na) contents (% DW) of leaves, stems and roots of *P. atlantica* seedlings

Classes	NaCl (mM)	Na		
		Leaves	Stems	Roots
C1	0	0.16^{d}	0.28^{b}	0.67^{cd}
	100	0.4^{bcd}	0.59^{a}	0.78^{abc}
	200	0.72^{ab}	0.69^{a}	0.79^{ab}
C2	0	0.2^{d}	0.27^{b}	0.68^{bcd}
	100	0.56^{abc}	0.59^{a}	0.85^{a}
	200	0.84^{a}	0.63^{a}	0.84^{a}
C3	0	0.24^{cd}	0.31^{b}	0.62^{d}
	100	0.43^{bcd}	0.58^{a}	0.79^{abc}
	200	0.62^{ab}	0.64^{a}	0.84^{a}

Columns having the same letter are not significantly different at $p \leq 0.05$.

Table 4. Sodium chloride effect on chlorides (Cl) contents (% DW) of leaves, stems and roots of *P. atlantica* seedlings

Classes	NaCl (mM)	Cl		
		Leaves	Stems	Roots
C1	0	0.47^{de}	0.36^{f}	1.42^{cd}
	100	3.67^{b}	1.18^{de}	0.83^{d}
	200	5.2^{a}	3.9^{a}	1.66^{cd}
C2	0	0.59^{cde}	0.36^{f}	1.07^{d}
	100	1.3^{cd}	1.66^{d}	2.25^{bc}
	200	1.54^{c}	2.7^{b}	4.26^{a}
C3	0	0.24^{e}	0.47^{ef}	1.18^{cd}
	100	0.59^{cde}	1.78^{cd}	2.96^{b}
	200	1.07^{cde}	2.48^{bc}	4.62^{a}

Columns having the same letter are not significantly different at $p \leq 0.05$.

IV – Discussion

The present study was conducted on one year seedlings in order to test the efficiency of mother tree early selection for salt tolerance at the germination stage. The results showed important variation within and between seedling classes for some growth parameters such as leaf number and fresh weight and axillary shoot number. This intra specific variation impede from distinctive behaviours of the three seedling groups for growth parameters. However, few differences were observed at 200 mM NaCl when results were expressed in percent of control: the leaf number reduction was the most important for C1 followed by C2 and then by C3 whereas the axillary shoot number decrease allowed a reverse classification of these plant classes.

Table 5. Sodium chloride effect on potassium (K) contents (% DW) of leaves, stems and roots of *P. atlantica* seedlings

Classes	NaCl (mM)	K		
		Leaves	Stems	Roots
C1	0	1.43^{abc}	1.62^{a}	1.28^{a}
	100	1.12^{c}	1.64^{a}	1.05^{a}
	200	1.36^{abc}	1.42^{a}	1.28^{a}
C2	0	1.44^{abc}	1.66^{a}	1.29^{a}
	100	1.28^{bc}	1.58^{a}	1.33^{a}
	200	1.74^{a}	1.6^{a}	1.04^{a}
C3	0	1.49^{abc}	1.43^{a}	1.03^{a}
	100	1.55^{ab}	1.38^{a}	0.99^{a}
	200	1.48^{abc}	1.5^{a}	1.06^{a}

Columns having the same letter are not significantly different at $p \leq 0.05$.

Mineral content analysis showed no significant differences in potassium content between *P. atlantica* classes which confirm the salt tolerance of this specie (Ferguson *et al*., 2005). All seedlings tested contained more sodium in roots than in leaves and stems under salt stress. As reported by Walker *et al.* (1987) and Picchioni and Miyamoto (1990) *P. atlantica* seems to accumulate Na^+ in roots to limit its ascension in shoots. When the leaf sodium accumulation of the three seedling classes was compared with control, C3 appears as the most effective in limiting Na^+ ascension to the leaves. Chloride contents varied the most between the three seedling classes with highest and lowest Cl^- accumulation in roots and shoots (leaves + stems) respectively for C3 seedlings and inversely for C1 ones. The C2 class had intermediate values. At the intra-specific level, it was reported that the genotypes which limit ascension of Na^+ and Cl^- in the leaves by their retention in the roots are the most salt tolerant (Zid and Grignon, 1991). The salt tolerance of the three seedling classes can be classified as follows: C3 > C2 > C1.

To conclude, it appears from this study that *P. atlantica* salt tolerance at germination stage of one mother tree seems not to be reproducible at one year old seedling stage. Consequently, mother trees early selection using this method needs more investigations to determine useful salt tolerance indicators.

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