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(Research Article)

Effect of salinity on germination capacity of laurel (*Laurus nobilis* L.)

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Abstract

In Tunisia, more precisely in semi-arid and arid areas, drought accentuated by the superficial evaporation of water leads to a gradual increase in salinity, which is a limiting factor in crop production. In these highly salty ecosystems, some plants grow naturally; however, various species show different levels of tolerance to salinity during their development. The germination of the seeds is the most sensitive stage to this abiotic stress. The effect of this severe stress is little known among Laurel (*Laurus nobilis* L.), which is a native species of natural vegetation of the Mediterranean region, well known for its medicinal, aromatic, forestry, ornamental and culinary properties, and rare in its natural habitat, often subject to overexploitation or threatened with risk of extinction in Tunisia. The objective of this work is to study the effects of different concentrations of NaCl of irrigation water on germination behavior of *laurus nobilis* L. The experiment was carried out in Tunis, the experimental design was entirely randomized with four treatments: 0, 50, 100, and 150 mM NaCl and five replications of 80 seeds, for each treatments. Salt stress caused a negative effect on the germination of laurel seeds especially for the highest concentrations of salt (150 mM NaCl).

Keywords: *Laurus nobilis L.*; NaCl; Salt stress; Irrigation; Germination

1. Introduction

Arid and semi-arid regions present about two-thirds of the earth's surface [1]. In these ecosystems, marked by frequent and severe droughts, salinity in the soil and water is one of the abiotic environmental stresses limiting crop production. Germination is a critical stage in the life cycle of plants in saline environment, then increase in salinity concentrations led to a reduction in seed germination percentage and delay germination process initiation, while salt stress can also cause complete inhibition of the germination process [2]. At this stage, plants are more sensitive to environmental stress than the other phases so the sensitivity of plant species to environments with high concentrations of salts in the soil, during the germination phase, may be critical to plant establishment [3]. In Tunisia, the medicinal and aromatic flora is much diversified. Five hundred species (out of a total of 2013, or 25% of species) are recognized as having medicinal and aromatic properties [4] which are grown generally in fragile environments (semi-arid and arid) and are still very little known. Laurel, *Laurus nobilis* L., is one of the most important woody perennial species grown in the Mediterranean area, and it is a member of the Lauraceae family which contains 32 genera and about 2000-2500 species [5]. It represents one of the most appreciated aromatic herbs with multiple properties. It is widely used in traditional medicine for the treatment of chronic sinusitis, bronchitis and flus [6]. Nevertheless, there is in the literature few information on the effects of salt stress on the emergence and growth of this species.

In contrary of many other species like *Melia azedarach* L. which is a multi-purpose tree species valued for bio-pesticide [7], timber [8] and ornamental use [9] and which was considered as a salt tolerant tree species [10] and *Thymus*

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broussonetii which behave to salinity as a moderately salt tolerant species [11], there is in the literature few information on the effects of salt stress on the emergence and growth of laurier.

Considering the importance of salinity on seed germination, the objective of this study is to evaluate the effects of different concentrations of NaCl on the germination of *Laurus nobilis* L.

2. Material and methods

2.1. Plant material and germination conditions

Seeds of laurel (*laurus nobilis* L.*)* were collected from the northwestern region of Tunisia. The same size seeds were sterilized for 5 min with sodium hypochlorite solution (10%) and then rinsed 3-5 times with distilled water. Seeds were placed in blister plates containing peat and sprinkled with different concentrations of NaCl: 0, 50, 100 and 150 mM. Five replications of eighty seeds were used for each treatment and plates were placed in greenhouse under natural light conditions and temperature between 16 °C and 35 °C. A seed was considered germinated by the emergence of 1 mm of the radicle. The counting of germinated seeds was done daily until the end of the experience. Seedlings were placed in a greenhouse at the National Agronomic Institute of Tunisia in Tunis (36°49′ N, 10°10′ E, 8 m s.l.), Tunisia. Seedling production was calculated based on the total number of emerged seedlings per total number of seeds in each treatment. After that, some seeds of each treatment were removed and their morphological traits were assayed.

2.2. Studied parameters

2.2.1. Germination parameters

After final count, final germination percent (FGP) and speed of germination (SG) were calculated using the following formulae [12, 13]:

$$FGP = S/T \times 100$$

$$SG = N1/D1+N2/D2+\ldots+Ni/Di$$

Where, S is the number of final germinated seeds, T is the total number of seeds, and Ni is the number of germinated seeds per day (Di).

2.2.2. *Vegetative parameters*

The number of leaves, shoot length, root length, and of five randomly selected seedlings from each replication were measured.

2.2.3. Seedlings dry weight

The dry weight of leaves, shoots and roots of five randomly selected seedlings were recorded after drying in oven at 80 °C to constant weight.

2.3. Statistical analysis

Results were analysed by ANOVA using SPSS 20.0. To separate treatments, Duncan's multiple range test was performed at P=0.05.

3. Results

3.1. Percentage and speed of germination

The germination percentage of laurel seeds under control and saline conditions (50, 100 and 150 mM NaCl) was investigated in table 1. For the control, the percentage of germination was 93%. Salinity affects germination and its percentage was reduced with NaCl concentration. On NaCl 100 mM, this parameter fell to 35%, representing only 37.6% of the control. On the highest concentration of NaCl (150 mM), the germination of seeds was completely inhibited. The germination speed of laurel seeds was also significantly affected by salinity ($P<0.001$) (Table 1). In fact, seedlings emerged more slowly in presence of NaCl. On NaCl 100 mM, the germination speed was 1.24 seed day^{-1} which represents only 12.7% of the control.

Table 1 NaCl effects on germination percentage and germination speed of *Laurus nobilis* seeds

NaCl (mM)	Germination percentage (%)	Germination speed (seed day^{-1})
0	93 a	9.76 a
50	52 b	4.01 b
100	35 c	1.24 c
150	0 d	0 d
Significance[a]	***	***

a *** = significant at P≤ 0.001. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤0.05).

3.2. Growth of the aerial part

The shoot length and the number of leaves for laurel seedlings were significantly affected by salinity (table 2). Shoot length was the highest for the control (8.57 cm) and decreased clearly with NaCl. On NaCl 100 mM, this parameter was reduced to 3.47 cm, representing 40.5% of the control. We remember that seedling production was completely inhibited on NaCl 150 mM. The number of leaves fell from 6 leaves per seedling for the control to only 2.3 leaves on NaCl 100 mM, this represent 38.3% of the control.

Table 2 NaCl effects on vegetative growth of *Laurus nobilis* seedlings

NaCl (mM)	Number of leaves	Shoot length (cm)	Root length (cm)
0	6.0 a	8.57 a	13.67 a
50	3.7 b	5.20 b	7.90 b
100	2.3 c	3.47 c	1.33 c
150	0 d	0 d	0 d
Significance[a]	***	***	***

[a] *** = significant at P≤ 0.001. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤0.05).

3.3. Growth of the root system

Salinity exerts a significantly inhibiting effect on the root growth of of *Laurus nobilis* L. seedlings (Table 2). In fact, root length was decreased in presence of NaCl. These parameters were the highest for the control, 13.67 cm. On NaCl 100 mM, the root length was 1.33 cm representing 9.7 % of the control

3.4. Fresh and Dry weights

The fresh weight of *Laurus nobilis L*. seedlings (whole plant, leaves, shoots and roots) was the highest for the control (0.66 g, 0.24 g, 0.31 g and 0.11 g, respectively) (Table 3). In presence of NaCl 100 mM, this parameter fell to 0.13 g, 0.09 g, 0.03 g and 0.01 g, respectively.

The dry weight of the different organs of the control seedlings was 0.29 g for the whole plant (0.23 g for the aerial part and 0.06 g for roots). On NaCl 100 mM, this parameter decreased for all organs and was 0.027 g for the whole plant (0.023 g for the aerial part and 0.004 g for roots), representing only 9.3% of the control (10% for the aerial part and 6.6% for roots).

Table 3 NaCl effects on fresh and dry weights of leaves, shoots and roots of *Laurus nobilis* seedlings

NaCl (mM)	Fresh weight (g per plant)				Dry weight (g per plant)			
	Leaves	Shoots	Roots	Whole plant	Leaves	Shoots	Roots	Whole plant
0	0.243 a	0.310 a	0.109 a	0.662 a	0.089 a	0.137 a	0.067 a	0.293 a
50	0.204 b	0.213 b	0.042 b	0.459 b	0.052 b	0.110 b	0.026 b	0.188 b
100	0.089 c	0.033 c	0.012 c	0.134 c	0.012 c	0.011 c	0.004 c	0.027 c
150	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
Significance[a]	***	***	***	***	***	***	***	***

[a] *** = significant at P≤ 0.001. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤0.05).

4. Discussion

Several studies have demonstrated that the high salt content in soil, especially sodium chloride (NaCl), may inhibit germination [14]. Accordingly, in the present study, our results showed that salt stress reduced the final germination percentage (FGP) of laurel seeds. This is in accordance with the results of [15] indicating that salt reduced the germination percentage of *Oryza sativa* L. [16] found the same result for *Phaseolus vulgaris* L. Our results were in accordance also with those of [14] showing that salinity affected significantly the germination of three ornamental species (*Petunia* x *hybrida*, *Torenia fournieri*, and *Tagetes patula).* Presumably, an excess of salt causes a reduction in soil water potential, leading to a less capacity for water absorption by seeds [14].

[17] Indicated that the osmotic effect due to salinity was the main inhibitory factor that reduced germination as noticed. This reduction of water potential and toxic effects of salts initially intervene in the process of water absorption by seeds, affecting the germination process [18]. In the same context, the high level of salt solution can significantly reduce seed germination and seedling growth, due to the combined effect of high osmotic potential and specific ion toxicity [19]. [20] founded that the high accumulation of Na^+ in saline soils causes the lowering of water potential and thus makes the plant unable to extract water from soil.

The speed of germination (SG) of *Laurus nobilis* seedlings was inversely related to salt concentration. Salt inducing a reduction in this parameter was reported in different types of rice (*Oryza sativa* L.) by [21]. [22] also observed the same results with *Medicago sativa.* The decreasing tendency of speed germination under salt stress might be mainly attributed to an osmotic stress [23].

The reduction of seedling height is a common phenomenon of many crop plants grown under saline conditions [24, 25, 26]. In our conditions, NaCl affected the shoot length of *Laurus nobilis* L. seedlings. Similarly, root length was also decreased by salinity. However, root length was more inhibited by NaCl than shoot length. In fact, on NaCl 100 mM, root length represents 9.7% of the control while shoot length represents 40.5%. [27] explain that important reduction in root length by salinity might be due to more inhibitory effect of NaCl on root growth than on shoot growth.

Shoot and root dry weights of laurel seedlings were affected by salinity. Similarly, [28] observed that shoot and root dry weights of rice were decreased with increasing salt stress. This confirms previous studies that suggested that salt stress reduced the biomass of tomato [29], pea [30], *Phaseolus* [31] and rice [32]. The significant dropped in root and shoot development may be due to toxic effects of NaCl as well as unbalanced nutrient uptake by the seedlings [15].Hence, results found by [33] confirmed this explication.

In the present study, as we noticed, root dry weight appeared to be more sensitive to salt stress than shoot. This was manifested by a higher percent reduction in root dry weight, which can indicate that the ability of seedlings to produce roots was more inhibited than producing shoots. This observation might also be due to partitioning energy to the root and this could be used as an indicator of salinity tolerance of a particular variety [15].

5. Conclusion

NaCl caused a negative effect on the germination stage of laurel seeds. Hence, we note a reduction in the final germination percentage and the germination speed, especially at NaCl 150 mM where the germination was totally inhibited. Moreover, the growth of aerial (shoot, leaves) and root parts was reduced by salinity. However, the growth of roots was clearly more affected by NaCl than the growth of the aerial part. Salt stress induced also a decrease in the fresh and dry weights of laurel seedlings concerning the whole plant, leaves, shoots and roots.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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