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Growth of young argan tree seedlings (*Argania spinosa L.* Skeels) in north-east of morocco under controlled conditions at different NaCl concentrations

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Abstract

This study is a contribution to assess the effect of salt on the development of young Argan tree seedlings *(Argania spinosa L.* Skeels). The experiments were conducted in pots under glass greenhouse at the National Institute of Agronomic Research of Oujda. The samples were subjected to salt treatment at different NaCl concentrations of 0, 1, 3, 5 and 7g/l. Herein, salt tolerance of the young seedlings was investigated by taking into account the morpho-metric and physiological parameters. The obtained data showed that the applied salt stress significantly affected the overall studied parameters, except for the height to diameter ratio that remained unaffected by the treatment. An increase of the mortality rate of the stressed samples was observed under higher NaCl concentrations. Indeed, the presence of 7g/l of NaCl in irrigation water solution led to a mortality rate of 71%. Also, the height and diameter of the young seedlings stems were found to be affected by the high NaCl concentrations. At the leaf level, the effect of the salt resulted in a decrease in the number of leaves and in lower levels of chlorophyll.

1. Introduction

Salinity is one of the major severe constraints that limit crop productivity in 40% of the land surface, notably in the Mediterranean region [1]. On average, the world loses 10 hectares of arable land per minute, from which 3 hectares are lost due to salinization [2]. It was shown that from 10 to 15% of irrigated areas (20 to 30 million hectares) suffered with varying degrees from salinization problems [2]. Furthermore, salty soils are widespread in arid and semi-arid lands, thereby limiting significantly the productivity of herbaceous and woody crops. These areas cover a large part of the countries of the southern fringe of the Mediterranean. In these regions, the low availability of water and the salinity of soils are among the major factors limiting crop production [3].

Morocco, which offers all variants of the Mediterranean climate, does not escape to this phenomenon, where drought observed since a long time clearly led to soil salinization process [4]. Both natural constraints: drought and salinity, have altered the ecosystem stability and are being among the major causes of soil desertification.

In General, different strategies may be attempted to overcome the salinity issue, namely the technique of drainage of salt excess. Nevertheless, these methods are not so affordable and require a large volume of water to leach the soils [5]. Therefore, the introduction of plant species which are tolerant to salt stress and of great socio-economic values is one of the possible approaches to rehabilitate soils.

Hence, the search for vegetation adapted to elevated salinity levels becomes imperative to expand the cultivation of plants in those areas. Among the highly valued species, *Argania spinosa* (*L.*) skeels have particularly gained a special interest and it is considered as a relic of the Tertiary Era. In fact, numerous studies highlighted its forest, ecological and socio-economic importance [6, 7, 8]. Particularier, this plant represents the second forest species in Morocco in terms of area, thereby being widespread across 828000 hectares [8]. More concretely, the Argan tree covers a coastal strip between the mouths of the river Tensift and the Oued Souss, and many of the areas of the Souss plains and watersheds of the High Atlas and Anti Atlas [9]. It is also localized in the mountains of BeniSnassen (northeastern Morocco) and in the upper valley of Oued Grou (near Rabat) and from the entire

Draa valley til the hamada of Tindouf [10]. In some regions, the Argan tree is also observed in the form of isolated feet, such as the Cap Blanc of El Jadida [11] and in the plain of Bou-Areg of Nador [12]. On the other hand, the domestication of this species in eastern Morocco was undertaken for the first time by the Biology Department of the Faculty of Sciences of Oujda since some years ago, and the first produced seedlings have began to fructify [13]. It was shown that the Argan tree had some tolerance to salinity at germination stage and at the first phases of its in vitro growth [14]. It's hence important to evaluate the behavior of young plants under salt stress conditions in order to assess their response towards various abiotic stresses, in similar proportions to those found in arid and semi-arid lands.

The present work aims at exploring the degree of tolerance of young Argan seedlings, of North-Eastern Morocco, when being subjected to salt stress in terms of their morpho-metric and physiological parameters.

2. Material and Methods

The study was carried out in a glass greenhouse at the agricultural station of the National Institute of agronomic research of Oujda. The experiment lasted for two months, from December (2012) to February (2013). The mean temperatures recorded in the greenhouses during the test period were oscillating between 4°C at night and 21°C during the day.

2.1. Plant material

The trial focused on Argan tree seedlings eight months old. These plants have been produced in a culture chamber. The seedlings were then transplanted in plastic pots, already filled with the background substrate, below which a layer of gravel (≈ 5cm) was placed for ensuring water drainage. Each pot contained about 3.5 liter of substrate, including 75% of peat and 25% of river sand.

After an acclimatization period of five weeks, the plants were subjected to salt stress by the application of various treatments using sodium chloride (NaCl).

2.2. Salt treatment

We studied the effect of five NaCl concentrations tested previously on the germination of seeds of the Argan tree [14]. Five weeks after the transplanting process, salt was brought progressively to the irrigation solution so as to mitigate the osmotic shock, until the final concentrations of 0g/l (T_0), 1g/l (T_1), 3g/l (T_2), 5g/l (T_3), and 7g/l (T_4) are reached. Amounts of water applied during the irrigation of young Argan tree seedlings were measured on the basis of the evapotranspiration process. Manual plant watering were performed twice a week so as to maintain the capacity of evapotranspiration at a constant rate of 100%, and to allow at the same time the salt leaching.

1.3. Test Methodology

To perform the tests, we adopted a random experimental methodology based on five treatments with increasing concentrations of NaCl (0, 1, 3, 5 and 7g/l). Each treatment was applied on 10 seedling samples, and was replicated 3 times (for each NaCl concentration). In short, the experiment was conducted using a total of 150 seedlings (10 seedlings per-treatment * 5 NaCl treatments * 3 replicate).

1.4. Studied parameters

Observations and measurements were taken monthly for eight weeks and focused, on one hand, on the mortality rate, height and stem diameter, and number of leaves per seedling, and on the other hand on the content of chlorophyll in the leaf. The dosage of chlorophyll pigments was conducted by measuring the optical density at 663 and 645nm of the supernatant obtained from the grinding of the leaves extracted from the control and stressed plants. Chlorophyll concentrations were defined by the formulas C (a + b) = (7.15 * DO663 - 18.71 * DO645) × V/M expressed as mg.g-1 F.M. (fresh matter) [15].

The obtained data were analyzed through a one way analysis of variance (ANOVA). Then the means were compared by the method of Benforonni based on the smallest significant difference, by using SPSS software. Correlations were determined between each of the selected parameters and concentrations of NaCl.

3. Results and discussion

3.1. Analyses of variance

The results obtained from the analysis of variance (Table 1) indicated that the salt treatment was significantly meaningful for the most studied factors, with the exception of the height to diameter ratio. Treatment effect explained more than 80% of total variability for the majority of the parameters except for H/D ratio where it explained 51%.

Table1: Analysis of variance for the studied morpho-physiological parameters.

variation Source	Df	Mean square					
		Mortality rate	Leaf Number	Stem Height	Stem Diameter	H/D ratio	Chlorophyll content
Treatment	4	2797.733***	2413.733***	9.111**	0.003*	0.458	0.380***
Replicate	2	147.467	76.067	1.027	0.000	0.089	0.000
Error	8	124.133	90.483	1.149	0.001	0.34	0.004

Df: Degree of freedom, H/D: Height to diameter ratio, * Significant at 0.05 probability level; ** Significant at 0.01 probability level; *** Significant at 0.001 probability level

3.2. Effect of salinity on the mortality rate

Increasing the salt concentration in irrigation water solution resulted in a significant increase in the mortality rate of young Argan tree seedlings (Figure 1). This increase changes from 0% in the absence of NaCl to 71% under the effect of 7g/l of salt. The concentration of 5g/l of NaCl caused a mortality rate of 50% for stressed seedlings. The comparison of the mortality rate averages revealed the existence of four classes (Table 2).

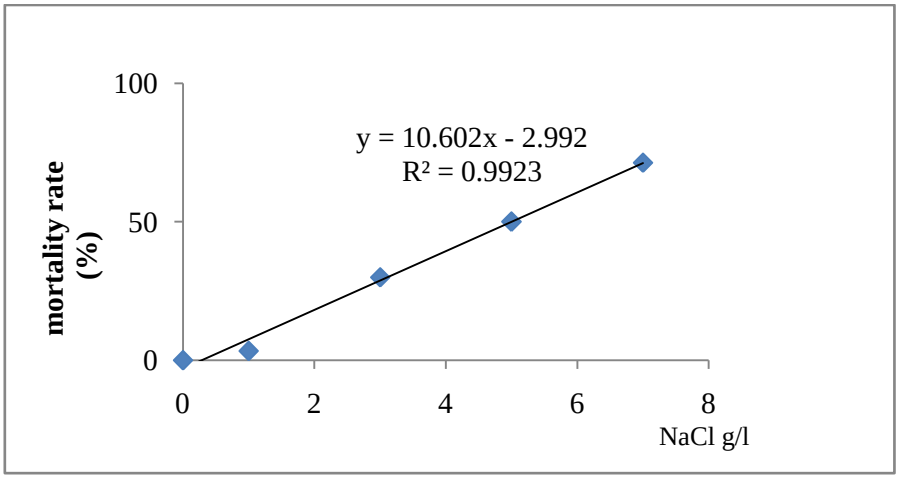


Figure 1: Effect of NaCl concentrations on mortality rate

Table 2: Mean comparison of the studied parameters

	Mortality rate (%)	Leaf number	Stem height (cm)	Stem diameter (cm)	H/D Ratio (cm/mm)	Chlorophyll content
T_0=0g/l	0.0000 d	82.0000 a	37.5942 a	0.4147 a	9.0798 a	1.0107 a
T_1=1g/l	3.3333 d	66.0000 a	37.9333 a	0.4200 a	9.08061 a	0.8700 b
T_2=3g/l	26.6667 c	39.3333 b	35.2533 b	0.3877 ab	9.1091 a	0.3900 c
T_3=5g/l	50.0000 b	25.6667bc	34.9533 b	0.3620 ab	9.6726 a	0.3013 cd
T_4=7g/l	71.3333 a	13.3333 c	33.9417 b	0.3433 b	9.9015 a	0.2253 d

H/D: Height to diameter ratio; Means for each character followed by the same letter are not significantly different according to LSD test at $P < 0.05$

3.3. Effect of salinity on the stem height

The Argan samples respond to different concentrations of NaCl by a very significant reduction in stem height growth (Figure 2). In this case, the observed mean height ranged between 33.9 cm (T_4) to 37.9 cm (T_1). In this regard, the stem height started to decrease since a concentration of 3g/l or higher was applied. At this concentration, we noticed a height reduction of 6%, while at 7g/l, the height decrease reached 10%.

Overall, the salt stress seemed to induce a necrosis of the seedlings apex, which was evidenced by growth arrest and reduction of the seedlings height. A comparison of the mean height of seedlings, suggests the presence of two subsets (Table 2). The first subset includes the samples related with the treatments T_4, T_3, and T_2, while the second concerns the T0 and T_1 treatments.

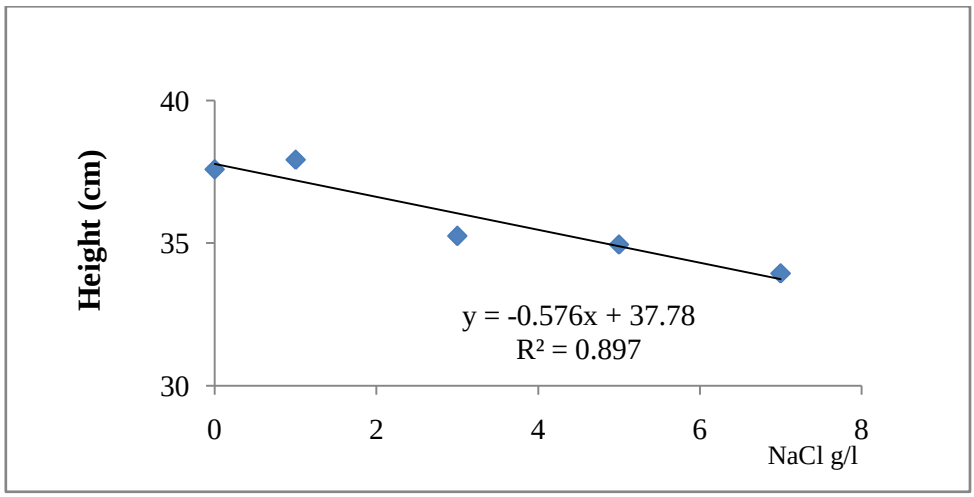


Figure 2: Effect of NaCl concentration on the stem mean length

3.4. Effect of salinity on the stem diameter

Increasing the NaCl concentration in the irrigation water solution significantly decreases the growth rate relative to the stem diameter (Figure 3). Indeed, the observed mean diameter ranged from 0.41 cm to 0.34 cm for the control sample and for the T_4 sample, respectively, hence a reduction of 19% between T_0 and T_4. Consequently, the salt stress seems to have a little impact on the growth diameter of the stressed sample. Average comparison test revealed two categories (Table 2). The first group consists of young seedlings of treatment T_4, T_3 and T_2, while the second is represented by T_3, T_2, T_1 and T_0.

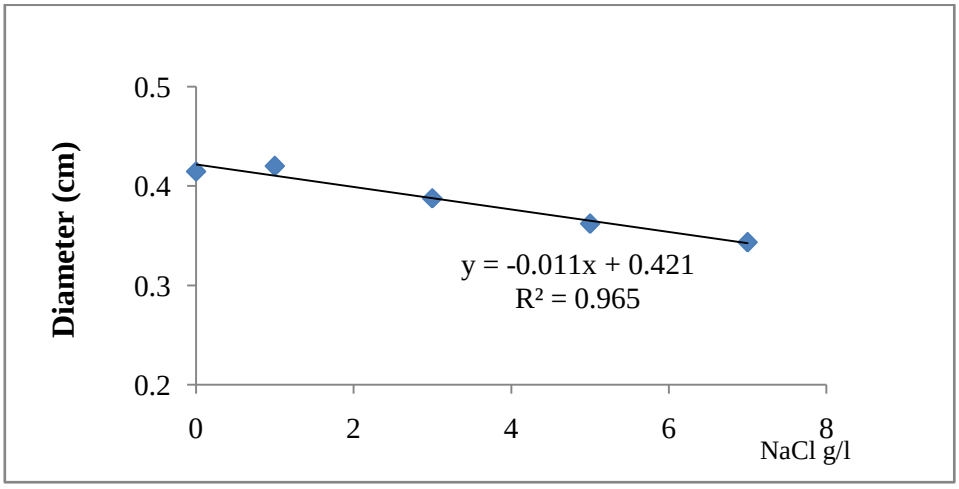


Figure 3: Effect of NaCl concentration on the stem diameter

3.5. Effect of Salinity on the stem height to diameter (H/D) ratio

The data related with the stem height to diameter ratio (H/D) show no meaningful effect of saline treatment on the studied Argan samples (Figure 4). In fact, only a slight increase from 9.07 for control sample to 9.9 for T_4 seedlings was observed in the H/D ratio. This can be explained by the fact that the applied stress acted more on the height rather than the stem diameter.

3.6. Effect of salinity on the leaf number

This parameter is considered as a good indicator of the assimilative capacity of the plant and its biomass productivity. In this sense, the effect of salinity on the Argan samples resulted in a significant decrease in the number of leaves (Figure 5). Indeed, when the NaCl concentration increased, the average number of leaves per seedling decreased. Concretely, by using a concentration of 1g/l of NaCl, the average number of leaves has experienced a 20% decrease compared to the unstressed sample. At 3g/l, the average number of leaves has dropped by 53% compared to the control sample. And at 5 and 7g/l, the leaves drop rates were 70 and 85%, respectively. The comparison of the average number of leaves of young Argan seedlings allowed underlining the presence of three classes (Table 2).

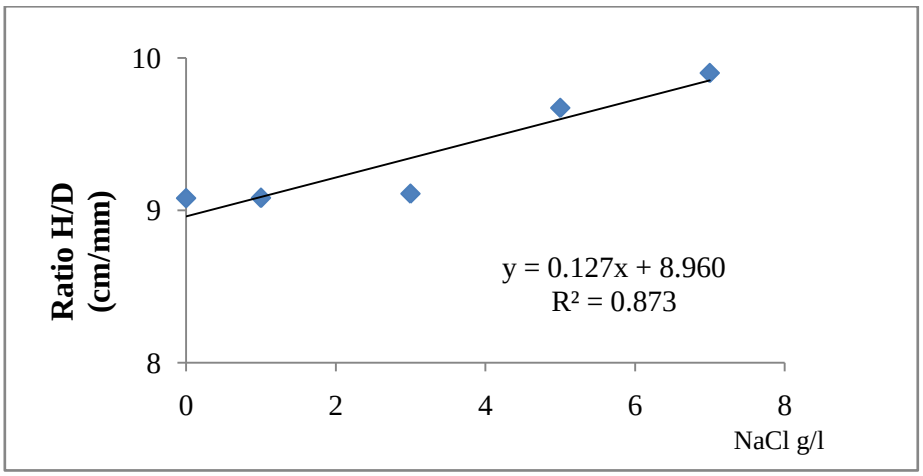


Figure 4: Effect of NaCl concentration on the height to diameter ratio (H/D)

The first class contains the leaves of the control sample and those of T_1 treatment. The second and third classes are represented by T_2 and T_4 respectively. However, the average number of leaves related with T_3 treatment was intermediate between T_2 and T_4. It should be noted that the number of leaves contained in the samples was greatly affected by the elevated concentrations of NaCl. Indeed, the damage induced by salt stress was noticed through yellowing and drops of the leaves. Thus, the seedlings of T_3 and T_4 treatments experienced an earlier leaf drop compared to seedling leaves under T_2 treatments.

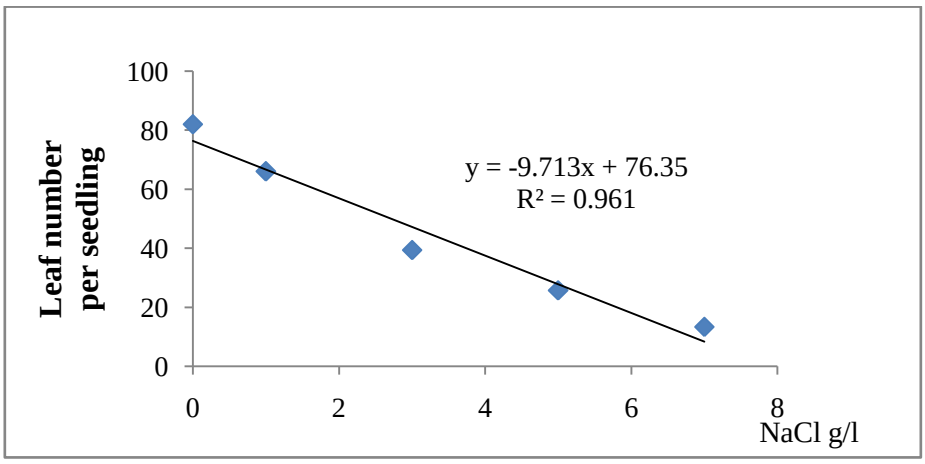
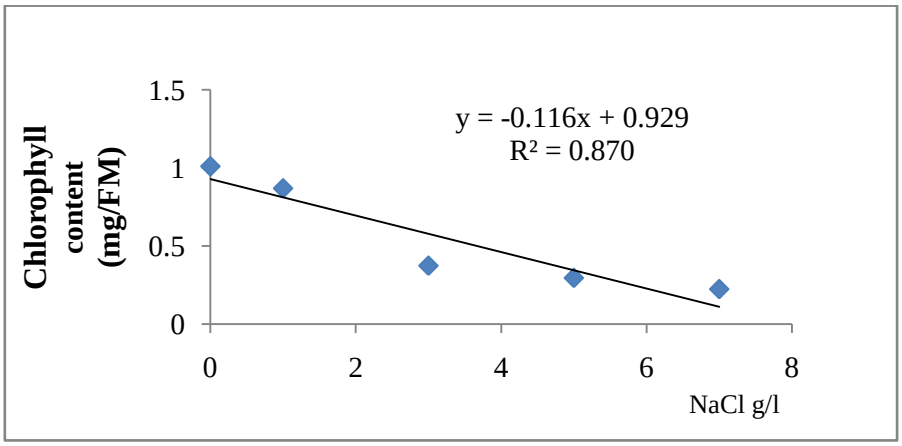


Figure 5: Effect of NaCl concentration on the mean number of leaves

3.7. Effect of salinity on chlorophyll content

The chlorophyll content was significantly reduced by the effect of salinity (Figure 6). Thus, in the unstressed plants, chlorophyll content was higher compared to that measured in plants treated with sodium chloride. The most significant reductions were observed in the presence of 3, 5 and 7g/l of NaCl. In fact, a decrease in the amount of chlorophyll of 61, 70 and 78% was noticed in the leaves subjected respectively to T_2, T_3 and T_4 treatments. The comparison test of the mean content of chlorophyll showed four sets (Table 2). Each set corresponds to one treatment, except for the T_3 related seedlings set whose content was intermediate between T_2 and T_4. The correlations between the parameters which were studied on the one hand and the various NaCl concentrations on the other were high, thus confirming the depressive effect of the saline stress on growth and development argan tree. The results of the depressive effect of salt stress on the growth and development of young Argan seedlings, in case of high salt stress, are consistent with those obtained in the same species [14, 16] and in other species, such as jojoba [17, 19], acacia [20], cereals [2, 25], banana [26] and *Elaeis guineensis Jacq* [27]. The effects of salinity on the growth of young Argan seedlings in greenhouses vary depending on the applied NaCl concentration. According to our results, a concentration of 5g/l of salt in the irrigation water decreased significantly all the studied parameters and resulted in 50% of mortality rate in the argan. The results of this study show that salinity negatively affects the height and diameter of the stem. Similar results were obtained by [28] on seedling of *Pistacia vera L.* that were submitted to in vitro salt stress and also on *Jatropha curcas L.* [29]. On one hand, our results demonstrate that the effect of salt stress on the height to

diameter ratio was not meaningful. In contrast, the growth in terms of height and diameter were strongly affected by salt. According to the standards reported by [30], the height to diameter ratio expressed in cm/mm should be lower than 8. This quotient (H/D ratio) calculated in case of all applied treatments exceed this threshold, which means that salt stress has no effect on this quotient.



FM: Fresh matter

Figure 6: Effect of NaCl concentration on the chlorophyll content

On the other hand, the leaves are the most sensitive tissues of the plants when subjected to stress. In this regard, the Argan tree seedlings experience a significant decrease in their number of leaves, compared to the untreated control sample, for treatment concentrations higher than 3g/l. These results are in good agreement with those of [29], which found that concentrations of 8 and 16g/l significantly reduce the number of sheets of two sources of *Jatropha curcas L*. In our case, Chlorophyll content underwent a sharp decrease since a concentration of 3g/l of NaCl, namely a drop of 61% compared to the control sample. These results are similar to those obtained by [31] across varieties of safflower. The damage observed in plant growth and its different bodies, when subjected to salt stress, is attributed to several causes, including the accumulation of Na^+ ion in foliar tissues, which is known to induce ionic damage in the plant tissues [32]. According to the same authors, salt stress inhibits the absorption of nutrients such as P and K; thereby affecting the plant growth and development. [33] confirms that the reduction in the absorption of (NO_3^-) ions is responsible for the reduction in growth. The slowdown in aerien growth can also be explained by the accumulation of some growth regulators in the plant tissues including abscisic acid and cytokinins induced by the salt treatment [34, 28]. Thus, the growth reduction of the aerial parts is an adaptive capacity [35]. It allows the plant to accumulate energy and resources for overcoming the stress, and facing the irreversible damage caused when the threshold concentration is reached. In several Eucalyptus species, when plants are exposed to high concentrations of NaCl, the roots accumulate much more sodium than the sheets [36]. The reduction in stem growth was also noticed in young seedlings of the Argan tree from concentration of 3g/l [14]. These results are in agreement with the work of [17] where the jojoba reacted by a reduction of its aerial part, in response to salt stress. In the red oak and American beech, the length of the stem was not affected by the presence of 0.44 g/l and 0.94 g/l of NaCl, respectively [38]. In contrast, [20] found the opposite in the *Acacia cyanophylla*. According to [39], salt has significant effects on several anatomical and morphological parameters such as the number and size of stomata, the density of trichomes and leaf size. Moreover, the green color of the leaves alleviated by the high concentrations of NaCl could be explained by a decrease in leaf chlorophyll pigments. This effect is probably owed to the decrease in the synthesis of chlorophyll, due to a change in the membrane structure of thylakoid [40]. Furthermore, this result can be explained by the formation of a proteolytic enzyme (chlorophyllase), which is responsible for the chlorophyll degradation [41]. The chlorophyll reduction may be linked with the sensitivity of its biosynthesis to sodium chloride; this latter has less effect on the chlorophyll biosynthetic pathway [42]. In this way, [43] reported that the detrimental effect of salinity on the levels of chlorophyll pigments is partially responsible for the reduction of carbohydrate synthesis.

Conclusion

Salt stress induces a depressive effect on the studied morpho-metric and physiological parameters of Argan tree seedlings. And, the level of salt tolerance depends on the stress intensity. On one hand, the presence of 7g/l of NaCl in irrigation water led to a mortality rate of 71%. Also, the height and diameter of the stem were found to decrease in function of the stress intensity. In contrast, the ratio H/D increased without significant relation with

the concentration of NaCl. On another hand, Chlorophyll content and the leaf number seemed to be highly sensitive to salt stress parameters.

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