# Role of Salt Stress on Seed Germination and Growth of Jojoba Plant Simmondsia Chinensis (Link) Schneider

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# Abstract

An extensive problem in agriculture is the accumulation of salts from irrigation water. Evaporation and transpiration remove pure water from the soil. This water loss concentrates solutes in the soil. When irrigation water contains a high concentration of solutes, salts can reach rapidly injurious levels vis-à-vis salt-sensitive species. One factor limiting jojoba plant (*Simmondsia chinensis* (Link) Schneider) domestication in the oriental areas of Morocco is the damage caused by salt effect since irrigation enhances salinity in plantation soils. The objectives of this study were to define the effect of various NaCl concentration levels on plant growth and seed germination. The tolerance to salinity of jojoba seed and seedlings was assessed at NaCl concentrations of 0, 1, 2, 3, 5, and 7 g l<sup>-1</sup> and the experiment was undertaken in the laboratory under greenhouse conditions. Results obtained show that more than half of the seeds germinated in the presence of 7g/l of NaCl; whereas 5g/l of NaCl inhibited completely the emergence of plumules. Concerning jojoba seedlings, 3 g/l of NaCl marked the start of negative affect on the growth.

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## Introduction

Environmental stresses are among the most limiting factors to crop plant productivity. Salinity is one of the most detrimental ones (Flowers et al. 1997; Boyer 1982). The progressive salinisation of irrigated land produces vast amounts of uncultivated soils (Ashraf, 1994). The deleterious consequences of high salt concentrations in the external solution of plant cells are hyperosmotic shock and ionic imbalance (Niu et al. 1995; Zhu et al. 1997). When salinity results from an excess of NaCl, homeostasis of not only Na<sup>+</sup> and Cl<sup>-</sup> but also K<sup>+</sup> and  $Ca^2$ + is disturbed (Serrano et al. 1999; Hasegawa et al. 2000; Rodriguez-Navarro 2000). Plant survival and growth are dependent on adaptation and tolerance that re-establish ionic homeostasis, thereby minimizing the duration of cellular exposure to disequilibria of ions by maintaining the osmotic balance and thus widening cultivated areas (Marshner 1986).

Plants tolerance toward salts is a complex phenomenon which affects several aspects of the plant. The germination rate and early growth under salty soils indicate clearly that the plant is tolerant to environmental stresses (Tal 1985). Halophyte germination is affected in two ways when seeds are exposed to saline conditions. Firstly, the high osmotic potential of the medium prevents the embryo from taking up water, and secondly, the toxic effect of some ions leads to embryo poisoning (Pollack and Waisel 1972). It is difficult to decipher the exact share that is caused by an excess of ions or that is due to water deficiency. Glycophyte species are generally affected by an excess of ions in spread mature leaves and by hydrous deficit in the young expanding ones (Greenway and Munnus 1980).

The jojoba, *Simmondsia chinensis* (Link) Schneider, Buxaceae, is an arid xerophytic shrub native to the Sonoran Desert of Arizona, northern Mexico. Jojoba is now grown commercially in Australia, Argentina, Chile, Peru, Egypt and Israel. Plantations in South Africa and India are also reported. Jojoba is covering a surface of about 8500ha hectares (Canoira 2006; IJEC 2008) and the world production of jojoba is expected to grow markedly over the next decade.

The jojoba requires minimum a of established techniques and maintenance in the first stages of its proliferation and it can make a contribution of a very long duration against the advance of the desert. It has deep root systems and grows in both arid and saline conditions (Brown et al. 1996; Jensen and Salisburry 1988). A large surface of saline desert, which is of very limited use, could be exploited by the introduction of jojoba. Indeed, shrubs of jojoba are growing satisfactorily with brackish water having 2000 ppm concentration of salts in the Pacific Ocean and in California.

It is dioecious specie giving male and female plants in same proportions however sex in jojoba was identified by using random amplified polymorphic DNA markers (Agrawal et al. 2007). Several researchers studied the resistance of the jojoba toward non-biotic stresses (Botti et al. 1996). Salinity was reported to cause reduction of the elongation and the thickening of stems (Bartolini et al., 1991; Franco et al. 1993); the reduction of the shoot system and leaf size as well as an increase in the thickening of leaves (Botti et al. 1996). Salinity also influenced cutinisation, reduced the development of vascular tissues and increased the density of the trichomes (Botti et al. 1998) and the chemical composition of fatty acid and fatty alcohol in the grain of jojoba (Kayani et al. 1990).

Most of the oriental Morocco land surface is arid or semiarid. Unfortunately these areas can only be made more productive by irrigation. A strong link with salinisation (Ghassemi et al. 1995), throws an immediate question over the sustainability of using irrigation to increase food production. Furthermore, given the amount by which jobs creation will have to be increased, it seems reasonable to introduce salt tolerant and drought resistant plants with economic values to this area to improve the sustainability of rural activities and thus tackle rural emigration.

We have undertaken the introduction of jojoba in the semiarid area of the oriental Morocco for the first time in 1996. In order to improve the domestication, it is necessary to study its resistance under non-biotic stresses. In the present study, we examine the effect of various levels of NaCl concentration on the germination and the growth of jojoba seedlings.

#### Material and Methods

Seeds used are from plants growing in arid land near Marrakech. The germination essay of seeds was carried out in 50 mm diameter Petri dishes on three layers of an absorbent cotton mattress moistened with different concentrations of NaCl solution. Petri dishes were sealed with a plastic film to prevent evaporation. An emerged radicle was the criterion for germination (Côme 1982) and the growth of the seedlings was followed under greenhouse conditions. The effects of (0, 1, 3, 5)and 7 g/l concentrations of NaCl on the germination of seeds were tested. In each essay, 30 seeds were disinfected by sinking them in bleach for 5 min. The grains were washed three times with distilled water. The seeds were placed in the Petri dishes and then incubated in a dark oven at 26°C. The Germination was monitored every 24 h for 20 days and seeds giving rise to the primary root and those emitting plumule were counted.

The effects of the NaCl concentrations cited above were tested on eleven five month old jojoba seedlings. The latter were transplanted into 0.5 litre black plastic hags filled with soil samples made of 50% fresh ground, 25% manure and 25% river sand. The seedlings were monitored under greenhouse conditions every week for 150 days. The mean temperatures maintained during the probation period ranged between  $14^{\circ}$ C to  $21^{\circ}$ C (night/day).

The watering of the seedlings was performed each two days in the beginning, and then it was reduced to once a week for the rest of the experiment. Leaves and internodes numbers as well as shoots lengths were measured every week for five months.

#### Statistical analyses

The statistical analysis was carried out by the test of comparison of means in order to detect the effect of salt on germination and growth parameters. In the same way, the correlation analysis was given between each one of these parameters and the NaCl concentrations.

#### **Results and Discussion**

The germination of jojoba seeds was affected negatively by NaCl starting from the concentration of 3 g/l, whereas the stimulated concentration of lg/lthis germination (Fig. 1). Comparison of the mean germination rates vis-à-vis various NaCl concentrations shows that the salt effect was raised when NaCl concentrations ranged from 5 to 7 g/l. These results revealed the depressive effect of strong salt concentrations and are in agreement with those observed in the case of the Argania spinosa plant (Reda Tazi et al. 2001). In the case of Hordeum vulgare and Atriplex halimus, the effect of NaCl was observed in situ starting from 10g/l (Sibi and Fakiri 2000; Ben Naceur et al. 2001) and 13 g/l (Belkhodja et al. 2004; Choukr-Allah et al. 1997) respectively. It is also noted that a concentration of 2g/l of NaCl stimulated the rate of germination of Atriplex halimus.

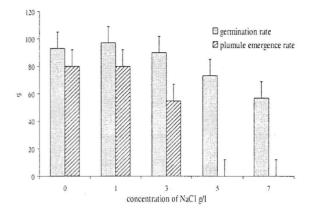


Figure 1. Effect of NaCl concentrations on the rate of plumule emergence

As salinity reached 3g/l of NaCl, the rate of the plumule emergence of jojoba seeds decreased by 50% and it was completely inhibited by NaCl concentration of 5g/l. In the meantime, the rate of germination decreased slightly to 73% compared to the control sample (Fig. 1) at 5g/l of NaCl. These results are in agreement with earlier reports on argan (Reda Tazi et al. 2001) and cereals (Malek-Maalej et al. 1998; Ben Naceur et al. 2001). Statistical analysis of results obtained for samples under salt stress showed significant control differences.

The monitoring of electrical conductivity at the start and end of the salinity treatment showed a considerable increase in this parameter for all media except for the control one where the final conductivity was slightly lower than the initial one (Table 1). This strong final electric conductivity can be explained by the cumulative effect of various solutions used for irrigation, which blocked the opening of the plumule. The incubation of samples with 7g/l NaCl solution resulted in salt accumulation at the soil surface and produced a salinity medium with concentration of 16g/l of NaCl.

	Initial electrical conductivity <sup>a</sup> (mmhos/cm)	Finale electrical conductivity <sup>a</sup> (mmhos/cm)
T <sub>0</sub>	0.42	0.32
T <sub>1</sub>	1.87	3.06
T <sub>2</sub>	5.4	6.6
T <sub>3</sub>	8.26	14.21
T <sub>4</sub>	11.78	23.91

Table 1: Initial and final electric conductivity in different Petri dishes

<sup>a</sup> Average values reported for N = 11, with R.S.D.< 5%

The effect of salt on the shoot system growth was highly perceptible. Indeed. significant statistical differences were observed in the case of samples treated with salt concentration of 3, 5 and 7g/l over the control one. Results indicated that length reduction over control of the stem was 25 and 69% for samples watered by NaCl solutions of 3 and 7g/l respectively (Fig. 2). It appeared clearly that the growth in length of the jojoba seedlings verv affected was by strong NaCl concentrations. These results are in agreement with those reported by Benzioni et al. (1992) where jojoba responded to soil salinity by reducing its shoot system. The growth reduction of the stem was also observed in the case of argan seedlings starting from the concentration of 3g/l (Reda Tazi et al. 2001).



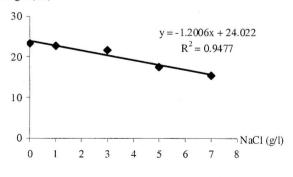


Figure 2. Effects of salt concentration on the mean shoot length

The effect of salinity on length of seedlings produced a reduction in the number of leaves and internodes. Statistical analysis showed that there was a salt effect on the number of leaves and internodes on NaCl treated plants over the control sample (Fig. 3 and 4). According to Botti et al. (1998) jojoba plants grown under high salt levels did not show much difference from those grown under non-saline conditions for most of the morphological and anatomical parameters such as number and size of stomata, density of trichomes, leaf size, branching characteristics and stem diameter. Only leaf and cuticle thickness showed a high tendency to increase under saline conditions.

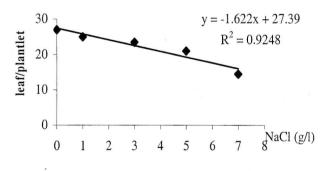


Figure 3. Effects of NaCl concentration on the mean number of leaves

It is important to note that saline stress during the drought period had no affect on ramification of young jojoba plants. In contrast, Botti et al. (1998) reported that saline stress depressed the ramification and diameter of adult stems of seedlings. In addition, the green colour of leaves was attenuated by high NaCl concentrations; this could be explained by a reduction of chlorophyll pigments.

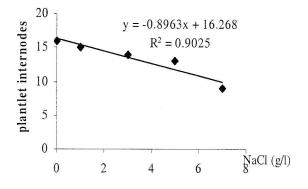


Figure 4. Effects of NaCl concentration on the number of internodes

It is worth noting that the growth reduction along with an increase in the damage of the foliar percentage could be explained by reduction in the hydrous potential which resulted from an increase in NaCl concentration in the cultural medium. Indeed, the toxicity of sodium and chloride ions was the principal cause of the inhibition of growth of several species, though the salt concentration was weak (Marshner 1986). The resistance of the crop plants to saline stress depends in part on their capacity to control the access of sodium in the leaves. The non halophytes accumulate much more sodium and/or chloride ions in foliar tissue even in the presence of weak salt concentrations (Downtown 1978; Greenway and Munns 1980). For several species of Eucalyptus, when the seedlings were exposed to strong NaCl concentrations, roots accumulated much more sodium than the leaves (Fathi and Part 1989; Van der Moezel et al. 1988).

The correlations between the parameters which were studied on the one hand and the various NaCl concentrations on the other were high (0.946 < r < 0.973), thus confirming the depressive effect of the saline stress on germination, growth and development of the jojoba plant. These results are in agreement

with those obtained on other species such as argan (Reda Tazi et al. 2001), acacia (Hatimi 1999) and cereals (Driouich and Rachidai 1996; Sibi and Fakiri 2000; Ben Naceur et al. 2001).

### Conclusion

The *in vitro* study of the germination of seed and the growth of the jojoba plant under saline stress enabled us to conclude that NaCl 1g/l stimulated the seeds germination. More than half of the jojoba seed germinated in medium with NaCl concentration up to 7g/l, whereas 5g/l of NaCl marks the start of the complete inhibition of the emergence of plumules. The salt seemed to affect the opening of the plumule rather than germination. The evolution of the growth and the development of the young seedlings are strongly affected by strong NaCl concentrations.

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