

Germination of jojoba (*Simmondsia chinensis* L) seeds under the influence of several conditions

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Rec. 20 Mar, 2012 Accpt. 2 May, 2012

Abstract

Our study indicates that jojoba is suitable plant for cultivation of the Egyptian marginal soils, in the desert area, where the seeds were germinated and grown in sandy soil of marginal fertility. To study the effect of NaCl and mannitol on seeds germination, jojoba seeds were placed on cotton layer flooded with solution containing different concentrations of them. Salinity stimulated seed germination, especially, when the seeds were subjected to relatively low concentration of NaCl (0.5 – 3 gm/l). Increase the concentration of NaCl over 3 gm/l resulted in inhibition of seed germination as well as radicle growth. Application of 2 or 3 gm/l mannitol in germination medium improved the seed germination, vice versa was detected under progressive increase of mannitol in germination medium. Mannitol as same as NaCl delayed seed germination of jojoba plant. On the other hand, jojoba seeds can be germinated in low frequency under high concentration of mannitol, up to 100 gm/l, when seeds were placed on three cotton layers just wetted by distilled water containing mannitol. Temperature may be the most critical factor during jojoba seed germination, therefore summer was the best season for seed germination; also, 30 °C was the best temperature degree for seed germination and emergence of radical in the shortest time.

Key words: Desert cultivation, Jojoba, mannitol, sodium chloride, seed germination, stress.

Introduction:

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a desert shrub which tolerates salinity and drought. The chromosome number of jojoba is $2n = 52$ (Weiss, 1983). Its natural life span appears to be between 100 and 200 years. Jojoba seeds contain a liquid wax of economic importance in industry (machine lubricant) as well as in medicine, where it can be used in cosmetics and anticancer compounds. Jojoba was used as a medicine for cancer, stomach ache, kidney disorders, easing childbirth and in tending wounds (Weiss, 1983). Jojoba has attracted interest as a landscape plant; also it can be used for soil conservation. The plant has a deep root system; therefore it can be used in highway and roadside plantings and hedges. It can also be used as a soil stabilizer in green belts around desert cities suffering from particulate air pollution. It is the only plant known that synthesizes liquid wax. The seeds contain about 50% of simple wax esters of mono-unsaturated fatty acids and alcohols.

Plantations are established by using seeds, seedlings, rooted cuttings, or plantlets produced from tissue culture (Roussos *et al.*, 1999; Roussos *et al.*, 2006; Mohasseb *et al.*, 2009). The male plants outnumber the females when raised from seeds (Harsh *et al.*, 1987). Jojoba plants obtained from seeds showed a high variability in most characteristics including yield because it is dioecious, and obligate cross-pollinated species (Gentry, 1958). Previous reports indicated that only a small proportion of the plant population (less than 1%) originating from seeds of native plants has the potential to produce economically acceptable yields (Purcell and Purcell, 1988; Ramonet-Razon, 1988). Therefore, comprehensive selection and breeding program was conducted in many countries all over the world to obtain elite cultivars.

Salinity is considered one of the most important factor restrict the horticultural production, especially in soils of the arid and semi-arid regions on the earth. Few economical plant species can be grown

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successfully in saline soil. It is worth to mention that the total area of arable land is gradually decreasing due to the progressive salinization of the soil (Botti *et al.*, 1998).

While jojoba is known as salt tolerant species (Jensen and Salisbury, 1988; Benzioni *et al.*, 1992). salt damage can occur and differences in response among clones had been observed (Rasoolzadegan *et al.*, 1980; Benzioni *et al.*, 1992). indicating the possibility of developing clones with increased levels of salt resistance or tolerance. Elongation and thickening of stems (Bartolini *et al.*, 1991). the total leaf area and leaf size (Bartolini *et al.*, 1991). shoot and leaf expansion, number of leaves and flowers were reduced in response to increased levels of salinity (Rasoolzadegan *et al.*, 1980; Benzioni *et al.*, 1992). Increasing in leaf thickness was also reported when jojoba plants were subjected to salt stress (Sa´nchez-Blanco *et al.*, 1991).

Benzioni *et al.*, 1999 reported that some clones exhibited excellent vegetative traits related to yield potential such as a high survival rate, rapid growth, extensive branching, high node density, high flower density, high percentage of fruit set, high seed weight, and high wax content in the seed. The clones also differed in their wax composition. Under Egyptian condition, jojoba maximally utilizes 50-70 liters of water weekly in summer and 10-30 liters in winter but when it irrigated by flooding, 12-15 irrigation times per year is needed. Mature shrubs characterize by their strong ability to survive without irrigation for long time where they can survive for a whole year without watering. Jojoba can also tolerate up to 3,000 p.p.m. without any impact to the yield. Therefore, Jojoba is considered one of the most practical and scientific solutions for desert plantation in Egypt. In spite of its importance, very few studies aim to understand the effects of abiotic stresses on the development and yield of the jojoba. This article covers the research on jojoba ecophysiology, with emphasis on the effects of water and salt stress on seed germination.

Material and Methods:

Plant material: For the experiments, seeds were obtained from the Egyptian Natural Oil Co. S.A.E., Ismailia Farm, Salam Zone, Manayef, Ismailia, Cairo, Egypt. The farm is

planted in Ismailia in 1991 and it is about 88,200^{sq.m} of jojoba plants, it was used for research and production.

Effect of soil type on seed germination: Thirty jojoba seeds were sown in plastic pots containing two Kg of soils composed from sand, soil or both according to the following table:

Soil structure	
Sandy soil	Clay soil
100%	0%
75%	25%
50%	50%
25%	75%
0%	100%

After 40 days percentage of seed germination and germination period were estimated. An emerged radicle was the criterion for germination (Côme, 1982). and the growth of the seedlings was laboratory or greenhouse conditions.

Effect of season on seed germination: Thirty jojoba seeds were sown in plastic pots containing two kg of soil containing 1 and 1, sand and clay soil, respectively. After 40 days percentage of seed germination and germination period were estimated.

Effect of temperature on seed germination:

Jojoba seeds were grown on cotton in glass jars contained 50 ml Hogland solution and incubated at 30°C, 40°C, and room temperature (maximum 18°C). Germination frequency, length of roots, length of shoots and number of leaves per shoot were determined after four, nine, and 21 days of seedling.

Effect of NaCl on seed germination:

Under sterilized condition, Jojoba seeds were grown on cotton in glass jars contained 50 ml of Hogland solution supplemented with several concentrations of NaCl (0, 0.5, 1, 2, 3, 4 g/l). Jars were incubated at 30°C. Germination frequency, length of roots, length of shoots and number of leaves per shoot were determined after four, nine, and twenty one days of seedling.

Effect of mannitol on seed germination:

Jojoba seeds were flooded on cotton layer in sterilized glass jars contained 50 ml Hogland solution supplemented with several concentrations of mannitol (0, 0.5, 1, 2, 3, 4 g/l). Jars were incubated at 30°C. Germination frequency, length of roots,

length of shoots and number of leaves per shoot were determined after four, nine, and twenty one days of seedling. In addition, jojoba seeds were placed on three cotton layers wetted with 50 ml and containing different concentration of mannitol. The percentage of seed germination was determined in 40 days.

Results and discussion:

The used jojoba seeds were usually smooth, brown to black in colour, their dimensions are 8 – 17 mm in length and 5-11 mm in cross-section. One hundred seed weight can vary from 61 - 157.8 gm/100 seed. Positive correlation was detected between seed size and oil content but the quality of the oil was exhibited very little variation regardless of the geographic origin of the seed (Yermanos, 1979). As was reported previously, the seeds contain little or no endosperm and consist mainly of the undifferentiated tissue of the cotyledons (Weiss, 1983). In this work, seeds were obtained after two months of harvesting date and they showed germination when they were subjected for suitable condition for seed germination. They were readily germinated in sandy or clay soil or in mixture from them under wide range of temperature from 18- 40 °C, it was in accordance with others studies (Gentry, 1958; Yermanos, 1982).

Data in this work (Table 1) indicated that sandy soil is the most suitable soil for seed germination of jojoba plant, where it is expressed the highest percentage of germinated seeds in short time. Therefore, our study indicates that jojoba is suitable

plant for cultivation of the Egyptian marginal soils, in the desert area, where the plant can grow in sandy soil of marginal fertility and needs little water. It withstands salinity and it does not seem to need fertilizers or other polluting chemical treatments. Consequently, jojoba can be generally cultivated in well-drained, coarse, desert soils, where the soil is composed of sandy alluviums and mixtures of gravels and clays derived from such igneous materials as granitics and volcanics. For all of the previous reasons, jojoba is recommended for cultivation in Egyptian desert.

Soil structure		Percentage of seed germination (%)	Germination period (day)
Sandy soil	Clay soil		
100%	0%	75.0	13
75%	25%	65.0	15*
50%	50%	62.5	17*
25%	75%	55.0	21*
0%	100%	45.0	23*

Table 1. Effect of soil type on percentage of seed germination and germination period.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution at 30°C at P < 0.05.

Temperature may be the most critical factor in growing jojoba. Jojoba is living in the bright desert sun and tolerates the extreme daily fluctuations of temperature which commonly range through -1 °C during the morning to daily extremes of 46 °C (shade readings). In our work, increase of temperature stimulated seed germination, shortened the time needed for emergence of radical (Table 2).

Age of seedling	Temperature of incubation	Seed germination freq.	Length of root	Shoot freq. (%)	Length of shoot
3 days	30°C	61	0.26	---	---
5 days	Room temperature	55*	0.2	---	---
7 days	30°C	72	0.4	---	---
	Room temperature	55	0.53	---	---
15 days	Room temperature	55	2	33	1.5
	30°C	72	7	66	0.8

Table 2. Effect of temperatures on seed germination frequency.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution at 30°C at P < 0.05.

Seed germination was influenced by temperature of the seasons. Seed germination in summer was higher than

winter (Table 3). Summer was the best season for seed germination it may be due to the highest temperature degree. Also, the shortest time of seed germination was detected when the seeds were subjected for the highest temperature in summer. Seedlings are more sensitive than mature

tree (Weiss, 1983). While seeding is sensitive to light frosts of -1 or -2°C below freezing, mature shrubs are known to tolerate temperatures as low as -9 °C. When temperatures reach 5°C flowers and terminal portions of young branches of most jojoba

plants are damaged. Wild jojoba plants can withstand very high temperature, cultivated cultivars showed maximum growth between 27 - 36 °C, but. Above 50 °C , the vegetative growth is suppressed, although not lethal (Weiss, 1983).

Parameter	Season			
	Summer	Autumn	Winter	Spring
Percentage of seed germination	77 ±3.81	63 ±2.00	58 ±3.81	73 ±2.50
Germination period / day	13 ±1.00	17 ±1.00	26 ±2.00	17 ±1.52

Table 3. Effect of seasons on percentage of seed germination and germination period.

Under germination condition, the number of germinated jojoba seeds increased with time (Table 4, 5 and 6). In four days, salinity delayed seed germination of jojoba seed. While, 61% of seeds showed seed germination on NaCl free medium, 57% of seeds showed seed germination under the influence of 0.5 gm/l NaCl (Table 4). With time on germination medium, salinity stimulated seed germination (Table 5 and 6) especially, when the seeds were subjected for germination in the presence of relatively low concentration of NaCl (0.5 – 3 gm/l). In this work, the negative effect of NaCl on seed germination was detected when the seeds were subjected for 4 gm/l NaCl. While jojoba is known as salt tolerant species (Jensen and Salisbury, 1988; Benzioni *et al.*, 1992), salt damage can occur and differences in response among clones had been observed (Rasoolzadegan *et al.*, 1980; Benzioni *et al.*, 1992). indicating the possibility of developing clones with increased levels of salt resistance or tolerance.

Relatively low concentration of NaCl (0.5-3 gm/l) stimulated seedling growth and resulted in the formation of higher fresh mass than control. On the other hand, germination of seeds on medium containing 4 gm/l NaCl retarded seedling growth with complete avoidance of shoots (Table 5 and 6). Elongation and thickening of stems (Bartolini *et al.*, 1991). the total leaf area and

leaf size (Bartolini *et al.*, 1991). shoot and leaf expansion, number of leaves and flowers were reduced in response to increased levels of salinity (Rasoolzadegan *et al.*, 1980; Benzioni *et al.*, 1992). Increasing in leaf thickness was also reported when jojoba plants were subjected to salt stress (Sa´nchez-Blanco *et al.*, 1991).

Conc. of NaCl (g/l)	Germination freq. (%)	DRS
Control	61	0.27
0.5	56.6*	0.17
1	38*	0.13
2	55.4*	0.6
3	44*	0.1
4	34.3*	0.1

Table 4. Effect of NaCl on seed germination after four days under germination condition.

* Means significantly different (t-test) from jojoba seeds cultured on hogland solution without salt at P < 0.05.

Increase the concentration of NaCl over 3 gm/l resulted in inhibition of seed germination as well as radicle growth (Table 5). Plumules were completely suppressed when 4 gm/l NaCl were used. These results were in agreement with previous report (Berrichi *et al.*, 2010). They were found that 5g/l of NaCl inhibited completely the emergence of plumules and, 3 g/l of NaCl marked the start of negative effect on the growth jojoba seedlings.

Conc. of NaCl (g/l)	Germination freq. (%)	Length of root (cm)	Shoot freq. (%)	Length of shoot (cm)
Control	66	1.7	16	0.5
0.5	61*	5.3	16	0.3
1	66*	3	16	0.4
2	83	11	33*	0.4
3	75	7.25	33*	0.5
4	41*	0.23*	---	---

Table 5. Effect of NaCl on seed germination after nine days under germination condition.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution without salt at P < 0.05.

Relatively low concentration of NaCl resulted in enhancement of seedling growth up to 3 gm/l NaCl (Table 6). The data also indicated that 4 gm/l of NaCl resulted in decreasing the radical length and inhibition of plumule formation. Botti *et al.*, (1998) reported that 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. On the other side they found that leaf and cuticle thickness showed a high tendency to increase under saline conditions.

Conc. of NaCl (g/l)	Germination freq. (%)	Length of root (cm)	Plumule formation freq. (%)	Length of shoot (cm)	No. of leaves per shoot	No. of shoots per seed
Control	69	2	33.3	0.8	2	1
0.5	66.6*	6.66	50	1	2	1
1	66.6*	3*	50	1	3	1
2	83*	15.3	42.2	4	6	1
3	83.3	14.3	33*	2.3	4	1
4	50*	0.73*	---	---	---	---

Table 6. Effect of NaCl on seed germination after fifteen days under germination condition.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution without salt at P < 0.05.

Mannitol as same as NaCl delayed seed germination of jojoba plant (Table 7). Application of 2 or 3 gm/l mannitol in germination medium improved the seed germination. Comparison between the effect of NaCl and mannitol indicated that incorporation of these both factors in relatively low concentration improved seed germination.

Conc. of mannitol (g/l)	Germination freq. (%)	Length of roots (cm)
Control	61	0.27
0.5	46*	0.1*
1	55*	1.8
2	46*	0.46*
3	33*	0.16*
4	27*	0.1*

Table 7. Effect of mannitol on seed germination after four days under germination condition.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution without mannitol at P < 0.05.

The data in this work indicated that the emergence of plumules were commenced after four days of subjecting the seeds for germination conditions (Table 8 and 9). The commencement of plumules was depend on the concentration of mannitol in the germination medium. While relatively low concentrations of mannitol (1 – 3 gm/l) stimulate plumule formation, 4 gm/l mannitol inhibit completely the emergence of seed plumule. The same results were obtained when 4 gm/l NaCl were used (Table 6).

Conc. of mannitol (g/l)	Germination freq. (%)	Length of roots (cm)	Plumule freq. (%)	Length of shoots (cm)
Control	66	1.7	16	0.5
0.5	55.5*	1.15*	16	0.5
1	55*	8.5	50	0.3
2	66*	7	33	0.2
3	83	3.3	25	1
4	50*	0.5*	---	---

Table 8. Effect of mannitol on seed germination after nine days under germination condition.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution without mannitol at $P < 0.05$.

Plumule formation were delayed under the influence of 4 gm/l mannitol where it was only commenced in two weeks (Table 9). On

the other hand, in two weeks, 4 gm/l mannitol stimulated the radical length in comparison to that of control. These data indicated that mannitol in concentration between 1 and 3 m/l stimulated both shoot length and the number of shoots per seed.

Conc. of mannitol (g/l)	Germination freq. (%)	Length of roots (cm)	Plumule freq. (%)	Length of shoots (cm)	No. of leaves per shoot	No. of shoots per seed
Control	66	2	33	0.8	2	1
0.5	55.5*	2	33	0.8	2	1
1	58*	14.6	50	3.16	2	2
2	83	9	33	1.1	2	1
3	83	7.66	83	1.81.8	2	3
4	50*	5.7	30*	0.66	---	1

Table 9. Effect of mannitol on seed germination of seeds placed on one layer of cotton flooded with Hogland solution for fifteen days.

* Means significantly different (t-test) from jojoba seeds cultured on Hogland solution without mannitol at $P < 0.05$.

Placing jojoba seeds on three cotton layers just wetted by germination medium created suitable condition for seed germination although the presence of high concentration of mannitol. Under these conditions, jojoba seeds were able to germinate and form plumule up to 100 gm/ mannitol. Comparison between data in tables 9 and 10 indicated that seed germination was strongly

affected by the presence of high water content in germination medium. It was expected since the plant strongly tolerates drought condition but it is sensitive for frost and water flooding. Consequently, jojoba has recently established as a crop in many arid and semi-arid regions of the world (Brown *et al.*, 1996), especially around the Mediterranean basin (Benzioni and Dunstone, 1986; Mills *et al.*, 1997) because it is drought and salt tolerant plant species.

Treatment	Mannitol concentration (gm/l)											
	0	10	20	30	40	50	60	70	80	90	100	110
Percentage of seeds cultured (%)	86	96	86	80	76*	63*	60*	56*	46*	43*	36*	20*
Germination period (day)	8	7	9	11	11	13	15	16	17	19	21	25
N. of shoot formation	18	27*	12*	10*	8*	7*	6*	5*	3*	2*	1*	0

Table 10. Effect of mannitol on seed germination of seeds placed on three layers of cotton and witted with distilled water solution for fifteen days.

* Means significantly different (t-test) from jojoba seeds cultured on distilled water without mannitol at $P < 0.05$.

The data of his work indicated that jojoba is the most suitable plant for the Egyptian conditions especially in desert area. Under Egyptian condition, jojoba maximally utilizes 50-70 liters of water weekly in summer and 10-30 liters in winter but when it irrigated by flooding, 12-15 irrigation times per year is needed. Mature shrubs characterize by their strong ability to survive without irrigation for long time where they can survive for a whole year without

watering. Jojoba can also tolerate up to 3,000 p.p.m. without any impact to the yield. Therefore, Jojoba is considered one of the most practical and scientific solutions for desert plantation in Egypt.

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الملخص العربي

إنبات بذور نبات الجوجوبا تحت تأثير العديد من الظروف

دلّت النتائج أن نبات الجوجوبا هو الاختيار الأمثل لزراعة التربة الفقيرة للصحاري المصرية لمقدرة النبات على الإنبات والنمو تحت تأثير تلك الظروف. لدراسة تأثير الملوحة والجفاف على النبات تم وضع البذور عائمة على محلول يحتوي تركيزات مختلفة من كلوريد الصوديوم والمانيتول. تمت ملاحظة أن التركيزات المنخفضة لكل من كلوريد الصوديوم والمانيتول يحسن من عملية الإنبات. على العكس من ذلك فإن التركيزات العالية منها كان لها تأثير مثبط على الإنبات وعلى نمو الجذور. ومن الجدير بالذكر أن بذور النبات تحملت التركيزات العالية من المانيتول عندما وضعت على ثلاث طبقات من القطن مبللة بمحلول المانيتول ولكن كانت بنسب منخفضة جداً. تأثر إنبات بذور الجوجوبا بدرجة حرارة الإنبات، وكانت درجة الحرارة من أهم العوامل التي تؤثر على الإنبات وبالتالي كان أفضل الفصول للإنبات هو فصل الصيف، أضف إلي ذلك أن تحضين البذور عند درجة حرارة 30 درجة مئوية زادة من الإنبات ونمو البادرات.