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Effect of Salt Stress on Chemical and Physiological Contents of Jojoba (*Simmondsia chinensis* (Link) Schneider) using *In vitro* Culture

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Abstract: Seawater (41000 ppm) was used at levels 0, 2000, 4000, 6000, 8000 or 10000 ppm with 3\4 MS medium and BAP at 1.0 mg \l to determine the tolerance of two jojoba clones (51 and 69) at *in vitro* multiplication stage for salinity. Number and length of shoots, number of leaves, leaf color degree and fresh weight of proliferated shoots was measured after 4 subcultures besides elements, free proline and amino acids contents. Clone 69 proved a more tolerant jojoba clone to different seawater levels. It accumulated less Na^+ and Cl^- ions in their shoots and more total amino acids than clone (51). Moreover, the increase in the amino acids glycine, aspartic, threonine, glutamic, alanine, isoleucine, leucine, tyrosine, lysine and proline in addition to ammonia due to salinity could play a positive role in tolerance of jojoba clone 69.

Key words: Amino acids · *In vitro* · Jojoba · Proline · Salinity · *Simmondsia chinensis*

INTRODUCTION

Selection of relatively resistant horticultural crops will be a necessity for the utilization of lands prone to Salinization, like arid and semi-arid areas, for horticultural production [1]. Jojoba or hohoba belongs to family simmondsiaceae. It is an evergreen, dioccious desert shrub, native of Sonoran and Baja deserts [2]. It tolerates salinity and drought. Its fruit is edible in its native habitat and it produces a unique and valuable liquid wax. Because of its sexual propagation, there is an extensive variation between clones. The response to salinity of jojoba nodal segments *in vitro* and that of the whole plant *in vivo* was similar, supporting the idea that *in vitro* screening of organs offers an efficient, inexpensive method of pre-selecting for salt tolerance [3]. Jojoba leaf nutrient content (N^{+3}, P^{+4} and K^+) decreased as salt levels increased, while Ca^{+2} and Na^+ were increased as will as proline content [4]. This study was carried out to determine the effect of salinity stress using seawater levels on *in vitro* multiplication stage of the two tested jojoba clones (51 and 69) and its effect on chemical composition of shoots.

MATERIALS AND METHODS

Effect of Different Levels of Seawater on Two Jojoba Clones: Seawater levels (41000 ppm) at 0, 2000, 4000, 6000, 8000 or 10000 ppm were used with 3\4 MS medium [5] and BAP at 1.0 mg\l to determine the tolerance of jojoba clones (51 and 69, from *in vitro* derived shoots) for salinity. Average number, length of shoot, number of leaves and fresh weight of proliferated shoots was determined after 4 subcultures. Also, leaf color degree was measured with a color chart (MunCell Color Co., Incorporated Baltimore 2, MD) from green to brown according to the following assessment of five colors:1- Brown 2- Orange 3- Greenish yellow 4- Yellow green 5- Moderate olive green.

Chemical Analysis

Determination of Elements Content: Jojoba shoots obtained through *in vitro* culture (after 4 subcultures with salinity stress) were taken and dried to constant weight in an oven at 50°C then ground. Thereafter, 0.5g of dried samples was digested using the H_2SO_4 and H_2O_2. The extracted samples were used to determine the following elements (gm/100g D.W): Nitrogen content was determined in the digested solution by the modified microkjeldahl method. Phosphorus content was determined calorimetrically. Potassium and sodium contents were determined against stander using flame-photometer. Calcium was determined using the plasma 400 emission spectrometer (0993-8778-REV B). Meanwhile, Cl^- was estimated according to the Mohr method.

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Determination of Free Proline Content: Proline was determined in shoots of *in vitro* jojoba clones 69 and 51, after four subcultures with salinity stress, according to the method recorded by Bates *et al*. [6].

Determination of Individual Amino Acids Content: Shoots of *in vitro* jojoba clones 69 and 51 were subjected to salt stress for 10 subcultures. Individual amino acids were detected in shoots using LC 3000 amino acid analyzer.

Statistical Analysis: Complete randomized design was adopted with 3 replicates for each treatment each replicate consisted of 3 jars. The data were statistically analyzed according to Duncan's multiple range test at 5% level of probability [7].

RESULTS AND DISCUSSION

Tolerance of Jojoba Clones to Different Seawater Levels: Data in Table 1 show that, seawater at the level 2000 ppm gave the highest significant average shoot number, while increasing salt level significantly decreased it. Similar results were obtained in average shoot length, leaf number and fresh weight. In addition, increasing seawater level more than 2000 ppm changed color of leaves from green to greenish yellow or even orange. Generally, seawater at the level 10000 ppm gave the lowest result for all parameters under investigation. Explants of clone 69 gave higher average shoot number; fresh weight and color degree than clone 51, while that of clone 51 gave higher leaf number. As for average shoot length, there was no significant difference between the two clones. In our study seawater at the levels 2000, 4000 and 6000 ppm promoted leaf expansion in the two clones (Fig. 1).

Effect of Seawater Levels on Some Elements Concentration of Two Jojoba Clones: Data in Table 2 show that, seawater levels affect significantly ions concentration (Na^+, Cl^-, K^+, N^{+3}, P^{+4} and Ca^{+2}) in *in vitro* jojoba shoots of clones 51 and 69. Regardless the control, the least Na^+ average concentration was achieved at the lowest salinity level (2000 ppm) which achieved the highest concentration of K^+ and Ca^{+2} and lower concentration of N^{+3} and P^{+4}. It is obvious that, shoots of clone 51 absorbed higher amounts of Na^+, Cl^-, $K^+$and N^{+3} but lower amounts of P^+ and Ca^{+2}than those of clone 69. The concentration of K^+ and Ca^{+2} significantly decreased as the salinity level increased in the medium, reached the lowest concentration at the salinity level 10000 ppm with the two clones. The concentration of Cl^- was greater at higher levels (8000 and 10000 ppm) than other levels.

Effect of Seawater Levels on Free Proline Concentration: Data in Table 3 show that, shoot proline content was significantly increased due to salinity effect, compared with the control. Clone 51 without salinity had higher average concentration of proline than clone 69. However, the increasing percentage in proline content due to salinity treatment in clone 69 was higher than that of clone 51. At seawater level 2000 ppm, clone 69 gave a significant higher percentage of increasing proline than that of clone 51.

Table 1: Tolerance of jojoba clones (51 and 69) to different seawater levels after 4 subcultures at multiplication stage

Level	Shoot number			Shoot length			Leaf number			Fresh weight			Color degree		
ppm	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean
Control	6.56b	6.28c	6.42B	1.45bc	1.35cde	1.4BC	4.31d	4.28d	4.30D	16.51g	21.81d	19.16C	4.00	5.00	4.50A
2000	6.5b	16.61a	11.56 A	1.53b	1.71a	1.62A	5.50a	4.85b	5.18A	23.17c	28.30a	25.74A	4.00	4.00	4.00A
4000	5.11d	4.11e	4.61C	1.42bcd	1.49bc	1.46B	4.88b	4.68c	4.78B	20.77e	25.82b	23.30B	3.67	4.00	3.84B
6000	3.92f	3.67g	3.80D	1.35cde	1.35cde	1.35BC	4.85b	4.28d	4.57C	14.87g	18.87f	16.87D	3.33	3.67	3.50BC
8000	1.39i	3.28h	2.34E	1.38cde	1.24ef	1.31CD	3.60e	4.27d	3.94E	5.23i	12.48h	8.86E	3.00	3.33	3.17C
10000	0.72k	1.11j	0.92F	1.26def	1.13f	1.2D	2.85f	2.95f	2.90F	2.34k	4.69j	3.52F	2.00	2.33	2.17D
Mean	4.03B	5.84A	--	1.4A	1.38A	--	4.33A	4.22B		13.75B	18.66A	--	3.33B	3.72A	--

Means with different letters within each parameter were significantly different at 5% level

Table 2: Effect of seawater levels on some elements concentration (mg/g dry weight) of two jojoba clones

Seawater	Na^+			Cl^-			K^+			N^+			P^+			Ca^+		
level	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean	Clone 51	Clone 69	Mean
Control	6.73 6.7k	6.0l	6.35F	0.020l	0.092e	0.056E	20.4c	18.0f	19.2B	31.6c	33.5a	32.55A	2.8f	3.7b	3.25C	4.0c	4.5a	4.25A
2000	17.6h	15.6i	16.6E	0.067d	0.105c	0.101C	22.8a	21.2b	22.0A	28.6d	21.5k	25.05E	2.8f	2.7f	2.75D	4.25b	4.5a	3.50B
4000	27.2d	24.0e	25.6C	0.068i	0.066j	0.067D	18.4e	19.2d	18.8C	24.5h	24.0i	24.25F	2.0h	3.2de	2.60E	3.75d	4.5a	3.18C
6000	46.4a	21.6f	34.0A	0.084g	0.029k	0.057E	18.4e	18.0f	18.2D	31.6c	25.9g	28.75C	3.1e	4.0a	3.55A	3.50e	3.0f	3.25C
8000	42.4b	20.8g	31.6B	0.267a	0.072h	0.170A	18.0f	18.4e	18.2D	32.5B	26.7f	29.60B	3.3d	3.5c	3.40B	3.50e	3.0f	3.25C
10000	32.4c	15.2j	23.8D	0.226b	0.087f	0.157B	10.2g	10.0h	10.1E	23.0j	27.4e	25.20D	2.2g	3.5c	2.85D	3.00f	3.0f	3.00D
Mean	28.78A	17.2B	--	0.127A	0.075B	--	18.03A	17.47B	--	28.63A	26.55B	--	2.7B	3.43A	--	3.67B	3.75A	--

Means with different letters within each element were significantly different at 5% level

Fig. 1: Effect of seawater level on shoot parameters of jojoba clones 51 and 69 at multiplication stage after 4 subcultures. 1= control, 2= 2000 ppm, 3= 4000 ppm, 4= 6000 ppm, 5= 8000 ppm and 6= 10000 ppm

Table 3: Effect of seawater levels on free proline concentration (mg/g fresh weight) in two jojoba clones after 4 subcultures

	Increasing percent		Proline concentration		
Seawater ppm	51	69	51	69	Mean
Control	--	--	3.24d	3.05e	3.15C
2000	2.16	13.44	3.31c	3.46b	3.39B
4000	9.88	13.77	3.56a	3.47b	3.52A
6000	10.18	13.77	3.57a	3.47b	3.52A
8000	10.80	14.43	3.59a	3.49b	3.54A
10000	10.80	14.75	3.59a	3.50b	3.55A
Mean	8.76	14.03	3.48A	3.41B	--

Means with different letters were significantly different at 5% level

Table 4: Effect of salinity stress on individual amino acids content (μg/g D.W.) of two jojoba clones after 10 subculture

	Seawater level					
	Control		2000 ppm		4000 ppm	
Amino acid	69	51	69	51	69	51
Aspartic	48.24	145.53	83.02	24.02	116.97	128.92
Therionine	190.55	239.65	372.64	--	550.69	66.60
Serine	110.16	437.47	--	136.45	325.29	77.70
Glutamic	37.79	87.15	50.94	85.98	180.15	70.87
Glycine	352.97	210.02	544.34	340.19	548.13	266.38
Alanine	284.62	257.08	424.53	268.22	567.77	90.50
Cystine	181.71	139.43	52.83	225.23	67.45	120.38
Valine	82.81	63.62	56.60	131.78	40.98	99.89
Methionine	586.13	169.06	--	133.65	92.21	70.01
Isoleucine	251.66	113.29	490.57	628.04	467.88	648.03
Leucine	342.51	193.46	745.28	669.16	737.67	620.70
Tyrosine	259.70	405.23	712.26	972.90	807.68	620.70
Phenylalanine	734.87	537.69	357.55	372.90	1833.94	177.59
Histidine	1096.68	729.41	819.81	461.68	3643.97	444.82
Lysine	1430.35	1606.10	1658.49	1257.94	1635.01	1073.21
NH_4^+	2818.09	2356.43	3356.60	2485.05	4174.17	3013.02
Arginine	4180.91	6274.51	3172.64	6704.67	4054.64	3453.58
Proline	15488.47	2989.61	15372.44	17242.95	24687.65	12543.15
Total amino acid	28478.22	16954.75	27814.89	32140.80	44532.26	23586.06

Effect of Salinity Stress on Individual Amino Acids Content: Data illustrated in Table 4 revealed that the control of clone 69 shoots had higher concentration of total amino acids than that of clone 51. The amino acids glycine, alanine, methionine, leucine, phenylalanine and histidine were presented at higher levels in control samples of clone 69 than that in clone 51. Moreover, these amino acids besides proline, arginine, lysine, ammonia and total amino acids were pronouncedly increased in shoots of clone 69 under salt stress than that in clone 51, especially at 4000 ppm concentration.

DISCUSSION

Lower levels of seawater did not inhibit growth of jojoba shoots especially 2000 ppm, while higher levels (8000 and 10000 ppm) decreased all parameter under investigation. Clone 69 seems to be more tolerant to salt stress than clone 51. These results are confirmed with the findings of Mills *et al.* [8]. They found that chloride salinity reduced growth and biomass production in salt sensitive jojoba clones, while in salt tolerant clones, its adverse effects were generally less pronounced or it even enhanced growth. Salinity significantly promoted leaf expansion in both types of clones. In addition, Roussos and Pontikis [9] found that fresh and dry weight of jojoba explants were increased until the medium salinity and decreased at high salinity treatments of NaCl during proliferation stage. Higher salt tolerant clone (69) accumulated less Na^+, Cl^-, K^+ and N^{+3} ions in their shoots and absorbed higher average concentration of P^{+4} and Ca^{+2} than clone 51. Similarly, Mills and Benzioni [3] stated that, sodium accumulation was highest in shoots of the salt sensitive jojoba clone 64 and lowest in most tolerant clone 154. Higher levels of seawater resulted in higher Na^+, Cl^- and P^{+4} concentrations and lower K^+ concentrations. Similar results were obtained in explants of kiwifruit with Sotiropoulos and Dimassi [10]. In our investigation, shoot proline content was significantly increased due to salinity effect. These results agree with the findings of Roussos and Pontikis [9] on jojoba *in vitro* shoots. The increasing percentage in proline content due to salinity treatment was higher in clone 69 than that in clone 51. Woodward and Bennett [11] stated that, shoot proline levels significantly increased in two salt tolerant clones of *Eucalyptus camaldulensis* when exposed to 100 mM NaCl in the shoot multiplication medium compared with other low tolerant clones. It was found that the much tolerance of *in vitro* jojoba clone 69 to salinity, expressed as shoot number, than that of clone 51 may due to the increase of total amino acids and ammonia under salt stress. Moreover, the increase in the amino acids glycine, aspartic, threonine, glutamic, alanine, isoleucine, leucine, tyrosine, lysine and proline in addition to ammonia, could play a positive role in tolerance of jojoba clone 69 when exposed to salinity. In this respect, Pulich [12] indicated that salinity stress directly affects NH_4 content regulation in the sea grass. Cusido *et al.* [13] found that as salinity increased the levels of free amino acids, especially of aspartic acid, glutamic acid and proline were increased in *Nicotiana rustica* plants. Further, Kumar *et al.*[14] stated that the salt tolerance of S1 mulberry cultivar was evident from the higher level accumulation of glycine betain. Recently, Momtaz *et al.*[15] recorded that amongst the amino acids that showed significant differences at high level of salt stress in egyptian cotton were alanine, proline, glutamine, aspargine and histidine. Pulich [12] indicated that proline functioned as an organic osmoticum, while alanine functioned as an osmoregulatory capacity.

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