EFFECT OF GA_3 TREATMENT ON OSMOTIC-STRESSED JOJOBA SEEDLINGS: II- CHEMICAL COMPOSITION

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Abstract: This Experiment was conducted in two seasons to investigate the effect of GA_3 on jojoba (*Simmondsia chinensis*, Link) seedlings which grown under osmotic stress. One-year old seedlings were grown in soil contains 0.0, 0.4 or 0.8% of NaCl + $CaCl_2$, (1:1 by weight) and irrigated at 60, 80 or 100% of FC. In order to alleviate water and/or salt stress plants were sprayed 5 times with GA_3 at 0, 50 and 100 ppm. Leaves content of the photosynthetic pigments, reducing and total sugars were significantly affected by water stress. In the first season, plants grown in soil irrigated up to 60% FC had 5.668 and 25.61 mg/g dry weight of reducing and total sugars respectively, these values were significantly higher than these of plants irrigated up to 100% FC. Percentage of N, P, K, Na and Ca were decreased by the gradual increase in FC. Highest salinity level resulted in the lowest values of the photosynthetic pigments, reducing and total sugars as well as N, P and K%. While Na and Ca% were increased with the gradual increase in the salinity level. Although, GA_3 treatment significantly reduced leaves content of photosynthetic pigments, it stimulated reducing and total sugars content. In the first season, leaves of 100 ppm GA_3-treated plants had 5.264 and 24.64 mg/g dry weight of reducing and total sugars, respectively. On the other hand the leaves % of N, P, K, Na and Ca of 100 ppm GA_3-treated plants were reduced to 2.590, 0.254, 2.705, 1.373 and 1.680%, respectively, which were significantly less than untreated plants. Overall, the highest values of photosynthetic pigments were obtained due to 60% FC, salinity and without applying GA_3. Whereas, highest leaves % of N, P and K being for plants treated with 60% FC, 0.0% salinity and 100 ppm GA_3. On the other hand highest values of reducing and total sugars were for plants treated with 100% FC, 0.0% salinity and 100 ppm GA_3. So that, it could be recommended to treats jojoba plants growing under water and/or salt stress with 100 ppm GA_3 to alleviate osmotic stress.

Key words: jojoba, *Simmondsia chinensis*, fixed oil, chemical composition, GA_3 , soil salinity.

Introduction

Jojoba, *Simmondsia chinensis* (Link) is native to the semiarid regions of southern Arizona, southern California and north-western Mexico. It is a woody evergreen shrub or small multi-stemmed tree grows to a height of 3 to 5m**.** Jojoba dry seed contains up to 50% of a light-gold colored liquid wax ester which characterized with high viscosity, high flash and fire point, high dielectric constant, high stability, low volatility and is little affected by temperatures up to 300°C (Undersander *et al*., 1990). This oil used in cosmetics, pharmaceutical, plastic, printing and lubricant industries. Since jojoba oil contains no cholesterol or triglycerides and is not broken down by normal metabolic pathways, it may become an important low-calorie oil for human consumption (Benzioni, 1997).

Since the 1970s, drought problems have become very serious with severe drought in many countries. So that, salinity and drought stress reduce growth and agricultural productivity more than any other environmental factors by reduction of water potential. However, the physiological mechanisms that mediates the response in each case may be different. Osmotic adjustment in response to water stress is a drought adaptation strategy which enable plants to cope with stress. Sugars and other metabolites will accumulate at least during the early stage of the drought condition (Thomas, 1999).

Although, jojoba has the ability to grow in harsh desert as well as salinity stress Rana *et al.* (2003) stated that increasing salinity from 1000 to 8000 ppm significantly reduced jojoba growth also, Tahir *et al*. (1992) and Baxter *et al*. (1998) indicated that germination and growth of jojoba plants as well as their production are controlled by salinity level. El–Tomi *et al.* (1984) irrigated jojoba seedlings after 40, 70 or 100% depletion of the available soil moisture. They found that there was no significant difference between treatments when the contents of N, P and K were expressed as percentages of dry weight but the effect was varied considerably when expressed as plant uptake especially under the 40 and 100% depletion regimes but Ca and Na decreased by increasing soil moisture.

Farahat (1990) recorded a reduction in leaves content of *Schinus molle, Schinus terebinthifolius* and *Myoporum acuminatum* of photosynthetic pigments, carbohydrates, N, P, K, Ca and Na with increasing irrigation intervals. Similar results were obtained by Shehata (1992) on *Cupressus sempervirens* and *Eucalyptus camaldulensis* seedlings using 40, 60 and 80% FC. However, El–Tantawy *et al.* (1993) irrigated *Eucalyptus camaldulensis* seedlings up to 40, 60 and 80% FC and found that photosynthetic pigments, total sugars and N, P and K% in the leaves were increased as soil moisture content decreased, while, leaf contents of N, P and K were increased by increasing water supply. Gradual increase in irrigation level (4.12, 5.50 and 6.88 $m^3/36.75\ m^2$) of *Khaya senegalensis* seedlings with and caused a reduction of photosynthetic pigments, reducing and total sugars as well as leaves % of N, P and K while, leaves content of N, P and K were increased (Sayed, 2001).

Irrigation of *Casuarina equisetifolia* seedlings with saline water (500 to 2000 mg 1^{-1}) decreased leaves content of total chlorophyll and carbohydrates (Asmaael, 1997). Abd El–Fattah (2001) reported that, 7000 mg l^{-1} of salinity significantly reduced chlorophyll content, soluble and non soluble sugar and N, P and K%, but increased Ca and Na% on Adhatoda, Hibiscus and Phyllanthus shrubs. Working on *Acacia saligna*, *Casuarina cunninghamiana* and *Eucalyptus camaldulensis*, El–Baha *et al.* (2003) found that increasing salinity level significantly decreased leaves content of N, P and K.

Sayed (2001) treated *Khaya senegalensis* seedlings with GA_3 at 25, 50 and 100 ppm and observed a reduction in leaves contents of photosynthetic pigments, reducing and total sugars. However, leaf contents of nitrogen, phosphorus and potassium were significantly increased due to GA_3 application. Similar findings were reported by Abdou (2001) on *Jacaranda ovalifolia* seedlings when GA_3 applied at 100 or 150 ppm. Mohammad (2003) found that treating *Acacia saligna* seedlings with GA_3 caused a reduction in photosynthetic pigments contents and leaf percentages of N, P and K, but increased N, P and K uptake.

Material and Methods

This experiment was conducted during the two successive seasons of (2003 and 2004) at Horticultural Department, Faculty of Agriculture Minia University. One-year old jojoba seedlings (33-35 cm height and 3.03–3.27 mm stem diameter) were transplanted into polyethylene bags filled with 6 kg of soil at the first week of March in both seasons. Physical and chemical properties of soil are shown in Table 1. Salinity treatment was conducted by mixing soil with equal weights of NaCl and $CaCl_2$ at 0.0, 0.4 and 0.8% before transplanting. Beside the control plants were irrigated to maintain 60, 80 and 100% of FC as described by El-Tomi *et al.* (1984). Plants was sprayed with GA_3 at 0, 50, and 100 ppm. on the first week of June, July, Aug., Sept., and Oct. of each season. Two or three drops of "Tween 20" was added as surfactant to the solution.

Table(1): Physical and chemical properties of experimental soil.

Soil characters	Value/type	Soil character		Value
Sand %	91.0	Available P %		3.50
Silt %	5.8	Ex. cations (mg/100 g soil)	Ca^{++}	3.80
Clay %	3.2		Mg^{++}	1.60
Soil type	Sandy		Na^{+}	2.00
Organic matter %	0.08		K^{+}	0.60
$CaCO_3$ %	12.71	DTPA Ext. (ppm)	Fe	1.20
PH (1: 2.5)	8.15		Cu	0.43
EC m mhos	1.09		Zn	0.30
Total N %	0.06		Mn	0.60

A spilt-spilt plot design with 3 replicates was followed in the experiment where, FC was distributed in the main plots, salinity levels in the sub-plots and GA_3 concentrations in the sub-sub-plots. Then, the experiment included 27 treatments, each treatment consisted of 5 plants. All plants were fertilized with 20 g of complete fertilizer 18-9-9 added in 4 equal portions during the first week of June, July, August and September on both seasons. At the first week of November plants were cut just above the soil surface. Chlorophyll a, chlorophyll b and carotenoids contents were determined in fresh leaves at the first week of Nov. (Fadl and Seri-Eldeen, 1978). Leaves were oven dried at 70° C to determine reducing and total sugars using Nelson's method (Zayed 1976). Nitrogen, P and K were estimated following Page *et al*. (1982) and Na and Ca% according to Jackson (1962). Data were statistical analysis according to Little and Hills (1978) using M-State-C computer program.

Results

Photosynthetic pigments

Tables 2, 3 and 4 show that the leaves contents of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were gradually increased for both seasons as the FC was sloping downward. In the first season, leaves of plants which irrigated to achieve 60% of FC had 2.268, 0.728 1.090 mg/g fresh weight of chlorophyll a, chlorophyll b and carotenoids, respectively. Whereas the equivalent values for plants irrigated up to 100% FC were 1.991, 0.613 and 0.961. Similar results were recorded in the second season. Same tables show that increase soil salinity to 0.4

or 0.8 % caused a significant reduction in chlorophyll a and b and carotenoids, in both seasons, in comparison with the unsalinized control seedlings. The seedlings which grown in unsalinized soil had the highest chlorophyll a, chlorophyll b and carotenoids content (2.254, 0.699 and 1.071 mg/g fresh weight respectively). The highest salinity level gave the lowest values of chlorophyll a, chlorophyll b and carotenoids than those of the unsalinized seedlings except the carotenoids in the first season.

Gibberellic acid caused a significant reduction in the contents of chlorophyll a and b and carotenoids in both seasons (except carotenoids in the first season) in regard to those of untreated seedlings (Tables 2, 3 and 4). GA_3-untreated seedlings had 2.185, 0.691 and 1.046 mg/g fresh weight of chlorophyll a and b and carotenoids, while seedlings which treated with 100 ppm of GA_3 had 2.100, 0.638 and 1.058 mg/g fresh weight. The interactions among investigated factors (irrigation, salinity and GA_3) were significant in both seasons except (irrigation x salinity), (irrigation x GA_3) and (irrigation x salinity x GA_3) in the first season for carotenoids and the second one for chlorophyll b. The highest contents of chlorophyll a, chlorophyll b and carotenoids (2.406, 0.824 & 1.145) and (2.146, 0.861 & 1.083 mg/g fresh weight) in the first and second seasons, respectively were for seedlings grown in unsalinized soil, irrigated up to 60% FC and untreated GA_3. The lowest contents of chlorophyll a, chlorophyll b and carotenoids in the first seasons (1.866, 0.557 and 0.912 mg/g fresh weight) respectively were for seedlings grown in soil with 0.8% salinity, irrigated up to 100% FC and treated with 100 ppm GA_3.

Leaves content of reducing and total sugars

Data in tables 5 and 6 indicate that both reducing and total sugars were gradually decreased by increasing the level of FC. In the first season. The plants which irrigated to maintain 100% FC had the lowest content of reducing and total sugars; (4.528 & 20.95 mg/g) dry weight, respectively. Whereas, the plants subjected to 60% FC had the highest reducing and total sugars content; (5.668 and 25.61 mg/g dry weight). Salinity significantly reduced the reducing and total sugars content of jojoba seedlings. In the first season, using 0.8% caused 16.44 and 14.67% reduction in the reducing and total sugars content compared with control plants. Similar results concerning the effects of water and salt stress were found in the second season, except that of salinity on reducing sugars.

There were significant differences in reducing and total sugars among treated plants at GA_3 levels (Tables 5 and 6). Reducing and total sugars were significantly and gradual increased due to GA_3 treatment up to

100 ppm in both seasons. Under any level of FC or salinity the plants treated with 100 ppm of GA_3 had significantly higher reducing and total sugars than untreated plants. Also the results showed that GA_3 had alleviated the diverse effect of salinity as there were insignificant difference in both of reducing and total sugars between GA_3 -untreated plants grown in soil with 0.0% salinity and 100 ppm GA_3 -treated plants grown in soil with 0.4% salinity. The interaction between different involved factors were significant in both seasons for reducing and total sugars except irrigation x salinity for reducing sugars in the second season. The highest values of reducing and total sugars in both seasons being for 100 ppm GA_3- treated plants grown in soil with 60% FC and 0.0 % salinity. Whereas, the lowest values for both parameters were for plants grown in soil with 0.8% salinity and irrigated up to 100% FC.

Leaves percentage of N, P and K

Tables 7, 8 and 9 show a gradual reduction in leaves percentage of N, P and K parallel by increasing FC level in both seasons. This reduction were significant for phosphorus and potassium for both seasons and nitrogen in the first season only. In general, plants irrigated at 100% of FC had the lowest percentage of N, P and K (2.521, 0.524 & 2.658%) and (2.397, 0.257 & 2.789%) in both seasons respectively. Percentage of N, P and K in the leaves of jojoba seedlings was gradually decreased by increasing salinity level. Significant differences were existed between each two successive salinity levels in both seasons (Tables 7, 8 and 9). Plants grown in soil with 0.8% salinity had the lowest percentage of N, P and K (2.537, 0. and 2.651%, in first season) and (2.282, 0.264 and 2.821%, in second season), respectively (Tables 7, 8 and 9).

Concerning GA_3 treatment, leaves percentages of N, P and K% were significantly and gradually reduced in both seasons as the concentration of GA_3 was sloping upward (Tables 7, 8 and 9). Under any level of FC or salinity N, P and K% were reduced with increasing applied GA_3 concentration. In respect to the interaction between different involved factors, they were significant for N, P and K% in both seasons except (irrigation x GA_3) and (salinity x GA_3) for N in the first season and (irrigation x salinity x GA_3) in the first season and (irrigation x salinity) and (irrigating x GA_3) in the second season for P. Overall, the highest values of N, P and K% (2.853, 0.346 & .058) and (.934, 0.353 and 3.122) in both seasons respectively being for plants irrigated up to 60% of FC irrigation x 0.0% salinity x 0.0 ppm GA_3 (Tables 7, 8 and 9). However, it should be noticed that leaves content of N, P and K leaves was gradually and significantly increased with increasing FC level and reducing salinity level in both seasons. This

was due to the considerable increase in leaves dry weight with the increase in FC and reducing salinity level (see part one).

Leaves percentage of sodium and calcium

Tables 10 and 11 show that reducing FC level caused significant increase in jojoba dry leaves of Na and Ca in both seasons. In the first season, the highest values of Na and Ca (1.492 and 1.785%) respectively being for plants irrigated up to 60 % FC. Whereas, the equivalent values for plants which irrigated up to 100% FC were 1.334 & 1.665%. The highest Na and Ca% in the leaves of jojoba plants (1.652 & 1.845% and 1.432 &1.719%) in both season, respectively were obtained due to the high salinity treatment (0.8 %). Also, increased salinity level significantly increased the leaves percentage of Na and Ca.

On the contrary to water and salinity stress, GA_3 caused significantly reduction in sodium and calcium percentage in the leaves of jojoba plants in both seasons except Na. in the second season. The high GA_3 concentration was more effective than the low concentration in the reduction of leaves percentage of Na and Ca. (Tables 10 and 11). There was a significant interactions among investigated factors except between FC and GA_3 for Na%, in both season and FC x salinity x GA_3 for Na%, in the second one. The highest values were obtained due to irrigation at 60% FC, 0.8% salinity and 0.0 ppm GA_3. While the lowest values of sodium and calcium percentage were due to irrigation at 100% FC, 0.0 salinity and GA_3 at 100 ppm.

Discussion

Water is the most limiting ecological resource for most trees, moreover lack of water eventually leads to catastrophic biological failures and death. As soon as soil-water content declines, trees become more stressed and begin to react to resource availability changes. However, the results presented herein show that jojoba leaves contents of photosynthetic pigments were increased under water stress. This reduction in photosynthetic pigments in jojoba seedlings which could be due to the reduction in fresh weight was similar to these findings by Sayed (2001). The reducing and total soluble sugar content which consider an important solutes in osmoregulation are accumulated during abiotic stress. This might reduce the osmotic potential and maintaining the integrity and function of membranes which can be damaged under stress of jojoba stressed seedlings (Parker, 1972). Thomas (1997) suggested that the reduction of photosynthesis under water stress is less than the reduction in the growth therefore, sugars and other metabolites will accumulate during drought condition. These results can be compared with earlier observations where the imposition of water stress has been related to

elevated levels of sugars in different tree and shrub species (Asmaael, 1997 and Abd el-Fattah, 2001). Also, water stress increased leave percentages of N, P, K, Na and Ca. These results were in close agreement with the findings reported on *Cupressus sempervirens* and *Eucalyptus camaldulensis* (Shehata, 1992), Adhatoda, Hibiscus and Phyllanthus (Abd El–Fattah, 2001) and *Acacia, Casuarina* and *Eucalyptus* (El–Baha *et al*., 2003).

Observed results showed that salinity reduced photosynthetic pigments, reducing and total sugars and N, P and K% of leaves, but Na and Ca% were increased by the gradual increase in the salinity level. The reduction in plant growth under salt stress is accompanied by a variety of metabolic dysfunctions in nonhalophytes, including reduction in photosynthetic pigments (Choudhary *et al*., 2003). Synthesis of carbohydrates as well as transport of photosynthetic products and their utilization in production of new tissues (Kozlowski, 1997). Salinity often upsets the nutritional balance of plants by osmotic effects of salts, competitive interactions among ions in the substrate, and effects on membrane selectivity. In addition to that, as root elongation slows, the amount of ions reaching the roots by diffusion decreases (Kozlowski, 1997). Salinity decreases uptake of K of *Casuarina equisetifolia* (Dutt *et al*., 1991). Under such conditions, fairly definite limits of accumulation of ions such as sodium have been observed to be associated with the development of toxic symptoms in certain plant species.

In general, GA_3 treatment reduced the photosynthetic pigments of stressed jojoba seedlings which might enhance vegetative growth even under osmotic stress. Leaves % of N, P, K, Na and Ca, were reduced following GA_3 treatment but reducing and total sugars were increased. Furthermore, under any level of FC or salinity plants GA_3 treatment significantly increased leaves content of reducing and total sugars. These results are similar to those previously found by Sayed (2001) on *Khaya senegalensis*, Abdou (2001) on *Jacaranda ovalifolia* and Mohammad (2003) on *Acacia saligna*. Kaur *et al*. (2000) suggested that increasing the reducing sugar content under water stress might be due to a greater invertase activity which might be stimulating in phloem unloading of sucrose, thereby enhancing the conversion of sucrose into hexoses. There were significant interactions among different involved factors, The highest values of photosynthetic pigments and leaves % of N, P and K were obtained due to 60% FC, 0.0% salinity and 0.0 ppm GA_3. However, the highest values of reducing and total sugars being for plants treated with 60% FC, 0.0% salinity and100 ppm GA_3.

Conclusion

Although, *Simmondsia chinensis* (Link) Schneider plant distinguished as a tolerant plant for water and salinity stress this study amid to investigate the effect of GA_3 on seedling stage which is more sensitive to stress than old plants. Observed results indicated that both of water and salt stress had significant effects on chemical compositions of jojoba plants. Spraying with GA_3 especially at 100 ppm was effective in improving N, P and K and total sugars leaves content, while, the photosynthetic pigments content were decreased. So that, it is recommended to grow jojoba plants in unsalinized soil and irrigate them up to 100% of FC. In case of salinity and/or water shortage spraying with 100 ppm GA_3 would help plants to cope with water or salt stress.

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تأثير المعاملة بحامض الجبريليك على شتلات الهوهوبا النامية تحت إجهاد اسموزى، 2: المحتوى الكيميائى

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أجريت هذه التجربة خلال الموسمين المتتاليين 2003 و 2004 بقسم البساتين بكلية الزراعة جامعة المنيا بهدف دراسة تأثير GA_3 على الشتلات الهوهوبا النامية فى ظروف سعة حقلية وملوحة متباينة. زرعت شتلات عمر سنة فى تربة تحتوى على تركيزات صفر ، 0.4 ، 0.8% من خليط كلوريد الصوديوم و الكالسيوم (بنسبة 1 :1 بالوزن)، ورويت الشتلات بمعدل 60، 80، 100٪ من السعة الحقلية. رشت النباتات 4 مرات خلال فترة النمو والتى أمتد لمدة عام بتركيز صفر ، 50 ، 100 جزء فى المليون من GA_3.

حدثت زيادة فى الصبغات المسئولة عن البناء الضوئى وكذلك محتوى الأوراق من السكريات الكلية والمختزلة والنسبة المئوية لعناصر N, P, K, Na وCa تحت ظروف الإجهاد المائى. و كانت أعلى القيم لجميع الصفات السابقة هى الناتجة من إمداد النباتات بمياه الرى بمعدل 100٪ من السعة الحقلية. لكن حدث نقص تدريجيً ومعنوي لطول الجذر ووزنها الطازج والجاف نتيجة للزيادة التدريجية فى مياه الرى. أظهرت النتائج أن جميع صفات نمو النبات المدروسة تاثرت سلبياً بمعاملة الملوحة. ولقد كان هذا النقص تدريجياً حتى مستوى الملوحة المرتفع (0.8 ٪).