Egypt. J. Bot. **Vol. 62**, No. 1, pp. 97-111 (2022)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Impact of Slight Changes in Water Potential of Culture Media on *in vitro* Shoot Multiplication, Esterase, and Protein Patterns of *Simmondsia chinensis* L.

A.M. Hassanein#, A. Galal, G. Saad, D.M. Soltan
Central Laboratory of Genetic Engineering, Sohag University, 82524 Sohag, Sohag, Egypt.

AMPLIFICATION of DNA of 10 jojoba shrubs using 7 RAPD primers indicated that seed propagation of jojoba results in high genetic variation. To produce true-to-type clones, we applied a micropropagation technique. Medium (MS) containing relatively low concentrations (1 or 2mg/L) of 6-Benzylaminopurine (BAP) did not stimulate shoot formation on jojoba nodal explants. The best shoot cloning on nodal explants was established on MS medium containing 3mg/L BAP and 0.1mg/L 1-Naphthaleneacetic acid (NAA). Decrease in water potential (Ψ) of medium after adding 0.5mg/L of mannitol slightly increased the number of formed shoots and further decreased the medium Ψ by increasing NaCl or mannitol concentration, resulting in retardation of shoot formation. Data indicated that shoot formation was more sensitive than shoot growth to changes in Ψ of the medium. Callus weight decreased with decreasing medium Ψ to less than −435.014 MPa using 1g/L of NaCl, while mannitol application to decrease Ψ of the medium up to 435.054 MPa (4g/L) significantly increased callus fresh weight. Jojoba calli clearly expressed the effects of reduced Ψ in MS medium on esterase and protein patterns.

Keywords: Drought, Esterase pattern, Jojoba, Micropropagation, Salinity, SDS PAGEs, Shoot multiplication, Water potential.

Introduction

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a shrub of the family Simmondsiaceae (Mills et al., 1997) and is a native shrub in the northern Mexico region and Sonoran Desert of Arizona. Jojoba is commercially grown in Egypt, South Africa, Israel, India, Australia, Argentina, Chile, and Peru. Jojoba seed stores wax in liquid form that makes up 40%–60% of its dry weight and its wax has several applications in manufacturing of pharmaceuticals, cosmetics, extenders, and antifoaming agents. In addition, jojoba wax is used as a good lubricant to conserve uniform viscosity over a wide range of temperatures of engines (Low & Hackett, 1981; Mills et al., 1997). Jojoba shrub is a long-lifespan plant (100–200 years), dioecious, evergreen, and wind pollinated and shrubs can grow up to 5m height. The total area of global arable land is continuously decreasing due to desertification from environmental pollution and climate change, as well as soil salinization. Plants, such as jojoba, have established metabolic changes to grow economically under conditions of low Ψ resulting from harsh environments (Al-Dossary et al., 2019; Singh et al., 2020).

As a dioecious plant species, jojoba production of true-to-type by seed propagation is not guaranteed. In general, shrubs obtained from seed sowing show varying shrub canopy, height, stem diameter, leaf area, node length, number of flowers, seed morphology, and shrub yield, as well as wax, protein, carbohydrate, and simmondsin content (Al-Soqeer et al., 2012; Hassanein et al., 2015a). Jojoba seeds express variation in germination under relatively high salt stress (Hassanein et al., 2015a). Germination of new harvest seeds is 80%–

#Corresponding author email: ahmed.hassanein@science.sohag.edu.eg
Received 24/11/ 2020; Accepted 01/07/ 2021
DOI: 10.21608/ejbo.2021.46723.1571
Edited by: Prof. Dr. Soliman A. Haroun, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt.

90%, but decreases progressively with time (Harsh et al., 2001). Consequently, clonal propagation of jojoba elite shrubs is an essential prerequisite to establish an economical farm (Chaturvedi & Sharma 1989; Hassanein et al., 2015a). To avoid genetic variation of seed propagation, rooting of semi-hardwood cuttings were recommended (Low & Hackett, 1981; Lee, 1988; Cao & Gao, 2003), but the maximum number of possible propagules is limited by shrub size. In addition, rooting of shoot cuttings is difficult and requires specific requirements (Palzkill & Feldman, 1993). Tissue culture techniques offer opportunities for cloning of thousands of jojoba shrubs from elite-selected individuals irrespective of shrub size and at any time of the year (Lee, 1988; Hassanein et al., 2015 a,b). *In vitro* propagated jojoba plants show vigorous growth than those obtained from seeds or shoot cuttings (Lee, 1988).

Esterases hydrolyze ester bonds in a wide range of substrates (Balen et al., 2004). In jojoba, esterase is generally involved in lipid metabolism and production of many organic molecules (Flowers et al., 2010; Radic & Pevaler-Kozlina, 2010). In addition, the "includer" phenomenon (Mills & Benzioni, 1992; Mills et al., 2001) is supported by esterases to enable jojoba to absorb water efficiently under conditions of low water potential (Ψ; osmotic) from surrounding environments (Lima et al., 2012; Reyes-Pérez et al., 2019; Akbari et al., 2020).

Under tissue culture conditions, Ψ of the medium is proportional to the concentration of its chemical components and greatly affects plant multiplication and growth (El-Sheekh et al., 2020). Cultured plant tissues can grow and multiply if Ψ of the medium is higher than that of the cultured plant organ or tissue (Kirkham & Holder, 1981; de Paiva Neto & Otoni, 2003). When additional organic or inorganic substances are added to the plant medium, Ψ and water flow into plant tissue both decrease. Establishing an equilibrium state between Ψ of the medium and callus results in callus death (Kirkham & Holder, 1981). Consequently, a specific Ψ is needed to establish within the cells of the cultured organs to absorb water, expand, divide, and show morphogenic potential (Cleland, 1977). Since seashores or desert plant species such as jojoba are characterized by low Ψ, they need high salt medium with relatively low Ψ, such as MS medium, for *in vitro* growth and multiplication (Lassocinski, 1985). When the chemical components of MS increase, inhibition of *in vitro* growth and multiplication of many plant species were retarded partially or completely (Thorpe et al., 2008).

Basic components of MS, SH, and B5 media in liquid form without sugar at 25ºC, resulting in Ψ values of −212, −153, and −143, respectively (Fujiwara & Kozai, 1995). Water potential of the medium can decrease two-fold, the first stage occurring after sugar addition and the second from conversion of 40–50g of sucrose into monosaccharides during autoclaving (Lazzeri et al., 1988). Addition of 3% sucrose to shoot multiplication medium results in reduced Ψ of a growth medium by −223 MPa (Thorpe et al., 2008). Information related to the effect of changing Ψ of the *in vitro* growth medium by changing the media type or their components, or from the addition of NaCl or mannitol, are not sufficiently available. Further, knowledge on which is more sensitive to slight change in Ψ of culture medium is scarce. In addition, this study aimed to investigate whether *in vitro* cultured shoots or calluses are better to study alterations in gene expression of jojoba under various Ψ levels of culture media.

Materials and Methods

Shoot culture establishment

Used jojoba seeds were obtained from the Egyptian Natural Oil Company, Ismailia, Egypt. The farm of this company was used for research and production. Seeds were germinated and grown in plastic pots under room condition for one year. Laboratory grown plants were used for DNA analysis using 7 RAPD primers for establishing the *in vitro* culture. For sterilization, jojoba nodal explants were treated with Clorox for four minutes followed by 70% EtOH for a further four minutes. Jojoba nodal explants were then carefully washed by sterilized distilled water three times. After sterilization, the lateral ends of each nodal cutting were discarded to make 1.2–1.5cm long cuttings. Jojoba nodal explants were cultured in glass jars containing MS medium (Murashige & Skoog, 1962) with 3% sucrose and BAP concentration (1–5mg/L).

In all studied experiments, the pH of the medium was adjusted to pH 5.8. Medium was solidified with 8g/L agar. For each treatment in all studied experiments, 30 nodal explants in five glass jars were cultured. Jojoba cultures were incubated

in the tissue culture room with 16h daily light at 100µmol m^{-2} s^{-1} and 29± 1ºC. After four weeks, shoot frequency (%), number of shoots/explant, length of the obtained shoots (cm), number of leaves/shoot, and number of nodes/shoot were estimated.

DNA extraction

Young leaves of soil grown jojoba plants (100mg) were collected and grinded in a mortar in liquid nitrogen and 1,000µL of CTAB buffer. Mixture was transferred into 1.5mL Eppendorf tubes containing 2µL of RNAase. Mixture was incubated at 65ºC for 30min and mixed with 500µL of 24:1 Chloroform-Isoamyl alcohol to form emulsion. The emulsion was centrifuged for 5–10min at 13,500rpm. The top-aqueous solution was estimated and transferred into 1.5mL Eppendorf tubes. Consequently, one volume of cold isopropanol was added and incubated in the freezer for 45–60min. After centrifugation for 10 min at 12,000rpm, the supernatant was carefully discarded leaving the obtained DNA pellet at the base of the Eppendorf tube. The Eppendorf was then supplemented with 700µL of cold 70% ethanol and centrifuged for 1min. Ethanol alcohol was removed and the obtained DNA pellet was dried on a hot plate at 55ºC. The DNA pellet was resuspended in 100µL TE buffer for 1h at 55ºC before use.

DNA amplification

DNA amplification reactions were performed in a reaction mixture (25µL) containing 1X PCR buffer, 2mM $MgCl_2$, 0.2mM dNTPs, 25pmol primer, 1U Taq DNA polymerase, and 30 ng of template DNA. The amplification reaction was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems). The apparatus was programmed to perform 35 cycles, each one with four steps: denaturation at 94ºC for 45sec, annealing at 36ºC for 50sec, elongation at 72ºC for 1min, and extension for 7min at 72ºC. The PCR products were resolved by electrophoresis (95 volts) using 1.5% agarose gel containing EthBr (0.5ug/mL) in 1X TBE buffer. In this study, seven RAPD primers (A-03, A-04, A-08, A-13, C-02, K-02, and at-08) were used.

Induction of shoot formation on MS medium containing different growth regulators in different concentrations

Jojoba nodal cuttings of soil or *in vitro* grown plants were sterilized and used for induction of *in vitro* shoot formation on high salt medium (MS) containing different BAP concentrations (1–5mg/L). Further, jojoba nodal segments were cultured in MS medium containing different concentration of BAP (1–4mg/L) in combination with two NAA concentrations (0.1–0.2mg/L) for four weeks.

Cloning for long-term periods on MS medium containing different BAP concentrations (1–3mg/L) in combination with 0.1 mg/L NAA

In vitro formed shoots on nodal cuttings of soil grown plant were subcultured 12 times on MS medium supplemented with different BAP concentrations (1–3mg/L) in combination with NAA (0.1mg/L) for four weeks. An increase in the number of shoots obtained after the 1^{th}, 2^{th}, 3^{th}, 8^{th}, 9^{th}, 10^{th}, 11^{th}, and 12^{th} subculture was estimated. Consequently, the total number of shoots/explants after 12 subcultures under the influence of each treatment for 48 weeks were determined.

The effect of changing levels of water potential on frequency of shoot formation and shoot growth

To study the effect of Ψ exerted by different types of media, we used the basal medium of MS (Murashige & Skoog, 1962), SH (Schenk & Hildebrandt, 1972), and B5 (Gamborg et al., 1968), each containing 3mg/L BAP and 0.1mg/L NAA and each giving –212, –153, and –143MPa, respectively (Fujiwara & Kozai, 1995). In addition, Ψ of each medium decreased due to the addition of 3% sucrose equivalent to –223 MPa (Thorpe et al., 2008). Further, the effect of Ψ exerted by changing the MS medium strength (half, full, one and half, or double MS strength) containing 3% sucrose on shoot formation and shoot growth was investigated. In this study, further reductions in medium Ψ were established when the medium was supplemented with 0.5, 1, 2, 3, or 4.0mg/L NaCl or mannitol, giving –0.038, –0.071, –0.143, –0.214, and –0.286MPa, or –0.007, –0.014, –0.029, –0.043, and –0.054MPa, respectively (Neto et al., 2004).

The Ψ of each treatment equated to Ψ of medium components + Ψ of 3% sucrose + Ψ of the used concentration of NaCl or mannitol. The water potential of agar (–0.578MPa; Buah et al., 1999) was neglected where all treatments were supplemented with 8g/L agar irrespective of media type or its strength.

Jojoba shoots grown on MS medium containing different concentrations of NaCl or

mannitol were subjected for esterase and SDS-PAGE analysis.

Callus formation

To obtain jojoba calli, 0.5cm long sections of the first leaf of soil grown plants were cut and sterilized as described previously. Sections were then placed on MS medium containing 3% sucrose, 0.14mg/L BAP + 1mg/L NAA and 0.11mg/L 2,4-D. To induce callus formation, cultures of leaf segments were incubated under dark condition at 30± 2ºC for two months and weight/callus was determined. To study the effect of lowering Ψ of the growth medium on callus growth, 0.2g of calli were transferred to grow on MS media supplemented with 3% sucrose and different concentrations of NaCl or mannitol (0.5, 1, 2, 3, and 4g/L). Fresh weight/callus was estimated after ten days. Ten day-old calli were also used for SDS-PAGEs and esterase analysis.

Esterase detection

For natural protein extraction, 1g of jojoba callus was ground in a bowl of ice containing 1mL of protein extraction buffer (0.002M cysteine; 0.04M Tris-HCl; pH 7.0). After centrifugation at 15,000g and 4ºC for 15min, the obtained supernatant was carefully isolated. For esterase analysis, native PAGE gel was prepared using 7.5% acrylamide. The prepared protein samples were loaded onto gel wells for electrophoresis at 12mA per gel for 6h at 4ºC using run buffer (0.192 M glycine and 0.025M Tris-HCl, pH 8.9). The obtained esterase bands were visualized using staining mixture containing α- and β-naphthyl acetate and fast blue RR salt (Brewer, 1970).

SDS-PAGEs

For SDS-PAGE, we used 11% acrylamide gel. A volume of obtained extract containing 40µg of protein was mixed with an equal volume of buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.125 M Tris-HCl, pH 6.8). The mixture was incubated in a water bath at 96ºC for 90sec, after which the supernatant was carefully obtained by micropipette and transferred into gel wells. Electrophoresis was run using buffer containing 0.192M glycine, 0.025M Tris, and 0.1% SDS at 12mA/ gel for 6h at 4ºC. The separated protein bands were visualized using Coomassie Brilliant Blue Dye.

Statistical analysis

The obtained data were analyzed by LSD test at the 5% level of significance at $P< 0.05$.

Results

To generate a dendrogram showing the genetic relationships among ten jojoba shrubs obtained from seed sowing (Fig. 1), seven RAPD markers were used, two of which are included in Fig. 2. The obtained dendrogram grouped the ten shrubs into two main clusters, but shrub 1 was isolated in a separate stem. The largest cluster consisted of seven jojoba genotypes and the second cluster comprised only two jojoba shrubs. The dendrogram shows the existence of genetic variation between the ten studied shrubs, whereby shrubs 3 and 4 were closer to each other and both were remote from shrub 1.

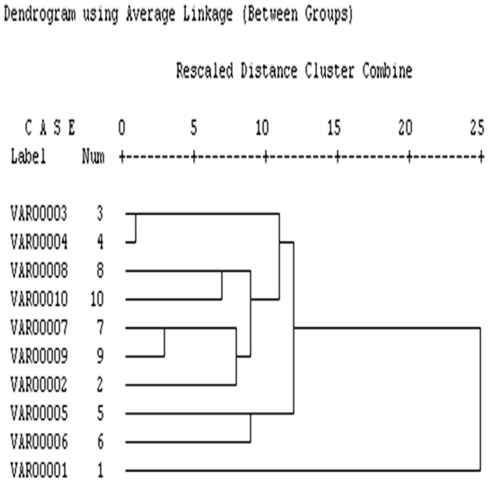

Fig. 1. Cluster generated from MVSP computer software program of Nie & Lie (1979) and clustered by unweighted pair group method based on arithmetic mean (UPGMA) using 7 RAPD markers for 10 jojoba plants obtained from seeds

For induction of *in vitro* shoot formation on jojoba explant for short term culture, we studied the effect of different BAP concentrations for four weeks. Nodal segments of soil grown jojoba plants were used as a source of explants. BAP free MS medium as well as MS medium containing 1 or 2mg/L BAP did not stimulate shoot formation on cultured explants. Shoot formation and shoot growth were detected when the BAP concentration increased more than 2mg/L (Table 1). The best results for shoot formation frequency and shoot number/explants were obtained when 5mg/L BAP was used. The determined growth parameters of formed shoots under the influence of 4 mg/L BAP were the same as 5mg/L BAP.

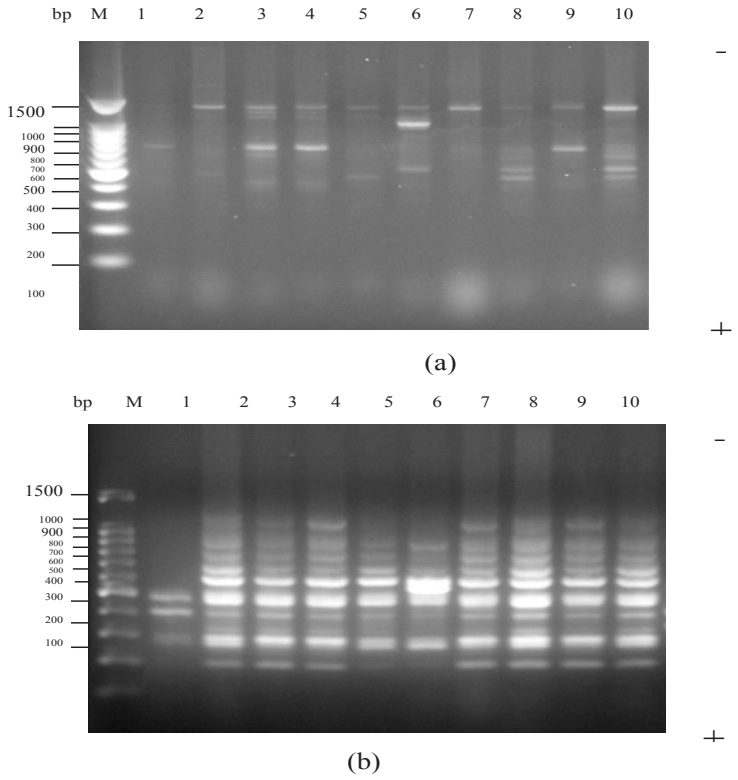

Fig. 2. Photographs show genetic variation between ten jojoba plants using the primers: (a) A-03 and (b) A-04

TABLE 1. Shoot formation on nodal segment of soil grown jojoba plants as influenced by different concentrations of BAP four weeks

BAP con. (mg/L)	Shoot frequency (%)	No. shoots/ explants	Length of shoot	No. of leaves/ shoot	No. of nodes/ shoot
0.0 to 2.0	0	0	0	0	0
3	10*	1*	0.4*	2*	1*
4	40*	2*	1.2	4	2
5	50	3	1.2	4	2

* Means significantly different (t-test) from intermodal explants cultured on MS medium supplemented with 5mg/L BAP at P< 0.05.

The response of cultured nodal explants under varying BAP concentrations was modulated when used in combination with NAA. All used BAP concentrations (1–5mg/L) in combination with NAA (0.1 or 0.2mg/L) induced shoot formation on cultured nodal explants, but with different values (Table 2). Generally, regeneration frequency on MS medium containing different BAP concentrations in combination with 0.1 mg/L NAA was higher than those in combination with 0.2mg/L NAA. The highest number of shoots/explants and number of nodes/shoots were obtained on MS medium containing 3mg/L BAP and 0.1mg/L NAA. Conversely, the lowest regeneration frequency was recorded on 1mg/L BAP combined with 0.2mg/L NAA.

Under long-term culture for 48 weeks (12 subcultures), a valuable number of jojoba shoots/ explant were obtained on MS medium containing 2 or 3mg/L BAP combined with 0.1mg/L NAA (Table 3 and Fig. 3). Decreasing BAP concentration less than 2mg/L negatively affected the number of formed jojoba shoots/explant.

Induced shoot formation due to application of selected combination of growth regulators (3mg/L BAP and 0.1mg/L NAA) was influenced by Ψ exerted by media type, where three types of media (MS, SH, and B5) were used (Table 4). In four weeks, the highest shoot number and shoot growth were detected when explants were cultured on the lowest Ψ medium (MS). Since the water potential of SH was the same as B5, they expressed the same low number of shoots/explant.

TABLE 2. Shoot formation on nodal explants of soil grown jojoba plants as influenced by different concentrations of BAP and NAA for four weeks

GRs con. (mg/L)		Shoot freq. (%)	Number of shoots/ explants	Length of shoot (cm)	No. of leaves/ shoot	No. of nods/ shoot
BAP	NAA					
1	0.1	86.9	2	1.99	5.6*	4*
2	0.1	90.5	1.33	2.4	6.66	3.33*
3	0.1	85.7	2	2.4	6.66	5
4	0.1	57.14	1*	1.1*	3.33*	1.33*
1	0.2	51.66	1.33	1.13*	4.33*	2*
2	0.2	64.3	1.66	1.26*	4*	2.33*
3	0.2	85.7	2	0.8*	3.33*	1.33*
4	0.2	86.3	1.33	1.33*	3.33*	1.66*

* Means significantly different (t-test) from intermodal explants cultured on MS medium supplemented with 3mg/L BAP+ 0.1mg/L NAA at P< 0.05.

TABLE 3. Number of the obtained jojoba shoots after long term culture (12th subculture) three weeks each as influenced by 0.1mg/L NAA and different concentrations of BAP

GRs conc. (mg/L)		No. of subcultures							
BAP	NAA	1st	2nd	3rd	8th	9th	10th	11th	12th
3	0.1	2	8	32	58	93	192	446	740
2	0.1	1.33	6*	27*	97*	161*	326*	401*	717*
1	0.1	2	2.6*	6*	63*	65*	160*	189*	234*

* Means significantly different (t-test) from shoots cultured after 12 sub culture at P< 0.05.

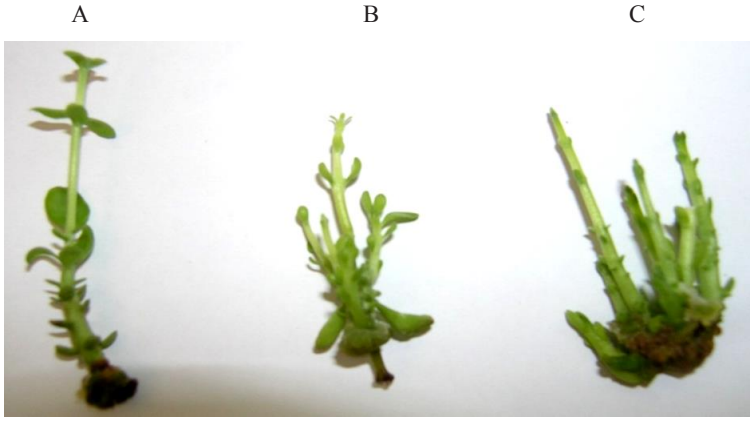

Fig. 3. Photograph shows *in vitro* shoot growth after 12 subcultures: (A) Shoot grown on MS medium supplemented with 1mg/l BAP + 0.1mg/L NAA; (B) Shoots grown on MS medium supplemented with 2mg/L BAP + 0.1mg/L NAA and (C) Shoots grown on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA

TABLE 4. Effect of basal medium type on shoot multiplication and growth of in vitro grown shoots on MS, SH, and B5 media supplemented with 3mg/L BAP+ 0.1mg/L NAA for four weeks

Medium type	No. of shoots/ explants	Length of shoot (cm)	Number of leaves/ shoot	Number of nodes/ shoot
MS	4	5.1	7.3	5.6
SH	1*	4.6*	6.3	5.6
B5	1*	5.3	5*	5

* Means significantly different (t-test) from jojoba shoots cultured on full MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA at P< 0.05.

The Ψ of MS medium changed with altered MS medium strength. When nodal segments of *in vitro* grown shoots were cultured on MS medium under different medium strengths (0.5, 1.0, 1.5, and 2.0), each one containing 3mg/L BAP, 0.1mg/L NAA, and 3% sucrose (Table 5), they expressed different values for shoot number/explant and shoot growth. The data indicated that the number of formed shoots significantly decreased when MS components were more or less than that of full-strength MS components (−435MPa).

Induced shoot formation on MS medium supplemented with 3mg/L BAP and 0.1mg/L NAA was influenced by decreasing Ψ of the medium after adding certain NaCl (Table 6) or mannitol (Table 7) concentrations. In comparison to Ψ of the basal MS medium containing 3% sucrose (−435MPa), a slight decrease in the medium Ψ due to the addition of 0.5g/L of mannitol (−435.007MPa) was associated with an increase in the number of shoots/explant. Further reductions in medium Ψ less than −435.007MPa from 0.5gm/L mannitol or −435.038MPa from 0.5gm/L NaCl resulted in a significantly reduced number of shoots/explant and growth of formed shoots. The data indicate that shoot formation was more sensitive than shoot growth in reducing Ψ of the medium.

Callus formation on leaf explants was determined when explants were placed on MS medium containing 0.14mg/L BAP + 1mg/L NAA and 0.11mg/L 2,4-D under dark conditions at 30±2ºC for two months. Callus weight decreased with decreasing medium Ψ lower than −435.071MPa when 1g/L of NaCl was included in the MS medium containing 3% sucrose. Application of mannitol to decreasing Ψ of the medium up to −435.054MPa (4g/L) significantly increased callus fresh weight (Table 8).

We found changes in esterase expression of shoots with a gradual decrease in medium Ψ due to gradually increasing NaCl (Fig. 4) or mannitol (Fig. 5) concentrations. Each level of Ψ changes in the medium expressed specific esterase patterns. Medium containing 0.5g/L NaCl (Lane 2) showed two additional esterase isoenzyme forms (EST-1 and EST-2) compared with the control (Lane 1). Esterase patterns indicated that two esterase bands (EST-4 and EST-5) and four forms (EST-8, EST-9, EST-10, and EST-11) disappeared when 2 or 4g/L NaCl and 1 or 3g/L NaCl were used, respectively. Esterase expression of jojoba was not effectively influenced by reducing Ψ of the medium due to increases in mannitol concentrations within the specified range (Fig. 5). Two esterase bands (EST-7 and EST-17) disappeared under the influence of 2g/L mannitol (Lane 4).

We explored esterase expression of jojoba calli under different medium Ψ due to the presence of different concentrations of NaCl (Fig. 6) or mannitol (Fig. 7). Compared with NaCl free medium (control), medium containing 0.5–3.0g/L expressed two additional esterase isoenzyme forms (EST-1 and EST-8), which later disappeared when jojoba calli were cultured on MS medium containing 4g/L NaCl. Further, two isoenzyme forms (EST-7 and EST-10) disappeared in calli cultured on MS medium containing 3.0 or 4.0g/L NaCl (−435.286MPa). When jojoba calli were subjected to successive lowering of medium Ψ due to increasing mannitol concentrations (Fig. 7), the number of detected esterase isoenzyme forms increased where six- faint new bands (EST-1, EST-2, EST-9, EST-10, EST-11, and EST-12) were detected, but one isoenzyme form (EST-4) disappeared.

The number of protein patterns in the SDS-PAGE of jojoba shoots under different medium Ψ levels after applying varying mannitol concentrations are shown in Fig. 8. SDS-PAGE indicated that several polypeptides with apparent molecular weight ranging from 7 to 200kDa were determined. Under the influence of Ψ of MS medium containing 3% sucrose and mannitol in different concentrations (−435.007 [0.5g/L] to −435.054 MPa [4g/L]), four new polypeptides were detected (135.3, 124.6, 85, and 77kDa), while two polypeptides disappeared (90 and 80kDa).

Growth parameters (Table 8) and SDS-PAGE data (Fig. 9) indicate that callus fresh weight showed no negative effects when cultured on Ψ of MS medium containing 3% sucrose and relatively low concentrations of NaCl up to −435.214 MPa (3g/L NaCl). Further, these conditions resulted in an increase in staining intensity of some polypeptide bands (Fig. 9, Lanes 5 and 6) and the new appearance of others (58, 43.8, 16.8, 12.5, and 10kDa). Protein expression stability was demonstrated under used concentrations of mannitol in MS medium containing 3% sucrose (−435.007 to −435.054MPa). Under these conditions, expression of two new polypeptides of 73 and 138kD were detected (data not shown).

In general, esterase or SDS-PAGE patterns of calli were more expressive than those of plant shoots regarding the effect of reduced medium Ψ on *in vitro* cultured tissues.

TABLE 5. Effect of basal medium strength on shoot multiplication and growth of *in vitro* grown shoots, media were supplemented with 3mg/L BAP + 0.1mg/L NAA for four weeks

MS strength	No. of shoot/ explant	Length of shoot (cm)	No. of leaves/ shoot	No. of nodes/ shoot	Shoot fresh weight (g)
Half	3*	3.2*	7.3	5*	0.1*
Full	8	5.5	8	4	0.2
One and half full	3*	5	8	5*	0.2
Double full	3*	7.8*	8	5*	0.24

* Means significantly different (t-test) from jojoba shoots cultured on full MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA at P< 0.05.

TABLE 6. Effect of various concentrations of NaCl on *in vitro* shoot multiplication and shoot growth. Shoots were subcultured on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA and several concentrations of NaCl for three weeks

NaCl conc. (g/L)	No. of shoots/ explant	Length of shoot (cm)	No. of leaves/ shoot	No. of nods/ shoot	shoot fresh weight (g)
0	8	4.8	8	5	0.11
0.5	8	5.8*	12.3*	6*	0.14
1	4*	6.6*	8	6*	0.14
2	1.66*	6*	9*	6.6*	0.12
3	2.66*	5	8	5	0.12
4	2*	5.3	8.6*	6*	0.1

* Means significantly different (t-test) from jojoba shoots cultured on MS medium supplemented with 3mg/L BAP +0.1mg/L NAA without NaCl for three weeks at 29±1ºC at P< 0.05.

TABLE 7. Effect of various concentrations of mannitol on *in vitro* shoot multiplication on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA for three weeks

Conc. of mannitol (g/L)	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ shoot	No. of node/ shoot	Shoot fresh weight (g)
0	8	4.8	8	5	0.18
0.5	9	6*	12*	6*	0.18
1	3*	5	10*	5	0.18
2	2*	5	10*	5	0.17
3	1*	3.5*	8	4*	0.16
4	1*	3*	8	4*	0.18

* Means significantly different (t-test) from jojoba shoots cultured on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA without mannitol under tissue culture condition at P< 0.05.

TABLE 8. Callus fresh weight/leaf explant under the influence of different concentrations of NaCl or mannitol. Leaf explants were cultured on MS medium with 0.14mg/L BAP + 1mg/L NAA and 0.11mg/L 2, 4-D

Conc. NaCl or mannitol (g/L)	NaCl	Manniol
0.0 Conrol	**0.63**	
0.5	0.63	0.66
1	0.64	0.64
2	0.54	0.72*
3	0.61	0.72*
4	0.49*	0.70*

* Means significantly differed (t-test) from callus placed on MS medium without NaCl or mannitol at P<0.05.

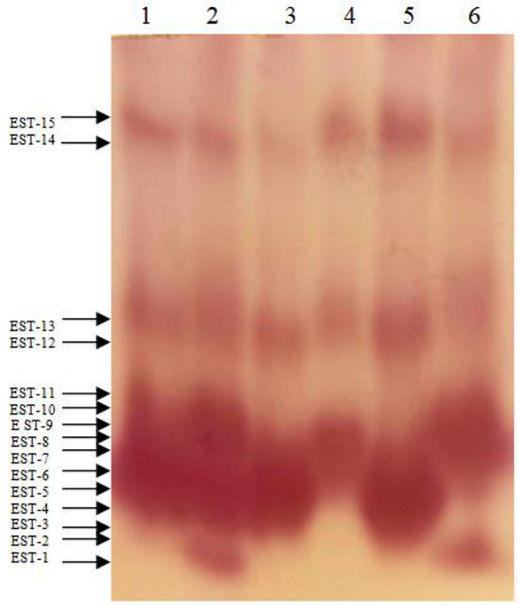

Fig. 4. Native gel electrophoresis of esterase isoenzyme pattern of jojoba microshoots grown for three weeks on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA and 0, 0.5, 1, 2, 3 and 4g/L NaCl, lanes 1, 2, 3, 4, 5 and 6, respectively

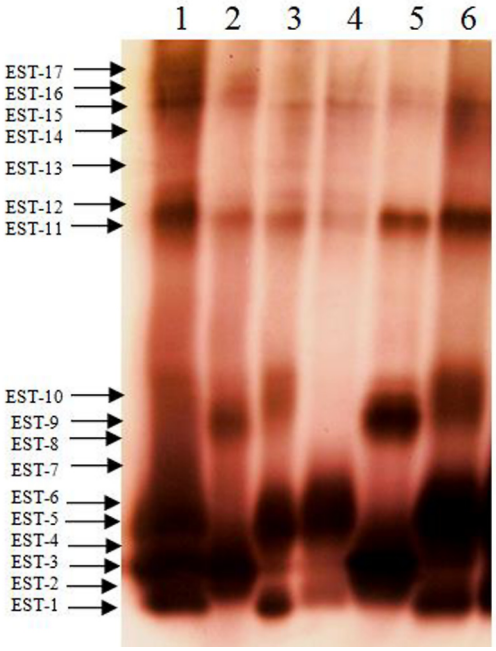

Fig. 5. Native gel electrophoresis of EST isoenzyme pattern of jojoba shoots grown for three weeks on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA and 0.0, 0.5, 1, 2, 3 and 4g/L mannitol, lanes 1, 2, 3, 4, 5 and 6, respectively

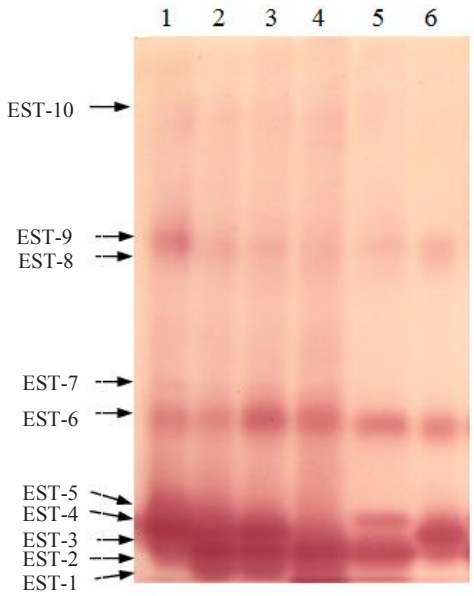

Fig. 6. Native gel electrophoresis of esterase isoenzyme pattern of jojoba calli grown for ten days on MS medium supplemented with 0.56mg/L BAP + 1mg/L NAA + 0.11mg/L 2, 4 D and different concentrations of NaCl: 0.0, 0.5, 1, 2, 3, and 4g/L NaCl, lanes: 1, 2, 3, 4, 5 and 6, respectively

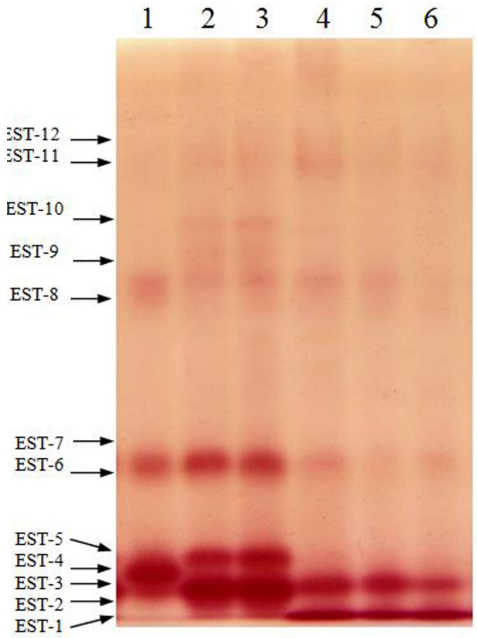

Fig. 7. Native gel electrophoresis of esterase isoenzyme pattern of jojoba calli grown in dark for ten days on MS medium supplemented with 0.56mg/L BAP + 1mg/L NAA + 0.11mg/L 2, 4 D and different concentrations mannitol: 0.0, 0.5, 1, 2, 3, and 4g/L ; lanes 1, 2, 3, 4, 5 and 6, respectively

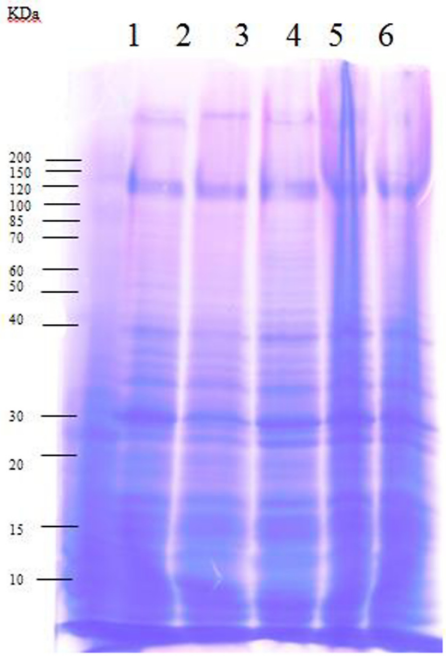

Fig. 8. SDS-PAGE of jojoba shoots grown for four weeks on MS medium supplemented with 3mg/L BAP + 0.1mg/L NAA and 0.0, 0.5, 1 , 2, 3 and 4g/L mannitol, lanes 1, 2, 3, 4, 5 and 6, respectively

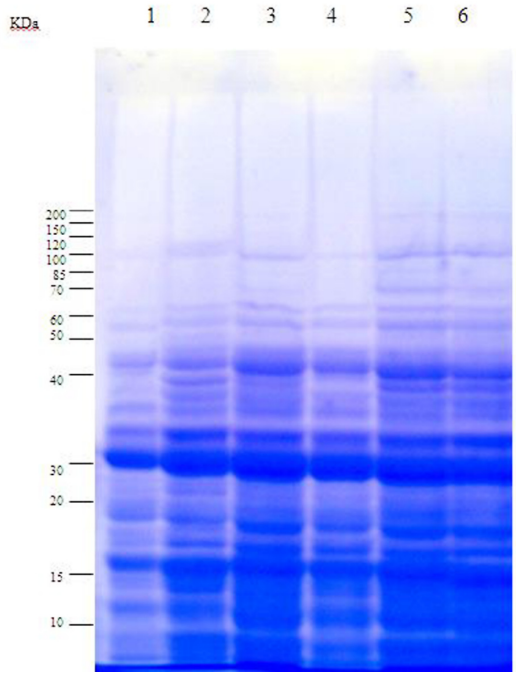

Fig. 9. SDS-PAGE of jojoba calli grown for ten days on MS medium supplemented with 0.56mg/L BAP + 1mg/L NAA + 0.11mg/L 2, 4 D and different concentrations of NaCl: 0.0, 0.5, 1, 2, 3, and 4g/L NaCl, lanes: 1, 2, 3, 4, 5 and 6, respectively

Discussion

Genetic variation stimulation is desirable when exploring selection of specific mutants (Govindaraj et al., 2014), but it should be avoided if true-to-type clones are needed. Jojoba seed propagation results in high genetic variation (Hassanein et al., 2015a) for dioecious plant species. Kumar et al. (2012) confirmed that a low percentage of seed-originating jojoba shrubs can produce high and desirable quality of jojoba liquid wax. DNA-based genetic markers, such as RAPD, were used in this study and others to detect genetic variation in jojoba (Amarger & Mercier 1996). The obtained dendrogram of seven RAPD markers revealed large variation among jojoba shrubs grown from seeds, as previously reported (Amarger & Mercier, 1996). Consequently, safe methods to clone elite jojoba shrub are needed, a method that can be used to avoid genetic variations of sexual-propagated shrubs and their negative effects on commercial production. Here, vegetative propagation (Kumar et al., 2011) or micropropagation (Hassanein et al., 2015a,b) can be used.

Application of *in vitro* cloning is recommended in jojoba (Hassanein et al., 2015b; Hassan et al., 2019) and other plant species (Salem, 2020). Application of 3–5mg/L BAP resulted in shoot formation on nodal cuttings of soil grown jojoba plants. The capacity of BAP to induce shoot formation in *in vitro* cultured explants and growth of formed shoots may be attributed to the ability for plant tissues to metabolize BAP and produce natural hormones, such as zeatin, within cultured tissue (Malik et al., 2005; Rai et al., 2010). When low BAP concentrations (1 or 2mg/L) were applied, shoot formation on cultured jojoba explant was not detected, which may be due to insufficient synthesis of natural hormones. Conversely, these concentrations (1 or 2mg/L) used in combination with 0.1 or 0.2mg/L NAA stimulated shoot formation. Under short- or long-term culture, the highest shoot cloning was obtained when MS medium with 3mg/L BAP + 0.1mg/L NAA was used. The number of shoots/explants and nodes/shoots were the essential prerequisite for shoot multiplication during *in vitro* propagation of jojoba and other plant species (Hassanein et al., 2015b; Salem 2016, 2020).

Induced shoot formation on cultured jojoba nodal explants was influenced by Ψ exerted

by MS (−435MPa), SH (−376MPa), or B5 (−366MPa) medium, each of them containing 3% sucrose (Thorpe et al., 2008; El-Sheekh et al., 2020). The best result was obtained using the lowest Ψ medium (MS). In addition, induced shoot formation and growth of obtained shoots were influenced by lowering the Ψ of medium following addition of different NaCl or mannitol concentrations, as previously reported (Neto et al., 2004; Thorpe et al., 2008; El-Sheekh et al., 2020). Cultured jojoba shoots can multiply and grow when water Ψ of cultured tissues was lower than the surrounding medium. A specific Ψ is needed to establish within cells of cultured tissue and reveal cell division and morphogenesis (Cleland, 1977). In this study, the water Ψ outside cultured plant materials was caused by a medium component, sucrose, and NaCl or mannitol concentrations. When Ψ of MS medium was lower than −435MPa after adding mannitol or NaCl, the medium became more suitable for jojoba callus growth, especially when established by mannitol up to −435.054MPa (4g/L). Incompatible Ψ of the MS medium was more effective on shoot formation than shoot growth. While reduced medium Ψ due to the addition of 1gm or more of NaCL or mannitol retarded shoot formation, it enhanced shoot growth, especially when mannitol was used. Stress tolerant plants, such as jojoba, are characterized by low water potential and require medium with relatively low water potential, such as MS for shoot multiplication and growth (Lassocinski, 1985).

We studied esterase patterns of cultured plant shoots and calli. Multiple forms of esterases and their differential expression under applied conditions indicate their important role in several physiological-biochemical mechanisms (Coppens & Dewitte, 1990), including cell wall elasticity (Tamás et al., 2005) and acclimatization to abiotic or biotic stresses (Sasidharan et al., 2011). In jojoba, the number of esterase isoenzyme forms due to reduced medium Ψ (following addition of NaCl or mannitol) in plant shoots was higher than that of calli. Each level of Ψ lower than the MS medium after adding certain concentrations of mannitol or NaCl showed a specific esterase pattern. It was clear that under different concentrations of mannitol or NaCl in culture medium, callus was better than *in vitro* cultured shoot to study esterase expression in jojoba.

Compared with the esterase isoenzyme pattern of MS medium containing 3 g/L sucrose (−435MPa), reducing its Ψ following addition of 0.5–3.0g/L NaCl resulted in expression of two additional isoenzyme forms (EST-1 and EST-8), but six new isoenzyme forms (EST-1, EST-2, EST-9, EST-10, EST-11, and EST-12) were detected under applied mannitol concentrations on jojoba calli. Consequently, the growth of jojoba calli on mannitol-containing medium was better than that of NaCl, which may be due to the supportive role of six esterase isoenzyme forms on mannitol-containing medium. In addition, NaCl concentrations expressed lower Ψ than corresponding mannitol concentrations. Further, toxicity of Na^+ ions in NaCl containing medium negatively affected callus growth. An increase in the number of isoenzyme forms and/or increase in staining intensity of specific isoenzyme bands can indicate increasing enzyme activity (Hassanein, 1999, 2004). Detecting new esterase isoenzyme forms under abiotic conditions have also been reported (Hasssanein, 1999; Bekheet et al., 2006), which are related to the genotype and morphogenic phase (Martinelli & Gianazza, 1996) and used as a biochemical marker (Coppens & Dewitte, 1990). In jojoba, esterase is involved in lipid catabolism serving as a carbon source for synthesis of new molecules (Flowers et al., 2010; Radic & Pevaler-Kozlina, 2010). The role of esterases (Radic & Pevaler-Kozlina, 2010) and "includer" phenomenon (Mills & Benzioni, 1992; Mills et al., 2001) may help jojoba plant absorb water and use it efficiently under non extreme Ψ of the surrounding medium, whereby MS is the ideal environment for jojoba *in vitro* culture. In plants, esterases combined with other enzymes are involved in salinity defense mechanisms (Lima et al., 2012; Reyes-Pérez et al., 2019; Akbari et al., 2020).

In jojoba, stability in protein patterns was the primary texture phenomenon for an increasing number of polypeptide bands under stress conditions. SDS-PAGE patterns changed following reduced Ψ when incorporating mannitol or NaCl in the culture media, resulting in expression of specific polypeptides (Hassanein 1999; Win & Oo, 2017). These changes in protein patterns may be an important part of biochemical processes that make a plant species, such as jojoba, more fit for the harsh environment (Singh et al., 1985; Wang et al., 2019). The appearance of new polypeptide bands and disappearances of others were detected in jojoba and other plants (Amini

et al., 2006). The relationship between reduced Ψ levels and expression of new polypeptide bands suggests that modulating gene expression may be involved in the ability for plant cells to survive, multiply, and grow under more negative Ψ caused by abiotic stress in harsh environments (Hassanein, 1999; Bekheet et al., 2006; Hassanein et al., 2015b).

In conclusion, high salt medium (MS) with an Ψ value of −435MPa is recommended for *in vitro* culture of jojoba explants. Jojoba shoot formation was more sensitive to changes in Ψ of the culture medium than growth of formed shoots. Callus was a better indicator than shoot culture for investigating the molecular effects of abiotic stresses on *in vitro* cultured jojoba explants.

Conflicts of interest: No conflicts of interest have been declared.

Author contribution: AMH, GS and DMS proposed the idea. GS and DMS designed the experimental work, and performed the experimental measurements. AMH analyzed and interpreted the data and wrote the manuscript. DS performed the calculations and statistical analysis, participated with AMH in analyzing and interpretation of the data, revised the manuscript, checked, and adjusted the plagiarism. AMH acted as a corresponding author. AG revised the manuscript. All authors drafted the manuscript, read and approved the final manuscript.

Ethical approval: Not applicable

References

Akbari, M., Katam, R., Husain, R., Farajpour, M., Mazzuca, S., Mahna, N. (2020) Sodium chloride induced stress responses of antioxidative activities in leaves and roots of *Pistachio Rootstock*. *Biomolecules*, **10**, 189; doi:10.3390/biom10020189

Al-Dossary, O., Kharabian, A., Al-Mssallem, I., Furtado, A., Henry, R. (2019) Transcriptomics analysis for the detection of novel drought tolerance genes in Jojoba (*Simmondsia Chinensis*). *Proceedings*, **36**, 135; doi:10.3390/proceedings2019036135

Al-Soqeer, A., Motawei, M.I., Al-Dakhil, M., El-Mergawi, R., Al-Khalifah, N. (2012) Genetic variation and chemical traits of selected new Jojoba (*Simmondsia chinensis* (Link) Schneider) Genotypes. *Journal of the American Oil Chemists' Society*, **89**, 1455-1461.

Amarger, V., Mercie, L. (1996) Molecular analysis of RAPD DNA based markers: their potential use for the detection of genetic variability in jojoba (*Simmondsia chinensis* L. Schneider). *Biochimie*, **77**(12), 931–936.

Amini, F., Ehsanpour, A.A., Hoang, Q.T., Shin, J.S. (2006) Protein changes in tomato under *in vitro* salt stress. *Russian Journal of Plant Physiology*, **54**(4), 464- 471.

Balen, B., Krsnik-Rasol, M., Zadro, I., Simeon-Rudolf, V. (2004) Esterase activity and isoenzymes in relation to morphogenesis in *Mammillaria gracillis* Pfeiff tissue culture. *Acta Botanica Croat*, **63**(2), 83–91.

Buah, J.N., Kawamitsu, Y., Sato, S., Murayama, S. (1999) Effects of different types and concentrations of gelling agents on the physical and chemical properties of media and the growth of banana (Musa spp.) *in vitro*. *Plant Production Science,* **2**(2), 138-145.

Bekheet, S.A, Taha, H.S., Solliman, M.E. (2006) Salt tolerance in tissue culture of onion (*Allium cepa* L.). *Arab Journal of Biotechnology*, **9**, 467-476.

Brewer, G.J. (1970) "*Introduction to isoenzymes Techniques*". Academic Press, New York, San Francisco, London.

Cao, B., Gao, H.D. (2003) Technology of cutting propagation of *Simmondsia chinensis* (Link) Schneider (in Chinese). *Journal of Nanjing Forest University*, **27**, 62-66.

Chaturvedi, H.C., Sharma M. (1989) *In vitro* production of cloned plants of Jojoba (*Simmondsia chinensis* (Link) Schneider) through shoot proliferation in long term culture. *Plant Science*, **63**, 199–207.

Cleland, R.E. (1977) The control of cell enlargement. *Symposia of the Society for Experimental Biology*, **31**, 101-116.

Coppens, L., Dewitte D. (1990) Esterase and peroxidase zymograms from barley (*Hordeum vulgare* L.) callus as a biochemical marker system of embryogenesis and organogenesis. *Plant Science*s, **67**, 97–105.

de Paiva Neto, V.B., Otoni, W.C. (2003) Carbon sources and their osmotic potential in plant tissue culture: does it matter? *Scientia Horticulturae*, **97**(3–4), 193-202.

El-Sheekh, M.M., Morsi, H.H., Hassan, L.H.S. (2020) Assessment of the optimum growth medium and the effect of different light intensities on growth and photosynthetic pigments of *Chlorella vulgaris* and *Scenedesmus arvernensis*. *Egyptian Journal of Botany*, **60**(2), 395-404.

Flowers, T.J., Galal, H.K., Bromham, L. (2010) Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology*, **37**, 604-612.

Fujiwara, K., Kozai, T. (1995) Physical microenvironment and its effects. In: "*Automation and Environment Control in Plant Tissue Culture*", Aitken-Chistie, J., Kozai, T., Smith, M.A.L. (Eds.). Kluwer Acadmic Publisers, pp. 319-369.

Gamborg, O.L., Miller, R.A., Ojima, K. (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Researches*, **50**, 151-158.

Govindaraj, M., Vetriventhan, M., Srinivasan, M. (2014) Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International,* **4**. https://doi.org/10.1155/2015/431487

Harsh, L.N., Tiwari, J.C., Bohra, M.D., Tripathi, D. (2001) Standardization of agronomic practices of jojoba cultivation in arid regions. In: *Proceeding of national seminar on production, marketing and processing of jojoba* (*Simmondsia chinensis*). *Jaipur*, *India*, (abstr.); pp. 31–32.

Hassan, S.A.M., Zaied, N.S., Hassan, H.S.A., Ahmed, D.M.M. (2019) Effective protocol for *in vitro* shoot production through flower bud explants of jojoba (*Simmondsia chinensis*). *Plant Archives*, **19**(1), 2631-2636.

Hassanein, A.M. (1999) Alterations in protein and esterase patterns of peanut plants in response to salinity stress. *Biologia Plantarum*, **42**, 241-248.

Hassanein, A.M. (2004) Effect of relatively high concentration of mannitol and sodium chloride on regeneration and gene expression of stress tolerant (*Alhagi graecorum*) and stress sensitive (*Lycopersicon esculentum* L) plant species. *Bulgarian Journal of Plant Physiology*, **30**, 19-36.

Hassanein, A.M., El-Sherbeeny, G.R., Kalid, A.G., Gaboor, G.M. (2015a) Seed propagation increases genetic variation and micropropagation to multiply selected shrub with desirable characters. *Journal of International Scientific Publications*, **3**, 325-339.

Hassanein, A.M., Galal, A., Soltan, D.M., Saad, G.k. (2015b) Effect of medium strength and activated charcoal on in vitro shoot multiplications and growth of jojoba. *Journal of Environmental Studies*, **14**, 81-90**.**

Kirkham, M.B., Holder, P.L. (1981) Water, osmotic, and turgor potentials of kinetin-treated callus (Cactus, Echinopsis turbinate). *HortScience,* **16**(3), 306-307.

Kumar, S., Mangal, M., Dhawan, A.K., Singh, N. (2011) Assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensis* (Link) Schneider using RAPD and ISSR markers. *Acta Physiologia Plantarum*, **33**(6), 2541–5.

Kumar, S., Mangal, M., Dhawan, A.K., Singh, N. (2012) Biotechnological advances in jojoba [*Simmondsia chinensis* (Link) Schneider]: recent developments and prospects for further research. *Plant Biotechnological Reports*, **6**(2), 97–106.

Lassocinski, W. (1985) Chlorophyll-deficient cacti in tissue cultures. *Acta Horti*culture, **167**, 287-293.

Lazzeri, P.A., Hildebrand, D.F., Sunega, J., Williams, E.G., Collins, G.B. (1988) Soybean somatic embryogenesis: interactions between sucrose and auxin. *Plant Cell Reports*, **7**, 517-520.

Lee, C.W. (1988) Application of plant biotechnology for clonal propagation and yield enhancement in jojoba. *Proc. 7th Int. Conf. Jojoba and Its Uses,* Phoenix, Arizona USA, 102-111.

Lima, M.G., Lopes, N.F., Zimmer, P.Z., Meneghello, G.E., Mendes, C.R., do Amarante, L. (2012) Enzyme expression in indica and japonica rice cultivars under saline Stress. *Acta Scientiarum Biological Sciences Maringá*, **34**, 473-481.

Low, C.B., Hackett, W.P. (1981) Vegetative propagation of jojoba. *California agriculture*, **35**, 12-13.

Malik, S.K., Chaudhury, R. Kalia, R.K. (2005) Rapid *in vitro* multiplication and conservation of *Garcinia indica*: A tropical medicinal tree species. *Scientia Horticulturae*, **106**, 539–553.

Martinelli, L., Gianazza, E. (1996) Biochemical changes during regeneration of Sunflower (*Helianthus annuus* L.). *Electrophoresis*, **17**, 191–197.

Mills, D., Benzioni, A. (1992) Effect of NaCl salinity on growth and development of jojoba clones. II. Nodal segments grown *in vitro*. *Journal of Plant Physiology*, **139**, 737–741.

Mills, D., Wenkart, S., Benzioni, A. (1997) Micropropagation of *Simmondsia chinensis* (Jojoba). In: "*Biotechnology in Agriculture and Forestry*", Bajaj, Y.P.S. (Ed) , Vol **40**. High-Tech and Micropropagation -Verlag, Berlin, Heidelberg. Springer.; VI, pp. 370–393.

Mills, D., Zhang, G., Benzion, A. (2001) Effect of different salts and of ABA on growth and mineral uptake in jojoba shoots grown *in vitro*. *Journal of Plant Physiololgy*, **158**, 1031–1039.

Murashige, T., Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiolgia Plantarum*, **15**, 473–497.

Nei, M., Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* USA, **76**, 5269-5273.

Neto, N.B.M., Saturnino, S.M., Bomfim, D.C., Custódio, C.C. (2004) Water stress induced by mannitol and sodium chloride in soybean cultivars. *Brazilian Archive of Biology and Technology,* **47**, 521-529.

Palzkill, D.A., Feldman, W.R. (1993) Optimizing rooting of jojoba stem cuttings: effects of basal wounding, rooting medium and depth of insertion in medium. *Journal American Oil Chemistry Society*, **70**, 1221–1224.

Radic, S., Pevalek-Kozlina, B. (2010) Differential esterase activity in leaves and roots of *Centaurea ragusina* L. as a consequence of salinity. *Periodicum biologorum*, **112**, 253–258.

Rai, M.K., Asthana, P., Jaiswal, V.S., Jaiswal, U. (2010) Biotechnological advances in guava (*Psidium guajava* L.): Recent development and prospects for future research. *Trees*, **24**, 1–12.

Reyes-Pérez, J.J., Ruiz-Espinoza, F.H., Hernández-Montiel, L.G., de Lucía, B., Cristiano, G., Murillo-Amador, B. (2019) Evaluation of glycosyl-hydrolases, phosphatases, esterases and proteases as potential biomarker for NaCl-Stress tolerance in *Solanum lycopersicum* L. *Varieties Molecules,* **24**, 2488; doi:10.3390/molecules24132488

Salem, J.M. (2016) *In vitro* propagation of *Moringa oleifera* L. under salinity and ventilation conditions. *Genetics and Plant Physiology*, **6**(1-2), 54-64.

Salem, J.M. (2020) Effects of anti-ethylene compounds on vitrification and genome fidelity of *Stevia rebaudiana* Bertoni. *Egyptian Journal of Botany*, **60**(2), 519-535.

Sasidharan, R., Voesenek, L.A.C.J, Pierik, R. (2011) Cell wall modifying proteins mediate plant acclimatization to biotic and abiotic stresses. *Critical Reviews in Plant Sciences*, **30**(6), 548-562.

Schenk, R.U., Hildebrant, A.C. (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell culture. *Candian Journal of Bottany*, **50**, 199-204.

Singh, A.K., Dhanapal, Sh., Yadav, B.S. (2020) The dynamic responses of plant physiology and metabolism during environmental stress progression. *Molecular Biology Reports*, **47**, 1459–1470.

Singh, N.K., Handa, A.K., Hasegawa, P.M., Bressan, R. (1985) Proteins associate with adaptation of cultured tobacco cells to NaCl. *Plant Physiology*, **79**, 126-137.

Tamás, L., Huttová, J., Mistrík, I., Šimonovičová, M., Široká, B. (2005) Aluminum induced esterase activity and isozyme pattern in barley root tip. *Plant Soil Environment*, **51**, 220–225.

Thorpe, T.A., Yeung, E., De Klerk, G. (2008) The Components of Plant Tissue Culture Media ll: Organic Additions, Osmotic and pH Effects, and support system. In: "*Plant Propagation by Tissue Culture*", E.F. George et al. (Eds.), 3rd ed. Springer, pp.115–173.

Wang, X., Gao, F., Bing, J., Sun, W., Feng, X., Ma, X.,

Win, K.T., Oo, A.Z. (2017) Salt-stress-induced changes in protein profiles in two black gram (*Vigna mungo* L.) varieties differing salinity tolerance. *Advances in Plants & Agriculture Research*, **6**(6), 00239.

Zhou, Y., Zhang, G. (2019) Overexpression of the jojoba aquaporin gene, ScPIP1, enhances drought and salt tolerance in transgenic arabidopsis. *International Journal of Molecular Sciences*, **20**, 153; doi:10.3390/ijms20010153

تأثير التغيرات الطفيفة لجهد الماء في الأوساط الغذائية على الاكثار الدقيق، أشكال التعبير الجيني لانزيم الاستيريز والبروتينات لنبات الجوجوبا

أحمد محمد حسانين، عبد الناصر جلال، غادة سعد، ضياء محمد سلطان

المعمل المركزي للهندسة الوراثية- قسم النبات والميكروبيولوجي- كلية العلوم- جامعة سوهاج- 82524 سوهاج- سوهاج- مصر.

تكبير الحامض النووي الديؤكسي ريبوز لعشر شجيرات من نبات الجوجوبا باستخدام سبعة بادئات تكبير عشوائية (رابد) أوضح أن هناك اختلافات جينية كبيرة في نباتات الجوجوبا الناتجة بالاكثار العادي باستخدام البذور.

للحصول على نسخ صحيحة جينيًا مماثلة النبات الأم تمامًا، استخدمنا تقنية الاكثار الدقيق. الوسط المغذي موراشيجي وسكوج المضاف إليه تركيزات منخفضة نسبيًا (1 أو 2 مجم/لتر) من البنزيل أمينو بيورين لم يحفز تكوين الأفرع على القطع العقدية للجوجوبا. أما الوسط المغذي موراشيجي وسكوج المزود بتركيز 3 مجم/لتر من البنزيل أمينوبيورين و 0.1 مجم/لتر من نفثالين حمض الخليك، كان هو الأفضل لإعطاء نسخ أفرع خضرية على المستقطعات العقدية لنبات الجوجوبا. تقليل الجهد المائي للوسط المغذي بعد إضافة 0.5 مجم/لتر من المانيتول أسفر عن زيادة بعض الشئ (بسيطة) في عدد الأفرع المتكونة، كما أن تقليل الجهد المائي إلى حد أبعد بزيادة تركيز ملح كلوريد الصوديوم أو المانيتول، كان نتيجته إعاقة وتأخر تكوين الأفرع الخضرية. أشارت النتائج التي تم الحصول عليها في هذه الدراسة أن تكوين الأفرع الخضرية كان أكثر حساسية للتغيرات في الجهد المائي للوسط المغذي المنماه عليه من نمو تلك الأفرع. انخفض وزن الكالاس بتقليل الجهد المائي للوسط المغذي إلى أقل من 435.014 MPa - باستخدام 1 جم/لتر من كلوريد الصوديوم، بينما استخدام المانيتول لتقليل الجهد المائي بنفس القيمة السابقة وهي 435.054 MPa (4 جم/لتر) للوسط المغذي، زود الوزن الطازج للكالاس بشكل معنوي. كالاسات الجوجوبا عبرت بشكل واضح عن تأثيرات الجهد المائي للوسط المغذي موراشيجي وسكوج على التعبير الجيني وأشكال إنزيم الاستيريز والبروتين.