Induction of somatic embryogenesis and DNA fingerprinting of Jojoba

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ABSTRACT

*In-vitro induction of somatic embryogenesis and regeneration ability of jojoba [*Simmondsia chinensis *(Link) Schneider] plant were investigated using two different types of explants, i.e., immature zygotic embryos and leaf disks, and different culture media. Compact embryogenic callus was induced on Murashige and Skoog (MS) medium supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and sucrose. Embryogenic callus was developed from immature zygotic embryos on MS medium containing 1.0 mg/l 2,4-D, 0.1 mg/l 6-benzyl aminopurine (BA) and 4% sucrose. Induced calli were subcultured on MS medium supplemented with (4% or 6%) sucrose and 0.1, 0.5 and 1.0 mM kinetin for plant regeneration. Somatic embryos at the globular, heart-shaped, torpedo, and cotyledonary stages were developed. Embryogenic callus and hence different stages of somatic embryos were developed from leaf segments on MS medium supplemented with 3%, 4% or 6% sucrose, 1.0 mg/l 2, 4-D and 0, 0.1, 0.5 or 1.0 mg/l BA. Leaf-derived embryogenic calli did not mature on any of the maturation/germination media examined even after 4 weeks of culture. Random amplified polymorphic DNA (RAPD) technique was used to investigate the patterns and distribution of genetic variability in natural field-grown cuttings of jojoba plants. Five oligonucleotide primers were used to screen five randomly selected jojoba samples for analysis. Total DNA extracted from field-grown cutting leaves was used as template in the PCR reaction. The five primers used showed a highly polymorphic nature of the studied plants. It is concluded that jojoba plants in the natural habitate of Egypt may belong to different genotypes, none of them could differentiate to complete plantlets by tissue culture.*

Key words: *Jojoba, callus induction, somatic embryogenesis, RAPD.*

INTRODUCTION

The jojoba family (*Simmondsiaceae*) has only one genus, *Simmondsia*, consisting only one species, jojoba (*Simmondsia chinensis* (Link) Schneider). Earlier, jojoba was considered as an isolated member of the Box family (*Buxaceae*), but now it is regarded as sufficiently distinct to be placed in a separate family. Jojoba is found in coastal and cis-montane southern California east to central Arizona and south to Sonora and Baja California (Munz, 1974; Yermanos, 1974). The species is dioecious, relies on winds for successful pollination (Niklas and Buchmann, 1985) and the seedlings cannot be sexed until the first flower buds appear 9 to 24 months after sowing (Dunstone and Begg, 1983). Although jojoba plants start producing fruits in

the plant's life estimated to be 100 years (Verbanic, 1986). Plants are extremely tolerant to drought (Al-Ani *et al.*, 1972) and their foliage, the meal remaining from seed pressing, contain 30% protein consisting of 17 amino acids, 7 of which are essential (AA). After treatment, the meal becomes suitable for utilization as cattle, sheep, goat and fish feed and/or organic fertilizers. The large seeds have been used locally in North America as a food source by indigenous people (Brooks, 1978).

The most noteworthy feature of jojoba from a human perspective is the unusual liquid wax that makes up the storage reserve of its seeds. This substance, a fatty acid ester of a long-chain alcohol, is unique in the plant kingdom. It has chemical and rheological properties similar to those of sperm whale oil, making it useful in a host of applications (Brooks, 1978). The oil concentration of the seed at maturity stage ranges from 38 to 62% of seed dry weight (Miwa and Spencer, 1976). Oil is obtained by mechanical pressure or by solvent extraction (Verbanic, 1986). The unique oxidative stability of the natural jojoba oil, the pleasant feel of the oil on skin and hair, the biodegrade ability, non petroleum based and renewable product are reasons for its valued use in cosmetic industry (Brown, 1994; Wisniak, 1987). Some plants in the natural habitate appear to be genetically predisposed to be more productive than others, making selection for higher yield possible (Nord and Kadish, 1974; Yermanos, 1974).

Jojoba is considered one of the most practical solutions for desert plantation in Egypt. Heat, drought and salt tolerance, lesser possibilities for infection, lesser need for fertilizers, and generous financial income, are certainly the most encouraging goals to plant jojoba in Egypt (El Moguy, 2002). Since May 1991 El Moguy started the first practical steps to introduce jojoba to Egypt by planting the first jojoba field surrounded by date palms with the technical help of an agricultural expert, and the help of the FAO coordinator (El Moguy, 2002).

Tissue culture techniques have been applied only to a limited extent in jojoba. Compared to many other plants, it is more difficult to obtain somatic embryogenesis and plant regeneration from jojoba. Micropropagation by *in-vitro* seedling culture (Roussos *et al.*, 1999), shoot regeneration *via* organogenesis and somatic embryogenesis (Hamama *et al.*, 2001) were achieved only recently in jojoba.

RAPDs are dominant molecular markers developed by Welsh and McClelland (1990) and Williams *et al.* (1990). They are random pieces of DNA amplified from the genome by a PCR-based technique. Amplified DNA fragments may be visualized on gel, and bands scored as presence / absence character states. RAPD profiling is being increasingly used in population surveys because of the ease of methodology and the numerous polymorphic distinguishable (Stewart and Excoffier, 1996; Hussein *et al.*, 2005; Hussein *et al.*, 2006; Hussein and Younis, 2006; Hussein *et al.*, 2006).

The objectives of the present study are: (1) to describe a rapid and efficient protocol for somatic embryogenesis in jojoba and (2) to assess the polymorphic nature of field-grown plant cuttings based on RAPD markers.

MATERIALS AND METHODS

Plant material

Jojoba immature zygotic embryos (1-2 mm long) and mature leaves (2-5 cm in length) were collected from different plants and 30 cuttings grown in the Jojoba farm of Mr. Nabil Elmogy and The European Egyptian Farm for the Medicinal Plants of Mr. Hossny Abu Elyazeed, Cairo, Egypt.

Seed sterilization and explant culture

Immature zygotic embryos and leaves (2-5 cm long) were chosen and surface sterilized by the following procedure: seeds and leaves were initially sterilized for 30 sec using 70% alcohol, then immersed in 10% (v/v) commercial bleach for 10 min and finally rinsed five times in sterile distilled water. Immature zygotic embryos (1-2 mm) were dissected to 2-3 explants and mature leaves (2-5 cm) were cut into 3 x 3 mm segments.

Induction, proliferation and maintenance of embryogenic calli

Both pieces of immature zygotic embryos and leaf segments were cultured, 10 embryo pieces or 10 leaf segments (placed on their adaxial sides) per 100 x 15 mm Petri dish, were cultured on MS media (Murashige and Skoog, 1962) supplemented with various concentrations and combinations of 2, 4-D (0.5, 1.0 or 2.0 mg/l), BA (0, 0.1, 0.5 or 1.0 mg/l) and sucrose (2, 3, 4, 6 or 15 %). Cultures were maintained in darkness for 28 days at 22°C, and then embryogenic calli with high frequency of embryogenesis were chosen for the next subculture. Continuous subcultures were carried out in the same way for three times. Subsequently, embryogenic calli were subcultured every 15 days on MS medium supplemented with 4% sucrose, 0.5 mg/l 2, 4-D and 0.5 mg/l B.A.

Differentiation of somatic embryos and maturation

High frequency embryogenic calli were chosen and transferred onto embryo-differentiation medium composed of MS medium supplemented with different concentrations of kinetin (0.1, 0.5 or 1.0 mg/l) and sucrose (4 or 6%) for the induction and development of somatic embryos.

All media were solidified with 2 g/l gelrite and the pH was adjusted to 5.8 before autoclaving at 121°C for 15 min. Embryogenic calli were incubated at 22°C ± 2°C under a light intensity of approximately 2000 Lux provided by cool white fluorescent lamps with 16 h photoperiod.

DNA isolation and PCR amplification

DNA was extracted from field-grown cutting leaves using the modified CTAB protocol described by Porebski *et al.* (1997). Five random 10-mer primers (OP-A3, OP-C5, OP-D5, OP-M5 and OP-N4) were used in this study for DNA amplification. The sequences of the primers are shown in Table (1). The polymerase chain reactions (PCR) were carried out in 25 µl volume containing 50 ng of genomic DNA template, 30 pmoles/µl primers, 0.2 µM each of dATP, dCTP, dGTP and dTTP, 10 x buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, and 2 units of Taq polymerase (AB gene).

Table (1): The sequence of the five decamer arbitrary primers used in RAPD analysis.

Primer	Sequence
OP-A3	5' AGTCAGCCAC 3'
OP-C5	5' GATGACCGCC 3'
OP-D5	5' TGAGCGGACA 3'
OP-M5	5' GGGAACGTGT 3'
OP-N4	5' GACCGACCCA 3'

PCR amplifications were carried out in a Biometra T1 thermal cycler programmed as follows: an initial strand separation at 94 °C (5 min) followed by 40 cycles with the following temperature profile: 94°C (1 min), 36°C (1 min), 72°C (1.5 min) and a final extension at 72°C (7 min). Amplification products were visualized by electrophoresis in 1.5% agarose gels stained with ethidium bromide then photographed with a Polaroid camera and the DNA banding patterns were scored.

The data statistics

A minimum of five plates were cultured per treatment with ten explants per plate. To detect significant differences between treatments, data were subjected to analysis of variance. Duncan's multiple range test at the 5% probability level was used to separate mean differences when significant treatment effects were detected (Duncan and Beverly, 1955).

Band recording

The bands which are clear and can be repeated were recorded as "1", otherwise "0".

RESULTS AND DISCUSSION

Induction and proliferation of callus from immature zygotic embryos

In the present study eight different MS-based media were used to investigate the effect of different concentrations of 2,4-D, BA and sucrose on the induction of somatic embryogenesis in jojoba. Different concentrations of 2,4-D (0.5 or 1.0 mg/l), BA(0, 0.1, 0.5 or 1.0 mg/l) and sucrose (2, 3, 4, 6 or 15%) were tested for callus initiation and proliferation. As shown in Table (2), callus formation was induced from immature zygotic embryos within three weeks in all media. The results revealed that five media composition, i.e., C3, C4, C5, C6 and C8 have succeeded to induce 100% callus formation as yellow and brown somatic embryos in all stages (Fig. 1). This was followed by C7 (76.67%) and C2 (33.33%). While, medium C1 (MS containing 2% sucrose, 1 mg/l 2,4-D and without BA) produced the lowest percentage of calli with somatic embryos (0.001%). The former five media with different concentrations of 2, 4-D, BA and sucrose expressed 100% embryogenic callus induction. This result constitutes the significant importance of decreasing the concentration of 2,4-D avoids somaclonal variation (Mangolin *et al.*, 2002). Therefore, it could be concluded that medium C8 (containing 0.5 mg/l 2,4-D) is the best choice for embryogenic callus induction from jojoba immature zygotic embryos. These results are in agreement with those reported by Hamama *et al.* (2001) and Wang and Janick (1986).

Table (2): Frequencies of embryogenic calli obtained from immature zygotic embryos on eight MS media supplemented with different concentrations of sucrose, 2,4-D and BA.

Media code	Sucrose %	2,4-D (mg/l)	BA (mg/l)	Embryogenic calli%
C 1	2	1.0	0	0.001^{B}
C 2	15	1.0	0	33.33^{B}
C 3	3	1.0	0.1	100^{A}
C 4	3	1.0	1.0	100^{A}
C 5	4	1.0	0.1	100^{A}
C 6	6	1.0	0.5	100^{A}
C 7	2	0.5	0.5	76.67^{A}
C 8	4	0.5	0.5	100^{A}

No. of plates /treatment = 5

Means with the same letter are not significantly different at 5% probability level

The results in Table (2) also revealed that increasing the sucrose concentration from 2% to 3% or 4% results in higher percentage of embryogenic calli, while 15% sucrose led to a significant decline in the percentage of embryogenic calli. Thus, the optimum embryogenic callus induction could be obtained by optimizing the concentration of sucrose in the culture medium. In this respect, Nobre *et al.* (2004) found that sucrose concentrations of 87.6–146.0 mM gave the highest multiplication rates and improved shoot growth of the Mediterranean species *Viburnum tinus* L. Moreover, Sandra and Natoniel (2004) examined the effect of different pH and sucrose concentrations on *in vitro* propagation of *Nephrolepis biserrata*. Inhibition of shoot and leaf regeneration was observed with the pH adjusted to 3, 5, 7 and 9 in the absence of sucrose. On the other hand, when sucrose was added to the medium, the number of shoots increased, reaching the maximum average values of 51.25 and 38.25 shoots per explant at pH 5 and 7, respectively. Concerning the level of BA, the results in (Table 2) revealed that supplementing the medium with BA at a concentration ranging from 0.1 to 1 mg/l significantly increased the percentage of embryogenic calli. These results are in good agreement with those of Takeshi *et al.* (1985).

Callus maturation and somatic embryogenesis from immature zygotic embryos

Following induction, the calli were transferred to MS medium supplemented with different concentrations of sucrose (4% or 6%) and kinetin (0.1, 0.5 or 1.0 mg/l) for callus maturation (Table 3).

Table (3): Frequencies of somatic embryogenesis from embryogenic calli on six MS media supplemented with various levels of kinetin and sucrose.

Media code	Sucrose %	Kinetin (mg/l)	Somatic Embryogenesis %
M 1	4	0.1	0.001^{B}
M 2	4	0.5	100.0^{A}
M 3	4	1.0	0.001^{B}
M 4	6	0.1	0.001^{B}
M 5	6	0.5	100.0^{A}

No. of plates /treatment = 5

Means with the same letter are not significantly different at 5% probability level.

The results revealed that 100% of the calli cultured on the two media M2 (MS + 4% sucrose + 0.5 mg/l kinetin) and M5 (MS + 6% sucrose + 0.5 mg/l kinetin) produced somatic embryos. The difference between the two media was in the quantity of somatic embryos which was more in M2 medium. All the embryogenic development stages, i.e., globular, heart-shaped, torpedo, and cotyledonary stages developed on these maturation media (Fig. 1B). This result is in agreement with those reported by Tawfik and Noga (2002) as they found that the presence of kinetin in the callus induction medium for cumin (*Cuminum cyminum* L.) seedlings with 2,4-D enhanced both the callus proliferation and the subsequent differentiation of the embryos.

Induction and proliferation of callus from leaves

The results of callus induction ability from leaves using different concentrations of sucrose (2, 3, 4 or 6%), 2, 4-D (1.0 or 2.0 mg/l) and BA (0, 0.1, 0.5 or 1.0 mg/l) are summarized in Table (4).

Table (4): Frequencies of embryogenic calli from leaf tissue on six MS media supplemented with different concentrations of sucrose, 2,4-D and BA.

Media code	Sucrose %	2,4-D (mg/l)	BA (mg/l)	Embryogenic calli %
L 1	2	1	0.0	0.0010[B]
L 2	2	2	0.0	0.0007[B]
L 3	4	1	0.0	100.00[A]
L 4	3	1	0.1	100.00[A]
L 5	3	1	1.0	100.00[A]
L 6	6	1	0.5	100.00[A]

No. of plates /treatment = 5

Means with the same letter are not significantly different at 5% probability level.

The leaves developed callus within 10-36 days after culture. The maximum callus induction was achieved on media L3, L4, L5 and L6 which induced 100% embryogenic calli. Very low frequencies of callus induction were observed in L1 and L2 media. The calli of these two media also showed low growth rates. Variations in callus induction ability among the tested media indicated that the difference in response can be attributed to the use of a low concentration of sucrose (2%) in L1 and L2 media. These results are in agreement with the results reported by Murashige and Tucker (1969). They found that the best sucrose concentration to increase both explants proliferation and sustained callus growth was from 4% to 6%. It is worth to note that leave explants cultured on L3 medium produced 100% embryogenic calli directly without passing through a friable callus stage. This observation was also reported by Aly *et al.* (2006). Moreover, the results in Table (4) revealed that L3 medium composed of MS medium supplemented with 4% sucrose and 1.0 mg/l 2,4-D was enough to produce 100% embryogenic calli. This reveals that the addition of BA is not necessary to obtain high frequency of embryogenic calli. The same result was noticed by Green and Philips (1975) as they studied the effect of 2,4-D for inducing callus in maize without cytokinins. Similar findings were reported by Lazer *et al.* (1988) as they initiated callus from immature embryos of wheat on MS medium supplemented with 2 mg/l 2,4-D alone. This conclusion contradicts the observation reported by Fujimura and Komamine (1975) when they found that BA is an essential factor for the promotion of somatic embryogenesis in carrots. Embryogenic calli from leaves were characterized by globular structures, smooth, shiny and creamy or green color in all media (Fig. 2A).

Effect of different hormones treatments on the induction and maturation of embryogenic calli from leaves

As shown previously, it is needful to increase the sucrose percentage up to 4% or 6% with 1 mg/l 2,4-D to induce 100% embryogenic calli (Table 4). To investigate the effect of supplementing the MS media containing low concentration of sucrose (2% or 3%) with different hormones in the production of embryogenic calli or somatic embryos. Six media with different combinations of hormones (2,4-D, BA, NAA and kinetin) were assayed. From the data presented in Table (5), it can be noted that B1 (KS + 2% sucrose, 1 mg/l 2,4D and 0.1 mg/l BA) and B2 (MS + 2% sucrose, 1 mg/l 2,4D and 0.25 mg/l BA) media succeeded to induce 63% and 60% embryogenic calli, respectively.

Table (5): The effect of different types of hormones with 2% or 3% sucrose in producing embryogenic calli and somatic embryos using leaf tissue as explants.

Media code	Sucrose %	2,4-D (mg/l)	NAA (mg/l)	BA (mg/l)	Kinetin (mg/l)	Somatic Embryogenesis %	Callus Description
B 1	2	1.0	0.0	0.10	0.0	63.000^{A}	compact turned to embryogenic yellow
B 2	2	1.0	0.0	0.25	0.0	60.000^{A}	compact turned to embryogenic yellow
B 3	2	0.0	0.1	0.10	0.0	0.001^{C}	compact, white and adventitious roots
B 4	2	0.0	0.1	0.25	0.0	0.001^{C}	compact, white and adventitious roots
B 5	3	0.5	0.0	0.00	0.0	0.001^{C}	compact and white
B 6	3	0.0	0.0	0.50	0.5	30.000^{B}	compact, friable turned to somatic embryogenesis and globular yellow

No. of plates /treatment = 5

Means with the same letter are not significantly different at 5% probability level.

From these data it can be concluded that there are no significant differences between the two above-mentioned treatments. This confirms the conclusion that the BA concentration does not have a noticeable effect on promoting embryogenic calli. These results are in agreement with those recorded by Hamama *et al.* (2003) who worked on jojoba leaf tissues to get somatic embryos. When the medium was supplemented with NAA (0.1 mg/l) instead of 2,4-D (B3 and B4 media) only a very low percentage (0.001%) of embryogenic and compact calli with adventitious roots were produced (Table 5). These results indicate that the 2,4-D in 1 mg/l concentration is an essential factor in the induction of embryogenic calli in jojoba.

On the other hand, using 0.5 mg/l kinetin and 0.5 mg/l BA with 3% sucrose (B6 medium) resulted in yellow somatic embryos with globular and early cotyledonary stages (Table 5 and Fig. 2B). This indicates that when using 3% sucrose, the addition of kinetin (0.5 mg/l) is an essential factor to produce somatic embryos in jojoba.

DNA fingerprinting of Jojoba

Molecular markers are efficient tools for genotype identification and estimation of relatedness through DNA fingerprinting. Short primers of arbitrary nucleotide sequence, called RAPD markers, can be used to amplify genomic DNA segments, and that polymorphism can be detected between the amplification products of the different isolates through examination of agarose gel (Williams *et al.*, 1990). We used five random primers, as RAPD markers, for analysis of genomic DNA diversity among different five jojoba field-grown cutting leaf samples (P1, P2, P3, P4 and P5).

RAPD markers profile and densitograms results of jojoba samples were mentioned in Fig. (3) and Tables (6 and 7), respectively. The results revealed that 47 reproducibly scorable genomic DNA bands were generated by five random primers. The RAPD profiles were generated using five different decamer primers. The amplified DNA fragments obtained in this study ranged from 4100 to 280 base pairs (bp) in size. The number of amplified DNA fragments was scored for each primer, the highest number of amplified DNA fragments was 10 (primers OP-D5 and OP-N4). While, the other three primers amplified 9 DNA fragments, with an average of 9 amplicons per primer across the 5 jojoba samples. The overall DNA patterns generated by primers OP-A3 and OP-M5 were five polymorphic bands. There were 6 polymorphic bands out of 9 scorable genomic DNA bands of the five jojoba leaf samples amplified by primer OP-C5, while 7 polymorphic bands out of 10 reproducible genomic DNA bands generated by primers OP-D5 and OP-N4 (Table 6).

Table (6): RAPD amplification patterns among the five jojoba samples using five random decamer primers.

Primer	Amplicon size (bp)	Presence or absence of bands P1	P2	P3	P4	P5	Number of polymorphic band
	2000	1	1	1	0	1	
	1302	1	1	1	1	1	
	984	1	1	1	1	0	
	942	0	0	0	0	1	
OP-A 3	790	1	1	1	1	1	5
	597	1	1	1	1	1	
	489	1	1	1	1	1	
	330	0	1	1	0	0	
	290	1	1	1	0	1	
	3200	1	1	1	0	1	
	1200	1	1	1	0	1	
	1170	0	0	0	1	0	
OP-C 5	1000	1	0	1	0	1	
	750	1	1	1	1	1	6
	620	1	1	1	1	1	
	513	0	1	0	1	1	
	390	1	1	1	0	0	
	280	1	1	1	1	1	
	2800	1	1	1	0	1	
	2160	1	0	0	0	0	
	1680	1	1	1	0	1	
	1290	1	0	0	0	0	
OP-D 5	820	1	0	1	1	1	7
	700	1	1	1	1	1	
	610	1	1	1	1	1	
	430	1	1	0	1	1	
	360	1	0	0	0	0	
	310	1	1	1	1	1	
	2360	1	1	1	0	1	
	2050	1	1	1	0	1	
	1780	1	1	1	1	1	
	910	1	1	1	1	1	
OP-M 5	830	1	1	1	1	1	5
	700	1	1	1	1	1	
	660	1	1	1	0	0	
	410	1	0	0	1	0	
	330	1	1	1	0	0	
	4100	0	1	1	0	1	
	3020	1	1	1	0	1	
	1750	1	1	1	0	1	
	1000	1	1	1	0	1	
OP-N 4	820	1	1	1	1	1	7
	730	1	1	1	1	1	
	580	1	1	1	1	1	
	410	0	0	1	1	0	
	365	0	0	0	0	1	
	290	0	1	1	0	0	

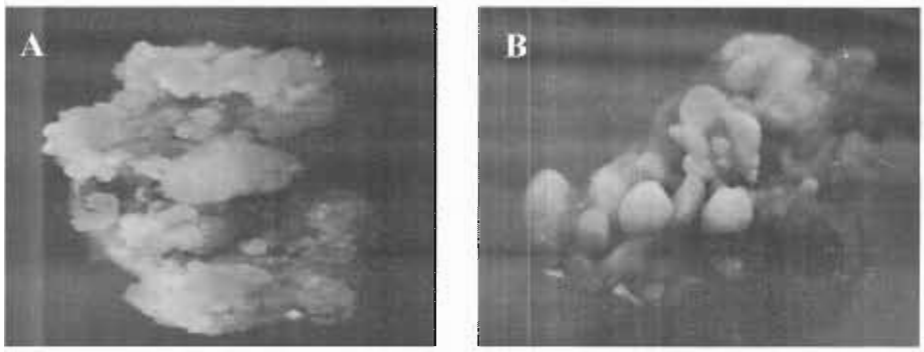

Fig. (1): Induction of embryogenic calli and somatic embryos of jojoba using immature embryos as explants. (A) callus in the globular and torpedo stages on the induction media. (B) somatic embryos in cotyledonary stage on the maturation medium.

Fig. (2): Induction of embryogenic calli and somatic embryos of jojoba plant using leaf segments as explants. (A) embryogenic calli in the globular stage on the induction medium using 2,4-D and BA. (B) and (C) somatic embryos in the globular, torpedo and early cotyledonary stages on the induction medium using kinetin and BA.

The summary of RAPD polymorphism detected between the five leaf samples generated by five random oligonucleotides primers was shown in Table (7). The polymorphic bands percentage (number of polymorphic amplicons / total number of amplicons) for primers OP-A3, OP-C5, OP-D5, OP-M5 and OP-N4 were 55%, 67%, 70%, 55% and 70%, respectively. The five primers amplified a total of 47 amplicons, seventeen of them were monomorphic and thirty fragments were polymorphic with an average of 64% polymorphism (Tables 7).

Table (7): Total number of amplicons, monomorphic and polymorphic amplicons as revealed by RAPD primers among the five jojoba samples.

Primer	Total Number of Amplicons	Polymorphic amplicons	Monomorphic amplicons	% of Polymorphism
OP-A3	9.0	5.0	4.0	55%
OP-C5	9.0	6.0	3 0	67%
OP-D5	10	7.0	3.0	70%
OP-M5	9.0	5.0	4.0	55%
OP-N4	10	7.0	3.0	70%
Total	47	**30**	17	**64%**

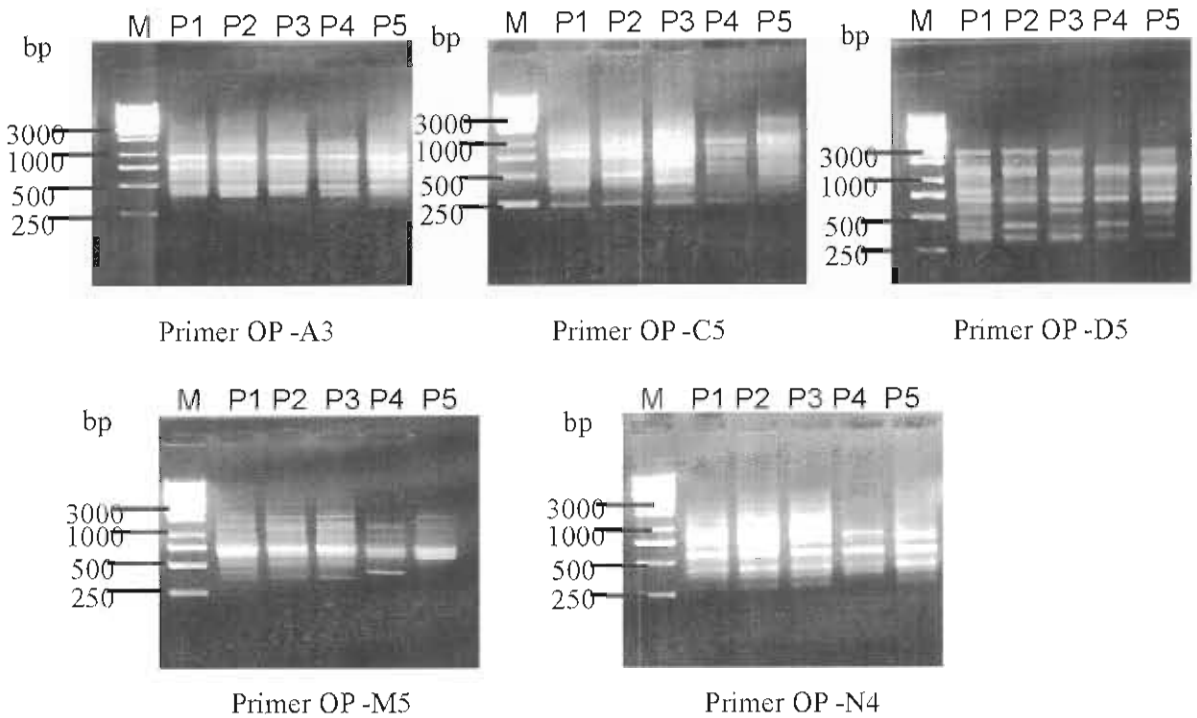

Fig. (3): RAPD profiles of five jojoba leaf samples amplified with 5 RAPD primers, M: 1Kbp ladder DNA molecular marker.

In conclusion, the different banding patterns produced from RAPD markers show genetic variation among the five jojoba field-grown cutting leaf samples. The RAPD data showed 64% polymorphism among the five samples and the high value of variation most probably is due to the fact that jojoba is an out-breeding plant species. Thus, the genetic variation among jojoba plants was clearly correlated with the genotype of each sample. These results are in agreement with Amarger and Mercier (1996) as they used homologus 1.7 kbp probe and a 20-mer probe targeting the 17S and 25S coding region of the nuclear ribosomal DNA, and reported significant differences among jojoba individuals grown from seeds of unknown origin.

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الملخص العربي

استحداث الأجنة الجسدية ودراسة البصمة الوراثية فى نبات الهوهوبا

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فى هذا البحث تم دراسة استحداث الأجنة الجسدية والقدرة على إعادة التكشف فى نبات الهوهوبا فى أنابيب الاختبار المعملية ، ولقد تم استخدام كل من الأجنة غير الناضجة وأقراص من الأنسجة الورقية وتجربتها على مجموعة من المزارع البيئية، وقد أظهرت النتائج أنه تم استحداث الكالس المضغوط على بيئة MS والمضاف إليها تركيزات مختلفة من 2,4-D والسكروز · وقد تطور الكالس الجنيني من الأجنة الزيجوتية غير الناضجة على بيئة MS التي تحتوي على تركيز ١ ملجم / لتر من 2,4-D وتركيز ١، ملجم / لتر من مركب BA بالإضافة إلى ٤% سكروز · ولقد تم نقل وإعادة استزراع الكالس المستحدث على بيئة MS والتي تحتوي على سكروز بتركيزات ٤-٦% بالإضافة إلى تركيزات ٠،١ ، ٠،٥ ، ١،٠ ملليمول من مركب الكينيتين وذلك للحصول على إعادة التكشف النباتي · وقد أظهرت النتائج الحصول على أجنة جسدية ذات الشكل الكروي والقلبي والتربيدي والفلقي · كما تم الحصول على مراحل مختلفة من الكالس الجنيني عندما استخدمت الأنسجة الورقية على بيئة MS المزودة بتركيزات مختلفة من السكروز (٣-٤-٦%) ومركب 2,4-D (١ملجم/ لتر) وهرمون BA (٠،١ ، ٠،٥ ، ١،٠ ملجم / لتر) · ومن الملاحظ أن الكالس الجنيني لم يتطور إلى المراحل المختلفة عند نقلها إلى بيئة مزارع الإنضاج النباتي حتى ولو تركت لمدة ٤ أسابيع ·وقد تم تطبيق تكنيك تفاعل البلمرة المتسلسل على مجموعة من النباتات المختلفة وذلك لدراسة مدي التباين الوراثي فيها، وفى هذا الصدد تم استخدام خمسة باداءت عشوائية الترتيب النيوتيدي· وأظهرت النتائج ان نباتات الهوهوبا النامية في البيئة الصحراوية المصرية على درجة عاليه من التباين الوراثي، مما يستنتج معه أنها من المحتمل أنها تشمل مجموعة من التراكيب الوراثية المختلفة، لم ينجح أي منها في التمايز الى نبيتات كامله تحت الظروق المعملية·