Pak. J. Bot., 40(4): 1467-1471, 2008.

ESTIMATION OF FIXED OILS FROM VARIOUS EXPLANTS AND *IN VITRO* CALLUS CULTURES OF JOJOBA (*SIMMONDSIA CHINENSIS*)

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Abstract

The present investigation was conducted for the estimation of fixed oils from various explants and respective callus cultures in jojoba (*Simmondsia chinensis*). Leaves, nodes, internodes, shoot apices and cotyledons were used as explants. MS media containing different concentrations of growth regulators were used for callus induction. Quantitative estimation of oils of different explants revealed that cotyledonary explant contained highest amount of oil. Leaves contained second highest amount, followed by shoot apices, nodes and internodes. Comparison of oil yield from explants with six or nine weeks-old callus cultures indicated that explants had highest amount of oil content followed by nine and six weeks old callus cultures.

Introduction

Jojoba (*Simmondsia chinensis*) belongs to the Simmondsiaceae family. It is a dioecious, highly saline-tolerant woody shrub of arid, semiarid or marginal lands (Hogan, 1979). In Pakistan, jojoba was formally introduced during the 1980's at Arid Zone Research Station, Pakistan Agricultural Research Council at Bahawalpur. The future of jojoba seems to be bright in Pakistan as it has successfully been cultivated in many areas like Bahawalpur, D. I. Khan, Karachi and Quetta. The solution for the drought-hit areas thus seems to be the use of crops better suited to arid regions, such as jojoba (Ahmad, 2001).

In view of its economic value, jojoba is gaining rapid popularity and research work on several aspects is going on (Hassan, 2003). The seeds of jojoba contain about 50% oil by weight. Jojoba oil is called 'liquid golden oil' due to its golden color. Hydrogenation of jojoba oil produces crystalline wax, similar to bee wax, canderilla, and cornuba. Jojoba oil resembles sperm whale oil and is notably resistant to degradation by bacteria. It is a natural high temperature and high-pressure lubricant (Yermanos, 1979). So it could also be used as lubricant for high-pressure machinery and other industrial purposes (Sardana & Batra, 1998). Passerini & Lombardo (2000) reported that jojoba oil contained specific lipids that can be used to formulate cosmetics. Jojoba oil is also useful in treatment for skin diseases such as eczema, acute acne, skin cancer, psoriases, sores, wounds and kidney malfunctions (Naqvi & Ting, 1990). It is edible and contains simmondisin, which depresses appetite. It does not grow rancid and may become suitable for vegetable oil.

As jojoba is a dioecious species, usually there are an equal number of male and female plants in the field. Hence from the viewpoint of seed production, more male plants reduce the chance of large number of seeds being available for oil extraction. Furthermore, it takes nearly 5 to 6 years for seeds to be available for oil extraction. Plant

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tissue culture techniques provide an answer to this problem in two ways. Firstly, it may ensure the desired ratio (10:90) of male / female jojoba plants through micropropagation. Consequently, the seed production may be enhanced. Secondly, in *vitro*-grown callus and zygotic embryos contain oil, which if extracted by suitable methods (Zia *et al.*, 2007) can minimize our dependence on seeds for oil production (Lee, 1988; Gabr, 1993).

With the above background in view, the present investigation aims at oil extraction and comparison of the amount of oil extracted from various explants and respective callus cultures. The work will eventually pave way for obtaining large amount of oil from callus cultures and independence from seed availability for oil production.

Materials and Methods

The explants were taken from 6 months to 1 year-old jojoba plants that were growing at Arid Zone Research Station of PARC, Bahawalpur and from Botanical Gardens, University of the Punjab, Lahore. Apical buds (0.5-1.0 cm), nodal segments (0.5-1.0 cm), internodal segment (1 cm), leaves (0.5-1.0 cm^2) and cotyledon (width: 0.2 cm; height: 0.8 cm) were used as explants. To disinfest, all explants were treated separately with 0.6 % NaClO containing 0.1 % Tween-20 for 20 minutes (Aftab *et al.*, 2008). Explants were thoroughly rinsed at least five times with autoclaved distilled water before inoculation. MS (Murashige & Skoog, 1962) medium supplemented with various growth regulators, i.e., indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA), 3-indolebutyric acid (IBA), 6-benzylamino purine (BAP), N-isopentenylamino purine (2ip) and gibberellic acid (GA_3) were used for the inoculation of disinfested explants. The growth response was determined on the basis of fresh weight of callus cultures at day 30. Three arbitrary classes for growth response, i.e., 'slow', 'better' or 'best' were designated to calluses weighing 25-50, 51-150 or over 150 mg respectively at day 30 of callus induction. The calluses were subcultured at 4-week interval and were grown and maintained for 9 weeks. The culture room conditions were 25 ± 1 °C with 16 h photoperiod at 35 μmol m^{-2} s^{-1} from cool white fluorescent tube lights. For the quantitative estimation of oils, Soxhlet Apparatus with hexane as a solvent was used. The intact plant portion (explant) and callus weighing 5-10 g was taken and several siphons were run at 70 °C until all oil contents were extracted. Five consecutive transparent siphons indicated complete extraction. Finally, the hexane was evaporated and remaining oil sample was weighed.

Results

Callus induction: The response of different explants of jojoba to callus induction is depicted in Table 1. It is quite evident from the data that the callus could be achieved from all the explants albeit inoculated at different growth regulator levels. There was a sharp difference between the callus induction responses of different explants ranging from 15 (shoot apices) to 30 days (internodes and leaves, in different media). From the data it also became clear that callogenic response could be enhanced in all explants with the manipulation of mainly the growth regulators in a culture medium.

Comparison of oils obtained from intact plant parts (explants) and six or nine weeks old callus cultures: The oil yields from intact plant parts and their respective callus cultures were compared. It was found that callus cultures of both six or nine week's age

had comparatively low amount of oil as compared to the explants. Cotyledonary calluses of 6 and 9 weeks age contained 7.68 and 8.24 % oil contents respectively whereas the oil yield of cotyledonary explants was 15.026 %. Although the two amounts (7.68% from six-weeks-old and 8.24 % from 9 weeks-old callus cultures) were rather comparable with one another, a substantial difference between these values compared with cotyledonary explants was observed. Similar was the case with other explants and respective callus cultures (Table 2).

Table 1: Callogenic response of Nodal, internodal, leaves, shoot apices and cotyledon segments/explants of *Simmondsia chinensis* in MS medium supplemented with various growth regulators

MS Medium	Growth Regulators Supplemented	Explants									
		Node		Internode		Leaves		Shoot Apices		Cotyledon	
		CI	CR	CI	CR	CI	CR	CI	CR	CI	CR
MSI	MS+BAP 10^{-2} M + IAA 10^{-5}M	16	+++	30	+	30	+	28	+	26	+
MSII	MS+NAA 10^{-5} +2ip 10^{-5}	25	+	15	+++	26	++	22	++	21	++
MSIII	MS+2ip 10^{-5} + NAA 10^{-6}	27	+	21	++	20	+++	24	++	24	++
MSIV	MS+2ip 10^{-6}M + NAA 10^{-5}	29	+	23	++	23	++	15	+++	25	++
MSV	MS+ 2ip 10^{-6} + NAA 10^{-6}	30	+	25	++	25	++	25	++	18	+++

CI: Callus induction (in days), **CR:** Callogenic response **+++:** Comparatively best growth response*, **++:** Better growth response, **+:** Slow growth response, ***:** The basis for the determination of growth response is given in Materials and Methods section.

Table 2: Comparison of oil content obtained from intact plant parts/explants with their respective six and nine weeks old callus cultures in *Simmondsia chinensis*

Sample	Oil content from explants		Oil content from 6 weeks-old callus cultures		Oil content from 9-weeks old callus cultures	
	Weight (g)	%	Weight (g)	%	Weight (g)	%
Leaves	0.8394	8.394	0.334	3.34	0.634	6.34
Node	0.2868	2.868	0.0186	0.186	0.125	1.25
Internodes	0.1638	1.638	0.0641	0.641	0.071	0.71
Apices	0.5631	5.361	0.0305	0.305	0.1062	1.062
Cotyledon	1.5026	15.026	0.768	7.68	0.824	8.24

Discussion

In the present investigation, effect of various concentrations of growth regulators supplemented to MS medium were observed on callus induction and proliferation from five explants, i.e. leaves, nodes, internodes, shoot apices and cotyledonary segments. The best response for callus induction from cotyledons was obtained in MS medium supplemented with 2ip and NAA (both at 10^{-6} M). Callus induction from various explants has also been reported by Arce & Jordan (1988) in jojoba plant. The callus formation was 100% when nodal segments were cultured on MS medium + 0.1 mg/L BAP + 0.3 mg/L NAA + 0.01 mg/L GA_3. Callus was also produced by Rossi (1996) from the nodal segments of jojoba on MS + 10 mg/L BA or kinetin alone or in combination with 0.2 mg/L IBA. In another study, Sardana & Batra (1998) produced callus from leaf explants of jojoba on MS + 1.0 mg/L NAA and 3.0 or 5.0 mg/L BAP. Jabeen (1999) reported that when shoots formed on MS medium (MS + 2.0 mg/L BAP) were subcultured on B_5 + 1.0 mg/L 2, 4-D + 0.1 mg/L kinetin, callus formation was observed in 14 days. On the other hand, leaves produced callus when inoculated on B_5 medium + 2, 4-D + kinetin (0.1

mg/L). A protocol was developed for the induction, maturation and germination of somatic embryos from leaf tissue of jojoba **(**Hamama *et al*., 2001). In their work, explants were placed on their adaxial sides in Petri dishes and maintained in darkness on half-strength Murashige and Skoog basal medium (MS/2). Combinations of 2,4-D (1.35–4.52 μM) with BAP (1.33–4.43μM) and 2 synthetic cytokinins, N- (2-chloro-4pyridyl)-N′-phenylurea (1.21–4.03μM) or (E)-6-[3-(trifluoromethyl)-but-2-enylamino] purine (1.11–3.71μM) resulted in formation of embryogenic cultures and somatic embryos. It is therefore concluded that auxins and cytokinins both play an important role in regulating the induction and proliferation of callus. It was observed in the present investigation that different explants showed differences in callus induction as regards time and proliferation rate. The cultured explants comprise of various tissues, either meristematic, parenchymatous or others less differentiated cells. So, the type of cells exposed to growth regulators perhaps determines the nature of callus. Diverse group of cells comprising callus, on the other hand, are also triggered differently by the same hormone (Fitch & Moore, 1990).

In this study, jojoba oil was not only obtained from seeds (cotyledons) but also from various explants and callus cultures. It was observed that among all explants, cotyledons contained highest amount of oil content, i.e., 15.026 %. Leaves contained 8.394 % oil followed by apices with 5.361 % oil yield. Nodes and internodes also contained oil, albeit a lower quantity, i. e., 2.868 and 1.638 % respectively. Lee *et al*., (1983) also observed that cotyledons contained fair amount of wax bodies (up to 17.2 %). Lee & Thomas (1985) obtained cotyledonary structures arising from asexual embryos in jojoba. These cotyledonary structures contained wax bodies and liquid wax identical to that of seed. Chretien *et al*., (1990) cultured stem explants of jojoba *in vitro* under conditions favoring either shoot or callus development. Under all experimental conditions, jojoba tissues contained high amount of lipids. Later Gabr (1993) also obtained i*n vitro* production of jojoba liquid wax from somatic embryos. In another study, Palmer *et al*., (1994) presented an overview for *in vitro* oil production from different sources including jojoba. Our results partially corroborate their findings since increase in callus age resulted in increased quantity of oil. When comparison of oil yield from explant with that obtained from respective callus cultures of either six or nine weeks age was made, it was observed that explants contained higher amount of oil contents. Furthermore, nine weeks-old callus cultures had higher amount of oil as compared to six weeks-old callus.

It is evident from the data discussed so far that explants contained a reasonable amount of oil content. The yield of secondary products from explants was generally higher than the derived callus cultures. The explant usually retains the capacity to synthesize products identical to that in intact plant or has the capacity to produce in increased quantities. Biosynthesis of secondary products is best reported in callus and suspension cultures and altered metabolism is also observed during organ differentiation. Consequently, yield of secondary products may either decrease or even increase further (Roja *et al*., 1987). Reported oil yields from various cultures also vary between different samples and it is unknown whether this reflects environmental or genetic differences between cultures (Allan *et al*., 1998).

From the present investigation, it is thus observed that jojoba oil is present in explants and callus cultures in quite a reasonable amount. This indicates the possibility to use plant tissue culture techniques to obtain jojoba oil. Further qualitative analysis of oil obtained from in vitro cultures and a comparison with seed oil is also important. We are attempting to work out this aspect by using thin layer chromatography (TLC) and allied qualitative techniques. In view of increasing commercial demand of jojoba oil, the results from the present investigation foresee further refinement and standardization of plant cell and tissue culture protocols to provide an alternative means for oil recovery.

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(Received for publication 21 April, 2008)