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RESEARCH ARTICLE

MICROPROPAGATION OF *Simmondsia Chinensis* (Link.)SCHNEIDER: A REVIEW ON THE REGENERATION POTENTIAL OF DIFFERENT EXPLANTS

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ABSTRACT

Micropropagation technique is a powerful tool of biotechnology which can be used in crop improvement and meets the demand for availability of uniform clones in large numbers for economically important crops. *Simmondsia chinensis* (Link.) Schneider is an oil-yielding, eco-friendly plant of the family *Simmondsiaceae*. The seed contains a unique liquid-wax about 50-60%of their dry weight which is used in pharmaceuticals, cosmetics, lubricant industries and treatment for skin cancer and wound healing properties. Propagation of *S. chinensis* is through seeds, seedlings, stem cutting, grafting and micro propagation technique. Problems in direct seed cultivation in field due to male biased population which have male to female ratio is 5:1. Vegetative propagation via. cutting, grafting have limitation due to seasonal process and difficulty of rooting in *S. chinensis*. Hence, successful regeneration of plants by micropropagation technique offers excellent opportunities for the improvement of *S. chinensis* and protocols providing regeneration and multiplication from various vegetative parts of the plants have been tested with varying degree of success. In this review article we have assimilated the recent literature pertaining to the new developments in the field of micropropagation of *S. chinensis* using different explants such as shoot tip, nodal segment, leaf, immature zygotic embryos, micropropagated shoots for rooting and the effect of various additives on *S. chinensis*.

INTRODUCTION

Simmondsia chinensis (Link) Schneider is an arid and semi-arid evergreen shrub (Llorente and Apostolo, 2013). It belongs to the family *Simmondsiaceae*.It is commonly known as Jojoba and Hohoba. It is an economically important, perennial and dioecious shrub with male and female flowers on separate plants (Tyagi and Prakash, 2004). It is evergreen and wind pollinated shrub reaching a height of 3 to 5 meters with thick and leathery green leaves. Themale flowers are yellowish-green color and found in cluster form while the female flowers are pale-green color and singly borne at each node of leaf, as shown in Figure 1(a) and (b). It has also been reported that male plants have a smaller canopy than females (Gentry, 1958; Kohorn, 1994). Its natural life span appears to be between 100 to 200 years (Ayanoglu, 2000). Plants blooms in winter season and plants ripen their acorn shaped seeds in summer. Seeds of *S. chinensis* are dark brown in color and wrinkled in shape, as shown in Figure 1(c). Seeds size varies from 1 cm to 2.5 cm among seed-lots (Yermanos, 1979). Seeds of *S. chinenesis* contains about 50–60% of a light yellow color liquid-wax which is uniquein the plant kingdom. The liquid-wax is a fatty acid ester of a long-chain alcohol (Abu-Arabi *et al*., 2007). It has similar chemical and rheological properties to the sperm whale oil, which is an endangered species (Brooks, 1978; Low and Hackett, 1981). The liquid-wax hashigh potential applications in cosmetics (Passerini and Lombardo, 2000), anti-foaming agents and plastic industries (Reddy and Chikara, 2010), leather industry (Radwan *et al*., 2007), medicinal usesfor treatment in skin cancer, eczema, psoriases, acute acne, sores, kidney malfunction (Naqvi and Ting, 1990) and wound healing properties (Ranzato, 2011).The liquid-wax, also, resembles human sebum and can help in prevent dry and oily skin. It has promising physical properties, such as high viscosity index, fire points, high dielectric constant, high stability and freezing point that can be used as a lubricant for high-pressure machinery and electric insulators (Sardana and Batra, 1998; Agrawal *et al*., 2007). It is edible and contains simmondsin, which depress appetite. It does not rancid and may is suitable for vegetable oil (Aftab *et al*., 2008). Because of all the above properties, *S. chinensis* is a potential seed oil crop and also also useful in preventing wind erosion in desert regions (Hosseini *et al*., 2011). *S. chinensis* has a deep root system and is, therefore, drought resistant. It can also tolerate extreme range of temperature from -5 to 54^{o}C (Bhardwaj *et al.,* 2010). A mature shrub tolerates temperatures as low as -10°C, but seedlings are sensitive to frosts (Orwa *et al*., 2009). It can be grown in all types of soils which are well drained and have an average pH between 7 and 8.5 (Hall, 1996).

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Geographical distribution of *S. chinensis*

It is the native to Sonoran desert (Gila desert) of South Western USA and Northern Mexico. It is also cultivated in other countries including Israel, Argentina, Peru and Morocco

(Gentry, 1958; Tobares*et al*., 2004; Benzioni *et al*., 1995). In India, it was introduced in 1965 at Central Arid Zone Research Institute (CAZRI), Jodhpur, from Israel (Harsh *et al*., 1987). *S. chinensis* being developed as a plantation crop in the arid regions of Western India due to the commercial benefits of the unique waxy content of its seeds (Agarwal *et al*., 2011).

Propagation

Propagation of *Simmo10ndsia chinensis* is through seeds, seedlings, rooted cuttings and plantlets produced from micropropagation technique (Bashir *et al*., 2007c). Several attempts have been made to propagate *S. chinensis* vegetatively through stem cuttings (Palzkill, 1988;Bashir *et al*., 2001; Bashir *et al*., 2007a), grafting (Assaf; 1990; Shah and Bashir, 2000; Bashir *et al*., 2006), air-layering (Alcaraz and Ayala-Rocha, 1982; Reddy, 2003; Bashir *et al*., 2005), and tissue culture raised plantlets (Roussos *et al*., 1999; Gao and Cao., 2001; Agrawal *et al*., 2002; Bashir *et al*., 2007b; Singh *et al*., 2008; Kumar *et al*., 2010; Llorenteand Apostolo, 2013) with a limited success.

Problems of direct seed cultivation

Plants raised through seeds and seedlings are slow growing and require three to four years to reach the flowering stage (Singh *et al*., 2008). Direct seeded plantation having a male biased population which having male to female ratio is 5:1. Being dioecious in nature, a seeded plantation of *S.chinensis* has genetic heterogeneity and low average yield (Al-Obaidi *et al*., 2012).

Disadvantages of vegetative propagation

Commercial plantations of *S. chinensis* are mainly established using cuttings, grafting and air-layering, to ensure female plants however, resulting in a narrow genetic diversity that may cause the low seed yield (Dunstone *et al*., 1988). Although jojoba is a difficult to root plant, yet propagation through cuttings is the most commonly used asexual method with limited success (Palzkill and Feldman, 1993; Bashir *et al.,* 2009).

Micropropagation

Micropropagation is one of the key tools of plant biotechnology that exploits the totipotent nature of plant cells, a concept proposed by Haberlandt (1902). In recent years, micropropagation has been successful in raising plants *in vitro* to a commercial level in many plant species (Chandra and Mishra, 2003). Multiple shoots can be produced *in vitro* and these can be developed into true-to-type plantlets by regenerating their roots. Successful regeneration of plants from tissue culture offers excellent opportunities for the improvement of *S. chinensis* and, in recent years, various reports have been published on regeneration of *S. chinensis* through direct orindirect organogenesis and embryogenesis. There are various reports available on micropropagation of *S. chinensis* using different explants such as shoot tip, nodal segment, leaf, immature zygotic embryos, micropropagated shoots for rooting and the effect of additives on *S. chinensis*.

Shoot tip explants culture for *in vitro* propagation

Morel and Martin (1952) developed the technique of apical meristem culture for the production of virus-free Dahlia. George Morel (1952) was the pioneer in applying shoot tip culture for micropropagation of orchid Cymbidium and the first detailed protocol for *in vitro* propagation of Cymbidium starting with meristem culture published by Wimber (1963). The explant of meristem tip culture may either be the apical meristematic dome or the apical dome with few leaf primodia. Generally, It is agreed that meristem shoot tip between 0.2 to 0.5 mm most frequently produced virus free plants and this method is referred to as meristem tip culture (Chawla, 2010). Scaramuzi and D'Ambrosio (1988) attempteddirect organogenesis by using apical, axillary and epicotyls explants cultured on MS medium supplemented with BAP alone or with IBA and IAA concentrations.*In vitro* plant regeneration from apical meristem explants of mature *S. chinensis* plant by using MS (Murashiage and Skoog, 1962) medium with 2iP has been described but the multiplication rate was low and the rooting percentage ranged between 20 and 95% depending on the clone,with IAA and IBA auxins (Mills *et al*., 1997). Attempts have been made to propagate jojoba by using shoot tip explants *in vitro* on MS media containing different concentration of PGRs such as BA, NAA and IBA (Sardana and Batra, 1998). Roussous *et al*., (1999) studied that shoot tip explants from*in vitro* grown 8-10 weeks old seedling were cultured on a modified DK (Driverand Kuniyuki, 1984) medium supplemented with various concentration of BA (4.4, 8.9, 17.8, 26.6, 35.5 µM) alone and with $AgNO_3$3 mg/L. Shoot proliferation were achieved with a maximum number of shoots 15.2 per explants and for induction of rhizogenesis, IBA, IAA alone and IBA plus NAA treatments. Only IBA 49.2 µM plus NAA 53.7 µM gives the 64 % rooting and survival of rooted plantlets under mist conditions was also high as 90%.

Synthetic seed: Apical and axillary buds about 3 mm long each from *in vitro* grown seedling explants were used for encapsulations performed with 6 % sodium alginate and 100 mM calcium chloride solution into the sterilized petri-dish. Encapsulated buds gives the best morphogenic response and percentage of conversion into plantlets on medium MS Supplemented with BAP 1.0 mg/L, IAA 3.0 mg/L and adenine sulfate 40 mg/L as a additive and gelled with 0.8% bacteriological agar with success rate 73%.Further, culturing of encapsulated buds on peat moss: sand (2:1) moistened with MS liquid medium had the lowest conversion percentage 48% and regenerated plantlets (Hassan, 2003). Alginate-encapsulation beads of shoot tip explants *in vitro* grown derived from nodal explants of *S. chinensis* for germplasm exchange and distribution. For encapsulation of shoot tip explants, a solution of 3% sodium alginate and 100 mM of calcium chloride was found most suitable for formation of synthetic seed. Shoot sprouting from encapsulated shoot tip beads on MS medium with 0.8% agar and rooting were observed on MS medium containing 1 mg/L IBA with 0.8% agar as solidifying agent. Only 70% encapsulated shoot tip beads converted into plantlets (Kumar *et al*., 2010). Prakash *et al*. (2003)showed the differential influence on male and female explants of different adjuvants, activated charcoal (AC), casein hydrolyzate (CH), coconut water (CW), polyvinylpyrrolidone (PVP) and triiodobenzoic acid (TIBA) has been assessed on the shoot production potential of shoot tip explants *in vitro* grown derived from nodal segments of male and female genotypes of *S. chinensis*. The explants were cultured on MS medium supplemented with different concentration of AC (0.05, 0.1, 0.5, or 1.0%), CH (50, 250, 500, 750, or 1000 mg/L), CW

Figure 1. (a) Male Flower (b) Female Flower(c) Seeds of *S. chinensis*

Table 1. Summary of work done on micropropagation of *Simmondsiachinensis*

Explant type	Medium used	Plant Growth Regulators (PGRs)	Additives	Results	References
Immature Zygotic embryo	MS	BA, NAA	-	Embryogenesis	Lee and Thomas, 1985
Leaf and Immature Zygotic embryo	MS	2,4-D, BA	-	Embryogenesis	Wang and Janick, 1986
Apical, Axillary and Epicotyl	MS	BAP, IBA, IAA	-	Organogenesis	Scaramuzi and D'Ambrosio, 1988
Nodal segment	MSH	BAP, IBA, NAA	CA	Organogenesis	Chaturvedi and Sharma, 1989
Micropropagated shoots	½ MS	NAA	AC	Rooting	Kacker*et al*., 1993
Micropropagated Shoots	MBN	NAA		Rooting	Rugini*et al*., 1993
Micropropagated shoot	½ MS	IBA	-	Rooting	Apostolo*et al*., 1996,
Apical and Nodal segment	MS	2iP, IAA, IBA	-	Organogenesis	Mills *et al*., 1997
Micropropagated shoots	-	IBA	Cocultivation with Agrobacterium rhizogenes	Rooting	Benavides and Radice, 1998
Shoot tips	MS	BA, NAAA, IBA	-	Organogenesis	Sardana and Batra, 1998
Micropropagated shoots	½ MS	IAA, IBA, NAA	-	Rooting	Elhag*et al*., 1998
Nodal segment	MMS	BA, IBA	-	Organogenesis	Llorente and Apostolo, 1998
Shoot tip and Nodal segment	MDK	BA, IBA, NAA	$AgNO_3$	Organogenesis	Roussos *et al*., 1999
Shoot bunches	MS	BA, IBA	AC	Rooting	Khanam*et al*., 1999
Nodal segment	MS	ZT, NAA, IBA, IAA	-	Organogenesis	Gao and Cao, 2001
Leaf Segment	½ MS	BAP, N,N-phenylurea	-	Embryogenesis	Hamama*et al*., 2001
Micropropagated shoots	MMS	IBA	α- and β-cyclodextrins	Rooting	Apostolo*et al*., 2001
Nodal segment	MS, ½MS	BA, IBA,	AC	Organogenesis	Agarwal*et al*., 2002
Shoot apical and axillary bud	-	-	6% Sodium alginate and 100 mM $CaCl_2$	Synthetic seed	Hassan, 2003
Shoot buds	MS	BA, IBA	TIBA, AC, CH, CW, PVP	Organogenesis	Prakash*et al*., 2003
Micropropagated shoot	MS	IBA	-	Rooting	Tyagi and Prakash, 2004,
Leaf and immature zygotic embryo	MS	2,4-D, 6% Sucrose	-	Embryogenesis	Gaber*et al*, 2007
Nodal segment	MS	BA, IBA,NAA, IAA	-	Organogenesis	Bashir *et al*., 2008
Nodal segment	MS	BAP, NAA, IBA	Adenine	Organogenesisi, Invitro flowering	Singh *et al*., 2008
Leaf and Zygotic embryo	MS	2,4-D, BA, 4% Sucrose	-	Embryogenesis	Mohammed *et al*, 2008
Nodal segment	MS	BAP, NAA	-	Organogenesis	Kumar *et al*, 2009
Nodal segments	MS	BAP, IBA	CH, AC	Organogenesis	Mohasseb*et al*, 2009
Shoot tips	-	-	3% Sodium alginate + 100mM $CaCl_2$	Synthetic seed	Kumar *et al*., 2010
Shoot tip	MS, WPM, B5	2iP, Kin, BA, IAA, IBA	-	Organogenesis	Abass., 2010
Shoot tips	MS	BA, Kin	-	Shooting	Ekta*et al*., 2012
Nodal Segment	MS+B5	BA, IBA, GA3	-	Organogenesis	Llorente and Apostolo, 2013

(5, 10, 15, or 20%), PVP (0.005, 0.025, 0.075, or 0.10 µM) and TIBA (1, 5, 10, or 20 µM)alone or along with optimum levels of BA; 10 µM for male and 20 µM for female explant. In female explants, BA with TIBA promoted shoot multiplication while in male explants, BA along with PVP, increased the response of male shoot multiplication. Abass (2010) studied the effect on shoot tip explants were cultured on MS, WPM and B5 media at different strength of salts such as double full strength, one and half full strength, one and quarter full strength, full strength,3/4,1/2,1/4 and 1/8strength alone. For shoot multiplication , the effect of MS and 1.25 WPM media supplemented with 2ip conc. (0.1, 0.2, 0.4, 0.8 and 1.6 mg/L), BA conc. (1.75, 3.5, 7,0 mg/L), Kin and IAA at different conc. (0.5, 1.0, 1.5, 2.0, 2.5 mg/L), rooting observed with the effect of IBA at conc. 4.9, 9.9, 19.9 mg/L with MS full and 1.25 WPM full strength medium and 60% survival of plantlets in peat:sand (1:2 v/v). Menghani *et al*. (2012) studied the shoot tip explants taken from 5-year old plant cultured on MS medium supplemented with BAP and Kin conc. 0.5, 1.0, 2.0, 3.0 mg/L each were used. The best results at the conc. of BAP 2.0 mg/L than the kin.

Nodal segment explants culture for *in vitro*propagation

Nodal segment have appeared to be the most favored choice for explants, due to the absence of apical dominance and the presence of axillary buds at a more advanced stage of development (Amin and jaiswal, 1987).Various reports available on *in vitro* propagation by using mature as well as immature nodal segment explants are discussed below. **C**haturvedi and Sharma (1989) developed a protocol for *in vitro* proliferation of male and female mature plants by using nodal segments explants inoculated on modified SH (Schenk and Hildebrandt's, 1972) medium supplemented with BAP 1 mg/L and IAA, formed 5-8 shoots per explants Further multiplication at BAP 0.5 mg/L and 90% shoots were rooted in IBA 7 mg/L, NAA 1 mg/L and caffeic acid 1 mg/L induced tap-root like system. The survival rate was 80% in humid conditions. Mills *et al*. (1997) described the protocol for *in vitro* regeneration of nodal segment explants of mature plant by using MS medium supplemented with 2iP but multiplication rate slow and rooting percentage as low as depends upon clone and specific concentration of rooting hormones of IBA and IAA. It has been reportd by Llorente and Apostolo (1998) that the nodal segment explants taken from the mature *S. chinensis* plant propagated on modified MS medium supplemented with BA 1 mg/L conc. gives a 4.6 folds increase in shoot numbers in 30 days and rhizogenesis induced on modified MS medium with IBA 3 mg/L in only 25% shoots. Survival rate of tissue culture raised plantlets in an organic mixture, agrosoil^{TM}. Roussous *et al*. (1999) observed shoot proliferation on modified DKW (Driver and Kuniyuki, 1984) medium supplemented with BA plus $AgNO_3$ Conc. from *in vitro* grown nodal segments explants taken from 8-10 weeks old seedlings and rooting percentage was 64% by using NAA, IBA and IAA with90% survival rate of acclimatized plantlets. Nodal explants of juvenile stage used for *in vitro* propagation on MS medium supplemented with Zeatin and NAA at different concentration for multiplication of shoots and for root induction, Full strength of MS medium with different concentration of auxins, IAA and IBA (Gao and Cao, 2001). Agrawal *et al*. (2002) devloped an effective prot ocol for *in vitro* shoot production through nodal explants from 18-20 years old female plant of *S. chinensis*. Explants were cultured on MS medium supplemented with BA 20 µM showed 100% differentiation of shoots and 85% of the shoots induced the roots with a treatment IBA 50 µm given prior treatment of semi-solid MS medium containing IBA 10 µM+0.5 % Activated charcoal+BA 1µM. plantlets hardened in Soilrite and transfer to soil.

Bashir *et al*. (2008) attempted *in vitro* propagation of nodal segments for six promising genotypes PKJ-1, PKJ-2, PKJ-3, PKJ-4, PKJ-5, and PKJ-6 were cultured on MS medium supplemented with BA in combination with different conc, of NAA, IBA and IAA for shoot formation and found that best conc. BA 5.55 µM+IAA 7.1 µM for shoot development and rooting in PKJ-3 and PKJ-6 genotypes by using BA 5.55 µM+IBA 6.1 µM or NAA 6.7 µM and maximum survival percentage of plantlets was 63.33 during acclimatization in greenhouse. Among all six genotypes, PKJ-3 performed the best response in *in vitro* shooting and rooting. An efficient micropropagation protocol was developed by Singh *et al*. (2008) using nodal segment explants of mature male and female genotypes of S. chinensis cultured on MS medium supplemented with BAP 4.44 µM and adenine 88.8 µM showed improvement in shoot multiplication rate and 20% *in vitro* flowering in male culture after increase in the KNO3 concentration. Root induction in micropropagated shoots, treatment in liquid medium supplemented with IBA 49.0 µM, NAA 5.40 µM and IAA 5.71 µM and resulted 92% rooting was achieved on ½ MS medium supplemented with 1.37 µM chlorogenic acid, 1% activated charcoal and 2% sucrose concentration and 99% establishments of *in vitro* produced plantlets. A combined effect of MS supplemented with BAP 2.0 mg/L and NAA 0.5 mg/L was found to be very effective in establishment and multiplication off nodal explants of mature plant of *S. chinensis* (Kumar *et al*., 2009). *In vitro* clonal propagation of *S. chinensis* by Mohasseb *et al*. (2009) using nodal segment explants of 5-year old plant on MS medium supplemented with BAP 1 mg/L + CH 250 mg/L and rooting induced by using ¼ MS medium plus IBA 0.5 µM treatment gives 82% results with 70% survival of plantlets in soil. Llorente and Apostolo (2013)studied the single-nodal explants which were cultivated on MS salts+B5 vitamins medium supplemented with BA 11.1 µM, IBA 0.5 µM and GA3 1.4 µM but best shoot proliferate in BA 4.4 µM concentration and rooting was achieved on ½ MS salts and B5 vitamins with IBA 14.7 µM during 7 days and plantlets were acclimatized in humid conditions on soil: peat: perlite (5:1:1) substrate.

Leaf Explants culture for *in vitro* propagation

Wimber (1965) pioneered leaf tissue culture and gave the detailed report on production of protocorm-like body (PLBs) from Cybidium leaves. Leaf explants are easy to obtain, unlike shoot tips and do not require the sacrifice of the mother plant and their availability is not restricted to any season like inflorescence explants. A few reports available on leaf explant culture in S. chinensis for induction of direct and indirect somatic embryogenesis (Wang and Janick, 1986, Hamama *et al*., 2001; Gaber *et al*., 2006, Mohammed *et al*., 2008). The first report on leaf explants culture by Wang and Janick (1986) for induction of somatic embryogenesis using MS medium supplemented with 2,4-D concentration and MS medium with

2,4-D and BA concentration for maturation and germination of Somatic embryos. Hamama *et al*. (2001) developed a protocol for induction, maturation and germination of somatic embryogenesis in leaf segments derived from *in vitro* grown shoot of female plant S. chinensis inoculated on ½ MS medium combination of 2,4-D conc. (1.35-4.52 µM)combination with BA conc. (1.33-4.43 µM) and two synthetic cytokinins, N-(2-chloro-4-pyridyl)-N'-phenylurea (1.21-4.03 µM) or (E)-6-[3-(trifluoromethyl)-but-2-enylamine]purine (1.11-3.71 µM) resulted in formation of somatic embryogenesis and for maturation and germination of somatic embryos were achieved using NAA 3.75 µM or IBA 3.44 µM in combination with BA (0.44 0r 1.33 µM) or F3iP (0.37 or 1.11 µM). Gaber *et al*. (2006) reported the induction of Somatic embryogenesis in mature leaf explants (2-5 cm in length) on using MS medium supplemented with 2,4-D 1.0 mg/L, BA 0.1 mg/L and 4% sucrose and for regeneration of somatic embryos , induced calli sub-cultured on MS supplemented with 4% or 6% sucrose and Kin 0.1, 0.5, and 1.0 mM to produced globular, heart-shaped, torpedo and cotyledonary stages of embryos but leaf derived embryos did not mature at any conc. of medium. Mohammed *et al*. (2008) described the protocol for induction of somatic embryogenesis in *S. chinensis* from mature leaf explants were cultured on MS basal medium supplemented with concentration of 2,4-D 0.4 µM, BA 4.4 µM and 4% sucrose but did not maturation of leaf derived somatic embryos and with the induction of essential polysaturated fatty acid, linoleic acid, alpha-linoleic acid with higher value of long chain saturated fatty acid palmitic acid and monosaturated fatty acid oleic acid influenced by induction of growth regulators.

Zygotic Embryos explants culture for *in vitro* propagation

Embryo culture has been reported to account for a higher level of recovery of plants than that obtained by conventional seed germination (Biggs *et al*., 1986) because embryo culture provides aseptic seedling material; cultured embryos can serve as a host for screening plants for diseases resistance (Sharma and Bedi, 1990). Considerable interest is focused on embryo culture in establishing a direct somatic embryogenesis system capable of yielding embryoids reproducibly and in large numbers, amenable to mechanical harvesting (Maheswaran and Williams, 1984). A few reports on zygotic embryos are available for *in vitro* somatic embryogenesis (Lee and Thomas, 1985, Wang and Janick, 1986, Gaber *et al*., 2006, Mohammed *et al*., 2008). A first report on somatic embryogenesis produced by Lee and Thomas (1985) by using immature zygotic embryos for induction of somatic embryos on MS basal medium supplemented with BA and NAA but no germination of somatic embryos. Wang and Janick (1986) showed that induction of somatic embryos from immature zygotic embryos cultured on MS medium with 2,4-D and germination of somatic embryos on full strength of MS medium supplemented with 2,4-D and BA and showed that *in vitro* accumulation of wax upto 39% on a dry weight basis. *In vitro* induction of somatic embryogenesis reported by Gaber *et al*. (2006)from immature zygotic embryos (1-2 mm long)incubated on MS medium containing 2,4-D 1.0 mg/L and BA 0.1 mg/L with 4% sucrose content and for regeneration of somatic embryos stages on 4-6% sucrose and 0.1, 0.5, and 1.0 mM kin concentration were used. Mohammed *et al*. (2008) developed a protocol for direct somatic embryogenesis from immature zygotic embryos (1-2 mm length) were surface sterilized by 70% ethanol followed by 10% commercial bleach for 10 minutes and inoculated on modified MS medium supplemented with 0.4 µM 2,4-D, 2.2/4.4 µM BA and 4% Sucrose and zygotic embryo derived somatic embryos developed to the globular, heart, torpedo and cotyledonary stages. Besides being useful for clonal multiplication, *S. chinensis* regeneration through somatic embryogenesis may also be useful for genetic transformation (Kim and Liu, 1999) and to develop new products from jojoba oil (Benzioni, 1995).

Micropropagated Shoots explants culture for rooting

Kacker *et al*. (1993) incubated the micropropagated shoots in dark in a liquid medium ½ strength MS containing NAA 10 mg/L for 72 h for initiation of root and then transfer into ½ MS with 2500 mg/L activated charcoal and observed 80% rooting in cultures. Rugini *et al*. (1993) inoculated the shoots explants on modified Bourgin and Nitsch medium alone or combination with NAA 0.93 mg/L concentration. The micropropgated shoots cultured on ½ MS medium containing 3.0 mg/L IBA gives 31.08% rooting in 70 days by Apostolo *et al*. (1996).Elhag *et al*. (1998) observed rooting on ½ MS and MS with IBA, whereas, root induction on *in vitro* shoot of S. chinensis after 60 days co-cultivation with Agrobacterium rhizogenes alone or with IBA found by Benavides and Radice (1998). Khanam *et al*. (1999) obtained successful rooting from micropropagated shoots on rooting media containing BA 0.2-2.0 mg/L, IBA 2 mg/L and activated charcoal 5000 mg/L. Apostolo *et al*. (2001) developed a protocol for *in vitro* rooting of *S. chinensis* shoots on modified MS medium containing 0.03-0.5 mM α- and β-cyclodextrins with 0.015 mM IBA for 7 week treatment, rooting percentage increased 30-60%.Tyagi and Prakash (2004) observed *in vitro* rhizogenesis in 44-67% cultures given a treatment of IBA 10 mg/L for 20 minutes. Bashir *et al*. (2007) studied that root initiation from *in vitro* derived shoots of PKJ-3 by using MS medium supplemented with IBA 0.5 mg/L was most effective treatment to attained longer and higher percentage of rooted shoots.

Effect of Additives on *S. chinensis* tissue culture

A large number of natural as well as synthetic additives like coconut water (CW), casein hydrolysate (CH), activated charcoal (AC), triiodobenzoic acid (TIBA), polyvinylpyrrolidone (PVP) (Prakash *et al*., 2003), silver nitrate ($AgNO_3$) (Roussous *et al*., 1999), adenine (Singh *et al*., 2008), AC and CH (Mohasseb *et al*., 2009), caffeic acid (CA) (Chaturvedi and Sharma, 1989) and AC (Agrawal *et al*., 2002) are commonly added to medium for propagation of S. chinensis. The influence of different adjuvants, AC, CH, CW, PVP and TIBA has been assessed on the shoot production potential of the nodal segments from male and female *S. chinensis* plants (Prakash *et al*., 2003). AC alone proved beneficial for morphogenic response in male as well as female explants in comparison to basal medium containing AC and BA whereas, CH proved inhibitory for shoot differentiation in male as well female explants and PVP also showed inhibitory effect on female explants. In female explants, TIBA promoted shoot multiplication as compared to male explants. Mohasseb *et al*. (2009) used CH in shoot multiplication and AC in rooting experiment showed positive effect in shoot proliferation as well

as rhizogenesis. AC induced root initiation and elongation was observed when explants cultured on MS medium fortified with 0.5, 1.0, 2.0 and 3.0 g/L AC. Roussous *et al*. (1999)observed the maximum number of nodes per shoot by adding the silver nitrate in the MDK medium, caused a significant differences in shoot length which could be explained by the added presence of nitrate and its uptake by the explants. Chaturvedi and Sharma *et al*. (1989) used caffeic acid as additive in rooting medium showed positive effect to produced tap-root like system, whereas, adenine incorporated with BAP showed also positive effect on shoot multiplication observed by Singh *et al*. (2008). Influenced of activated charcoal in rooting medium along with IBA and BA proved helpful in both for preventing callus and induce rooting in *S. chinensis* observed by Agrawal *et al*. (2002).

Abbreviations

AC-Activated Charcoal, B5-Gamborge medium, BAP-6-benzylamino purine, CA-caffeic acid, CH-Casein Hydrolysate, 2,4-D-2,4-dichlorophenoxyacetic acid, GA3-gibberellic acid, MBN-modified Bourgin and Nitsch medium, MS-Murashiage and Skoog medium, MDK-modified Driver and Kuniyuki medium, MSH-modified Schenk and Hidebrandt medium, MMS-modified Murashiage and Skoog Medium, IAA-indole-3-acetic acid, IBA-indole-3-butyric acid, NAA-1-Naphthaleneacetic Acid, ZT-Zeatin, Kn-Kinetin, 2iP-6-(y,y-Dimethyiallylamino) purine, $AgNO_3$-Silver Nitrate, WPM-Woody Plant medium

Conclusion and Future Perspectives

Improvement programs of *Simmondsia chinensis* by micropropagation technique of biotechnology are of great interest throughout the world. Seed propagated plants are highly heterogynous and thus exhibits tremendous variability in morphological as well as yield contributing characters. Experiments conducted by various researchers indicate the necessity of selecting desirable female plants, to enhance yields. There is much research works on tissue culture technique by using different male and female explants with limited success. In this review, most of the reports showed that explants where taken from plants of unspecified age and sex, which is of little significance as male and female plants show differential response to the same propagation protocol. Major problem in seed propagation is that *S. chinensis* is a dioecious plant where male and female ratio is 5:I and sex cannot be determined until flowering stage (3-4 years after cultivation). There is need for an early stage detection of male and female plants preferably at seed or early seedling stage. A favorable ratio of male to female plants can enhance yield and save resources. Therefore, there is an immediate need to create superior genotypes, particularly, female plants for successful implementation of *S. chinensis* as a sustainable commercial crop.

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REFERENCES

Abass, N.H. 2010. The influence of some growth regulators on proliferation and rooting *in vitro* of propagated jojoba. *Australian J. of Basic and Applied Science*., 4:45-53.

Abu-Arabi, M.K., Allawzi, M.A. and Al-Zoubi, A.S. 2007.Adsorption of phenol from aqueous solutions on jojoba nuts residue. *Chemical Engineering and Technology*, 4:493-500.

Aftab, F., Akram, F. and Iqbal, J. 2008.Estimation of fixed oils from various explants and *in vitro* callus culture of Jojoba (*Simmondsia chinensis*). *Pak. J. Bot.*, 40:1467-1471.

Agarwal, M., Shrivastava, N. and Padh, H. 2011. Development of sex linked AFLP markers in *Simmondsia chinensis*. *Plant Breed*., 130:114-116.

Agrawal, V., Prakash, S. and Gupta, S.C., 2002.Effective protocol for *in vitro* shoot production through nodal explants of *Simmondsia chinensis*. *Biol Plant*., 45:449-453.

Agrawal, V., Sharma, K., Gupta, S., Kumar, R. and Prasad, M. 2007. Identification of sex in *Simmondsia chinensis* (Jojoba) using RAPD markers. *Plant Biotechnol. Rep*., 1:207-210.

Alcaraz, M.L. and Ayala-Rocha. 1982. Asexual reproduction of jojoba by air layering. *Hort. Science*., 17:893.

Al-Obaidi, J.R., Suliman, B.K. and Al-Ani, N.K. 2012.Molecular-based marker for sex determination of jojoba *in vivo* and *in vitro* Iraqi cultivats RAPD-PCR technique. *Scientific Research and Essays*, 7:522-527.

Amin, M.N. and Jaiswal, V.S. 1987. Rapid clonal propagation of guava through *in vitro* shoot proliferation on nodal explants of mature trees. *Plant Cell Tissue Organ Cult*., 9:235-243.

Apostolo, N.B., Llorente, L.H., Princen and C. Rossi. 1996. Rooting and acclimatization of micropropagated jojoba seedlings. Proceedings of the 9th International Conference on Jojoba and its uses, 25-30 September, 1994, Catamarca, Argentina, pp:47-49.

Apostolo, N.M., Brutti, C., Ferrarotti, S.A., Llorente, B.E. and Krymkiewicz, N.L. 2001. Stimulation of root development with cyclodextrins on jojoba shoots *in vitro*. *In Vitro Cell Dev. Biol. Plant*, 37:414-418.

Assaf, S.A. 1990. Changing of male jojoba plants to females by grafting.*Jojoba Happenings*, 18:2.

Ayanoglu, F. 2000. Jojoba [*Simmondsia chinensis*(Link.) Schneider] and its cultivation in Turkey. I. Morphology, production and cultivation of jojoba. *Anadolu*., 10:18-23.

Bashir, M.A, Rashid, H. and Anjum, M.A. 2007c.*In vitro* shoot multiplication of six promising strains of jojoba (*Simmondsia chinensis*). Biotechnology, 6:309-315.

Bashir, M.A., Ahmad, M. and Anjum, M.A. 2005.Response of six promising jojoba strains to air layering.*Biosci. Res*., 3:172–177.

Bashir, M.A., Ahmad, M. and Anjum, M.A. 2006.Propagation of six promising jojoba strains through veneer grafting.*Int. J. Agric. Biol*., 8:482–484.

Bashir, M.A., Anjum, M.A. and Rashid, H. 2007d. *In vitro* root formation in micropropagated shoots of Jojoba (*Simmondsia chinenesis*). Biotechnology, 6:465-472.

Bashir, M.A., Anjum, M.A., Chaudhry, Z. and Rashid, H. 2009. Response of jojoba (*Simmondsia chinensis*) cuttings to various concentrations of auxins. *Pak. J. Bot*., 41:2831-2840.

Bashir, M.A., H. Rashid and M.A. Anjum. 2007b. *In vitro* shoot initiation from nodal explants of jojoba (*Simmondsia chinensis*) strains. Biotechnology, 6: 165-174. 2840.

Bashir, M.A., Iqbal, A.M., Asad, M.N., Rana, M.Y. and Shah, S.F.H. 2001.Propagation of jojoba through cuttings. *J. Animal and Plant Sci*., 11:24-29.

Bashir, M.A., M. Ahmad and M.A. Anjum. 2007a. Effect of various potting media on growth of rooted jojoba (*Simmondsia chinensis*) cuttings. *Int. J. Agri. Biol.,* 9: 147-151.

Bashir, M.A., Muhammad, A.A. and Hamid, R. 2008. *In vitro* propagation of some promising genotypes of jojoba (*Simmondsia chinensis*). *Afr. J. Biotech*., 7:3878-3886.

Benavides, M.P. and Radice, S. 1998. Root induction in *Simmondsia chinensis* (Link.) Schneid. using *Agrobacterium rhizogenes*. *Biocell*, 22:109-114.

Benzioni, A. 1995. Jojoba domestication and commercialization in Israel. *Hortic. Rev*., 17:234–266.

Bhardwaj, M., Uppal, S., Jain, S., Kharb, P., Dhillon, R. and Jain, R.K. 2010. Comparative assessment of ISSR and RAPD marker assays for genetic diversity analysis in jojoba [*Simmondsiachinensis* (Link.) Schneider]. *J. Plant Biochem. Biotechnol*., 19:255-258.

Biggs, B.J., Smith, M.K. and Scott, K.J. 1986. The use of embryo culture for the recovery of plants from cassava (*ManihotesculentaCrantz*) seeds.*Plant Cell Tissue Org. Cult*., 6:229-234.

Bourgin, J. P. and Nitsch, J.P. 1967. Obtention de *Nicotiana*haploides à partirdétaminescultivées*in vitro*.*Ann. Physiol. Veg*., 9:377-382.

Brooks, W.H. 1978. Jojoba- A North American desert shrub; its ecology, possible commercialization and potential as an introduction into other arid regions. *J. Arid Environ*., 1:227-236.

Chandra, R. and Mishra, M. 2003. Comprehensive micropropagation of horticultural crops.International Book Distributing Company, Lucknow UP, India.

Chaturvedi, H.C. and Sharma, M. 1989. *In vitro* production of cloned plants of jojoba (Simmondsiachinensis (Link) Schneider) through shoot proliferation in long term culture.*Plant Sci*., 63:199-207.

Chawla, H.S. 2010. Introduction to Plant Biotechnology. Oxford and IBH Publishing Company Pvt. Ltd., New Delhi, India.

Driver, J.A. and Kuniyuki, A.H. 1984.*In vitro* propagation of paradox walnut rootstock. *Hortic. Sci*., 19:507-509.

Dunstone, R.L. 1988. The reproductive cycle of jojoba, In: A.R. Baldwin (ed.). proc. 7th Intl. conf. on Jojoba and its uses.*Amer. Oil Chem. Assn. Champaign*, III:50-59.

Elhag, H., El-Olemy, M.M., Mossa, J.S., Tag-El-Din, S.S., Al-Zoghet, M.F. and Al-Alsheikh, A.M.A. 1998.*In vitro* propagation of jojoba. In: Program abstract of the annual conference on new crops and new uses: biodiversity and sustainability, *Phoenix, AZ*.

Gaber, A., El-Maraghy, H.M.M., Aly, M.A.M., Rashed, N.A.K. and Gamal El-Din, A.Y. 2006.Induction of somatic embryogenesis and DNA fingerprinting of jojoba. *Arab. J. Biotech*., 10:341-354.

Gamborg, O.L., Miler, R.A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell. Res*., 50:151-158.

Gao, H.D. and Cao, B. 2001. Study on technology of tissue culture of *Simmondsiachinensis* (Link.) Schneider (in Chinese). *J. Jiangsu For Sci. Technol*., 28:12-14.

Gentry, H.S. 1958.The natural history of jojoba (*Simmondsiachinensis*) and its cultural aspects.*Econ. Bot*., 12:261-295.

Haberlandt, G. 1902. Culturversuchemitisolierten. *Pflanzenze llen Sitzungsber Akad Wiss Wien Math Nat. Cl. III Abt*., 1:69-91.

Hall, N.1996. Mallee Gross Margins1996-97 Agriculture Victoria, SwanHill, Victoria.

Hamama, L., Baaziz, M. and Letouze, R. 2001.Somatic embryogenesis and plant regeneration from leaf tissue of jojoba. *Plant Cell Tissue Organ Cult*., 65:109-113.

Harsh, L.H, Tewari, J.C., Patwal, D.S. and Meena, G.L. 1987.Package of Practices for Cultivation of Jojoba (*Simmondsiachinensis*) in Arid Zone, CAZRI, Jodhpur, India, pp. 1-19.

Hassan, N.S. 2003. *In vitro* propagation of jojoba [*Simmondsiachinensis (Link) Schneider*] through alginate-encapsulated shoot apical and axillary buds. *Int. J. Agric. Biol*., 5:513-516.

Hosseini, F.S., ShahsavandHassani, H., Arvin, M.J., Baghizadeh, A. and Mohammadi-Nejad, G. 2011.Sex determination of jojoba (Simmondsiachinensis cv. Arizona) by random amplified polymorphic DNA (RAPD) molecular markers. *African J. of Biotechnology*, 10:470-474.

Jacoboni, A. and Standardi, A. 1987. Tissue culture of jojoba (*Simmondsiachinensis*Link.). *ActaHortic*., 212:557-560.

Kacker, N.L., Joshi, S.P., Singh, M. and Solanki, K.R., 1993.*In vitro* regeneration of female plants of Simmondsiachinensis (Link) Schneider (Jojoba) using coppice shoots. *Ann. Arid Zone*, 32:175-177.

Khanam, A., Rao, Y.B.N. and Farook, S.A. 1999.Standard *in vitro* protocol for high frequency mass micropropagation of jojoba (*Simmondsiachinensis* (Link) Schneider). *Adv. Plant Sci*., 12:361-366.

Kim, S.W. and Liu, J.R. 1999.Somatic embryogenesis and plant regeneration in zygotic embryo cultures of balloon flower.*Plant Cell Tissue Organ Cult*., 58:227-230.

Kohorn and Loraine, U. 1994. Shoot Morphology and Reproduction in Jojoba: Advantages of Sexual Dimorphism. *Ecology*, 75:2384-2394.

Kumar, S., Rai, M.K., Singh, N. and Mangal, M. 2010. Alginate-encapsulation of shoot tips of jojoba [*Simmondsiachinensis* (Link.) Schneider] for germplasm exchange and distribution.*Physiol Mol. Biol. Plants*, 16:379-382.

Kumar, S., Singh, N. and Mangal, M. 2009.Micropropagation of *Simmondsiachinensis*(Link.)Schneider through enhanced axillary branching from nodal segments. *J. Plant Biol*., 36:75-81.

Lee, C.W. and Thomas, J.C. 1985. Jojoba embryo culture and oil production.*Hortic. Sci*., 20:762-764.

Llorente, B. and Apostolo, N.M. 1998.Effect of different growth regulators and genotype on *in vitro* propagation of jojoba. *N. Z. J. Crop Hortic. Sci*., 26:55–62.

Llorente, B.E. and Apostolo, N.M. 2013.*In vitro* propagation of jojoba. Methods in *Molecular Biology*, 994:19-31.

Lloyd, G. and McCown, B.H. 1980. Commercially feasible micropropagtion of mountain laurel, *Kalmia latifolia*by use

of shoot tip culture. Proceed. Int. Plant Propagation Soc., 30:421-427.

Low, C.B. and Hackett, W.P. 1981. Vegetative propagation of jojoba.*Calif. Agric*., 35:12-13.

Maheshwaran, G. and Williams, E.G. 1984. Direct somatic embryoid formation on immature embryos of *Trifoliumrepens, T. pratense* and *Medicagosativa* and rapid clonal propagation of *T. repens*. *Ann. Bot*., 54:201-221.

Menghani, E., Sudhanshu, Khan, S. and Soni, M. 2012. *In vitro* cloning of *Simmondsiachinensis*(Link.) schni.using BAP and Kinetin. *World J. of Pharmaceutical Research*, 2:103-111.

Mills, D., Wenkart, S. and Benzioni, A. 1997. Micropropagation of *Simmondsiachinensis* (Jojoba). In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, High-tech. and micropropagation VI. Springer, Berlin, 40:370-393.

Mohammed, A.M., AlyEssam, A., AmerWasef, A., Al-Zayadneh, A.E. and Negm, E. 2008. Growth regulators influence the fatty acid profiles of *in vitro* induced jojoba somatic embryos. *Plant Cell Tissue Organ Cult*., 93:107-114.

Mohasseb, H.A.A., El-Bahr, M.K., Adam, Z.M., Moursy, H.A. and El-Din Solliman, M. 2009.*In vitro* clonal propagation of jojoba (*Simmondsiachinensis*(Link.) schn.).*Australian J. of Basic and Applied Sciences*, 3:3128-3136.

Morel, G. and Martin, C. 1952.Guerison de dahlias atteintsd'umeMaladie a Virus.*C. R.Acad. Sci., Paris*, 235:1324-1325.

Murashige, T. and Skoog, F. 1962.A revised medium for rapid growth and bioassay with tobacco tissue cultures.*Physiol. Plant*, 15:473-497.

Naqvi, H.H., and Ting, I.P. 1990. Variability in seed characteristics of unselected and selected jojoba populations.*Hort. Science*, 25:364.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. 2009. Agroforestree Database: A tree reference and selection guide version 4.0. (http://www.worldagroforestry. org/sites/treedbs/treedatabases.asp)

Palzkill, D.A. 1988. Propagation of jojoba by stem cutting. In: Baldwin AR (ed) Proc. 7th Int. Conf. on Jojoba and its uses. Am. Oil Chem. Chemists Assoc., Champaign, 86-101.

Palzkill, D.A. and Feldman, W.R. 1993. Optimizing rooting of jojoba stem cuttings: effects of basal wounding, rooting medium and depth of insertion in medium. *J. Amer. Oil Chem. Soc*., 70:1221-1224.

Passerini, E. and Lombardo, P. 2000. Cosmet. News, 22:396-398.

Prakash, S., Agrawal, V. and Gupta, S.C. 2003. Influence of some adjuvants on *in vitro* clonal propagation of male and female jojoba plants. *In Vitro Cell Dev. Biol. Plant,* 39:217-222.

Prakash, V., Agrawal, S. and Gupta, S.C. 2002.Effective protocol for *in vitro* shoot production through nodal explants of Simmondsiachinensis.*Biol. Plant*, 45:449-453.

Radwan, M.S., Ismail, M.A., Elfeky, S.M.S. and Abu-Elyazeed, O.S.M. 2007. Jojoba methyl ester as a diesel fuel substitute: Preparation and characterization. *Applied Thermal Engineering*, 27:314-322.

Ranzato, E., Martinotti, S. and Burlando, B. 2011. Wound healing properties of jojoba liquid wax: An *in vitro* study. *J. of Ethnopharmacology*, 134:443-449.

Reddy, M.P. and Chikara, J. 2010. Biotechnology advances in jojoba (*Simmondsiachinensis*). *In: Ramawat KG (ed) Desert plants.* Springer, Berlin, pp 407-42.

Reddy, Y.N. 2003.Effect of different concentration of auxins on rooting and root characters of air and ground layers of jojoba [*Simmondsiachinensis* (Link.) Schneider]. *Ethiop. J. Sci*., 26:155-159.

Roussos, P.A., Tolia-Marioli, A., Pontikis, C.A. and Kotsias, D. 1999.Rapid multiplication of jojoba seedling by *in vitro* culture.*Plant Cell Tissue Organ Cult*., 57:133-137.

Rugini, E., Jcoboni, A. and Luppino, M. 1993. Role of basal shoot darkening and exogenous putrescine treatment on *in vitro* rooting and endogenous polyamine changes in difficult to root woody species. *Sci. Hort*., 53:63-72.

Sardana, J. and Batra, A. 1998.*In vitro* regeneration of jojoba (*Simmondsiachinensis*): a plant of high potential. *Adv. Plant Sci*., 11:143-146.

Scaramuzzi, F. and D'Ambrosio, A. 1988.Organogenesis and propagation *in vitro* of *Simmondsiachinensis*(Link.)Schn. (jojoba) from vegetative fragments. *Acta Hortic*., 227:411-413.

Schenk, R.U. and Hildebrandt, A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot*., 50:199-204.

Shah, S.F.H. and Bashir, M.A. 2000.Propagation of jojoba through veneer grafting and T-budding.*J. Agric. Res*., 38:165-169.

Sharma, N.K. and Bedi, S. 1990. Effect of Phytophthorapalmivora on zygotic embryos of Papaya *in vitro*.*Annals of Botany*, 66:597-603.

Singh, A., Reddy, M.P. and Patolia, J.S. 2008.An improved protocol for micropropagation of elite genotype of *Simmondsiachinensis*(Link.) Schneider. *Biol. Plant*, 52:538-542.

Tobares, L., Frati, M., Guzmanc, C. and Maestri, D. 2004.Agronomical and chemical traits as descriptors for discrimination and selection of jojoba (*Simmondsi achinensis*) clones. *Ind. Crops Prod*., 19:107-111.

Tyagi, R.K. and Prakash, S. 2004. Genotype and sex specific protocols for *in vitro*micropropagation and medium term conservation of jojoba. *Biol. Plant*, 48:119-123.

Wang, Y.C. and Janick, J. 1986. Somatic embryogenesis in jojoba.*J. Am. Soc. Hortic. Sci*., 111:281-287.

Wimber, D.E. 1965. Additinal observations on clonal multiplication of cymbidiums through culture of shoot meri-stems. *Cymb. Soc. News*, 20:7-10.

Wimber, D.E. and Prensky, W. 1963.Autoradiography with meiotic chromosomes of the male newt (Triturusviridescens) using H3 thymidine. *Genetics*, 48:1731-1738.

Yermanos, D.M. 1979. Jojoba-A crop whose time has come. *California Agriculture*, 33:4-11.

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