



IN VITRO CALLUS INDUCTION IN SIMMONDSIA CHINENSIS

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Abstract

Present research investigations were studied role of various growth regulator like BAP, KIN, IBA, 2,4-D and IAA on in vitro callus formation. Various explants like apical shoots, nodal and axillary leaves inoculated on MS (Murashige and Skoog, 1962) medium supplemented with various concentration of phytohormones like, BAP (6-Benzylaminopurine), KIN, IBA, 2,4-D and IAA (Indole 3-acetic acid). MS medium contain BAP in various concentrations viz, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l combination with IBA or IAA to produce minimum average percentage of calli. These explants were inoculated on MS medium supplemented with different concentration of KIN like 1.0, 2.0, 3.0, 4.0 and 5.0mg/l combination with IBA or IAA. Apical shoot and axillary leaves were inoculated on MS medium supplemented with different concentration BAP and 2, 4-D these result were recorded maximum percentage of callus induction.

KEYWORDS: -in vitro, *Simmondsia chinensis*, 2, 4-D, callus.

Introduction:

Simmondsia chinensis belong to family Simmondsiaceae (jojoba family), has only 1 genus, *Simmondsia*, which consists of only one species, jojoba, *S. chinensis*. Once considered an isolated member of the box family (Buxaceae), jojoba is now regarded as sufficiently distinct to be placed in its own family. Jojoba is found from coastal and cismontane southern California east to central Arizona and south to Sonora and Baja California (Munz 1974). It is a characteristic plant of upland shrub communities in the

Sonoran and Colorado Deserts and is also quite common as a component of chaparral. The scientific name of *Simmondsia chinensis*, Jojoba does not originate in China; the botanist Johann Link, originally named the species *Buxus chinensis*, after misreading a collection label "Calif" as "China". Jojoba is a sparsely branched, decumbent to erect shrub that grows to 2 or rarely 3 m in height. Its large (2- to 4-cmlong), opposite, entire leaves are evergreen, leathery, and dull gray. Plants are extremely tolerant of drought (Al-Ani and others 1972) and their foliage is a source of nutritious forage for sheep, goats, and cattle, as well as for wild ungulates and smaller browsers such as rabbits. The large seeds have been used locally as a food source by indigenous people. In large quantities, the seed meal is toxic to many mammals, and the indigestible wax acts as a laxative in humans. The Seri, who utilize nearly every edible plant in their territory, do not regard the beans as real food and in the past ate it only in emergencies. 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, which make it useful in a host of applications.

Jojoba oil is used as a replacement for whale oil and its derivatives, such as cetyl alcohol. The ban on importing whale oil to the US in 1971 led to the discovery that jojoba oil is "in many regards superior to sperm oil for applications in the cosmetics and other industries. Jojoba oil is found as an additive in many cosmetic products, especially those marketed as being made from natural ingredients. In particular, such products commonly containing jojoba are lotions and moisturizers, hair shampoos and conditioners. Or, the pure oil itself may be used on skin, hair, or cuticles. Jojoba oil is a fungicide, and can be used for controlling mildew. Like olestra, jojoba oil is edible but non-caloric and non-digestible, meaning the oil will pass through the intestines unchanged and can cause a stool condition called steatorrhea. Jojoba biodiesel has been explored as a cheap, sustainable fuel that can serve as a substitute for petroleum diesel.

MATERIALS AND METHODS

Jojoba seedlings were grown in the greenhouse of botanical garden; these plants were used as experimental material. Plants were grown in the beds. The temperature in the greenhouse varied from 28 to 32°C. No artificial light was provided. Various explants were used for establishing present works, including apical shoots, nodal and axillary leaves. All these explants were taken from six month -old plants. above explants (0.5–0.8 cm) taken from 1st to 3rd node from the apical region of lateral branches, these were surfacesterilized with 0.01%

(w/v) mercuric chloride for 2–3 min, washed 3– 4 times with sterile double distilled water and inoculated on agar solidified MS medium supplemented with different concentrations of BAP (6-Benzylaminopurine), KIN, IBA, 2,4-D and IAA (Indole 3- acetic acid) either alone or in combination. The pH of the medium was adjusted to 5.8 before the autoclave. Cultures were maintained at $25\pm 1^{\circ}\text{C}$ with a 16-h photoperiod with 40 mol m^2/s provided by cool white fluorescent tubes light. In vitro culture was sub cultured after 25 days on the original callus inducing medium, for the mass production.

Culture conditions

After the inoculation culture tubes and culture vessels were transfers to culture room under a 16 h photoperiod supplied by cool white fluorescent tubes light and $21 \pm 0^{\circ}\text{C}$ temperature. At least ten cultures were raised for each treatment. Data were measured after 30days of five replicate for, callus induction of Mean (μ) values.

RESULTS AND DISCUSSION

The explants did not show any activity of callus initiation when cultured on control medium (without PGRs).

Induction of callus from various explants

The response of different explants, like apical shoots, nodal segment and axillary leaves of jojoba for callus induction was variously recorded, when these explants (0.5 cm) individually inoculated on MS medium in combination of different growth regulators viz, BAP, KIN, IBA, 2,4-D and IAA either alone or with combination. After 15 days all these explants response to callus induction but these are show sharp difference to growth regulator levels for the callus induction responses of different explants. MS medium contain BAP in various concentrations viz, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l combination with IBA or IAA (1.0 mg/l) to produce minimum average frequency of calli. These explants were inoculated on MS medium supplemented with different concentration of KIN like 1.0, 2.0, 3.0, 4.0 and 5.0mg/l combination with IBA or IAA. Apical shoot and axillary leaves were inoculated on MS medium supplemented with different concentration BAP alone with 2, 4-D all these explants shows maximum percentage of callus induction as shown table 1. The best response for callus initiation from apical leaves was observed in MS medium supplemented with 0.8 mg/l BAP combination with 0.4 mg/l 2, 4-D. the average percentage of callus initiation were recorded, when nodal segment and leaves explants inoculated on MS medium supplemented with 0.6 mg/L BAP + 0.4 mg/L IAA.

Table 1. Showing effect of various growth hormones on callus induction using axillary leaves and nodal segment as an explants

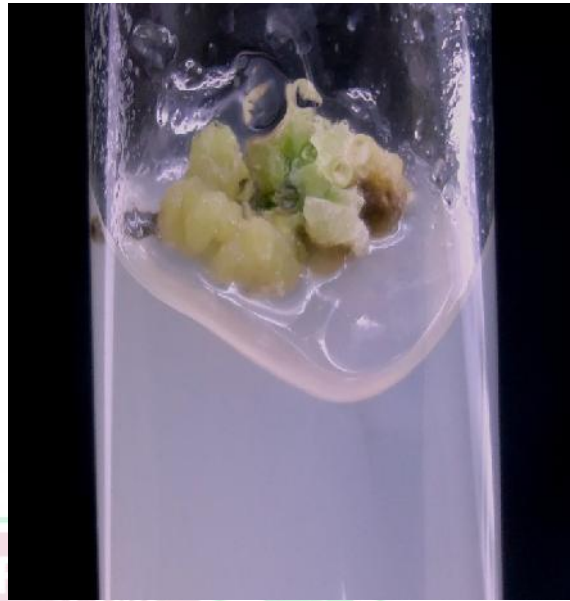
Explants	Concentration of PGR (mg/l)				Frequency of Callus induction
	BAP	KIN	IAA	2,4-D	
Axillary leaves	0.0	0.0	0.0	0.0	-
	0.0	0.0	0.0	0.2	++
	0.5	0.2	1.0	0.2	+
	1.0	0.2	1.0	-	++
	1.5	0.2	1.0	-	+
	0.2	-	-	0.2	+
Nodal segment	0.4	-	-	0.4	++
	0.6	-	-	0.6	++++
	0.8	-	-	0.8	+++
	1.0	-	-	1.0	++

(+) Callus formation less or more

Fig.1 Callus induction from different explants in Jojoba (*Simmondsia chinensis*)



Callus from Axillary leaves



callus from Nodal Explants

All these explants inoculated on MS medium supplemented with various concentration KIN and Auxin these culture show minimum response for callus initiation. MS medium + various concentration of 2, 4-D 0.2 mg/l, 0.4mg/l, 0.6 mg/l, 0.8 mg/l, and 1.0 mg/l shows average percentage of callus initiation were recorded. Similar results were found in case of *Portula cagrandiflora* L. Callus proliferation had predominate response on the media containing 5 μ M BAP alone or in combination with 5 μ M NAA. Formed calli in these treatments were green and friable but were not able to regenerate shoots (Yaghoob et al. 2010). It has been demonstrated that adenine, adenosine cytokinin like activity and when they are added to the culture medium they help improve growth or to reinforce the response normally attributable to cytokinin action. In this sense, adenine stimulates somatic embryogenesis and caulogenesis, enhances growth of isolated meristem tips, induces proliferation of axillary shoots in shoot cultures and promotes adventitious shoot formation indirectly from calli or directly from explants (Van Staden et al. 2008).

REFERENCES

Al-Ani H.A., Strain B.R., Mooney H.A. 1972. The physiological ecology of diverse populations of the desert shrub *Simmondsia chinensis*. *Journal of Ecology* 60: 41–57.

D.J. Undersander, E.A. Oelke, A.R. Kaminski, J.D. Doll, D.H. Putnam, S.M. Combs, and C.V. Hanson (1990). "*Jojoba*". *Alternative Field Crops Manual*.

Islam, R., A. Hoque, M. Khalekuzzaman and O.I. Joarder, 1997. Micropropagation of *Azadirachta indica* A. Jus s . from explants of mature plants . *Plant Tissue Cult.*, 7: 41–46.

Munz P. A. 1974. A flora of southern California. Berkeley, University of California Press. 1086.

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

Steven J. Phillips, Patricia Wentworth Comus (eds.) (2000). *A Natural History of the Sonoran Desert*. University of California Press. pp. 256–257. ISBN 0-520-21980-5.

VanStaden, J.; Zazimalova, E. and George, E.F. (2008). .Plantgrowth regulators II: ytokinins, their analogues and antagonist. *Plant Propagation by Tissue Culture*. 1: 205-226.

Yaghoob Safdari and Seyed Kamal Kazemitabar 2010. Direct shoot regeneration, callus induction and plant regeneration from callus tissue in mose rose (*Portulacagrandiflora* L.) plant. *P.O.J.* 3(2):47-51.

