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IN VITRO SHOOT MULTIPLICATION IN SIMMONDSIA CHINENSIS (LINK) SCHN.).

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Abstract

Simmondsia chinensis seedling explants were cultured on a MS medium, supplemented with various concentrations of 6-benzyladenine viz, 1.0, 1.5, 2.0, 2.5, 3.5, 4.0, 4.5 and 5.0 mg/l alone and in combination with silver nitrate. Shoot proliferation was successful at all the concentrations tested, with 3.0 mg/l BAP and lower concentration of IAA maximum number of shoots per original explant. Shoots produced during the proliferation stage were treated with -naphthalene acetic acid, indole-3-butyric acid and indole-3-acetic acid to induce rhizogenesis, reaching 64% rooting in some treatments. When the rooted explants were transferred to the mist system for acclimatization, 35% to 50% of them survived and continued to grow after a period of one month.

Key Words: *In vitro, Simmondsia chinensis , Shoot multiplication.*

Introduction

The *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.[10][11] 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 320C. No artificial light was provided. Various explants were used for establishing present works, including nodal segment, axillary shoots, etc. Axillary shoot and nodal segments were taken from six month -old plants while axillary shoots and root segment explants were removed from 10–15 day-old seedlings. Axillary shoots (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 KIN, BAP, IAA, and IBA either alone or in combination. The pH of the medium was adjusted to 5.8 before the autoclave. Cultures were maintained at 25±1ºC 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 multiplication-inducing medium. For the growth of axillary shoots arise from using the nodes of nodal segments as an

explants. Nodal segments (1–2 cm) were taken from lateral branches and surface-sterilized with 0.2% mercuric chloride and inoculated on agar-solidified MS medium supplemented with different concentrations of BA and KIN (alone or in combination). For root segments of 10–15 day old seedlings were cultured on the MS-based agar solidified medium supplemented with 2, 4-D (2 mg /l) and KIN (0.2mg /l) for callus formation. Tubes inoculated with root segments (0.8–1.0 cm) were incubated both in light and dark at 25ºC.

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 ± ^{0}C temperature. At least ten cultures were raised for each treatment.

Data record

Data were measured after 30days of five replicate for, shoot multiplication and shoot length Mean (μ) values ± SE.

RESULTS AND DISCUSSION

The explants did not show any activity of shoot initiation when cultured on control medium (without PGRs) or on those containing only an auxin at lower concentration.

Multiplication of Shoots

Small (0.5 cm) individual shoots from 5-week-old cultures inoculated on 1/2 MS+BAP (1.0mg/l) + IAA (0.5mg/l) these culture no effect of shoot multiplication. When these excised shoots were carefully transferred to full-strength MS medium supplemented with BAP on various concentration viz, 1.0, 1.5, 2.0, 2.5, 3.5, 4.0, 4.5 and 5.0 mg/l and lower concentration of IAA or IBA as shown table 1, for elongation of shoots and multiplication of shoots. Though each axillary shoot, which had grown fairly long, when these were cut into single node segments and transferred to fresh medium for further multiplication. The number of propagules obtained at the 3.0 mg/l BAP alone with lower concentration of IAA or IBA. And other multiplication treatments examined were various concentration cytokinin like BAP and KIN combination with Auxins IAA or IBA at lower concentration. At least 12-cultures were raised for each treatment and all the experiments were repeated at three times. Observations on number of cultures showing contamination, bud-break, shoot elongation and rooting were made at weekly intervals. Mean was calculated and is indicated by sign.

Table 1:- Effect of BAP for multiplication of nodal and shoot tip explants

Explants	Conc. of growth regulators (mg/L)			Shoot length (Mean ± SE)	% of shoot formation
	BAP	IAA	IBA		
Nodal	1.0	0.5	0.2	1.02 ± 0.08	40
	1.5	0.5	0.2	1.17± 0.10	55
	2.0	0.5	0.2	2.78± 0.11	63
	2.5	0.5	0.2	3.5± 0.12	90
	3.0	0.5	0.2	4.13± 0.10	95
Shoot tip	1.0	0.4	0.2	1.12±0.13	45
	1.5	0.4	0.2	1.74±0.12	60
	2.0	0.4	0.2	3.42±0.11	70
	2.5	0.4	0.2	4.18± 0.13	90
	3.0	0.4	0.2	4.64±0.08	95

*__After 30 days mean ± SE of 5 replicate__

Root initiation

For rooting, terminal 3 cm long portions of elongated shoots with 3–4 nodes were excised and cultured on MS medium supplemented with indole-3-butyric acid (IBA) or NAA in the range of 1 to 5 mg/l. MS+ 1.0 mg l IBA, MS+ 2.0 mg l IBA, MS+ 3.0 mg l IBA, MS+ 4.0 mg l IBA, MS+ 5.0 mg l IBA. Another treatments examined for rooting were MS basal medium with 5mg/l IBA combined with 0.5, 1.0,2 .0 and 3.0 g/l AC (Charcoal) respectively to simultaneously promote both elongation and rooting. Shoot proliferation was successful at the entire concentrations tested, with a maximum number of 10-15 shoots per original explants. When the rooted explants were transferred to the green house for acclimatization, 35% to 50% of them survived and continued to grow after a period of one month. MS+BAP 3mg /l + kin 1.5mg /l proved optimum for shoot multiplication but it did not facilitate Shoot growth. Therefore, 0.5 cm long individual shoots were excised carefully after 5 weeks and transferred to MS+BAP 1mg /l supplemented with 0.5mg/l IAA or IBA for elongation. On M S+BAP 1mg/l, the original shoot attained a length of 8.5 cm, with 8 nodes, after 5 weeks in 95% cultures Thus, eight-fold shoot multiplication every 5 weeks could be achieved on MS+BAP by cutting the solitary shoot into s ingle node segments and culturing them on fresh medium. This rate of shoot multiplication was maintained. More than 20 times the nodal explants were taken and used for shoot multiplication. Since every time the explants were taken from freshly formed in vitro shoots, therefore, we have not observed any significant difference (variation) in the results, this was observed. Islam *et al.,* (1997) used a medium with a lower level of BAP (0.1 mg/ l) for shoot elongation, suggesting that the shoots remained very small on the multiplication medium and required an elongation step to obtain shoots suitable for rooting.

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