World Journal of Pharmaceutical research
Volume 2, Issue 1, 103-111. Research Article ISSN 2277 – 7105

IN VITRO CLONING OF *SIMMONDSIA CHINENSIS* (LINK) SCHNI USING BAP AND KINETIN

Sudhanshu, Shakera Khan, Ekta Menghani[b*] and Mohit Soni[b]

[a]Suresh Gyan Vihar University Mahal, Jagatpura, Jaipur, India

[b]Mahatma Gandhi Institute of Applied Sciences, JECRC University, Jaipur, India

Article Received on
20 November 2012,

Revised on 12 December 2012,
Accepted on 26 December 2012

***Correspondence for Author:**

*** Dr Ekta Menghani**,
Women Scientist A, DST,
Department of Biotechnology,
MGIAS, JECRC University,
Jaipur, India
ektamenghani@yahoo.com

ABSTRACT

The study was conducted to initiate shoots from *in vitro*, from 5 year old plants of Jojoba. MS medium was supplemented with cytokines i.e., BAP and kinetin each at the rate of 0.5, 1.0, 2.0, 3.0 (mg / L) in the are experiment and at the rate of 0.5, 1.0, 2.0, 3.0 mg/L in the other experiment. In the 1st experiment, the lowest concentration gave slow response. The shoots differentiated from thick explants were healthier and these grow more vigorously, at 28 $\pm$ 2^0C on MS medium supplemented with 2.0 mg/L BAP. In the 2nd experiment kinetin was found to be less effective cytokines than BAP for shoot induction. The shoot bud differentiation was positively affected by incorporation of additives in medium. If the additive were not added, yellowing of shoots occurred.

Keywords : Jojoba, *Simmondsia chinensis*, Shoots, BAP - 6 – benzylaminopurime, Kinetin.

INTRODUCTION

Jojoba (*Simmondsia chinensis* (Link) Schneider) is a desert shrub which tolerates saline and alkyl soils and draught. The seeds contain a characteristic liquid wax which has economic importance in industry (machine lubricant) and medicines (e.g. cosmetics and anticancer compounds[1].

A major problem in seeds productions is that Jojoba is dioeciously plant where its sex in not easily determined prior to flowering (3-4 years from cultivation). Therefore, plant regeneration via tissue culture is an important tool in micro propagation, mutant selection and

genetic transformation. Tissue culture techniques have been applied only to a limited extent in Jojoba[2, 3].

Micro propagation of Jojoba offers a promising method for mass production of superior pathogen-free clones for commercial plantations[4]. The use of simple node, double node and three nodes cutting from different individuals of Jojoba by applying different plant hormones will increase the total number of prop gules obtained from stock plants[5]. Shoot tips established in tissue culture, however, will generally give rise to multiple shoots that can be rooted. Thus a single explants source, shoot tip, could conceivably provide thousands of new plantlets a year. Among the biotechnological techniques micro propagation is an area of practical aspects for large scale multiplication of elite planting material[6].

Kacker *et al*., 1993, [7] incubated the micropropogated shoots of jojoba in the dark in a liquid half strength MS medium containing 10 mg L^{-1} NAA for 72h (for early root initiations on subsequent medium) and then transferred to half strength MS rooting medium containing 2500 mg L^{-1} activated charcoal. They observed root initiation within a week. More than 80% shoots developed within a month. Rugini *et al*., 1943, [8] placed explants of jojoba for rooting on modified Bourgin and Nitsch medium with or without 0.93 mg L^{-1} NAA. None of the explants rooted in the absence of NAA. Apostolo *et al*., 1996, [9] obtained the 31.08% rooting after 70 days from micropropagated shoots cultured on half strength MS medium containing IBA at the rate of 3.0 mg L^{-1}. Elhag *et al*., 1998, [10] attempted rooting of microprogated shoots of jojoba on various media (e.g.) IAA, NAA, IBA, ½ MS or MS). Hassan, 2003, [11] achieved maximum frequency of conversion of encapsulated buds of jojoba into plantlets on MS + 1 mg L^{-1} BAP+40 mg L^{-1} adenine sulfate + 3 mg L^{-1} IAA. He observed after 35 days a well developed shoots roots on MS medium. The average length of the shoots 3.4 cm and that of the roots was 1.8 cm. Tyagi and Prakash, 2004, [12] reported that the pulse treatment of 10 mg L^{-1} IBA for 20 min caused *in vitro* rhizogenesis in 44-67% cultures of various jojoba genotypes tested.

The advantages of using asexual propagules in commercial jojoba plantations are that they provide uniform and predictable plant growth and yield[13]. Furthermore, jojoba in dioecious and cannot be sexed until flowers appear (usually 24 years from seed clonal propagation of clite individuals of known sexuality in necessary to ensure that the plants in commercial plots will be produce[14]. Thus micropropagation offers opportunities for the production of

thousands of elite plants from the selected stock plant[13]. Consequently, several investigations have attempted clonal propagation of jojoba tree[1, 2, 13]. Most of these reports deal with juvenile material or material of unspecified age, which is of little significance in clonal propagation of elite trees. Therefore the present is conducted on cloning of *S.chinensis* using BAP and Kinetin.

MATERIALS AND METHODS

1.1 Plant material and initiation of aseptic cultures.

- Explants were taken from semi-hard wood stem of 3 years old jojoba plant from Gyan Vihar botanical garden. Original plantlets in taken from Dhand jojoba farmhouse of AJORP, District Jaipur, Rajasthan.
- After removing leaves, the cuttings were thoroughly washed with 1% solution of savlon for 20 min and rinsed twice in sterile distilled water (SDW). All subsequent operations were carried inside a laminar air-flow cabinet. The clean cuttings were given a quick (30s) rinse in 70% ethanol, followed by two washings in sterile distilled water. These were then surface sterilized in 0.1% mercuric chloride ($HgCL_2$) solution for 13 min. and rinsed thrice with sterile distilled water. The cuttings were trimmed at both ends to expose fresh tissue before planting on Murashige and skoog (MS) medium (1962) containing 3% sucrose and gelled with 0.8% agar.
- The MS medium was supplemented with plant growth regulators. After adjusting the pH to 5.8, 20ml of medium was dispensed into each 150x25 mm. Borosil rimless glass tube. The culture tubes and jars were wrapped and autoclaved at 1.06 kg cm^{-2} and 121^0C for 15 min.
- Thermolabile compound, such as gibberellic acid (GA_3) was filter - sterilized and added to the autoclaved medium cooled to 50^0C medium was then dispensed into glass tubes inside a laminar air-flow cabinet.
- All the cultures were maintained in diffuse light (1000-2000 lx) and 16h photoperiod at 25±2 ^{0}C and 50 - 60% relative humidity.
- In the preliminary experiments, the explants were cultured on to full strength murashige and skoog basal medium. Subsequently MS medium was tested with BAP (1 micro mole) alone. The nodal explants with clusters of shoots produced by 5 week old primary cultures of nodal segments on ½ MS + BAP (1 micro mole) were transferred to ½ MS + kinetin for further shoot multiplication.

1.2 Amplification of Shoots:

- *In vitro* produced shoots were harvested and the mother explants were repeatedly transferred on the fresh medium. The fresh crop of shoots could be harvested from original explant upto four passage at an interval of 25-28 days.
- The shoots differentiated in culture were cut into 4-5 small clumps and cultured on fresh medium for the further multiplication. Subculturing was done every 4 weeks.
- For large scale amplification the culture were multiplied on medium containing commercial sugar cubes, cheaper source of agar-agar as gelling medium in 70x130 mm glass jar. BAP concentration were taken as 0.5 mg l^{-1}, 1.0 mg l^{-1}, 2.0 mg l^{-1}, 3.0 mg l^{-1}, and further transferred MS+ Kinetin. Kinetin concentrations were taken as 0.5 mg l^{-1}, 1.0 mg l^{-1}, 2.0 mg l^{-1}, 3.0 mg l^{-1}, etc. As we increase kinetin concentration rate of shoot per node increases.
- Regenerated shoots were rooted under *in vitro* and *ex vitro* conditions. The rooted plantlets were hardened under different regimes of the green house. Some of these were transferred to polybags containing garden soil, sandy soil and organic manure. The plantlets hardened in the green house were shifted to the nursery.

RESULT AND DISCUSSIONS

Table 1: M.S. media in combination with different hormones used for callogenesis and regeneration from *Simmondsia chinensis* (Link) Schneider.

Treatment no.	BAP(mg/l)	KINETIN(mg/l)
T_0	0.0	0.0
T_1	0.5	0.5
T_2	1.0	1.0
T_3	2.0	2.0
T_4	3.0	3.0

Table 2: Percentage callogenesis from nodal explants and shoots of *Simmondsia chinensis* (Link) Schneider on different hormonal combinations.

Treatment no.	Callogenesis(%)nodal explants	Callogenesis(%) shoots
T_0	0.0	0.0
T_1	62.0	87.0
T_2	30.0	78.0
T_3	63.7	67.0
T_4	64.0	62.0

In present research callus induction was observed from both nodal explants and shoots nodal explants of the plant were cultured on different concentration of hormones.The callus growth was observed to be remarkably affected by hormone treatment (Table -2) about slowly. Callus obtained on T_1were weak and developed very slowly.

As shown in Table 3, highest callus induction of about 64% was obtained on MS medium supplemented with BAP and Kinetin in T_4. It appeared that the presence of a high concentration of BAP enhanced the growth of the plant.Although this combination was used for regeneration but in this particular variety it produced good callus with fresh green color. Combination of BAP and Kinetin T_4 produced 68.2% direct regeneration from nodal segments and in T_3 5.2% produced direct regeneration from nodal segments. For regeneration, callus was subcultured and shifted to regeneration medium. Some nodal segments had directly shown regenerations. Some nodal segments had directly shown regeneration.T_1exihbited no indirect regeneration maximum direct regenerations was observed in T_4- as 68.2%. In T1 it was 2.4%, T_2=5.4%, T_3-5.2,T_4=68.2%. A rapid high frequency regeneration systems was established by using GA3 in the treatments of jojoba. As the kinetin concentration was increased, callus induction and was also increased with the callus being more green and bulky.

Best results were obtained by using T_4 about 68.2% nodal segments exhibited regeneration. When callus and shoots were transferred to regeneration medium, none of them showed regeneration (Table 3).

Table 3: Percentage regeneration frequency from nodal segments and shoots of *Simmondsia chinensis* (Link) Schneider on different hormonal combinations.

Treatment no.	Nodal segments		Shoots	
	DR regeneration	IDR Regeneration	DR regeneration	IDR regeneration
T_0	0.0	0.0	0.0	0.0
T_1	2,4	0.0	0.0	0.0
T_2	5.4	0.0	0.0	0.0
T_3	5.2	0.0	0.0	0.0
T_4	68.2	0.0	0.0	0.0

T=Treatment

DR=Direct regeneration =where explants become green and new shoot is produced.

IDR=Indirect regeneration =where explants first give callus.

Fresh and green calli obtained from nodal segments were subcultured on T_4 having different concentration of BAP and Kinetin. After 15 to 24 days of observation 31.25% calli showed rapid proliferation and 18% showed poor proliferation. Necrosis was observed in 17% calli (Table 4). Calli obtained from shoot segments were subcultured on T_4. Rapid proliferation was observed in 64% calli. Necrosis was obserbed in 5.6% calli (Table 4). None of the calli showed any root regeneration.

Table-4: Sub-culturing of calli obtained from nodal segments and shoots of *Simmondsia – chinensis* (Link) Schneider.

Exp no.	**No. of Sub cultured calli**	**Proliferation**			**Necrosis**	**Powdery callus**
		Good	**Average**	**Poor**		
		Subculturing	**Nodal segments**			
1.	22	10(45%)	8(36%)	2(9%)	1(4.5%)	1(4.5%)
2.	32	10(31%)	10(31%)	5(16%)	5(16%)	2(6.25%)
3.	42	10(24%)	10(24%)	10(21%)	10(24%)	2(4.7%)
Total	96	30(31.25%)	28(29%)	17(18%)	16(17%)	5(5.2%)
		Subculturing				
1.	32	24(75%)	2(6.25%)	2(6.25%)	2(6.25%)	2(6.25%)
2.	42	20(48%)	10(48%)	2(48%)	2(48%)	8(19.04%)
3.	32	24(75%)	2(6.25%)	2(6.25%)	2(6.25%)	2(6.25%)
Total	106	68(64%)	38(36%)	6(36%)	6(5.6)	12(11.3%)

If we see overall callogenesis from nodal explants in first experiment, 53% show no callus, 16% good callus, 7% medium callus, 7% poor callus, 7% show necrosis, 8% show powdery callus. In second experiment 36% show no callus, 7% show good callus, 7% medium callus, 15% poor callus. About 13% nodal segments exhibited no callus induction necrosis was observed in 10.3% calli. Good callus was obtained in 10.3% nodal segments. The highest callus induction frequency was obtained in T_4. In the third experiment out of 62 total cultures, 34% show no callus, 16% exihibit good callus, 16% medium callus, 16% exhibit good callus, 16% medium callus poor callus, 8% show necrosis 8% show powdery callus.

Figure 1: Callus formation in jojoba using kinetin concentration-3 mg/l

In case of overall callogenesis from shoots out of 20 total cultures, in the first experiment 2% show no callus, 2% show good callus, 2% show medium callus, 2% show no callus, 2% show medium callus, 2% show poor callus, 1% show necrosis, 1% show powdery callus.

In the second experiment out of 40 total cultures 25% show no callus, 0.2% show good callus, 0.5% show medium callus, 25% show poor callus, 12.5% show poor callus, 12.5% show necrosis, 12.5% show powdery callus. In the third experiment out of 60 total cultures, 2% show no callus, 13% show good callus, 17% show medium callus, 17% show poor callus, 16.7% show necrosis, 16.7% show powdery callus out of total cultures of 194 in case of nodal explants 41% show no callus,13% show good callus, 10.3% show medium callus, 13% show poor callus, 10.3% necrosis 14.43% show powdery callus.

Overall callogenesis from shoots out of 120.Total cultures, 16.7% show no callus, 16.7% good callus, 13.33% medium callus, 2% poor callus, 14.16% Necrosis, 14.16% powdery callus.

Table-5: Plant regeneration from Simmondsia chinensis (Link) Schneider.

Exp no.	Explant source Nodal segments	Total no.	Growth differentiation	Shoot formation
1.	22		20(91%)	2(1%)
2.	22		18(82%)	4(18%)
3.	22	66	16(72%)	6(27%)

SUMMARY AND CONCLUSION

As we see BAP and Kinetin concentrations were taken as 0.0 mg/l,0.5 mg/l,1.0 mg/l,2.0 mg/l,3.0 mg/l.Highest callogenesis obtained from nodal explants and shoots were by T_4 as

64.0% and T_1as 87.0% and percentage regenerations frequency from nodal segments and shoots of Simmondsia chinensis (Link) Schneider on different hormonal combinations are 68.2% from T_4 which is highest.Subculturing of calli obtained from nodal segments and shoots of Simmondsia chinensis (Link) Schneider. Good Subculturing were obtained. Plant regeneration from Simmondsia chinensis (Link) Schneider were obtained.

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