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In vitro Shoot Initiation from Nodal Explants of Jojoba (*Simmondsia chinensis*) Strains

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Abstract: Nodal segments of six promising strains of jojoba i.e., PKJ-1 to PKJ-6 were cultured *in vitro* on solidified MS medium supplemented with BA and Kinetin, alone or in combinations at different concentrations to initiate shoot growth. Different growth parameters regarding shoot initiation and growth were recorded during the experimental period of three months. The study revealed that BA (alone) was better than Kinetin (alone) or BA + Kinetin combinations. The lowest concentration of BA (1.25 mg L^{-1}) proved more effective for many shoot parameters and PKJ-3 strain was the most responsive to cytokinins alone or in combination. Some explants also developed callus at the highest level (5.0 mg L^{-1}) of BA (alone) or in combinations of BA with Kinetin. Many explants were vitrified at the highest level (5.0 mg L^{-1}) of Kinetin (alone).

Key words: Axillary bud cultures, cytokinins, genotypes, jojoba, micropropagation, *Simmondsia chinensis*

INTRODUCTION

Jojoba (*Simmondsia chinensis* Link. Schneider), monogeneric and monospecific member of family *Simmondsiaceae*, is a dioecious evergreen perennial desert shrub with natural lifespan over 100 years. Its seeds contain about 50% oil which is an alternative to sperm whale oil as well as famous for its utilization in cosmetics, lubricants and pharmaceuticals etc. Due to its unique oil properties, this valuable shrub has been focused by researchers and scientists globally. Jojoba as a cross-pollinated plant will not reproduce true to type by seed or sexual propagation. In order to maintain the characters for which it was selected, a single jojoba plant has to be asexually propagated. Asexual or vegetative propagation of jojoba can be accomplished by layering (Reddy, 2003), grafting (Bashir *et al*., 2006), cuttings (Singh *et al*., 2003) or tissue culture techniques (Tyagi and Prakash, 2004). Thus, a selected individual can be reproduced genetically identical that form a clonal population or cultivar. Among the biotechnological techniques, micropropagation is a technique for mass multiplication of selected planting material on a large scale within a short possible time. Thus a single explant source could conceivably provide thousands of new plantlets a year. With the success of raising *in vitro* plants, the micropropagation has reached a commercial level in many plant species in recent years (Chandra and Mishra, 2003).

Jacoboni and Standardi (1987) established cultures from apical, single node and binodal microcuttings taken from growing shoots of 4 year-old potted jojoba plants on MS medium supplemented with 1 mg L^{-1} Zeatin and 0.1 mg L^{-1} of Gibberelic acid (GA_3). New shoots 1.5-2.0 cm long arose from buds of microcuttings after 4, 5-6 and 9 weeks respectively. They obtained 3.5 cm long shoots with 3-4 nodes after 30 days of subculture from new shoots on MS + 2 mg L^{-1} Zeatin (ZT) + 2 mg L^{-1} GA_3 + 0.001 mg L^{-1} Naphthalene acetic acid (NAA). Kacker *et al.* (1993) produced axillary shoots on MS medium supplemented with 0.5 mg L^{-1} Kinetin and 1 mg L^{-1} Benzyladinine (BA) within a month of culture from nodal segments of female plants of jojoba. Llorente *et al.* (1996) cultured nodal segments of jojoba on MS medium containing BA or Kinetin at the rate of 1 mg L^{-1}, alone or in combination with 0.2 mg L^{-1} Indolebutyric acid (IBA). They observed that the shoot growth was significantly greater in BA than Kinetin treatments, but the presence of BA caused callus formation at the base of shoot tips and light hyperhydricity in 30% of explants. The presence of IBA did not affect shoot growth and leaf numbers. They also observed clonal differences in responses. Elhag *et al.* (1998) cultured shoot-tip explants of jojoba on MS or B5 basal medium supplemented with combination of Indole Acetic Acid (IAA) and BA at 0, 0.3 and 3.0 mg L^{-1} each in a factorial arrangement. After 56 days in culture, they

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recorded a significant genotypic effect and a medium composition (growth regulators) effect. The highest number of newly formed shoots per explant was produced by female plants F_3 and F_2. The male plants (M_1, M_2 and M_3) were the least responsive. The higher BA/IAA ratio favored shoot multiplication with 3.0/0.0 and 3.0/0.3 mg L^{-1} combination producing the maximum response. Lower BA/IAA favored shoot elongation and callus production. Llorente and Apostolo (1998) propagated *in vitro*, the explants of jojoba from randomly selected plants on modified MS medium supplemented with 1 mg L^{-1} BA. There was a 4.6-fold increase in shoot numbers after 30 days of culture. The response of clones was highly variable. Sardana and Batra (1998) obtained complete plantlets of jojoba with 1 or 2 thick roots per shoot when shoot tips were cultured on MS medium supplemented with NAA and BA, both at 1.0 mg L^{-1} after an incubation period of 35-40 days. An increase in BA concentration suppressed rooting but increased shoot length of plantlets. Agraval *et al.* (1999) observed 3.5 shoots per explant in 80% male in the presence of 2.25 mg L^{-1} BA and 4.7 shoots per explant in 100% female in the presence of 4.5 mg L^{-1} BA added into MS or B5 medium. The other cytokinins did not improve morphogenic response over BA. Khanam *et al.* (1999) observed greater shoot proliferation from single stem segment of female jojoba plants on MS medium supplemented with 4 mg L^{-1} BA. Roussos *et al.* (1999) found successful shoot proliferation from the seedling explants of jojoba with a maximum number of 15.2 shoots per explant on a modified Driver Kuniyuki medium, supplemented with various concentrations of BA alone and in combination with silver nitrate. Agraval *et al.* (2002) reported that 11.5% nodal explants of female jojoba clone EC 33198 produced an average of 2.7 shoots when cultured on MS medium supplemented with 4.5 mg L^{-1} BA. Prakash *et al.* (2003) noted that BA in combination with different levels of Triiodobenzoic acid (TIBA) promoted shoot growth in female explants. However, BA alone proved to be the best for differentiation of shoots for male at 2.25 mg L^{-1} as well as for female at 4.5 mg L^{-1}. Tyagi and Prakash (2004) noted that the nodal explants of different genotypes as well as sex elicited differential requirements of BA for optimum shoot regeneration. The female nodal explants of genotype EC 99692 produced maximum shoots (10 shoots per explant) followed by the male genotype EC 171284 (9.3 shoot per explant) on MS medium supplemented with 2.25 mg L^{+} BA.

The present study was envisaged to find out the most effective cytokinin or the combination of cytokinins for *in vitro* shoot initiation from nodal explants and to observe genotypic responses of various jojoba strains.

MATERIALS AND METHODS

The studies were conducted in Tissue Culture Laboratory of Agricultural Biotechnology Institute, National Agriculture Research Centre, Islamabad during the year 2005.

Source of explants and its preparation: Branches were collected from 2 year-old clonally propagated (through cuttings) plants of promising six female jojoba strains PKJ-1 to PKJ-6 as characterized by Bashir *et al.* (2006). The branches were washed in running tap water for 30 min; the leaves were trimmed out, leaving the petiole intact. These were again washed in 1% detergent (w/v) solution, followed by four washing in distilled water. The branches were divided aseptically into nodal segments of 1.5-3.0 cm length. Subsequent surface sterilization was conducted in three steps as follows:

- Dipping the explants in ethanol (90%) for 10 sec.
- Dipping the explants in 50% Clorox (sodium hypochlorite = 2.62%) + Tween-20 (2 drops) solution and stirring for 15 min, followed by four washings with distilled sterile water each for 5 min.
- Dipping the explants in 0.1% (w/v) aqueous mercuric chloride solution and stirring for 15 min then washing 4 times in distilled sterile water.

All sterilization operations and cultural manipulation were carried out under Laminar Air Flow Hood.

Culture medium and culture conditions: The explants were placed on solidified MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose and 0.7% (w/v) agar and supplemented with different concentrations of cytokinins, alone or in combinations according to treatments as given below:

In one experiment, the medium was supplemented with following concentrations of BA and Kinetin.

- 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin
- 2.5 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin
- 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin

The experiment was laid out in factorial CRD with 3 replications and 2 factors i.e., strains and combinations.

In other experiment, MS medium was supplemented with either Benzyladenine (BA) or Kinetin alone, each at the rate of 1.25, 2.5 and 5.0 mg L^{-1}. The experiment was laid out in factorial Completely Randomized Design (CRD) with 3 replications and 3 factors i.e., strains, cytokinins and concentrations.

The pH of the media was adjusted to 5.7±0.1 using either 0.1 N NaOH or 0.1N HCl prior to adding 0.7% (w/v) agar. Media were dispensed in 10 mL aliquots into culture tubes (2.5×15 cm), which were plugged with non-absorbent cotton wrapped in one layer of cheese cloth. Media were autoclaved at 121°C and 1.05 kg cm^{-2} for 20 min. The cultures were incubated under a 16 h photoperiod in cool, white fluorescent light of Philips tubes with light intensity of 55 μmol m^{-2} sec^{-1} at 25±2°C. The cultures were maintained by subculturing at 4 weeks interval to fresh medium with the same composition.

Data recording and statistical analysis: Initially ten explants were cultured under each treatment per replication of each experiment, keeping 1 explant in a test tube. Discarding the contaminated cultures, the following data were recorded from remaining clean cultures only.

Number of days to bud sprout: The cultured explants were observed vigilantly during the experimental period that lasted over three months. The count of days started from the date of culturing to the date of sprouting of axillary buds of explants. The days were averaged over number of explants per treatment per replication.

Length of primary shoot (cm): The lengths (cm) attained by the primary shoots within 3 months that arose from the explants was recorded and averaged over number of explants per treatment per replication.

Number of nodes per primary shoot: The number of nodes carrying the primary shoot of each explant was recorded and averaged over number of explants per treatment per replication.

Number of shoots produced per explant: The number of shoots arose from the explant within 3 months was recorded and averaged over number of explants per treatment per replication.

Percentage of sprouted explants: At the end of 3rd month from first culturing, the percentage of sprouted explants was recorded by the given formula and averaged over number of explants per treatment per replication.

$$\text{Percentage of sprouted explants} = \frac{\text{No. of sprouted explants}}{\text{No. of clean cultures}} \times 100$$

All parameters were recorded from ten initially cultured explants (discarding contaminated cultures and considering only clean cultures) and averaged over number of explants per treatment per replication. Data collected were subjected to Fisher's Analysis of Variance Technique and treatment means were compared by using Duncan's Multiple Range test at 5% probability (Steele and Torrie, 1984).

RESULTS AND DISCUSSION

Number of days to bud sprout: The combinations of cytokinins and the strains significantly affected the number of days to bud sprout (Table 1). The combination consisting of 1.25 mg L^{-1} BA+ 1.25 mg L^{-1} Kinetin took minimum days (31.65) to bud sprout, while the combination consisting of 2.5 mg L^{-1} BA+ 2.5 mg L^{-1} Kinetin took maximum days (43.68) to bud sprout as some cultures on this combination developed callus that might have caused delay in bud sprouting. The explants of PKJ-3 sprouted earlier taking minimum days (32.02), followed by those of PKJ-6 (34.03 days). The explants of PKJ-2 delayed in bud sprouting taking maximum days (41.69) and it remained statistically at par with those of PKJ-5 (41.25 days). The interaction between combinations and strains was statistically non-significant.

Number of days to bud sprout was significantly affected by the cytokinins, their concentrations and strains (Table 2). The explants in response to BA took significantly fewer days (24.57) than that of Kinetin (53.65). The lowest concentration (1.25 mg L^{-1}) of cytokinins was better than that of the other two concentrations as explants took minimum days (31.50) for bud sprouting, while the highest concentration (5.0 mg L^{-1}) of cytokinins caused delay in bud sprouting up to 47.39 days. The reason of delay in bud sprouting could be the callus formation in some cultures at the highest concentration of BA. The explants of PKJ-3 took the minimum time (33.98 days) to bud sprout, followed by PKJ-6 (35.54 days). While the explants of PKJ-2 took the maximum time (44.34 days) to bud sprout and it was statistically at par with PKJ-5 (42.82 days). The differences shown by the strains for this parameter might be due to variation in endogenous levels of growth regulators. The interaction between cytokinins and their concentrations was significant (Table 3). The explants in response to 2.5 BA mg L^{-1} sprouted earliest of all taking minimum number of days (20.44), but it was statistically alike with 1.25 mg L^{-1} BA (21.43 days). Both were more effective than that of 5.0 mg L^{-1} BA (31.83 days). While, 5.0 mg L^{-1} Kinetin caused maximum delay (62.95 days) to bud sprout. As the concentration of Kinetin increased it has the suppressing effect on the buds to sprout. The other reason of delay in bud sprouting might be vitrification at the highest concentration of Kinetin (Fig. 1). It was noted that Kinetin in combination with BA showed a slight

Table 1: Shoot parameters as affected by cytokinins (in combination) × strains interaction

Cytokinins (mg L^{-1})		Jojoba Strains						
BA	Kinetin	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	Average
No. of days to bud sprout								
1.25	1.25	31.25	35.75	26.92	31.92	35.25	28.83	31.65c
2.50	1.25	37.50	41.17	30.57	35.33	40.58	33.50	36.44b
2.50	2.50	42.83	48.17	38.58	44.92	47.92	39.75	43.68a
Average		37.19b	41.69a	32.02d	37.39b	41.25a	34.03c	
Length of primary shoot (cm)								
1.25	1.25	3.87	2.69	4.28	3.15	2.77	3.82	3.43a
2.50	1.25	3.04	2.46	3.85	2.82	2.56	3.56	3.10b
2.50	2.50	2.40	2.01	2.94	2.20	1.97	2.72	2.37c
Average		3.10b	2.36c	3.69a	2.72c	2.43c	3.36ab	
No. of nodes per primary shoot								
1.25	1.25	3.25ab	2.25cde	3.58a	2.42cd	2.08de	3.08b	2.78a
2.50	1.25	2.17cde	1.50f	3.33ab	2.50c	1.67f	3.25ab	2.40b
2.50	2.50	1.42fg	1.25g	2.25cde	1.33fg	1.17g	2.00e	1.57c
Average		2.28c	1.67d	3.06a	2.08c	1.64d	2.78b	
No. of shoots produced per explant								
1.25	1.25	2.92bc	2.33ef	3.33a	2.75cd	2.42de	3.17ab	2.82a
2.50	1.25	2.17f	1.58g	3.25ab	2.58de	1.83g	3.08abc	2.42b
2.50	2.50	1.25h	1.00h	1.83g	1.25h	1.17h	1.67g	1.36c
Average		2.11b	1.64c	2.81a	2.19b	1.81c	2.64a	
Percentage of sprouted explants								
1.25	1.25	53.33	40.00	60.00	40.00	33.33	53.33	46.67a
2.50	1.25	40.00	26.67	46.67	40.00	26.67	46.67	37.78b
2.50	2.50	40.00	20.00	40.00	26.67	26.67	33.33	31.11c
Average		44.44a	28.89b	48.89a	35.56b	28.89b	44.44a	

Means sharing similar letter(s) in a group for each parameter are non-significant at $\alpha = 5\%$ (DMR test)

Fig. 1: Vitrification of an explant on MS medium supplemented with 5.0 mg L^{-1} Kinetin

improvement over Kinetin alone, while BA alone at the rate of 1.25 or 2.5 mg L^{-1} remained better than that of combination with Kinetin. The results of the present study supported the findings of Lee (1988), Llorente *et al.* (1996) and Llorente and Apostolo (1998) who described that Kinetin, as a cytokinin source, was inferior to BA. The results also confirmed the findings of Kacker *et al.* (1993) who produced axillary shoots on MS medium + 0.5 mg L^{-1} Kinetin + 1 mg L^{-1} BA within a month of culture from nodal segments of female plants of jojoba. However, the data recorded by Elhag *et al.* (1998) revealed a significant genotypic effect and a medium composition (growth regulators) effect after 56 days in shoot-tip explants of jojoba on MS or B5 basal medium containing a combination of IAA and BA at 0, 0.3 and 3.0 mg L^{-1}.

Length of primary shoot (cm): The effect of cytokinins combinations and strains on the parameter under study was found significant, however, the interaction between combinations and strains was statistically non-significant (Table 1). The combination of 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin was significantly better (3.43 cm) than the other two combinations, because lower concentrations of cytokinins (in combinations) favoured the growth of primary shoot compared to higher ones. The explants of PKJ-3 attained the maximum shoot length (3.69 cm) and remained at par with that of PKJ-6 (3.36 cm), while the explants of PKJ-2 attained the minimum one (2.36 cm) and were at par with that of PKJ-5 (2.43 cm). Overall, the explants of PKJ-3 attained the maximum shoot length (4.28 cm) in response to combination of 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin, while those of PKJ-5 attained the minimum length (1.97) in response to combination of 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin.

The data presented in Table 2 revealed that the length of primary shoot was significantly increased by BA (4.39 cm) as compared to Kinetin (1.46 cm). The minimum concentration of cytokinins (1.25 mg L^{-1}) caused the maximum length of primary shoot (3.67 cm) and vice versa. The explants of PKJ-3 significantly attained the maximum shoot length (3.49) among other strains due to its genotypic response. Interaction between cytokinins and their concentrations (Table 3) exhibited that BA concentrations, 1.25 or 2.5 mg L^{-1}, were more effective and statistically similar in effect as these resulted in greater length of primary shoot (4.81 and 4.79 cm, respectively) than that of 5.0 mg L^{-1} BA (3.56 cm). It was probably due to the earlier sprouting of explants at both the concentration of BA than the highest concentration of BA. As the concentration of Kinetin increased it reduced the shoot length gradually, because the buds sprouted late on media containing Kinetin. The strains significantly reacted to the cytokinins (Table 4). The explants of PKJ-3 produced the longer primary shoot (5.02 cm) in response to BA, while the explants of PKJ-2 produced the shorter primary shoot (1.07 cm) in response to Kinetin. Length of primary shoot was affected significantly by the interaction of strains and concentrations (Table 5). The explants of PKJ-3 developed longer shoot (4.38 cm) at 1.25 mg L^{-1} that was statistically at par with PKJ-6 (4.13 cm) for same concentration, while the explants of PKJ-5 developed the shorter shoot (1.77 cm) at 5.0 mg L^{-1} that was not statistically different from that of PKJ-2 (1.82 cm) at same concentration. Interaction among three factors was statistically significant (Table 6). The maximum length of primary shoot (5.47 cm) was recorded in the explants of PKJ-3 cultured on the medium supplemented with 1.25 mg L^{-1} BA (Fig. 2) that was at par with the explants of same strain for 2.5 mg L^{-1} BA (5.33 cm), while the minimum shoot length (0.60 cm) was recorded by the explants of PKJ-2 cultured on the medium supplemented

Fig. 2: Shoot formation from nodal explant of PKJ-3 on MS medium supplemented with 1.25 mg L^{-1} BA

Table 2: Shoot parameters as affected by cytokinins, their concentrations and strains

Cytokinins	No. of days to bud sprout	Length of primary shoot (cm)	No. of nodes per primary shoot	No. of shoots produced per explant	Percentage of sprouted explants
B = BA	24.57b	4.39a	3.49a	3.77a	58.15a
K = Kinetin	53.65a	1.46b	1.16b	1.34b	39.26b
Conc. mg L^{-1}					
C_1 = 1.25	31.50c	3.67a	2.88a	2.19c	60.00a
C_2 = 2.50	38.44b	2.92b	2.40b	2.83a	45.56b
C_3 = 5.00	47.39a	2.19c	1.69c	2.66b	40.56c
Strains					
S_1 = PKJ-1	37.87c	2.99c	2.32b	2.52c	46.67b
S_2 = PKJ-2	44.34a	2.47e	1.75d	2.08d	36.67c
S_3 = PKJ-3	33.98d	3.49a	2.89a	3.15a	61.11a
S_4 = PKJ-4	40.06b	2.83d	2.30b	2.51c	50.00b
S_5 = PKJ-5	42.88a	2.54e	1.96c	2.14d	40.00c
S_6 = PKJ-6	35.54d	3.24b	2.74a	2.95b	57.78a

Means sharing similar letter(s) in a group for each parameter are non-significant at α = 5% (DMR test)

Table 3: Shoot parameters as affected by cytokinins × concentration interaction

Cytokinins × concentration	No. of days to bud sprout	Length of primary shoot (cm)	No. of nodes per primary shoot	No. of shoots produced per explant
B×C_1	21.43e	4.81a	4.09a	3.03b
B×C_2	20.44e	4.79a	3.79b	4.19a
B×C_3	31.83d	3.56b	2.59c	4.09a
K×C_1	41.60c	2.54c	1.67d	1.35cd
K×C_2	56.45b	1.04d	1.02e	1.46c
K×C_3	62.95a	0.82e	0.80e	1.22d

Means sharing similar letter(s) for each parameter are non-significant at α = 5% (DMR test)

Table 4: Shoot parameters as affected by cytokinins×strains interaction

Cytokinins ×strains	Length of primary shoot (cm)	No. of nodes per primary shoot	No. of shoots produced per explant	Cytokinins ×strains	Length of primary shoot (cm)	No. of nodes per primary shoot	No. of shoots produced per explant
B×S_1	4.56c	3.58b	3.84c	K×S_1	1.43h	1.06f	1.19f
B×S_2	3.86e	2.67d	3.09d	K×S_2	1.07j	0.82g	1.06f
B×S_3	5.02a	4.18a	4.56a	K×S_3	1.96f	1.60e	1.75e
B×S_4	4.28d	3.51b	3.78c	K×S_4	1.38hi	1.08f	1.24f
B×S_5	3.84e	2.98c	3.07d	K×S_5	1.24i	0.94fg	1.21f
B×S_6	4.76b	4.02a	4.29b	K×S_6	1.72g	1.46e	1.60e

Means sharing similar letter(s) for each parameter are non-significant at $\alpha = 5\%$ (DMR test)

Table 5: Shoot parameters as affected by concentration×strains interaction

Conc. × strains	Length of primary shoot (cm)	No. of shoots produced per explant	Conc. × strains	Length of primary shoot (cm)	No. of shoots produced per explant
C_1×S_1	3.74c	2.16f	C_2×S4	2.75f	2.69cde
C_1×S_2	3.01e	1.71g	C_2×S5	2.63fg	2.59cde
C_1×S_3	4.38a	2.86bc	C_2×S6	3.11e	3.16a
C_1×S_4	3.58c	2.05f	C_3×S1	2.19h	2.69cde
C_1×S_5	3.21de	1.81g	C_3×S2	1.82i	2.03f
C_1×S_6	4.13a	2.56d	C_3×S3	2.72f	3.28a
C_2×S_1	3.06e	2.71cde	C_3×S4	2.17h	2.79cd
C_2×S_2	2.56fg	2.49e	C_3×S5	1.77i	2.02f
C_2×S_3	3.36d	3.32a	C_3×S6	2.49g	3.12ab

Means sharing similar letter(s) for each parameter are non-significant at $\alpha = 5\%$ (DMR test)

with 5.0 mg L^{-1} Kinetin, possibly because the buds sprouted at the latest of all and had minimum time for the growth of primary shoot. Kinetin in combination with BA showed improvement as compared to Kinetin alone, while BA (alone) at the rate of 1.25 or 2.5 mg L^{-1} remained better than combination with Kinetin. Strong negative linear correlation between the length of primary shoot and the number of days to bud sprouting was recorded (Table 7). The results showed the superiority of BA (alone) at the rate of 1.25 or 2.5 mg L^{-1} over the other concentrations of cytokinins or combinations of cytokinins and the combination attempted by Jacoboni and Standardi (1987) who used Zeatin and GA_3 instead of BA or Kinetin. However, Elhag *et al*. (1998) reported that lower BA/IAA favored shoot elongation and callus production.

Number of nodes per primary shoot: The combinations of cytokinins, strains and the interaction between combinations and strains were statistically significant in effect for the parameter under study (Table 1). The combination consisting of 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin, produced significantly more nodes per primary shoot (2.78) than the other two combinations, because lower concentrations of cytokinins (in combinations) have already favoured earliness of sprouting and length of primary shoot that resulted in more number of nodes. The explants of PKJ-3 attained the maximum nodes (3.06) as it had sprouted earlier and attained maximum length of primary shoot among strains. The same strain attained the highest number of nodes in response to combination of 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin, while PKJ-5 attained the lowest number of nodes (1.17) and it was at par with that of PKJ-2 (1.25) in response to combination of 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin.

The number of nodes per primary shoot was significantly affected by cytokinins, concentration and strains (Table 2). The explants cultured on media containing BA, produced significantly higher number of nodes per primary shoot (3.49) than that of Kinetin (1.16), because of delay in bud sprouting and shorter primary shoot from explants cultured on media containing Kinetin. Maximum number of nodes per primary shoot (2.88) was recorded on the lowest concentration of cytokinins and vice versa. The explants of PKJ-3 had the maximum number of nodes per primary shoot (2.89), followed by those of PKJ-6 (2.74), both were statistically at par with each other. The explants of PKJ-2 had the minimum number of nodes per primary shoot (1.75) as it was slowest in bud sprout and had the shortest primary shoot. Interaction between cytokinins and concentrations was statistically significant (Table 3). The explants in response to 1.25 mg L^{-1} BA, produced maximum number of nodes per primary shoot (4.09), while those in response to 5.0 mg L^{-1} Kinetin did the minimum one (0.80). This parameter was also affected significantly by the interaction between cytokinins and strains (Table 4). The explants from PKJ-3 gained the maximum number of nodes per primary shoot (4.18) in response to BA due to earliness in sprouting and longer primary shoot, but it remained statistically at par with those of PKJ-6 (4.02) for same cytokinin. The explants from PKJ-2 gained the minimum number of nodes per primary shoot (0.82) in response to Kinetin due to maximum delay in bud sprouting and shorter primary shoot, but it remained statistically at par with those of PKJ-5 (0.94). As Kinetin in combination with BA showed improvement in time of bud sprouting and length of primary shoot compared to

Kinetin (alone) previously, hence, number of nodes also increased. As 1.25 mg L^{-1} BA (alone) remained better for time of bud sprouting and length of primary shoot compared to its combination with Kinetin previously, hence it expressed the same pattern for the number of nodes. It is also obvious from the coefficient of correlation obtained that the number of nodes per primary shoot was positively correlated with the length of primary shoot (Table 7). Previously, Jacoboni and Standardi (1987) obtained 3.5 cm long shoots with 3-4 nodes from new shoots on MS + 2 mg L^{-1} Zeatin (ZT) + 2 mg L^{-1} GA_3 + 0.001 mg L^{-1} NAA i.e., with a different combination of growth regulators to that used in the present study.

Number of shoots produced per explant: The number of shoots per explant was significantly affected by the combinations of cytokinins and the strains (Table 1). The combination 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin produced significantly more number of shoots (2.82) than the other two combinations, possibly because this combination of cytokinins has already favoured early bud sprouting, increased length of primary shoot and more number of nodes per primary shoot, so also boasted the number of shoots per explant. Combination 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin resulted in minimum number of shoots per explant (1.36) as this combination delayed bud sprouting, decreased length of primary shoot and reduced number of nodes per primary shoot. Regarding strains, the explants from PKJ-3 developed maximum number of shoots per explant (2.81) which showed statistical similarity with those from PKJ-6 (2.64), while the explants from PKJ-2 developed minimum number of shoots per explant (1.64) which remained statistically at par with those from PKJ-5 (1.81). The interaction between cytokinins combinations and strains was statistically significant. The explants of PKJ-3 produced the maximum number of shoots (3.33) in response to 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin, as it had sprouted earlier, carried longer primary shoot and more nodes per primary shoot under this combination. While those of PKJ-2 produced the minimum number of shoots (1.00) in response to combination 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin.

The parameter was significantly affected by cytokinins, their concentrations and strains (Table 2). Number of shoots produced per explant was significantly greater in BA (3.77) than that of Kinetin (1.34). The middle concentration of cytokinins (2.5 mg L^{-1}) produced the maximum number of shoots (2.83), followed by the highest concentration (5.0 mg L^{-1}) with 2.66 shoots per explant. As regard the strains, the explants of PKJ-3 produced the maximum number of shoots (3.15), while those of PKJ-2 did the minimum ones (2.08) and remained statistically at par with PKJ-5 (2.14 shoots). Interaction between cytokinins and concentrations for the parameter under study was statistically significant (Table 3). BA at the rate of 2.5 or 5.0 mg L^{-1} (4.19 and 4.09 shoots, respectively) were more effective (both with similar effect) than that of 1.25 mg L^{-1} BA (3.03 shoots). All concentrations of Kinetin produced less number of shoots than that of BA. The response of strains to the cytokinins differed significantly (Table 4). The explants of PKJ-3 produced maximum number of shoots (4.56) in response to BA, followed by those of PKJ-6 (4.29) for same cytokinin, while the explants of PKJ-2 produced the minimum number of shoots (1.06) in response to Kinetin which was statistically alike with those of PKJ-1, PKJ-4 and PKJ-5 for same cytokinin. The shoot number was also affected significantly by the interaction of strains and the concentrations used (Table 5). The explants of PKJ-3 produced maximum number of shoots (3.32) at the middle concentration of cytokinins, followed at the highest concentration (3.28). The explants of PKJ-6 ranked 2nd with 3.16 shoots at middle concentration and 3.12 shoots at the highest concentration of cytokinins. All these four remained statistically at par with each other. Interaction among three factors for this parameter was also statistically significant (Table 6). Maximum number of shoots (5.0) was produced by the explants of PKJ-3 at the highest concentration of BA that was at par with those of same strain at middle concentration of BA (4.87) and those of PKJ-6 (4.80) at the highest concentration of BA (Fig. 3 and 4). All these three were statistically similar to each other. Both strains gave good response to the

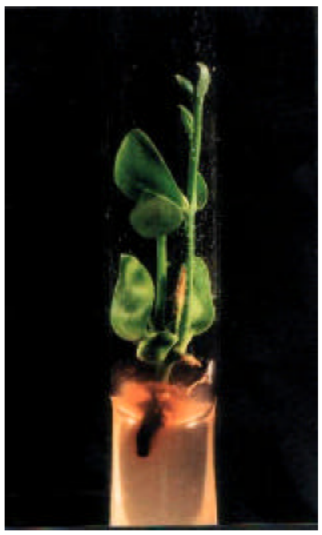

Fig. 3: Shoot formation from nodal explant of PKJ-3 on MS medium supplemented with 2.5 mg L^{-1} BA

Table 6: Shoot parameters as affected cytokinins×concentration×strains interaction

Cytokinins× concentrations ×strains	Length of primary shoot (cm)	No. of shoots produced per explant	Cytokinins× concentrations ×strains	Length of primary shoot (cm) per explant	No. of shoots produced
$B\times C_1\times S_1$	5.06b	3.07i	$K\times C_1\times S_1$	2.41ij	1.25lmno
$B\times C_1\times S_2$	4.11e	2.33j	$K\times C_1\times S_2$	1.92k	1.08no
$B\times C_1\times S_3$	5.47a	3.80gh	$K\times C_1\times S_3$	3.29fg	1.92k
$B\times C_1\times S_4$	4.73c	2.93i	$K\times C_1\times S_4$	2.43i	1.17mno
$B\times C_1\times S_5$	4.30de	2.53j	$K\times C_1\times S_5$	2.11jk	1.08no
$B\times C_1\times S_6$	5.17ab	3.53h	$K\times C_1\times S_6$	3.08gh	1.58kl
$B\times C_2\times S_1$	5.08b	4.20def	$K\times C_2\times S_1$	1.03mn	1.22lmno
$B\times C_2\times S_2$	4.43cd	3.87fgh	$K\times C_2\times S_2$	0.69op	1.11no
$B\times C_2\times S_3$	5.33ab	4.87ab	$K\times C_2\times S_3$	1.40l	1.78k
$B\times C_2\times S_4$	4.55cd	3.93efg	$K\times C_2\times S_4$	0.95mno	1.45lmn
$B\times C_2\times S_5$	4.31de	3.73gh	$K\times C_2\times S_5$	0.95mno	1.44lmn
$B\times C_2\times S_6$	5.04b	4.53bcd	$K\times C_2\times S_6$	1.18lm	1.78k
$B\times C_3\times S_1$	3.54f	4.27de	$K\times C_3\times S_1$	0.83nop	1.11no
$B\times C_3\times S_2$	3.05gh	3.07i	$K\times C_3\times S_2$	0.60p	1.00o
$B\times C_3\times S_3$	4.26de	5.00a	$K\times C_3\times S_3$	1.18lm	1.56klm
$B\times C_3\times S_4$	3.57f	4.47cd	$K\times C_3\times S_4$	0.76nop	1.11no
$B\times C_3\times S_5$	2.89h	2.93i	$K\times C_3\times S_5$	0.64op	1.11no
$B\times C_3\times S_6$	4.08e	4.80abc	$K\times C_3\times S_6$	0.90mnop	1.44lmn

Means sharing similar letter(s) for each parameter are non-significant at $\alpha = 5\%$ (DMR test)

Table 7: Coefficients of correlation for different shoot parameters studied under cytokinins (in combination) and cytokinins (alone)

Correlations	Cytokinins (in combination)	Cytokinins (alone)
Length of primary shoot-No. of days to bud sprout	-0.943	-0.986
No. of nodes per primary shoot-Length of primary shoot	+0.969	+0.985
No. of shoots per explant-No. of days to bud sprout	-0.953	-0.822

Fig. 4: Shoot formation from nodal explant of PKJ-6 on MS medium supplemented with 5.0 mg L^{-1} BA

highest concentration of BA. The minimum number of shoots (1.0 shoot) was produced by PKJ-2 in response to 5.0 mg L^{-1} Kinetin, expressing almost similar effect for other concentrations of Kinetin. It was also recorded that the number of shoots produced per explants was negatively correlated with the number of days to bud sprouting (Table 7). As Kinetin in combination with BA showed improvement in time of bud sprouting, length of primary shoot, number of nodes compared to Kinetin (alone) previously, so it also enhanced the number of shoots per explant. BA (alone) at three concentrations remained better as compared to its combination with Kinetin. Hence, it expressed the same pattern as did previously for already recorded parameters. The results obtained are in line with the findings of Llorente and Apostolo (1998), Agraval *et al*. (2002), Prakash *et al*. (2003) and Tyagi and Prakash (2004) who found significant increase in number of shoots by BA and differential response of genotypes to concentrations of BA. According to Llorente *et al*. (1996), BA was better in effect than Kinetin. But, according to Elhag *et al*. (1998), the higher BA/IAA ratio (3.0/0.0 and 3.0/0.3 mg L^{-1}) favored shoot multiplication with producing the maximum response and the highest number of newly formed shoots per explant was produced by female plants.

Percentage of sprouted explants: Although the combinations of cytokinins and the strains significantly affected the percentage of sprouted explants, but the interaction between the combinations and the strains was statistically non-significant (Table 1). The combination 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin increased significantly the percentage of sprouted explants (46.67) as compared to the other two combinations, because this combination, that contains lower concentrations of cytokinins, has already favoured early bud sprouting,

increased length of primary shoot, number of nodes per primary shoot and number of shoots per explant. The combination consisting of 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin had minimum percentage of sprouted explants (31.11) as it had previously minimum values for length of primary shoot, number of nodes and number of shoots per explant. The explants of PKJ-3 sprouted to the maximum (48.89%) which were statistically at par with PKJ-6 and PKJ-1 (44.44%), while the explants of PKJ-2 and PKJ-5 sprouted to the minimum (28.89%) and remained statistically at par with those of PKJ-4 (35.56%). Overall the explants of PKJ-3 sprouted maximum (60.00%) in response to the combination 1.25 mg L^{-1}BA + 1.25 mg L^{-1} Kinetin, as the explants had sprouted earlier, carried longer primary shoot, more nodes and number of shoots. While PKJ-2 had the minimum sprouted explants (20.00%) in response to combination of 2.5 mg L^{-1} BA + 2.5 mg L^{-1} Kinetin, because of minimum values of all previous parameters recorded.

Cytokinins, their concentrations and the strains significantly affected the sprouting of explants (Table 2). Maximum percentage of sprouted explants (58.15) was obtained when BA was added in culture medium and the minimum one (39.26) when Kinetin was added. The percentage of sprouted explants significantly increased to the maximum (60.00) at the lowest concentration of cytokinins and decreased to the minimum (40.56) at the highest concentration of cytokinins. As for as the strains are concerned, PKJ-3 led the other strains with 61.11% sprouted explants, followed by PKJ-6 with 57.78% sprouted explants. Both strains were statistically alike. While, PKJ-2 trailed the other strains with 36.67% sprouted explants, followed by PKJ-5 with 40.00% sprouted explants. Both strains were statistically at par with each other. Two way interactions and three way interactions among three factors were statistically non-significant for this parameter under study. As Kinetin in combination with BA showed improvement in time of bud sprouting, length of primary shoot, number of nodes and number of shoots per explant as compared to Kinetin (alone), that is why it also enhanced percentage of sprouted explants. BA (alone) at three concentrations remained better as compared to its combination with Kinetin. Hence, it depicted almost the same pattern as did for parameters recorded previously. The sequel of the present study was in conformity with the findings of Kacker *et al.* (1993), Llorente *et al.* (1996), Llorente and Apostolo (1998), Agrawal *et al.* (1999), Agraval *et al.* (2002), Prakash *et al.* (2003) and Tyagi and Prakash (2004), all of them found highly variable response of clones to cytokinins or combinations of cytokinins.

Callus formation: Some explants also developed calluses at the highest level (5.0 mg L^{-1}) of BA (alone) or in combinations of BA + Kinetin. Previously, Llorente *et al.* (1996) had also observed callus formation in the presence of BA and light hyperhydricity in 30% of explants. Sardana and Batra (1998) also noted callus in leaf explants cultures on MS + 1.0 mg L^{-1} NAA + 3 or 5 mg L^{-1} BA. However, Elhag *et al.* (1998) reported that lower BA/IAA favored callus production.

CONCLUSIONS

This study describes a protocol for direct shoot initiation *in vitro* from nodal explants of different strains of jojoba by manipulation of cytokinins alone or combinations of cytokinins at various concentrations. BA alone at 1.25 mg L^{-1} proved more effective for many shoot parameters except number of shoots. PKJ-3 strain was the most responsive to cytokinins alone or in combinations. Kinetin in combination with BA showed improvement over Kinetin alone. The combination consisting of 1.25 mg L^{-1} BA + 1.25 mg L^{-1} Kinetin significantly increased values of shoot growth parameters.

REFERENCES

Agraval, V., S. Prakash, A. Altman, M. Ziv and S. Izhar, 1999. Differential hormonal requirements for clonal propagation of male and female jojoba plants. Proceedings of the 9th International Congress of the International Association of Plant Tissue Culture and Biotechnology, Jerusalem, Israel, 14-19 June 1998, pp: 25-28.

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

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

Chandra, R. and M. Mishra, 2003. Comprehensive Micropropagation of Horticultural Crops. International Book Distributing Co. Lucknow, U.P. (India).

Elhag, H., M.M. El-Olemy, J.S. Mossa, S.S. Tag El Din, M.F. Al-Zoghet and A.M.A. Al-Alsheikh, 1998. *In vitro* propagation of jojoba. Program Abstracts of 1998 Annual Conference on New Crops and New Uses: Biodiversity and Sustainability, 8-11 November, 1998, Phoenix, Arizona, USA.

Jacoboni, A. and A. Standardi, 1987. Tissue culture of jojoba (*Simmondsia chinensis* Link). Acta Horticulturae, 212: 557-560.

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

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

Lee, C.W., 1988. Application of plant biotechnology for clonal propagation and yield enhancement in jojoba. Proceedings of the 7th International Conference on Jojoba and Its Uses, 17-22 January, 1988, Phoenix, Arizona, USA., pp: 102-111.

Llorente, B.N. Apostolo, L.H. Princen and C. Rossi, 1996. Micropropagation of jojoba: Effect of hormonal and clonal variation at the multiplication stage. Proceedings of the 9th International Conference on Jojoba and Its Uses, 25-30 September 1994, Catamarca, Argentina, pp: 50-52.

Llorente, B.E. and N.M. Apostolo, 1998. Effect of different growth regulators and genotypes on *in vitro* propagation of Jojoba. Newzealand J. Crop Hortic. Sci., 26: 55-62.

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

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

Reddy, Y.N., 2003. Effect of different concentration of auxins on rooting and root characters of air and ground layers of jojoba (*Simmondsia chinensis* (Link) C.K. Schneider). Ethopian J. Sci., 26: 155-159.

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

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

Singh, K.J., H. Nayyar, A. Dutta and K.K. Dhir, 2003. Rhizogenetic studies of jojoba: Hormone effect, rooting medium and seasonal variation. Indian Forester, 129: 1405-1411.

Steele, R.G.D. and J.H. Torrie, 1984. Principles and Procedures of Statistics: A Biometrical Approach, McGraw Hill Book Int. Co., Singapore.

Tyagi, R.K. and S. Prakash, 2004. Genotypes and sex specific protocols for *in vitro* micropropagation and medium term conservation of jojoba. Biol. Plant., 48: 19-23.