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In vitro Shoot Multiplication of Six Promising Strains of Jojoba (Simmondsia chinensis)

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Abstract: Nodal segments, 1.5-3.0 cm long with 1 or 2 nodes taken from *in vitro* shoot cultures of six jojoba strains, were cultured on MS medium supplemented with 2.5 or 5.0 mg L^{-1} BA or 2.5 mg L^{-1} BA + 2.5 mg L^{-1} of one of NAA, IAA or IBA. Different growth parameters regarding shoot multiplication were recorded. BA at the rate of 2.5 mg L^{-1} caused the earliest sprouting of buds and produced the longest primary shoot, maximum number of nodes per primary shoot. However, BA at the rate of 5.0 mg L^{-1} produced the maximum number of shoots per explant. While the combination consisting of 2.5 mg L^{-1} BA + 2.5 mg L^{-1} IBA delayed the bud sprouting and produced the minimum number of shoots, the shortest primary shoot and the minimum number of nodes per primary shoot. Among the strains PKJ-3 led the other strains in performance, while PKJ-2 trailed in all parameters. The number of shoots and the length of primary shoot were significantly affected by the interactions between strains and growth regulators combinations.

Key words: Growth regulators, genotypes, jojoba, micropropagation, regeneration, Simmondsia chinensis

INTRODUCTION

Jojoba [Simmondsia chinensis (Link.) Schneider] belonging to family Simmondsiaceae, is a dioecious, longlived evergreen perennial desert shrub which grows wild in Sonoran Desert of Mexico and USA with natural lifespan over 100 years. Its seeds contain about 50% liquid wax that proved an alternative to sperm whale oil and is being utilized in cosmetics, lubricants and pharmaceuticals etc. Its deep tap root system helps to survive in drought conditions. So, it could be a prime plant for desert areas of Pakistan i.e., Cholistan, Thal and Thar. Due to its unique oil characteristics, this precious bush attracted the attention of researchers recently for its propagation methods throughout the world. Jojoba plantations are raised through seeds, seedlings, rooted cuttings and plantlets produced from tissue culture. Micropropagation is a technique of large scale mass multiplication of planting material within short possible time. 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). The advantages of using asexual propagules in commercial jojoba plantations are now widely known and appreciated. Superior clones of jojoba, when used in field production, will allow uniform, predictable plant growth and yield. The use of simple node, double node and three node cuttings from different individuals of jojoba

by applying different plant hormones will increase the total number of propagules obtained from a stock plants (Cao and Gao, 2003). However, the maximum number of possible propagules will still be limited to one or two thousands per year. Shoots established through tissue culture, however, may give rise to multiple shoots that can be rooted. Thus a single explant source could conceivably provide thousands of new plantlets a year.

Jacoboni and Standardi (1987) obtained 3.5 cm long shoots with 3-4 nodes after 30 days of subculture from new shoots on Murashige and Skoog (MS) medium $+2 \text{ mg L}^{-1} \text{ Zeatin} (ZT) + 2 \text{ mg L}^{-1} \text{ Gibbrellic acid } (GA_3) +$ 0.001 mg L⁻¹ Naphthalene acetic acid (NAA). However, Kacker et al. (1993) produced axillary shoots from nodal segments of female jojoba on MS + 1 mg L^{-1} Benzyladinine (BA) ± 0.5 mg L⁻¹ Kinetin within 30 days of culture. Llorente et al. (1996) observed clonal differences in response and significantly greater shoot multiplication rate on MS + 1 mg L^{-1} BA than that of MS + 1 mg L^{-1} Kinetin. BA in combination with 0.2 mg L⁻¹ Indolebutyric acid (IBA) did not enhance multiplication and number of leaves. Elhag et al. (1998) cultured 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 each in a factorial arrangement. After 56 days in culture, the data revealed 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 M₃) 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) reported highly variable response of jojoba clones and 4.6-fold increase in shoot numbers after 30 days for in vitro propagated explants on MS + 1 mg L⁻¹ BA. Agrawal et al. (1999) achieved proliferation in 80% of male explants with an average of 3.5 shoots per explant in the presence of 2.25 mg L⁻¹ BA and in 100% of female explants with an average of 4.7 shoots per explant in the presence of 4.5 mg L⁻¹ BA incorporated into MS or B5 media. The other cytokinins did not improve morphogenic response over BA. Khanam et al. (1999) observed that shoot proliferation from single stem segments of female jojoba was greatest on MS + 4 mg L^{-1} BA. Roussos et al. (1999) gained successful shoot proliferation of seedling explants of jojoba with a maximum number of 15.2 shoots per original explant on a modified Driver Kuniyuki medium supplemented with various concentrations of BA alone and in combination with silver nitrate. Gao and Cao (2001) reported that branch segments of asecptic jojoba seedlings cultured either on MS + 1.5 mg L⁻¹ Zeatin (ZT) $+ 0.2 \text{ mg L}^{-1} \text{ IBA or on MS} + 2 \text{ mg L}^{-1} \text{ ZT} + 0.5 \text{ mg L}^{-1}$ NAA resulted in successful cultures. However, the best culture medium for rooting was half strength MS + 1.0 mg L^{-1} IBA + 1.0 mg L^{-1} Indole Acetic Acid (IAA). Agrawal et al. (2002) recorded an average of 2.7 shoots from the nodal explants of female jojoba clone EC 33198 cultured on MS + 4.5 mg L⁻¹ BA. Percentage of nodal explants producing multiple shoots, increased significantly when in vitro raised shoots were used as explant source. Sardana and Batra (1998) cultured shoot tips on MS medium supplemented with NAA and BA, both at 1.0 mg L⁻¹. They obtained complete plantlets with 1 or 2 thick roots per shoot after an incubation period of 35-40 days. An increase in BA concentration suppressed rooting but increased shoot length of plantlets. Benzioni et al. (2003) reported that the jojoba shoots multiplied in ventilated boxes, elongated much faster, developed more and bigger leaves, had higher biomass and produced more propagules than shoots grown in closed boxes with low ventilation. The increase in shoot elongation ranged from 1.3 to 4.0 fold in different clones. Hassan (2003) reported that encapsulated buds exhibited best shoot development on MS medium supplemented with 1.0 mg L⁻¹ BAP, 40 mg L⁻¹ adenine sulfate and 3.0 mg L⁻¹ IAA. Prakash et al. (2003) found that in vitro raised male and female jojoba shoots exhibited differential morphogenic behavior under

the influence of various adjuvants. BA in combination with different levels of Triiodobenzoic acid (TIBA) promoted shoot multiplication in female explants. However, BA alone proved to be the best for differentiation of shoots in both male (2.25 mg L⁻¹) as well as female (4.5 mg L⁻¹) explants. Mills et al. (2004) observed differential response of jojoba clones to ventilation in growth and multiplication rate during multiplication stage. They advised to include Magenta boxes equipped with vented lids as the preferred growing vessels. Tyagi and Prakash (2004) reported 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 containing 2.25 mg L⁻¹ BA.

Basically jojoba is a slow growing plant; hence, it needs to be selected on the bases of high yield potential as well as on successful establishment through vegetative propagation. The seedling jojoba has genetic heterogeneity and variability in morphology, anatomy and physiology. Direct selection based on identifying potentially promising genotypes and their testing via in vivo and in vitro vegetative propagation techniques for quick and vigorous growth has not only improved the crop performance, but also fulfilled the demands of growers for large quantity of propagules. Literature indicates that various growth regulators, alone or in combinations, with different concentrations have been used for micropropagation of jojoba and differential response of genotypes for growth has been observed. The present investigation is an attempt to propagate available promising jojoba strains by testing different combinations of growth regulators and observing responses of jojoba strains to these growth regulators in terms of different growth parameters during in vitro shoot multiplication stage.

MATERIALS AND METHODS

The studies were conducted in Tissue Culture Laboratory of Agricultural Biotechnology Institute, National Agriculture Research Centre, Islamabad during 2005-2006.

Source of explants and its preparation: The nodal segments, 1.5-3.0 cm long with 1 or 2 nodes, excised aseptically from *in vitro* raised shoots of initial explants that were cultured on MS medium supplemented with $1.25 \,\mathrm{mg}\,\mathrm{L}^{-1}\,\mathrm{BA}$ or on MS $+ 1.25 \,\mathrm{mg}\,\mathrm{L}^{-1}\,\mathrm{BA} + 1.25 \,\mathrm{mg}\,\mathrm{L}^{-1}$ IAA (Fig. 1 and 2) of six strains as characterized



Fig. 1: Shoot initiation of jojoba on MS + $1.25 \text{ mg L}^{-1} \text{ BA}$



Fig. 2: Shoot formation of jojoba on MS + $1.25 \text{ mg L}^{-1} \text{ BA}$ + $1.25 \text{ mg L}^{-1} \text{ IAA}$

previously (Bashir *et al.*, 2006). All cultural manipulation was carried out under Laminar Air Flow Hood.

Culture medium and culture conditions: The explants were placed on solidified Murashige and Skoog medium (1962) containing 3% (w/v) sucrose and 0.7% agar and supplemented with different concentrations of BA alone and its combination with auxins (NAA, IAA and IBA) as given below:

- $MS + 2.5 \text{ mg L}^{-1} BA$
- $MS + 5.0 \text{ mg L}^{-1} BA$
- $MS + 2.5 \text{ mg L}^{-1} BA + 2.5 \text{ mg L}^{-1} NAA$

- $MS + 2.5 \text{ mg L}^{-1} BA + 2.5 \text{ mg L}^{-1} IAA$
- $MS + 2.5 \text{ mg L}^{-1} BA + 2.5 \text{ mg L}^{-1} IBA$

The pH of the media was adjusted to 5.7 using either 0.1 N NaOH or 0.1 N HCl prior to adding 0.7% (w/v) agar. Media were dispensed in 50 mL aliquots into culture flasks (250 mg L $^{-1}$), which were plugged with nonabsorbent 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 $^{-2}$ s $^{-1}$ at 25±2°C. Subculturing was carried out every 2 weeks on fresh shoot multiplication medium with the same composition.

Data recording and statistical analysis: The experiment was laid out in factorial Completely Randomized Design (CRD) with 3 replications and 2 factors i.e., growth regulators and the strains. Four explants of each strain were cultured in each flask per treatment per replication. The data were recorded on the following parameters.

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

Number of shoots produced per explant: The number of shoots arose from the explant within 2 months was recorded and averaged over number of explants per treatment per replication.

Length of primary shoot (cm): The length (cm) attained by the primary shoot that arose from the explant within 2 months 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.

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: Effect of growth regulators supplemented and the strains on the parameter under

study was found statistically significant. However, interaction between two factors was statistically nonsignificant (Table 1). The minimum time for bud sprouting (9.56 days) was taken by those explants cultured on solidified MS medium containing 2.5 mg L⁻¹BA, followed by the combination of 2.5 mg $L^{-1}BA + 2.5$ mg $L^{-1}IAA$ (13.17 days). On the other hand the maximum time (24.67 days) was taken by those explants cultured on MS medium containing 2.5 mg L⁻¹BA + 2.5 mg L⁻¹IBA. As regards the strains, the explants of PKJ-3 took the minimum time for bud sprouting (13.13 days), followed by PKJ-6 (14.40 days), both were statistically at par with each other. While PKJ-2 took maximum time (19.27 days) and it was not statistically different from PKJ-5 (18.53 days). The results obtained are partially in lines with the previous findings of Elhag et al. (1998) who recorded significant genotypic effect and a medium composition (growth regulators) effect after 56 days of culture on MS or B5 + BA + IAA. Previously, Kacker et al. (1993) used Kinetin instead of auxin in combination with BA and produced shoots on MS + 1 mg L^{-1} BA + 0.5 mg L^{-1} Kinetin within 30 days of culture. However, Jacoboni and Standardi (1987) obtained new shoots after 7 days of subculture from new shoots on MS + Zeatin + GA₃ + NAA i.e., very close in time to bud sprout by 2.5 mg L⁻¹ BA (9.56 days). Lower concentration of BA in combination with NAA may induce rooting as Sardana and Batra (1998) obtained complete plantlets with 1 or 2 thick roots per shoot from shoot tips of jojoba cultured on MS + 1 mg L^{-1} BA + 1 mg L⁻¹ NAA after an incubation period of 35-40 days. It may be inferred from the results that BA at the rate of 2.5 mg L⁻¹ is comparatively better to cause early bud

sprouting than BA at the rate of $5.0~{\rm mg~L^{-1}}$ or other combination of growth regulators used for shoot multiplication of jojoba.

Number of shoots produced per explant: The data presented in Table 2 indicate that the growth regulators, strains and their interaction significantly affected this parameter. Number of shoots was significantly greater by BA at the rate of 5.0 mg L^{-1} (14.06) than that of BA 2.5 mg L⁻¹ i.e., higher concentration of BA favoured increase in number of shoots. BA in combination with auxins did not perform satisfactory compared to BA alone. The strains PKJ-3 performed the best with 11.80 shoots per explant among the strains. The interaction between two factors was statistically significant due to differential response of strains to different growth regulators supplemented. Maximum number of shoots (19.00) was produced by PKJ-3 on the medium containing 5.0 mg L⁻¹ BA (Fig. 3), followed by PKJ-6 (17.67) on same medium and PKJ-3 (17.00) on the medium containing BA at the rate of 2.5 mg L⁻¹. All three were statistically at par with each other. The minimum number of shoots (3.33) was produced by PKJ-2 in the medium containing 2.5 mg L⁻¹ $BA + 2.5 \text{ mg L}^{-1}$ IBA that remained statistically at par with the other strains in same medium and with other strains except PKJ-3 and PKJ-6 on MS medium supplemented with $2.5 \text{ mg L}^{-1} \text{ BA} + 2.5 \text{ mg L}^{-1} \text{ NAA}$ and with PKJ-1. PKJ-2 and PKJ-5 on MS medium containing 2.5 mg L⁻¹ $BA + 2.5 \text{ mg L}^{-1} IAA$. BA at the rate of 5 mg L^{-1} showed superiority over the other combinations of growth regulators in this experiment as well as the combination attempted by Jacoboni and Standardi (1987) who obtained

Table 1: Number of days to bud sprout as affected by growth regulators, jojoba strains and their interaction

Growth regulators used (mg L ⁻¹)				Jojoba strai	Jojoba strains							
BA	NAA	IAA	IBA	PKJ-1	PKJ-1 PKJ-2 PKJ-3 PKJ-4 PKJ-5 PKJ-6 A							
2.5	-			9.33	12.67	7.00	9.67	10.33	8.33	Average 9.56e		
5.0	-	-	-	19.00	22.00	14.33	21.00	20.33	15.33	18.67b		
2.5	2.5	-	-	17.67	19.67	12.67	17.00	19.33	13.33	16.61c		
2.5	-	2.5	-	14.00	15.00	10.00	12.67	16.00	11.33	13.17d		
2.5	-	-	2.5	24.33	27.00	21.67	24.67	26.67	23.67	24.67a		
Average				16.87c	19.27a	13.13d	17.00bc	18.53ab	14.40d			

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

Table 2: Number of shoots produced per explant as affected by growth regulators, jojoba strains and their interaction

Growth reg	gulators used	(mg L ⁻¹)			Jojoba strains						
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	Average	
2.5	-	-	-	11.67bc	8.67d-f	17.00a	11.00b-d	11.33b-d	13.67b	12.22b	
5.0	-	-	-	13.33b	10.33с-е	19.00a	12.33bc	11.67bc	17.67a	14.06a	
2.5	2.5	-	-	6.33f-j	4.33ij	7.33f-h	5.67g-j	4.67h-j	6.67f-I	5.83d	
2.5	-	2.5	-	6.33f-j	5.33g-j	10.33с-е	7.33f-h	6.00f-j	8.00e-g	7.22c	
2.5	-	-	2.5	4.00ij	3.33j	5.33g-j	4.33ij	3.67ij	4.67h-j	4.22e	
Average				8.33c	6.40d	11.80a	8.13c	7.47cd	10.13b		

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





Fig. 3: Shoot multiplication of PKJ-3 on MS \pm 5.0 mg L^{-1} BA

Fig. 4: Shoot multiplication of PKJ-6 on MS $+ 2.5 \text{ mg L}^{-1}$ BA

Table 3: Length of primary shoot (cm) from explants as affected by growth regulators, jojoba strains and their interaction

Growth regulators used (mg L^{-1})				Jojoba strains						
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	Average
2.5	95	0.5	54	7.17c	5.33e	8.83a	6.83c	6.17d	7.83b	7.03a
5.0		8 .	30	3.43lm	3.23lm	3.77j-1	3.301m	3.27lm	3.70kl	3.45c
2.5	2.5	314	2	4.53f-I	3.83i-1	4.83e-h	4.43g-j	4.23h-k	4.70e-h	4.435
2.5	1001000 3/5	2.5	54	4.50g-I	4.23h-k	5.23ef	4.67f-h	4.30g-k	5.03e-g	4.66b
2.5		ti l	2.5	2.43n	2.10n	2.80mn	2.33n	2.17n	2.50n	2.39d
Avera	ge			4.41c	3.75e	5.09a	4.31cd	4.03de	4.75b	

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

3.5 multiplication rate on MS + 2 mg L^{-1} Zeatin + 2 mg L^{-1} GA₃ + 0.001 mg L⁻¹ NAA. The results are partially in conformity with the findings of Llorente et al. (1996), Llorente and Apostolo (1998), who observed clonal differences in responses and found that shoot multiplication rate was significantly greater on MS+ 1.0 mg L-1 BA than that of the other cytokinins/auxins in the media. The results supported Elhag et al. (1998) who obtained the highest number of newly formed shoots in explants from female plants by 3 mg L⁻¹ BA or 3 mg L⁻¹ $BA + 0.3 \text{ mg L}^{-1}$ IAA into MS or B5 media. The results were in accordance with the findings of Agrawal et al. (1999) who achieved 3.5 shoots per explant in male by $2.25 \text{ mg L}^{-1} \text{ BA}$ and $4.7 \text{ shoots in female by } 4.5 \text{ mg L}^{-1} \text{ BA}$ into MS or B5 media. Similarly, Khanam et al. (1999) observed higher shoot proliferation on MS + 4 mg L⁻¹ BA. However, Roussos et al. (1999) gained 15.2 shoots per explant by BA alone or BA + silver nitrate on Driver Kuniyuki medium, because they used seedling explant, replaced MS medium with DK medium and added silver nitrate (additional nitrogen source) with BA. So, number of shoots produced in vitro may depend upon source of explant (Gao and Cao, 2001; Agrawal et al., 2002), type of

explants (Hassan, 2003), type of media, growth regulators, their concentrations and supplements, genotypes (Prakash *et al.*, 2003; Tyagi and Prakash, 2004), type of vessels and cultural conditions (Benzioni *et al.*, 2003; Mills *et al.*, 2004).

Length of primary shoot (cm): Length of primary shoot was significantly affected by the growth regulators supplemented, the strains and the interaction between these two factors (Table 3). BA at the rate of 2.5 mg L^{-1} (7.03 cm) was better than BA at the rate of 5.0 mg L^{-1} (3.45 cm) as higher concentration of BA showed suppressing effect in time to bud sprout previously. The effect of BA + NAA or BA + IAA was the same on the length of primary shoot. Overall PKJ-3 with 5.09 cm shoot length led the other strains significantly expressing significant clonal differences among strains for this parameter. Interaction between two factors was statistically significant due to differential response of strains to different concentrations of growth regulators. The longest primary shoot (8.83 cm) was attained by PKJ-3 on the medium containing BA at the rate of 2.5 mg L⁻¹ as bud sprouted earlier and spared more time

Table 4: Number of nodes per primary shoot as affected by growth regulators, jojoba strains and their interaction

Growth regulators used (mg L ⁻¹)				Jojoba strains							
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	Average	
2.5	-	-	-	6.00	4.33	7.33	5.67	5.33	6.67	5.89a	
5.0	-	-	-	2.67	2.33	3.67	3.00	2.67	3.33	2.94c	
2.5	2.5	-	-	4.00	3.67	4.67	4.00	4.00	4.33	4.11b	
2.5	-	2.5	-	4.33	3.33	5.00	4.00	3.67	4.67	4.17b	
2.5	-	-	2.5	2.00	1.67	2.67	2.00	1.67	2.33	2.06d	
Average				3.80c	3.07d	4.67a	3.73c	3.47c	4.27b		

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

to attain maximum length on this medium, followed by PKJ-6 (7.83 cm) on the same medium (Fig. 4). While the shortest primary shoot (2.10 cm) was attained by PKJ-2 on the medium containing $2.5 \text{ mg L}^{-1} BA + 2.5 \text{ mg L}^{-1} IBA$ and it remained statistically at par with all strains on the same medium. The reason was that the explants of PKJ-2 on this medium had delayed to bud sprout sparing minimum time for elongation of shoot, resultantly got minimum length of primary shoot. The results of this experiment showed the superiority of BA (alone) at the rate of 2.5 mg L⁻¹ over the combination attempted by Jacoboni and Standardi (1987). The results partially contradicted the findings of Elhag et al. (1998) that the lower BA/IAA ratio favoured shoot elongation. The results also confirmed the fact that the increase in shoot elongation differ in different clones. However, it could be made faster in ventilated boxes than in closed vessels (Benzioni et al., 2003; Mills et al., 2004).

Number of nodes per primary shoot: The growth regulator supplements of the media and the strains were statistically significant, but the interaction between these two factors was non-significant because of similar trend of each strain in each media as shown by their average (Table 4). The shoots arising from the medium supplemented with BA at the rate of 2.5 mg L⁻¹ gained the maximum nodes (5.89), because the buds sprouted earlier, got maximum length of primary shoot previously on MS + 2.5 mg L⁻¹ BA. The shoots of PKJ-3 attained the maximum number of nodes per primary shoot (4.67) followed by PKJ-6 (4.27), while that of PKJ-2 attained the minimum ones (3.07). The shoots of PKJ-1, PKJ-4 and PKJ-5 were statistically at par with each other for this characteristic. Overall the shoots of PKJ-3 produced maximum nodes per shoot (7.33) on the medium containing BA at the rate of 2.5 mg L⁻¹, while those of PKJ-2 and PKJ-5 produced the minimum number of nodes per primary shoot (1.67) on the medium containing $2.5 \text{ mg L}^{-1} \text{ BA} + 2.5 \text{ mg L}^{-1} \text{ IBA}$. It seems that the number of nodes is positively correlated with the length of primary shoot and negatively correlated with the number of days to bud sprouting. The use of BA (alone) at the rate of 2.5 mg L⁻¹ proved better than the other treatments of this experiment and the combination tried by Jacoboni and Standardi (1987) who recorded 3-4 nodes per shoot at the end of subculture.

CONCLUSION

The use of BA (alone) proved better than BA in combinations with auxins. BA at the rate of 2.5 mg L⁻¹ caused early sprouting of buds that resulted in the longest primary shoot and maximum number of nodes per shoot. While BA at the rate of 5.0 mg L⁻¹ produced the maximum number of shoots per explant and showed suppressing effect on other parameters. The effect of BA in combination with NAA or IAA was almost similar for shoot multiplication. However, BA + IBA caused delay in sprouting of buds that resulted in the lowest values of the parameters i.e., number of shoots produced per explant, length of primary shoot and number of nodes per primary shoot. Response of jojoba strains PKJ-3 and PKJ-6 to *in vitro* shoot multiplication was comparatively better than other strains.

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