African Journal of Biotechnology Vol. 7 (21), pp. 3878-3886, 5 November, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

In vitro propagation of some promising genotypes of jojoba (*Simmondsia chinensis*)

Muhammad Azhar Bashir[1], Muhammad Akbar Anjum[2] and Hamid Rashid[3]

[1]Horticultural Research Station, Bahawalpur, 63100 Pakistan.
[2]University College of Agriculture, Bahauddin Zakariya University, Multan - 60800 Pakistan.
[3]Department of Bioinformatics, Mohammad Ali Jinnah University, Islamabad Campus, Islamabad, Pakistan.

Accepted 26 September, 2008

Nodal segments (1.5 - 3.0 cm long) of six promising jojoba genotypes were cultured *in vitro* on solidified MS medium supplemented with BA in combination with different concentrations of NAA, IAA or IBA for shoot formation. The plant growth regulators combination of 5.55 µM BA + 7.1 µM IAA proved the best for shoot initiation and subsequent growth. Some explants of the jojoba genotypes, PKJ-3 and PKJ-6, developed thick rootlets when BA (5.55 µM) was used in combination with IBA (6.1 µM) or NAA (6.7 µM). Many explants developed callus on the medium containing BA (11.1 µM) in combination with NAA (13.4 µM), IAA (14.3 µM) or IBA (12.2 µM). *In vitro* derived shoots were rooted by culturing on solidified MS medium containing IBA (6.1 µM), IAA (7.1 µM) or NAA (6.7 µM). The plantlets which developed roots in response to IBA had the maximum survival percentage (63.33) during acclimatization in greenhouse. However, survival of the plantlets in field conditions was independent of the genotypes and the time of planting. Among the genotypes, PKJ-3 performed the best in all the parameters studied.

Key words: Auxins, cytokinins, genotypes, jojoba, micropropagation, *Simmondsia chinensis*.

INTRODUCTION

Jojoba (*Simmondsia chinensis* Link. Schneider) is a long-lived evergreen perennial shrub with extensive deep tap root system which helps to withstand drought conditions. Its seeds contain about 50% saturated oil wax which is not only an alternative to sperm whale oil, but also well known for utilization in cosmetics, lubricants and pharmaceuticals etc. Jojoba, being dioecious plant, does not reproduce true-to-type by sexual propagation. In order to maintain the desirable characters, a single jojoba plant has to be asexually propagated. Thus, a selected individual can be reproduced genetically identical that forms a clonal population or cultivar. Superior clones of jojoba, when used in field production, will allow a uniform, predictable plant growth and yield. Asexual propagation of jojoba has been accomplished by layering (Reddy, 2003; Bashir et al., 2005), grafting (Bashir et al., 2006), cuttings (Singh et al., 2003; Bashir et al., 2007a) or tissue culture techniques (Tyagi and Prakash, 2004; Bashir et al., 2007b,c,d) with limited success. Although the use of single node, double node, and three node cuttings from different individuals of jojoba by applying different plant growth regulators (PGRs) will increase the total number of propagules obtained from a stock plant (Cao and Gao, 2003), yet the maximum number of possible propagules will still be limited to one or two thousands per year. In recent years, micropropagation has been successful in raising plants *in vitro* to a commercial level in many plant species (Chandra and Mishra, 2003). Multiple shoots can be produced *in vitro* and these can be developed into plantlets by regenerating their roots. Thus a single explant source, shoot tip or nodal segment could conceivably provide thousands of new true-to-type plantlets per year.

Attempts have been made by several workers to propagate jojoba *in vitro* on various culture media (Elhag et al., 1998; Agrawal et al., 1999; Agrawal et al., 2002) containing different concentrations of PGRs (Jacoboni

*Corresponding author. E-mail: mabashirbzu@yahoo.com. Fax: +92 62 9255432.

Abbreviations: BA, N^6-Benzyladenine; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **NAA**, α-napthalene acetic acid; **PGRs**, plant growth regulators.

and Standardi, 1987; Kacker et al., 1993; Sardana and Batra, 1998; Khanam et al., 1999; Roussos et al., 1999; Gao and Cao, 2001; Prakash et al., 2003; Tyagi and Prakash, 2004; Bashir et al., 2007b,c,d) with varying levels of success. Mostly cytokinins (BA, kinetin and zeatin) have been used in combination with auxins (NAA, IAA and IBA) or GA_3. The genotypes/clones vary in their response when cultured *in vitro* (Llorente et al., 1996; Llorente and Apostolo, 1998; Elhag et al., 1998). Even the explants of both male and female shoots exhibit differential morphogenic behaviour under the influence of various adjuvants (Prakash et al., 2003). In a previous study, BA (alone) was found better than kinetin (alone) or BA + kinetin for *in vitro* shoot initiation of jojoba. The lower concentration (5.55 µM) of BA proved more effective for many shoot parameters and the genotype PKJ-3 was the most responsive to *in vitro* conditions (Bashir et al., 2007b). The present investigation was envisaged to standardize combinations of BA + auxins for *in vitro* shoot formation from nodal explants and to find out the most appropriate combination while observing the genotypic responses. Furthermore, the current study focuses on the subsequent effect of auxins, which was used for *in vitro* root formation, on survival of the plantlets during acclimatization in greenhouse and in field conditions with respect to their time of planting.

MATERIALS AND METHODS

In vitro shoot formation

Six promising female jojoba genotypes PKJ-1, PKJ-2, PKJ-3, PKJ-4, PKJ-5 and PKJ-6 (propagated through cuttings) as characterized by Bashir et al. (2007a) were used in the present study. The branches taken from 2 year-old potted plants were first thoroughly washed in running tap water for 30 min then leaves were removed keeping the petiole intact. These were washed again in 1% detergent (w/v) solution, followed by four washings in distilled water. The branches were divided into 1.5 - 3.0 cm long nodal segments. Surface sterilization of explants was accomplished in three steps i.e. the explants first were dipped in 90% ethanol (v/v) for 10 s, then dipped in 50% Clorox (v/v) solution + 2 drops of Tween-20 and stirred for 15 min, followed by four washings with sterile distilled water, each for 5 min. Finally the explants were dipped in 0.1% mercuric chloride (w/v) solution, stirred for 15 min and given 4 subsequent washings in sterile distilled water each for 5 min. The surface sterilization of explants and their inoculation were conducted in a laminar air flow hood.

The explants were cultured on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose, 0.7 % (w/v) agar, and different PGRs combinations i.e. BA + an auxin (NAA, IAA or IBA) as detailed below.

I. Control (without PGRs)
II. 0.00 µM BA + 6.7 µM NAA
III. 5.55 µM BA + 6.7 µM NAA
IV. 11.1 µM BA + 6.7 µM NAA
V. 11.1 µM BA + 13.4 µM NAA
VI. 0.00 µM BA + 7.1 µM IAA
VII. 5.55 µM BA + 7.1 µM IAA
VIII. 11.1 µM BA + 7.1 µM IAA
IX. 11.1 µM BA + 14.3 µM IAA
X. 0.00 µM BA + 6.1 µM IBA
XI. 5.55 µM BA + 6.1 µM IBA
XII. 11.1 µM BA + 6.1 µM IBA
XIII. 11.1 µM BA + 12.2 µM IBA

The pH of the media was adjusted to 5.7 ± 0.1 using either 0.1N NaOH or 0.1N HCl prior to adding agar. Media were autoclaved at 121°C and 1.05 kg cm^{-2} for 20 min. Thermo-labile vitamins (thiamine-HCl and nicotinic acid) and growth regulators (e.g. IAA) were pre-sterilized through membrane filters and added to the autoclaved medium under aseptic conditions. Media were dispensed in 10-ml aliquots into culture tubes (2.5 x 15 cm), which were plugged with non-absorbent cotton wrapped in one layer of cheese cloth. The explants were cultured on the culture media under laminar air flow hood. Initially, ten explants were cultured under each treatment per replication, keeping 1 explant in a test tube.

The cultures were incubated under a 16 h photoperiod in cool, white fluorescent light of Philips tubes with a light intensity of 55 µmol $m^{-2}s^{-1}$ at 25 ± 2°C. The experiment was laid out in factorial Completely Randomized Design with 3 replications and 2 factors i.e., PGRs combinations and genotypes. The cultures were maintained by sub-culturing at 4 weeks interval onto fresh medium of the same composition. The data were recorded from clean cultures only on the following parameters; number of days to bud sprout, length of primary shoot (cm), number of nodes per primary shoot, number of shoots per explants and percentage of sprouted explants.

In vitro raised shoots were rooted by culturing on solidified MS medium containing 3% (w/v) sucrose and 0.7% (w/v) agar and supplemented with either NAA (6.7 µM), IAA (7.1 µM) or IBA (6.1 µM) as described by Bashir et al. (2007d).

Transference of plantlets to greenhouse for acclimatization

The cotton plugs of test tubes containing rooted plantlets were removed, tubes were filled with sterile distilled water and plantlets were gently taken out with forceps and placed in Petri dishes under laminar air flow hood. These were thoroughly washed to remove the agar, transferred to plastic pots containing sterile coarse sand, covered with polyethylene bags to minimize the loss of moisture and kept in a greenhouse for acclimatization (28/24°C day/night, 16 h photoperiod, 65% RH) for about 2 months. During acclimatization, the plantlets were irrigated with ½ strength Hoagland solution (Hoagland and Arnon, 1950) at weekly interval. The pots were arranged in Completely Randomized Design with three replications. The survival percentage of the plantlets of each jojoba genotype was recorded with respect to the auxin used during *in vitro* rooting.

Transference of plantlets to open field for establishment

Acclimatized in *vitro* produced plantlets were shifted to open field conditions of Jojoba Research Station, Bahawalpur (Pakistan). The plantlets were planted in Randomized Complete Block Design with 2 factors and 3 replications. The first factor was time of planting (October vs March) and the second was jojoba genotypes. Ninety plantlets were transferred each in October 2005 and in March 2006, keeping 5 plantlets of each genotype under each replication. The data were recorded about 5 months after the planting each time on survival percentage of the plantlets from each jojoba genotype with respect to the time of planting.

Statistical analysis

Data collected on different parameters in each experiment were subjected to Fisher's Analysis of Variance technique except the data in percentage which were subjected to arcsine transformation prior to statistical analysis. The treatment means were compared by

Table 1. Number of days to bud sprout as affected by jojoba genotypes and PGRs combinations.

PGRs (µM)				Jojoba genotype						Average
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	
5.55	6.7	-	-	20.75p-r*	28.33j-m	11.42uv	19.67p-s	27.58k-n	12.92t-v	20.11f
11.1	6.7	-	-	27.50k-n	32.75h-j	21.42o-r	27.58k-n	29.00j-l	24.67l-p	27.15e
11.1	13.4	-	-	33.33h-j	37.00f-h	27.11k-n	33.22h-j	37.78f-h	31.33i-k	33.30c
5.55	-	7.1	-	13.58t-v	18.83q-s	9.08v	14.83s-u	20.25p-r	10.75uv	14.56g
11.1	-	7.1	-	22.42n-q	23.75m-q	17.17r-t	23.17m-q	23.50m-q	18.67q-s	21.44f
11.1	-	14.3	-	30.89i-k	33.55h-j	29.44j-l	29.89j-l	32.67h-j	31.11i-k	31.26d
5.55	-	-	6.1	35.20g-i	40.13ef	26.13k-o	37.67f-h	41.53d-f	26.33k-o	34.50c
11.1	-	-	6.1	44.08c-e	46.67bc	37.75f-h	39.67e-g	43.83c-e	37.00f-h	41.50b
11.1	-	-	12.2	42.78c-e	52.67a	40.39ef	45.78b-d	50.11a	45.67b-d	46.23a
Average				**30.06b**	**34.85a**	**24.43d**	**30.16b**	**34.03a**	**26.49c**	

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

employing Duncan's Multiple Range test at 5% probability (Steel and Torrie, 1984).

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 i.e. 0.00 µM BA + 6.7 µM NAA, 0.00 µM BA + 7.1 µM IAA and 0.00 µM BA + 6.1 µM IBA. Hence, these treatments were excluded from statistical analysis of the parameters for convenience in data presentation.

Number of days to bud sprout

The PGRs combinations applied and the genotypes used significantly affected the parameter (Table 1). The explants cultured on the medium containing the PGRs combination of 5.55 µM BA + 7.1 µM IAA took the minimum days, while those cultured on the medium containing 11.1 µM BA + 12.2 µM IBA took the maximum days to bud sprout. The explants of PKJ-3 sprouted earlier taking the minimum number of days, followed by those of PKJ-6. The bud sprouting in the explants of PKJ-2 was delayed and they took the maximum number of days but remained statistically at par with those of PKJ-5. The interaction between the PGRs combinations and the genotypes was also found statistically significant. The explants of PKJ-3 took the minimum time to bud sprout when they were cultured on 5.55 µM BA + 7.1 µM IAA, followed by those of PKJ-6 on the same PGRs combination. The explants of PKJ-2 took the maximum time when cultured on medium containing 11.1 µM BA + 12.2 µM IBA and it was statistically at par with those of PKJ-5 for the same PGRs combination. The literature indicates that the number of days to bud sprout depends upon the type of cytokinins, or combination of cytokinins and auxins, their concentrations, composition of culture media, nature of explant, plant genotype and cultural conditions. Previously, Jacoboni and Standardi (1987) obtained the best results for meristematic activity in explants on MS medium containing 4.6 µM zeatin + 0.3 µM GA_3. In the present study, zeatin was replaced by BA and it was combined with auxins in various concentrations, resultantly an improvement was recorded. The results partially supported the findings of Elhag et al. (1998), who recorded a significant genotypic effect and a medium composition (PGRs) effect after 56 days of culture of shoot-tip explants of jojoba on MS or B5 basal medium containing a combination of BA at 0.0, 1.3 or 13.3 µM with IAA at 0.0, 1.7 or 17.1 µM.

Length of primary shoot (cm)

The data revealed that the parameter was significantly affected by the PGRs combinations, the genotypes and the interaction between these two factors (Table 2). The maximum length of primary shoot was attained on the medium containing 5.55 µM BA + 7.1 µM IAA, while the minimum length of primary shoot was recorded in response to 11.1 µM BA + 12.2 µM IBA. The explants of PKJ-3 resulted in the longest primary shoot, followed by those of PKJ-6, while the explants of PKJ-2 produced the shortest primary shoot, followed by those of PKJ-5. Regarding the interaction, the longest primary shoot was produced by the explants of PKJ-3 in response to the PGRs combination of 5.55 µM BA + 7.1 µM IAA (Figure 1) that was statistically at par with those of PKJ-6 for the same PGRs combination (Figure 2). The combinations of BA + auxins in lower concentrations were better as they initiated early bud sprouting, which provided more time to grow the shoot and attain maximum length than the higher concentrations. The explants of PKJ-2 gave the shoots with minimum length in response to PGRs combination of 11.1 µM BA + 12.2 µM IBA, followed by those of PKJ-5 for the same combination as both took

Table 2. Length of primary shoots (cm) as affected by jojoba genotypes and PGRs combinations.

PGRs (µM)				Jojoba genotype						Average
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	
5.55	6.7	-	-	4.71c-e*	3.98h-k	5.74ab	4.90cd	4.03g-k	5.61b	4.83b
11.1	6.7	-	-	3.73j-n	3.13m-r	4.45d-h	3.88i-k	3.44m-p	4.14f-j	3.80c
11.1	13.4	-	-	3.46lm-p	3.02o-r	4.01h-k	3.51k-p	2.85q-t	3.62j-n	3.41d
5.55	-	7.1	-	5.57b	4.91cd	6.25a	5.55b	4.84c-e	6.00a	5.52a
11.1	-	7.1	-	4.78c-e	4.58d-g	5.21bc	4.68c-f	4.45d-h	4.96cd	4.78b
11.1	-	14.3	-	3.56k-o	3.29m-q	3.74j-l	3.69j-m	3.43m-p	3.68j-m	3.57d
5.55	-	-	6.1	3.16m-r	2.62r-v	4.32e-i	3.68j-o	2.67r-u	4.40d-i	3.48d
11.1	-	-	6.1	2.42s-v	2.07vw	3.06n-r	2.83q-t	2.60r-v	2.98p-s	2.66e
11.1	-	-	12.2	2.20u-w	1.15x	2.64r-u	2.23u-w	1.72w	2.38t-v	2.06f
Average				**3.73d**	**3.19f**	**4.38a**	**3.88c**	**3.34e**	**4.20b**	

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

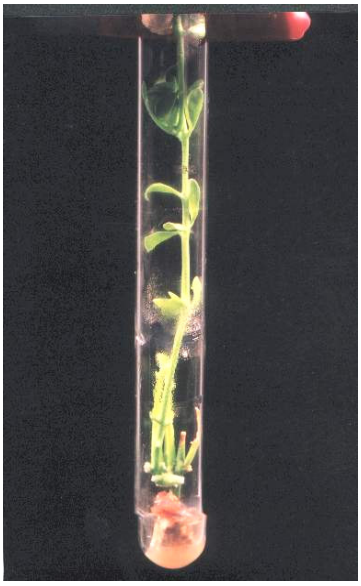

Figure 1. Shoot formation from an explant of PKJ-3 on MS medium containing 5.55 µM BA + 7.1 µM IAA.

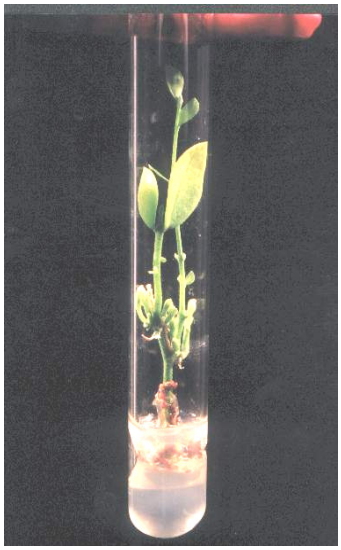

Figure 2. Shoot formation from an explant of PKJ-6 on MS medium containing 5.55 µM BA + 7.1 µM IAA.

more than 50 days to bud sprout that ultimately resulted in reduced shoot lengths. The results of the present study are in line with the findings of Elhag et al. (1998), who reported that lower BA/IAA ratio favoured shoot elongation. The results also partially supported the findings of Sardana and Batra (1998), who observed that an increase in BA concentration suppressed rooting but increased shoot length of plantlets as they obtained complete plantlet at 4.4 µM BA + 5.4 µM NAA. Complete plantlets were also obtained in the present study by the PGRs combinations of 5.55 µM BA + 6.1 µM IBA (Figure 3) and 5.55 µM BA + 6.7 µM NAA (Figure 4). According to Singh and Singh (2005), cytokinins generally do not act alone. In combination with auxins, these stimulate cell division even in non-meristematic tissues. In parenchyma, cell division occurs only when both auxins and cytokinins are present. Furthermore, the ratio of cytokinins to auxins controls cell differentiation and when the ratio is in the favour of cytokinins, shoot formation takes place, while for root formation it should be in favour of auxins. Type of vessels, cultural conditions and plant genotype may also affect the shoot length (Benzioni et al., 2003).

Number of nodes per primary shoot

The PGRs combinations, the jojoba genotypes and the interaction between the two factors significantly affected the number of nodes of primary shoot (Table 3). The PGRs combination of 5.55 µM BA + 7.1 µM IAA gave the maximum number of nodes, while the combination 11.1

Table 3. Number of nodes per primary shoots as affected by jojoba genotypes and PGRs combinations.

PGRs (µM)				Jojoba genotype						
BA	**NAA**	**IAA**	**IBA**	**PKJ-1**	**PKJ-2**	**PKJ-3**	**PKJ-4**	**PKJ-5**	**PKJ-6**	**Average**
5.55	6.7	-	-	3.67g-i*	2.75l-o	5.17a	3.92e-g	3.08j-m	4.75bc	3.89c
11.1	6.7	-	-	3.08j-m	2.33o-q	3.92e-g	3.25i-k	2.67m-p	3.58g-i	3.14d
11.1	13.4	-	-	3.00j-m	2.33o-q	3.44h-j	2.89k-n	2.22q-s	3.11j-m	2.83e
5.55	-	7.1	-	4.33de	3.83f-h	5.33a	4.33de	3.92e-g	5.08ab	4.47a
11.1	-	7.1	-	4.25d-f	3.75g-i	4.50cd	3.92e-g	3.75g-i	4.25d-f	4.07b
11.1	-	14.3	-	3.11j-m	2.56n-q	3.33i-k	3.22i-k	2.89k-n	3.11j-m	3.04d
5.55	-	-	6.1	2.13q-t	1.73tu	3.13j-l	2.33o-q	1.47u-w	3.07j-m	2.31f
11.1	-	-	6.1	1.67u	1.42u-w	2.50n-q	2.25p-r	1.83r-u	2.50n-q	2.03g
11.1	-	-	12.2	1.56uv	1.11w	2.33o-q	1.78s-u	1.22vw	2.11q-t	1.69h
Average				**2.98c**	**2.42e**	**3.74a**	**3.10c**	**2.56d**	**3.51b**	

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

Figure 3. Complete plantlet developed from an explant of PKJ-3 on MS medium containing 5.55 µM BA + 6.1 µM IBA.

Figure 4. Complete plantlet developed from an explant of PKJ-3 on MS medium containing 5.55 µM BA + 6.7 µM NAA.

µM BA + 12.2 µM IBA produced the minimum number of nodes per primary shoot. The explants of PKJ-3 led the other genotypes, while those of PKJ-2 trailed with the minimum number of nodes. The highest number of nodes was attained when the explants of PKJ-3 were cultured on the medium containing 5.55 µM BA + 7.1 µM IAA. However, this PGRs combination stood at par with that of 5.55 µM BA + 6.7 µM NAA for the same genotype. The lowest number of nodes was attained when the explants of PKJ-2 were cultured on the medium supplemented with 11.1 µM BA + 12.2 µM IBA that was at par with PKJ-5 for the same combination and also under the combination of 11.1 µM BA + 6.1 µM IBA. Number of nodes is directly correlated with shoot length. As bud sprouting was delayed in the explants of PKJ-2 and there was the minimum length of primary shoot in response to the combination of 11.1 µM BA + 12.2 µM IBA, it produced the lowest number of nodes. Maximum number of nodes means maximum propagules, therefore, the factors affecting length of shoot or number of shoots per explant may also affect the number of propagules.

Number of shoots produced per explant

The parameter was significantly affected by the PGRs combinations, the genotypes and the interaction between these two factors (Table 4). The PGRs combination of 5.55 µM BA + 7.1 µM IAA expressed significant superiority over all other combinations with maximum number of shoots per explant, while the combination 11.1 µM BA + 12.2 µM IBA produced the minimum number of shoots and stood statistically at par with that of 6.5 µM BA + 6.1 µM IBA. The combination of BA + IAA was better than BA

Table 4. Number of shoots produced per explant as affected by jojoba genotypes and PGRs combinations.

PGRs (µM)				Jojoba genotype						Average
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	
5.55	6.7	-	-	4.00i-k*	2.42r-u	5.50d	4.75e-g	3.67j-l	5.17de	4.25c
11.1	6.7	-	-	3.50k-n	2.92o-r	4.25g-i	3.58j-n	3.08n-q	3.92i-l	3.54e
11.1	13.4	-	-	3.22m-p	2.89p-r	3.67j-m	3.11n-q	2.67q-s	3.55j-n	3.18f
5.55	-	7.1	-	6.58c	4.83ef	8.50a	7.25b	6.25c	8.25a	6.94a
11.1	-	7.1	-	4.67e-g	4.33f-i	6.17c	4.42f-i	4.25g-i	5.42d	4.88b
11.1	-	14.3	-	4.08h-j	3.56j-n	4.56f-h	4.22f-i	3.44l-o	4.33f-i	4.03d
5.55	-	-	6.1	1.93u-x	1.40xy	2.40r-u	1.47xy	1.33y	2.20s-v	1.79h
11.1	-	-	6.1	1.58w-y	1.33y	2.58q-t	2.42r-u	2.08t-w	2.75p-r	2.13g
11.1	-	-	12.2	2.11t-w	1.33y	1.89u-x	1.67v-y	1.45xy	1.89u-x	1.72h
Average				**3.52c**	**2.78e**	**4.39a**	**3.65c**	**3.14d**	**4.16b**	

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

+ NAA, while the latter was better than that of BA + IBA in increasing number of shoots, possibly because IBA had more tendency to initiate roots (Agrawal et al., 1999; Khanam et al., 1999) that might delayed shoot initiation, even in combination with 11.1 µM BA. Auxins in lower concentrations were better in increasing number of shoots than those in higher ones, as it had already been noted in case of time to bud sprout, length of primary shoot and number of nodes per primary shoot. Earlier bud sprouting provided the maximum time to grow shoots resulting in greater shoot length and more number of nodes. The explants of PKJ-3 led with the maximum number of shoots per explant over all the genotypes, while those of PKJ-2 trailed with the minimum ones. Regarding the interaction, the explants of PKJ-3 produced the highest number of shoots in response to the PGRs combination of 5.55 µM BA + 7.1 µM IAA that was at par with those of PKJ-6 for the same combination (Figures 1 and 2). The explants of PKJ-2 produced the minimum number of shoots in response to combination of 11.1 µM BA + 12.2 µM IBA or 11.1 µM BA + 6.1 µM IBA, and those of PKJ-5 also did the same in response to 6.5 µM BA + 6.1 µM IBA. These results are partially in lines with the findings of Elhag et al. (1998), who recorded the highest number of newly formed shoots per explant in female plants compared to male ones. They also reported that higher BA/IAA ratio favoured shoot multiplication. BA alone has a significant role in increasing number of shoots from jojoba explants as compared to any other cytokinins although clonal differences exist in response to various concentrations of BA (Llorente et al., 1996; Llorente and Apostolo, 1998; Agrawal et al., 2002; Prakash et al., 2003; Tyagi and Prakash, 2004; Bashir et al., 2007b,c,d), yet the combination of BA + IAA performed well in the present study. Meyghani et al. (2005) also found that the number of shoots produced on the medium supplemented with 8.9 µM BA and 0.54 µM NAA (5.0 shoots per explant) was higher than the other PGRs combinations. According to Singh (2004), when the ratio of auxin to some plant constituents (particularly purines like adenine i.e. BA and kinetin) is low, the meristem tends to form bud and leaf primordia. However, the present findings contradicted to Scaramuzzi and D'Ambrosio (1988), who found that the shoot formation was best when the MS medium was supplemented with 44.4 µM BA + 27.9 µM IAA i.e. the combination consisted of very higher concentrations of growth regulators. 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), composition of media, nature of growth regulators, their concentrations, plant genotypes (Prakash et al., 2003; Tyagi and Prakash, 2004), type of vessels and cultural conditions (Benzioni et al., 2003; Mills et al., 2004).

Percentage of sprouted explants

The PGRs combinations, the genotypes and their interaction significantly affected the percentage of sprouted explants (Table 5). The PGRs combination of 5.55 µM BA + 7.1 µM IAA expressed significant superiority with the maximum percentage of sprouted explants over the other combinations, while that consisting of 11.1 µM BA + 12.2 µM IBA proved the inferior one. Auxins in lower concentrations were better in increasing the percentage of sprouted explants, as it had already been noted in case of time to bud sprout, length of primary shoot, number of nodes and number of shoots per explant. Although the genotype PKJ-3 led with maximum percentage of sprouted explants over all the genotypes, yet it remained statistically similar to PKJ-6. The minimum percentage of sprouted explants was recorded in PKJ-2, which was at par with that of PKJ-5. Regarding the interaction between the genotypes and PGRs combinations, the explants of PKJ-3 and PKJ-6 sprouted to the maximum in response to the combination of 5.55 µM BA + 7.1 µM IAA, which were at par with those of PKJ-4 for the same PGRs com-

Table 5. Percentage of sprouted explants as affected by jojoba genotypes and PGRs combinations.

PGRs (µM)				Jojoba genotype						Average
BA	NAA	IAA	IBA	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	
5.55	6.7	-	-	73.33c-e	66.67d-f	93.33ab	80.00b-d	66.67d-f	86.67a-c	77.78b
11.1	6.7	-	-	66.67d-f	53.33f-h	86.67a-c	66.67d-f	60.00e-g	80.00b-d	68.89c
11.1	13.4	-	-	46.67g-i	26.67jk	60.00e-g	40.00h-j	3333i-k	60.00e-g	44.44e
5.55	-	7.1	-	80.00b-d	73.33c-e	100.00a	86.67a-c	66.67d-f	100.00a	84.44a
11.1	-	7.1	-	73.33c-e	60.00e-g	93.33ab	66.67d-f	60.00e-g	86.67a-c	73.33bc
11.1	-	14.3	-	60.00e-g	40.00h-j	66.67d-f	46.67g-i	46.67g-i	60.00e-g	53.33d
5.55	-	-	6.1	53.33f-h	40.00h-j	80.00b-d	53.33f-h	46.67g-i	73.33c-e	57.78d
11.1	-	-	6.1	53.33f-h	26.67jk	60.00e-g	53.33f-h	33.33i-k	53.33f-h	46.67e
11.1	-	-	12.2	33.33i-k	20.00k	46.67g-i	33.33i-k	26.67jk	40.00h-j	33.33f
Average				**60.00b**	**45.19c**	**76.30a**	**58.52b**	**48.89c**	**71.11a**	

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

Table 6. Survival percentage of plantlets during acclimatization as affected by jojoba genotypes and auxins

Auxin	Jojoba genotype						Average
	PKJ-1	PKJ-2	PKJ-3	PKJ-4	PKJ-5	PKJ-6	
IBA	66.67a*	46.67a	80.00a	60.00a	53.33a	73.33a	63.33a
IAA	46.67a	26.67a	60.00a	40.33a	33.33a	53.33a	43.33b
NAA	40.00a	33.33a	60.00a	53.33a	33.33a	53.33a	45.56b
Average	**51.11b**	**35.56c**	**66.67a**	**51.11b**	**40.00c**	**60.00ab**	

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

bination. The explants of PKJ-2 got the lowest percentage of sprouted explants in response to the combination of 11.1 µM BA + 12.2 µM IBA and these remained at par with those of PKJ-5, PKJ-1 and PKJ-4 for the same combination and also at par with those of PKJ-2 and PKJ-5 under combination of 11.1 µM BA + 6.1 µM IBA. Agrawal et al. (2002) reported a very few number (11.5%) of nodal explants from female jojoba clone (EC 33198) that produced shoots when cultured on MS medium supplemented with BA at 20 µM. However, it was improved by the combination of BA + IAA in this experiment. Further improvement in this parameter could be achieved by subsequent use of *in vitro*-raised shoots as explant source (Agrawal et al., 2002). However, the present findings contradicted to Scaramuzzi and D'Ambrosio (1988), who found better shoot formation on MS medium supplemented with higher concentrations of BA + IAA. These differences were probably due to the genotypes and varying levels of endogenous growth regulators in the explants used in the two studies.

Root/callus formation

Some explants from PKJ-3 and PKJ-6 developed thick rootlets along with shoot growth on the medium containing 5.55 µM BA + 6.1 µM IBA or 5.55 µM BA + 6.7 µM NAA (Figures 3 and 4). These observations confirmed the findings of Sardana and Batra (1998), who obtained complete plantlets with 1 or 2 thick roots per shoot from cultured shoot tips on MS medium supplemented with 4.4 µM BA and 5.4 µM NAA. In the present study explants also developed callus on the medium containing 11.1 µM BA in combination with NAA (13.4 µM), IAA (14.3 µM) or IBA (12.2 µM). The PGRs combinations containing higher concentrations of both cytokinin and auxins caused callus formation in this study in contradiction to Scaramuzzi and D'Ambrosio (1988).

Survival percentage of plantlets during acclimatization

During acclimatization in greenhouse, survival percentage of the plantlets, produced *in vitro* from cultured shoots, was significantly affected by the jojoba genotypes and the auxins (NAA, IAA and IBA) used during *in vitro* rooting (Table 6). However, the interaction between the two factors (i.e. the genotypes used and the auxins applied previously) remained statistically non-significant. The plantlets of the genotype PKJ-3 showed the highest survival percentage, followed by those of PKJ-6 and both the genotypes behaved statistically alike. The genotypes PKJ-1 and PKJ-4 were in the middle with the same sur-

vival percentage of plantlets. The plantlets from PKJ-2 had the lowest survival percentage and remained statistically at par with those from PKJ-5. Regarding the auxins, IBA was found statistically different in effect from the other two auxins. The maximum survival percentage was recorded in those plantlets which developed their roots in MS medium containing IBA during *in vitro* root formation. The plantlets which developed their roots in MS medium containing NAA and IAA resulted in lower survival percentage and behaved statistically alike during acclimatization.

Plant genotype and *in vitro* applied auxins had prime role in the survival of plantlets during acclimatization. In the present study, the higher survival of the plantlets which developed their roots *in vitro* in response to IBA was due to their well developed root system (Bashir et al., 2007d). Among the genotypes, the plantlets of PKJ-3 had highest survival because of their well-developed root system, while those of PKJ-2 had the lowest one due to their poorly-developed root system compared to the other genotypes. Apostolo et al. (1996) also observed clonal differences in jojoba for rooting and acclimatization. Lee (1988) reported that tissue-culture-grown plantlets had a high mortality rate when they were transferred to soil medium because of insufficient plantlet acclimation (hardening) prior to transplanting. He urged on a gradual reduction of humidity after transplanting over a period of one week that might reduce plant mortality during the greenhouse phase. Previously, Birnbaum et al. (1985) developed the hardening-off procedures and the equipment to prepare plantlets produced *in vitro* for planting in the field. Meyghani et al. (2005) found the mixtures of peat and perlite at a ratio of 1:1 or 1:2 (v/v) as the most suitable media for transplanting or adaptation of jojoba plantlets.

Survival percentage of plantlets in field conditions

The survival of plantlets in field conditions varied from 33.33 to 53.33% and was not affected significantly by the genotypes, time of planting and their interaction. Although, the plantlets transplanted in October had slightly higher survival (48.89%) as compared to those transplanted in March (43.33) but the difference was statistically non-significant. The study revealed that survival of plantlets in field conditions was independent of the plant genotype and the time of planting. The poor survival of plantlets following field transplantation was probably due to reduction in the size of their leaves produced *in vitro*, as plantlets with larger and healthy leaves may adapt and grow faster in soil after transplanting (Lee, 1988).

REFERENCES

Agrawal V, Prakash S, Izhar S (1999). Differential hormonal requirements for clonal propagation of male and female jojoba plants. In: Altman A, Ziv M (eds) Plant biotechnology and *in vitro* biology in the 21st century. Proc. 9th Int. Cong., Int. Assoc. Plant Tissue Cult. Biotech., Jerusalem, Israel,14 - 19 June 1998, pp. 25-28.

Agrawal V, Prakash S, Gupta SC (2002). Effective protocol for *in vitro* shoot production through nodal explants of *Simmondsia chinensis*. Biol. Plant. 45: 449-453.

Apostolo NM, Llorente BE, Princen LH, Rossi C (1996). Rooting and acclimatization of micropropagated jojoba seedlings (in Spanish). Proc. 9th Int. Conf. Jojoba and Its Uses, Catamarca, Argentina, 25 - 30 September 1994, pp. 47-49.

Bashir MA, Ahmad M, Anjum MA (2005). Response of six promising jojoba strains to air layering. Biosci. Res. 3: 172-177.

Bashir MA, Ahmad M, Anjum MA (2006). Propagation of six promising jojoba strains through veneer grafting. Int. J. Agric. Biol. 8: 482-484.

Bashir MA, Ahmad M, Anjum MA (2007a). Effect of various potting media on growth of rooted jojoba (*Simmondsia chinensis*) cuttings. Int. J. Agric. Biol. 9: 147-151.

Bashir MA, Rashid H, Anjum MA (2007b). *In vitro* shoot initiation from nodal explants of jojoba (*Simmondsia chinensis*) strains. Biotechnololgy, 6: 165-174.

Bashir MA, Rashid H, Anjum MA (2007c). *In vitro* shoot multiplication of six promising strains of jojoba (*Simmondsia chinensis*). Biotechnology, 6: 309-315.

Bashir MA, Anjum MA, Rashid H (2007d). *In vitro* root formation in micropropagated shoots of jojoba (*Simmondsia chinensis*). Biotechnology, 6: 465-472.

Benzioni A, Mills D, Wenkart S, Zhou Y (2003). Effects of ventilation on the performance of jojoba (*Simmondsia chinensis*) clones: multiplication stage. Acta Hort. 616: 135-138.

Birnbaum E, Matias S, Wenkart S (1985). Vegetative propagation of jojoba by tissue culture. Proc. 6th Int. Conf. Jojoba and Its Uses, Beer-Sheva, Israel, 21- 26 October 1984, pp. 233-241.

Cao B, Gao HD (2003). Technology of cutting propagation of *Simmondsia chinensis* (Link) Schneider (in Chinese). J. Nanjing Forest. Univ. 27: 62-66.

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

Elhag H, El-Olemy MM, Mossa JS, Tag-El-Din SS, Al-Zoghet MF, Al-Alsheikh AMA (1998). *In vitro* propagation of jojoba. Program Abstracts of Annual Conference on New Crops and New Uses: Biodiversity and Sustainability, Phoenix, Arizona USA.

Gao HD, Cao B (2001). Study on technology of tissue culture of *Simmondsia chinensis* (Link) Schneider (in Chinese). J. Jiangsu Forest. Sci. Technol. 28: 12-14.

Hassan NS (2003). *In vitro* propagation of jojoba (*Simmondsia chinensis* L) through alginate-encapsulated shoot apical and axillary buds. Int. J. Agric. Biol. 5: 513-516.

Hoagland DR, Arnon DJ (1950). The water culture method for growing plants without soil. Circular California Agricultural Experimental Station, Berkeley. p. 347.

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

Kacker NL, Joshi SP, Singh M, Solanki KR (1993). *In vitro* regeneration of female plants of *Simmondsia chinensis* (Link) Schneider (Jojoba) using coppice shoots. Ann. Arid Zone 32: 175-177.

Khanam A, Rao YBN, Farook SA (1999). Standard *in vitro* protocol for high frequency mass micropropagation of jojoba (*Simmondsia chinensis* (Link) Schneider). Adv. Plant Sci. 12: 361-366.

Lee CW (1988). Application of plant biotechnology for clonal propagation and yield enhancement in jojoba. Proc. 7th Int. Conf. Jojoba and Its Uses, Phoenix, Arizona USA, pp. 102-111.

Llorente BE, Apostolo NM, Princen LH, Rossi C (1996). Micropropagation of jojoba: effect of hormonal and clonal variation at the multiplication stage. Proc. 9th Int. Conf. Jojoba and Its Uses, Catamarca, Argentina, 25 - 30 September 1994, pp. 50-52.

Llorente BE, Apostolo NM (1998). Effect of different growth regulators and genotypes on *in vitro* propagation of Jojoba. Newzealand J. Crop Hort. Sci. 26: 55-62.

Meyghani H, Ghazvini RF, Hamidoghli Y (2005). Micropropagation from stem segments of salt tolerant jojoba seedlings. J. Korean Soc. Hort. Sci. 46: 183-187.

Mills D, Zhou Y, Benzioni A (2004). Improvement of jojoba shoot multiplication *in vitro* by ventilation. In Vitro Cell. Dev. Biol.- Plant 40: 396-402.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
Prakash S, Agrawal V, Gupta SC (2003). Influence of some adjuvants on *in vitro* clonal propagation of male and female jojoba plants. *In Vitro* Cell. Dev. Biol.-Plant 39: 217-222.
Reddy YN (2003). Effect of different concentration of auxins on rooting and root characters of air and ground layers of jojoba (*Simmondsia chinensis* (Link) CK Schneider). Ethiop. J. Sci. 26: 155-159.
Roussos PA, Tolia-Marioli A, Pontikis CA, Kotsias D (1999). Rapid multiplication of jojoba seedlings by *in vitro* culture. Plant Cell Tissue Organ Cult. 57: 133-137.
Sardana J, Batra A (1998). *In vitro* regeneration of jojoba (*Simmondsia chinensis*): a plant of high potential. Adv. Plant Sci. 11: 143-146.
Scaramuzzi F, D'Ambrosio A (1988). Organogenesis and propagation *in vitro* of *Simmondsia chinensis* (Link) Schn (jojoba) from vegetative fragments. Acta Hort. 227: 411-413.
Singh DK (2004). Hi-Tech Horticulture. Geeta Somani Agrotech Publishing Academy, Udaipur, India.
Singh DK, Singh SK (2005). Physiology and Post-Harvest Management of Horticultural Crops. Geeta Somani Agrotech Publishing Academy, Udaipur, India.
Singh KJ, Nayyar H, Dutta A, Dhir KK (2003). Rhizogenetic studies of jojoba: hormone effect, rooting medium and seasonal variation. Ind. Forester, 129: 1405-1411.
Steel RGD, Torrie JH (1984). Principles and Procedures of Statistics: A Biometrical Approach. McGraw Hill Book Int. Co., Singapore.
Tyagi RK, Prakash S (2004). Genotypes and sex specific protocols for *in vitro* micropropagation and medium term conservation of jojoba. Biol. Plant. 48: 19-23.