African Journal of Biotechnology Vol. 11(62), pp. 12538-12545, 2 August, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.161
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Influence of kinetin on *in vitro* rooting and survival of banj oak (*Quercus leucotrichophora* L.)

Aseesh Pandey* and Sushma Tamta

Plant Tissue Culture and Molecular Biology Laboratory, Department of Biotechnology, Bhimtal Campus, Kumaun University Nainital, 263136 Uttarakhand, India.

Accepted 16 April, 2012

A study concerning the influence of cytokinins on shoot regeneration by using different stem segments derived from *in vitro* raised seedlings and their subsequent rooting was conducted in banj oak (*Quercus leucotrichophora L.*). Cytokinins play an important role in shoot regeneration and their multiplication. In the present study, cytokinins particularly kinetin (Kn) influenced *in vitro* rooting and subsequent survival of these *in vitro* raised plants in addition to shoot multiplication. It was found that the microshoots raised via kinetin treatment rooted very well (94.44%) without any basal callus formation in comparison to microshoots raised via other cytokinin treatments (that is, BA and 2-iP). In addition to this, these *in vitro* raised plants showed maximum survival rate (90%) during hardening process. On the basis of available literature this is a unique and significant study regarding the comparative effect of different cytokinins on *in vitro* propagation study of *Q. leucotrichophora* by using different stem segments, particularly the influence of kinetin *in vitro* rooting and survival of *in vitro* raised plants in addition to shoot multiplication. This significant study could be useful for large scale production of successfully hardened plants so that it would be helpful in conservation of this important Himalayan forest tree species.

Key words: *Quercus leucotrichophora*, acclimatization, cytokinins, *in vitro* rooting.

INTRODUCTION

Trees are the largest and among the most complex organisms in the world with millions of diverse life forms (Zobel and Talbert, 1984). Oaks are widely distributed and economically important tree species of the Himalayan region. This angiospermic genus of family Fagaceae is anemophilous (wind pollinated) with trees or shrubs, either deciduous or evergreen includes about 450 species (Chalupa, 1995). Nearly 400 species distributed throughout the temperate regions of the world (Johnson et al., 2002) and many as twenty three species in the Himalayan region, most of them are evergreen.Out of these, five evergreen species have been found growing in the central Himalaya between 1000 and 3600 m amsl (Champion and Seth, 1968). *Quercus leucotrichophora*, commonly known as banj oak, is a late succession and major forest forming tree species in central Himalaya. Palaeo-ecological evidence suggests that this species has been dominated for at least a million year in this part of Himalaya (VishnuMittre, 1974). *Q. leucotrichophora* forest is an important component of Himalayan ecosystem from biodiversity conservation viewpoint, maintenance of soil fertility and recharge of spring water. Being an important source of fodder, fuel wood and charcoal, severe biotic disturbance and the resultant harsh physical environment has inhibited the regeneration of *Q. leucotrichophora* in nature (Upreti et al., 1985). The over exploitation of this species for a long time has led to gradual degradation of its natural stands (Nautiyal et al., 2000). Trees do not bear acorns until these have attended approximately 25 years of age and good harvests are possible only at the interval of 2 to 5 years (Anonymous, 1965).

Tissue culture and micropropagation could be useful in overcoming aforementioned difficulties (Bisht et al., 1998). This technique has been applied successfully in several tree species. Some success has been achieved in micro-

*Corresponding author. E-mail: ashishpandey1986@gmail.com. Tel: 09458940052

propagation via auxiliary shoot prolifiration: *Quercus robur* and *Quercus petraea* (Vieitez et al., 1985; Favre and Juncker, 1987; Chalupa, 1988; San-Jose et al., 1990; Chalupa, 1990); *Quercus suber* (Pardos, 1981; Bellarosa, 1989; Manzanera and Pardos, 1990); *Quercus borealis* (Nkanka, 1982); *Q. shumardii* (Bennet and Davies, 1986); *Q. acutissima* (Sato and Mori, 1987); *Quercus serrata* (Ide and Yamamoto, 1987) and *Quercus rubra* (Schwarz and Schlarbaum, 1993). These reports of micropropagation clearly indicate that if appropriately applied, these methods can be a reliable and rapid means to effectively supplement efforts for the greening of the Himalaya. However, *in vitro* regeneration of central Himalayan oaks *Quercus floribunda* (Purohit et al., 2002a), *Q. leucotrichophora*, *Q. glauca* (Purohit et al., 2002c) and *Quercus semecarpifolia* (Tamta et al., 2008) has been reported from intact embryos and/or cotyledonary nodes and petiolar tube containing a primary shoot, respectively. Present communication describes potential of different stem segments (apical, middle and basal) derived from *in vitro* grown seedlings and effect of cytokinins 6-benzyle adenine (BA); 6-(α, α-dimethylallylamino)-purine (2-iP) and 6-furfuril- aminopurine (Kinetin; Kn) on shoot multiplication as well as on rooting in microshoots obtained via application of different cytokinins and further survival of these *in vitro* raised plants. So to develop a simple, more reliable and efficient procedure for large scale *in vitro* propagation of *Q. leucotrichophora* present work has been conducted.

MATERIALS AND METHODS

Seeds of *Q. leucotrichophora* were collected from elite plants growing at Nainital catchment area (1990 m amsl) of district Nainital, Uttarakhand, India. Seeds were separated from the cupule, washed under running tap water and disinfected by following the method given by Tamta et al. (2008). Since the use of sodium hypochlorite did not prevent contamination, mercuric chloride was used as surface disinfectant throughout the experiment.

Shoot regeneration and multiplication

After removing the seed coat from disinfected seeds, decoated seeds with intact embryos were inoculated on medium containing only sucrose (3.0%, w/v) and agar (0.8%, w/v) (water agar medium). After 1 week, contamination free seeds were transferred to woody plant (WP) (Lloyd and McCown, 1980) plant growth regulator (PGR)-free basal medium for further growth. After 4 weeks of inoculation (**Figure** 1b), the radicle and the cotyledons were removed carefully from well grown individual seedlings (height up to 4 to 5 cm) and excised into different segments (approximately 1.5 cm length), having at least one node in each segment. These stem segments (including apical, middle and basal segments) were transferred to WPM containing sucrose (3.0%, w/v), agar (0.8%, w/v), supplemented with various concentrations of 6-benzyleadenine (BA; 0.00-8.88 µM) (Table1) for shoot multiplication. Citric acid (20.0 mg/l) was added to control leaching of phenolic compounds and avoiding basal callus formation. Cultures were maintained at 25 ± 1°C under 16/8 h (light/darkness) photoperiod with an irradiance of 60 µmol$m^{-2}s^{-1}$ outside the culture flasks supplied by cool fluorescent tubes (Philips TL 40 W). These cultures were sub cultured every four weeks. Each treatment consisted of at least 27 explants (three explants/flask). Observations with respect to shoot number and shoot lengths were taken after every 7 days. A further study was conducted to explore the effect of same concentration (1 mg/l) of other cytokinins like Kn, 2-iP including BA also on shoot multiplication in WPM by taking the apical, middle and basal stem segments separately, derived from *in vitro* raised seedlings as explants (Table 2).

Rooting of microshoots

For induction of rooting in microshoots, a two-step method was used as followed by Tamta et al. (2008). Well-developed microshoots (2.0 to 3.0 cm in height with two or three leaves) were transferred in half strength WPM supplemented with indole-3-butyric acid (IBA; 50 or 100 µM) for 24 or 48 h (first step). After that the microshoots were transferred to PGR-free half strength WP basal medium (second step), solidified with 2.4% (w/v) clarigel. Cultures were initially placed in dark for five days, and then transferred to 16/8 h (light and dark) photoperiod. Twelve shoots were taken per treatment with three shoots per culture flask. The experiments were repeated twice. Half strength WPM devoid of IBA (in the first step) served as control.

Acclimatization of plantlets to soil

Shoots with well developed roots were taken out carefully from the flasks and the roots were gently washed under running tap water to remove traces of clarigel. After recording of data plantlets were transferred to small thermocol cups (12 x 8 cm) containing soil and farmyard manure (3:1, w/w); transparent polythene bags (15 x 10 cm) with small holes were inverted over these thermocol cups to maintain the humidity (Figure1f).These plants were placed inside growth chamber under 16 h photoperiod (60 µmol $m^{-2}s^{-1}$) at 25 ± 2°C temperatures and 60% relative humidity.Plants were watered on alternate days with 1/4 basal WPM devoid of sucrose and gradually acclimatized over a period of 1 month (Figure 1g) shows well rooted plants raised via Kn. The plants were then transferred to polythene bags (16 cm high; 8 cm diameter) containing garden soil and kept for further hardening inside a mist chamber for 8 weeks (Figure 1h). Well hardened plants were transferred to the earthen pots and kept in a polyhouse for 12 weeks (Figure 1i).

Statistical analysis

All experimental observations were recorded periodically. The data were analyzed on percent response, shoot formation and rooting by using one way ANOVA (SYSTAT of SPSS Inc., Chicago, USA) (Wilkinson, (1986). Least significant difference was calculated following the method of Snedecor and Cochran (1967). The effects of different concentrations of PGRs were quantified and the level of significance was determined by analysis of variance.

RESULTS AND DISCUSSION

Disinfection with mercuric chloride (0.1%; 10 min) resulted in 90% contamination-free seed germination within a week of culture on water agar medium (Figure 1a).

Shoot regeneration and multiplication

In the first experiment where mixed segments (basal,

Table 1. Effect of BA on shoot multiplication in mixed stem segments of *in vitro* raised seedling of *Q. leucotrichophora* on WPM.

S/N	BA Concentration (µM)	Average number of shoots/explant ±SE	Average shoot length (cm) ±SE	Length of longest shoot (cm) ±SE	Average number of leaves/ explant ±SE
1	0.00	0	0	0	0.92±0.42
2	2.22	1.11±0.29	1.29±0.28	1.34±0.33	1.33±0.33
3	4.44	3.56±1.12	2.77±0.61	4.52±1.42	4.78±1.81
4	8.88	2.67±0.70	2.06±0.81	3.44±1.32	3.78±1.22
LSD(P≤0.05)		0.024638*	0.042813*	0.022552*	0.113267ns

ANOVA

Source	Average number of shoot / explant			Average shoot length			Average length of longest shoot			Average number of leaves		
	Df	Mean square	F- ratio	Df	Mean square	F- ratio	Df	Mean square	F- ratio	Df	Mean square	F- ratio
Between Groups	3	7.532356	5.45*	3	48.7417	4.35*	3	12.89001	5.64*	3	10.51981	2.74ns
Within Groups	8	1.382875		8	11.2077		8	2.286725		8	3.842808	
Total	11			11			11			11		

Data were recorded after every 1wk of culture. All values are an average of 27 explants; individual treatments consisted of nine replicates, three explants per flask and the experiment was repeated twice with qualitatively similar results. BA: 6-Benzyladenine, LSD: Least-significant difference; Df: Degree of freedom, ns: Not significant; *: significant at 0.05 level. SE: Standard error of mean.

middle or apical) of *in vitro* raised seedlings having one node in each segment were inoculated on WPM supplemented with various concentrations of BA, maximum number of shoots per explant were recorded in WPM supplemented with 4.44 µM BA. Values for all the other parameters like average shoot length, length of longest shoot and average number of leaves per explant were also highest in this treatment (Table 1). The number of leaves was higher but not statistically different. The result of ANOVA shows that BA concentration significantly ($p = 0.05$) improved shoot number and shoot length (Table 1). On the basis of findings of this experiment WPM supplemented with 4.44 µM BA (that is, 1 mg/l) was considered to be the best responding medium for shoot multiplication (Table1). Based on the existing literature related to micropropagation, studies in different species of oaks like *Q. robur* (Chalupa, 1984, 1988; Vieitez et al., 1985; Favre and Juncker, 1987), *Q. shumardii* (Bennet and Davies, 1986), *Q. floribunda*, *Q. leucotrichophora* and *Q. glauca* (Purohit et al., 2002a, c), and *Q. semecarpifolia* (Tamta et al., 2008), WPM supplemented with BA was found to be the best medium for shoot regeneration and multiplication.

In the second experiment, where apical, middle and basal segments (at least one node in each segment) of *in vitro* raised seedlings were used separately as explants and cultured on WPM supplemented with the same concentration that is, 1 mg/l, of other cytokinins, results were quite interesting and are given in Table 2. When the explants were mixed (in the first experiment), calculated values for average number of shoots formed per explant were less but when the explants were categorized according to their position that is, apical, middle and basal, then it was observed that the basal segments with cotyledonary node were more generous in comparison to middle and apical segments. This trend was similar in all the three used cytokinins (Table 2).

Out of three used cytokinins, BA was again proved as the most effective cytokinin for shoot multiplication. Figure 1c shows shoot multiplication in basal segment in WPM supplemented with 4.44 µM BA. It was able to induce maximum number of shoots per explant (4.5 shoots/basal segment) with maximum average shoot length (2.32 cm), maximum average length of longest shoot (4.22 cm) and maximum average number of leaves

Table 2. Effect of same concentration of different cytokinins on shoot multiplication formation in different stem segments of *in vitro* raised seedling of *Q. leucotrichophora* on WPM.

Treatment (1mg/l)	Explant	Average number of shoots/explant ±SE	Average shoot length (cm) ±SE	Length of longest shoot (cm) ±SE	Average number of leaves/ explant ±SE
BA	Apical	1.67±0.17	1.94±0.28	2.67±0.58	2.78±0.46
	Middle	1.83±0.08	1.91±0.24	2.43±0.21	1.73±0.23
	Basal	4.50±0.66	2.32±0.37	4.22±0.66	5.25±0.63
Kn	Apical	1.33±0.22	1.34±0.33	1.73±0.43	2.42±0.30
	Middle	1.7±50.29	1.00±0.24	1.16±0.23	1.92±0.46
	Basal	2.90±0.93	1.75±0.29	2.34±0.46	4.83±1.17
2-iP	Apical	1.33±0.22	1.02±0.12	1.17±0.09	1.92±0.30
	Middle	1.92±0.22	1.09±0.15	1.34±0.26	1.75±0.66
	Basal	3.17±0.08	1.59±0.19	1.65±0.29	3.08±0.33
Control	Apical	-	-	-	-
	Middle	-	-	-	-
	Basal	0.17±0.17	0.17±0.17	0.28±0.28	0.92±0.42
LSD (P≤0.05)		0.009302*	5.81**	0.000572*	0.00989*

Anova

Source	Average number of shoots/ explant			Average shoot length (cm)			Length of longest shoot (cm)			Average number of leaves/ explant		
	Df	Mean square	F	Df	Mean square	F	Df	Mean square	F	Df	Mean square	F
Explant type	2	2.907769	7.48536	2	0.275313	19.7081	2	0.910009	5.745754	2	5.03210	10.6909
Treatments used	3	3.913507	10.0744	3	1.986074	142.172	3	4.595059	29.01299	3	4.62413	9.82423
Error	6	0.388461		6	0.01397		6	0.158379		6	0.47068	

Data were recorded after 6wks of culture different segments on WPM. All values are an average of 27 explants; individual treatments consisted of nine replicates, three explants per flask. , LSD: Least-significant difference; Df: Degree of freedom, *: significant at 0.05 level, **: significant at 0.01 level. SE: Standard error of mean.

(5.25) (Table 2). The induction of multiple shoots from the cotyledonary nodes of seedlings in response to BA has also been reported earlier (Purohit et al., 2002a) as well as in other tree species, including *Alnus glutinosa* L., Gaertn (Perinet and Lalonde, 1983), Pterocarpus marsupium Roxb (Tiwari et al., 2004), *Albizia odoratissima* L.f. (BENTH) (Rajeshweri and Paliwal, 2006); Macadamia tetraphylla (Mulwa and Bhalla, 2006); *Pterocarpus santalinus* L.f. (Rajeshweri and Paliwal, 2008) and *Aegle marmelos* (Nayak et al., 2007). BA was followed by 2-ip which was able to produce average number of shoots per basal segments up to 3.17. Kn responded least in terms of average number of shoots per explants (2.90 shoots/basal segment). Length of shoots was also maximum in BA supplemented medium (average shoot length- 2.32 cm and length of longest shoot- 4.22cm) followed by Kn (average shoot length- 1.75 cm and length of longest shoot- 2.34 cm) and 2-ip (average shoot length- 1.59 cm and length of longest shoot- 1.65 cm) supplemented medium. Juncker and Favre (1994) reported that 0.44 µM BA was best for auxiliary bud induction and shoot elongation than 0.49 µM 2-ip and 0.45 µM Zeatin in *Q. robur*. In the control, apical and middle segments were totally failed to produce shoots, however basal segment responds but it was not so good (only 0.17 shoots/segment) (Table 2).

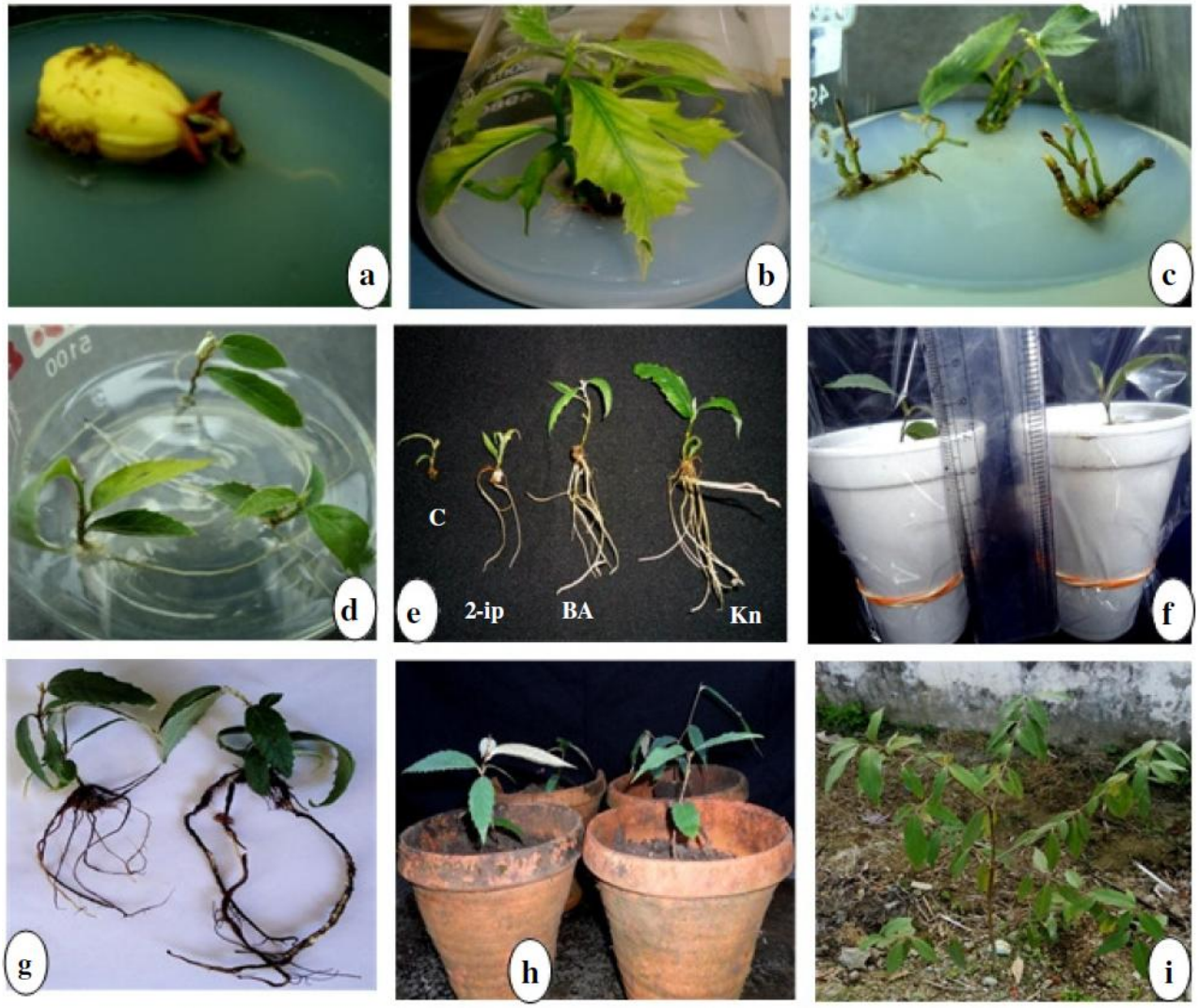

Figure 1. *In vitro* propagation of banj oak (*Q. leucotrichophora*). a) *In vitro* seed germination. b) Germinated seedling 4 wks after inoculation. c) Multiple adventitious shoots in WPM containing 4.44 µM BA after 8 wks of culture of basal segments. d) Well rooted plantlets on PGR-free, 1/2-strength WP medium. Photograph taken after 4 wks of induction of rooting. e) Rooting response of different treatments after 8 wks, C: control, prior to transfer to soil. f) Acclimatization of plantlets inside growth chamber in thermacol cups containing banj oak forest soil and manure (3:1) with 16/8h photo period and 250 degree celsius temperature. g) Well rooted plants raised via Kn (in shoot multiplication) and IBA (50 µM for 24 h, in rooting) treatment. h) Well acclimatize plants inside mist chamber. i) Well growing plant after 36 wks of transfer to the departmental garden.

Induction of rooting in microshoots

Prolonged exposure to auxins resulted in the formation of basal callus in *Q. leucotrichophora* and *Q.glauca* (Purohit et al., 2002c) and *Q. rubra* (Vengadesan and Pijut, 2009). Therefore, for induction of rooting in microshoots, a two-step method was used. This method of rooting was also found suitable in *Q. suber* (Manzanera and Pardos, 1990); temperate bamboo (Bag et al., 2000); *Q. glauca, Q. floribunda* and *Q. leucotrichophora* (Purohit et al., 2002a); *Q. rubra* (Vengadesan and Pijut, 2009). To observe the further role of cytokinins (used for shoot multiplication) if any on rooting, micro shoots raised by the application of different cytokinins, rooted separately by applying the aforementioned two step method. Results of this experiment are presented in Table 3. Although both the used concentration of IBA (50.0 and 100 µM) induced rooting in all microshoots, whether they were produced via BA, 2-iP or Kn treatment but the data for percent rooting, number of roots, average length of roots, plant height and average number of leaves varied in these three types of microshoots (Table 3). Microshoots failed to root, when IBA was absent from the first step medium (control). Lower concentration of IBA (50.0 µM) for 24 h gave maximum

Table3. Effect of short treatment with IBA on *in vitro* rooting of *Q. leucotrichophora* microshoots obtained via different cytokinins.

S/N	Source of explant	IBA (µM)	Treatment time (h)	% rooting±SE	Average number of roots /shoot±SE	Average root length (cm)±SE	Average length of longest root (cm)±SE	Plant height (cm)±SE	Average number of leaves/ plant ±SE	Basal Callusing
1	Microshoots obtained via BA treatment	50	24	38.89±10.24	0.94±0.26	0.99±0.33	1.16±0.41	1.79±0.52	1.05±0.26	+
			48	27.78±10.24	0.88±0.27	0.91±0.23	0.98±0.24	1.50±0.35	1.15±0.25	+
		100	24	66.66±12.17	1.11±0.16	0.98±0.12	1.88±0.29	2.31±0.23	1.5±0.76	+
			48	38.89±10.24	0.89±0.31	0.66±0.16	0.83±0.18	1.81±0.14	1.41±0.14	+
2	Microshoots obtained via 2iP treatment	50	24	44.44±11.11	0.89±0.27	2.80±0.70	3.04±0.80	1.77±0.36	2.11±0.63	++
			48	61.11±10.24	1.28±0.20	2.33±0.47	3.57±1.10	2.51±0.16	2.44±0.29	++
		100	24	38.89±5.56	0.61±0.19	0.96±0.15	1.01±0.11	1.89±0.14	1.81±0.27	++
			48	33.33±8.61	0.67±0.19	1.40±0.44	1.49±0.49	2.49±0.18	1.83±0.14	++
3	Microshoots obtained via Kn treatment	50	24	94.44±5.56	4.50±0.44	5.45±0.50	7.76±0.83	3.15±0.15	3.31±0.13	-
			48	50.00±7.45	1.89±0.53	2.49±0.33	3.41±0.26	2.71±0.19	2.74±0.19	-
		100	24	72.22±10.24	1.65± 0.39	3.19±0.61	3.17±0.41	2.44±0.17	2.89±0.25	-
			48	66.66±8.61	1.16±0.14	2.29±0.33	2.51±0.37	2.28±0.14	2.50±0.21	-
4	Control	00	00	0	0	0	0	0.91±0.46	1±0.51	-

Microshoots obtained via different cytokinins (2.0–3.0 cm in height with two or three leaves) were subjected to 24 or 48-h short treatment separately with various concentrations of IBA before subculture to PGR-free 1/2-strength WPM. Root formation was scored 8 wks later although root induction started after one wk of treatment. Each treatment was carried out in triplicate and each flask contained three microshoots (n=9). + presence of basal callusing, - plants free from basal callusing , ±SE slandered error of mean.

rooting (94.44%) without any basal callus formation in those shoots which were generated via Kn treatment (Table 3 and Figure 1d). Same treatment was able to induce only 38.89% rooting in microshoots produced by BA treatment. However, when 100.0 µM IBA applied for 24 h, it was able to induce rooting up to 66.66% in microshoots produced via BA treatment, whereas the rooting percentage was again higher in microshoots which were produced via kinetin treatment (72.22% rooting, IBA 100.0 µM for 24 h) (Table 3). This was quite noticeable or interesting that IBA when applied for 24 or 48 h, every time induced rooting, but the percentage of rooting changed as the source of microshoots changed means, same concentration of IBA, same treatment time but microshoots produced via application of different cytokinins resulted into different responses in terms of rooting (Table 3). Overall responses of microshoots produced via kinetin treatment was good in terms of rooting percentage, average root number, average length of longest root, total plant height as well as average number of leaves in comparison to other microshoots produced via BA or 2-ip treatments (Figure 1e). Although up to 66.66% rooting was observed in microshoots obtained via BA treatment but the values for average number of roots (1.11), average root length (0.98 cm), length of longest root (1.88 cm) and plant height (2.31 cm) were very less in comparison to the microshoots obtained via Kn treatment where the values for percent rooting (94.44%), average root number per shoot (4.50), average root length (5.45 cm), average length of longest root (7.76 cm), plant height (3.15 cm) and average number of leaves (3.31) were much more higher (Table 3).

Acclimatization and field establishment

A disadvantage of micropropagation is the high mortality rate during the transfer of *in vitro* raised

plants to *ex vitro* conditions (Lovato et al., 1999). In the present study, *in vitro* rooted plants were successfully acclimatized inside a mistchamber (25°C, 70% RH) with about 90% survival. Again, plants raised via application of Kn (in shoot multiplication) and IBA (in rooting) grew well with 90% survival, whereas plants produced via BA and 2-iP showed only 60 and 55% survival, respectively. It may be due to lack of vascular connection because BA and 2-iP raised microshoots showed higher basal callusing during rooting while Kn raised microshoots were free form basal callusing and cytokinins have been shown to stimulate development of the vascular system and to promote greater translocation of nutrients (Aloni, 2001). Same types of results were also reported by Vengadesan and Pijut (2009) in *Q. rubra.*

Conclusion

Findings of this study concluded that for shoot multiplication basal segments with cotyledonary node from *in vitro* germinated seedlings were the best explants. Although maximum number of shoots was produced via BA treatment followed by 2-ip and Kn, shoots obtained via Kn treatment responded very well in further steps of regeneration like rooting and acclimatization in comparison to microshoots obtained via BA treatment. Therefore, WPM supplemented with Kn could be suggested as the best shoot multiplication medium instead of BA supplemented WPM as reported by earlier workers (Purohit et al., 2002c). On the basis of available literature, this is a unique and significant study regarding the comparative effect of different cytokinins on *in vitro* propagation study of *Q. leucotrichophora* by using different stem segments, particularly the influence of Kn in *in vitro* rooting and survival of *in vitro* raised plants in addition to shoot multiplication. This study could be useful for large scale production of successfully hardened plants within one year so that it would be helpful in conservation of this important Himalayan forest tree species.

REFERENCES

Aloni R (2001). Foliar and axial aspects of vascular differentiation: Hypotheses and evidence. J. Plant Growth Regul. 201: 22-34.

Anonymous (1965). Silvics of Forest Trees of the United States. USDA Misc. Publication, p. 271.

Bag N, Chandra S, Palni LMS, Nandi SK (2000). Micropropagation of Dev-ringal (*Thamnocalamus spathiflorus*)-atemperate bamboo, and comparison of *in vitro* propagated plants and seedlings. Plant Sci. 156: 125-135.

Bellarosa R (1989). Oak (Quercus spp). In: Bajaj YPS (ed). Biotechnology in agriculture and forestry, Springer, Berlin. 5: 387-401.

Bennet LK, Davies FT (1986). *In vitro* propagation of *Quercus shumardii* seedlings. Hort. Sci. 21(4): 1045-1047.

Bisht MS, Vyas P, Bag N, Palni LMS (1998). Plant Tissue Culture and Molecular Biology-Application and prospects, Narosa Publishing House, New Delhi, pp. 126-170.

Chalupa V (1984). *In vitro* propagation of oak (*Quercus robur* L.) and linden (*Tilia cordata* Mill.). Biol. Plant. 26: 374-377.

Chalupa V (1988). Large scale micropropagation of Quercus robur L. using adenine-type cytokinins and thidiazuron to stimulate shoot prolification. Biol. Plant, 306: 414-421.

Chalupa V (1990). Plant regeneration by somatic embryogenesis from cultured immature embryos of oaq (*Quercus robur* L.) and linden (*Tilia cardata* Mill). Plant Cell Rep. 9:398-401.

Chalupa V (1995). Somatic embryogenesis in oak (*Quercus* spp.). In: Jain S, Gupta P & Newton R (eds) Somatic Embryogenesis in Woody Plants. Angiosperms. Kluwer Academic Publishers, Dordrecht, Netherlands, 2: 67-87.

Champion HG, Seth SK (1968). A revised survey of the forest types of India. Government of India Publications, New Delhi, India.

Favre JM, Juncker B (1987). *In vitro* growth of buds taken from seedlings and adult plant material in *Quercus robur* L. Plant Cell Tissue Org. Cult 8: 49-60.

Ide Y, Yamamoto S (1987). *In vitro* plantlet regeneration from axillary buds of juvenile seedlings of konara (*Quercus serrata*). J. Jpn. Soc. 69: 109-112.

Johnson PS, Shifley SR, Rogers R (2002). The ecology and silviculture of oaks. CABI, New York, p. 503.

Lovato PE, Schuepp H, Trouvelot A, Gianinazzi S (1999). In Mycorrhiza (eds Varma A and Hook B). Springer-Verlag. pp. 443-468.

Lloyd G, McCown B (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by shoot-tip culture. Comb. Proc. Int. Plant Prop. Soc. 30: 421-427

Manzanera JA, Pardos JA (1990). Micropropagation of juvenile and adult *Quercus suber* L. Plant Cell Tiss. Org. Cult. 21: 1-8; Doi: 10. 1007/BF00034484.

Mulwa RMS, Bhalla PL (2006). *In vitro* plant regeneration from immature cotyledon expiants of Macadamia (*Macadamia tetraphylla* L. Jhonson). Plant Cell Rep. 2512: 1281-1286.

Nayak P, Behera PR, Manikkannan T (2007). High frequency plantlet regeneration from cotyledonary node cultures of *Aegle marmelos* (L.) Corr. *In Vitro* Cell. Dev. Biol. Plant, 43: 231-236; Doi: 10. 1007/s11627-006-9013-6.

Nautiyal S, Rao KS, Maikhuri RK, Negi KS (2000). Apne hi ghar main kho gai mukhmar. Envis, Bull. Himalayan Ecol. Dev. 8: 83-84.

Nkanka BK (1982). Influence de la vitamine E sur la multiplication vegetative *in vitro* de l, *Eucalyptus rudis* Endl, Larix x eurolepis Henry et de *Q. borealis* Michx. Bull. Rech. Agron. Gembloux. 17(3): 219-226.

Pardos JA (1981). *In vitro* plant formation from stem pieces of *Q.suber*. In: AFOCEL (ed) Coll Int. Culture *in vitro* des essences forestieres, IUFRO, Fontainebleau, France, pp.186-190.

Perinet P, Lalonde K (1983). *In vitro* propagation and nodulation of actinorhizal host plant, *Alnus glutinosa* (L.) Gaertn. Plant Sci. Lett. 29: 9-17.

Purohit VK, Palni LMS, Nandi SK, Rikhari HC (2002a). *In vitro* plant regeneration through cotyledonary nodes of *Quercus floribunda* Lindl. ex A. Camus (Tilonj oak), a high value tree species of central Himalaya. Curr. Sci. 833: 101-104.

Purohit VK, Tamta S, Chandra S, Vyas P, Palni LMS, Nandi S K (2002c). *In vitro* multiplic.ation of *Quercus leucotrichophora* and *Q. glauca*: important Himalayan oaks. Plant Cell Tiss Org Cult 69:121-133.

Rajesweri V, Paliwal K (2006). *In vitro* propagation of Albizia odoratissima L.f. (benth.) From cotyledonary node and leaf nodal explants. *In Vitro* Cell Dev. Biol. Plant 42; 399-404.1054-5476/06

Rajesweri V, Paliwal K (2008). *In vitro* plant regeneration of red sanders (*Pterocarpus santalinus* L.f.) from cotyledonary nodes. Ind. J. Biotechnol. 7: 541-546.

Sato T, Mori N (1987). Saito A. *In vitro* plantlet propagation from epicotyl segments of young seedlings of kunugi (*Quercus acutissima*). J. Jpn. For. Soc. 69: 113-117.

San-Jose MC, Vieitez AM, Ballester A (1990). Clonal propagation of juvenile and adult trees of sessile oak by tissue culture techniques. Silvac. Gen. 39: 50-55.

Snedecor GW, Cochran WG (1967). Statistical methods. Oxford and IBH Publishing Co, New Delhi.

Schwarz OJ, Schlarbaum SE (1993). Axillary bud proliferation of 2 North American oak species: *Quercus alba* and *Quercus rubra*. Ann. Sci. For. 150: 340-343.

Tiwari S, Shah P, Singh K (2004). *In vitro* propagation of *Pterocarpus marsupium* Roxb.: An endangered medicinal tree. Ind. J. Biotechnol. 3:

422-425.
Tamta S, Palni LMS, Purohit VK, Nandi SK (2008). *In vitro* propagation of brown oak (Quercus semecarpifolia Sm.) from seedling explants. *In Vitro* Cell.Dev. Biol. Plant 44:136-141.
Upreti N, Tewari JC, Singh SP (1985). The oak forest of Kumaun Himalaya (India): Composition diversity and regeneration. Mountain, Res. Dev. 5(2)163-174.
Vengadesan G, Pijut PM (2009). *In vitro* propagation of northern red oak (*Quercus rubra* L.). *In Vitro* Cell. Dev. Biol. Plant, 45: 474-482.
Vieitez AM, San-Jose MC, Vieitez E (1985). *In vitro* plantlet regeneration from juvenile and mature *Q. robur*. J. Hortic. Sci. 60(1): 99-106.
Vishnu-Mittre (1974). Plant remains and climate from the late Harappan and other Chalcolithic cultures of India - A study in inter-relationships. Geophytology, 4: 46-53.
Wilkinson LL (1986). SYSTAT: The system for statistics. Systat, Evanston.
Zobel B, Talbert J (1984.). Applied forest tree improvement. Wiley, Prospect Heights.