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In vitro PROPAGATION OF CHINAR (*Platanus orientalis* L.) USING NODE AND INTERNODE EXPLANTS

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ABSTRACT

The aim of present study was to standardize a micropropagation protocol for Chinar (*Platanus orientalis* L.) using nodes and internode segments as explant materials. The sterilization treatment with 0.1% mercuric chloride for 10 min showed highest survival percentage in nodal explants (40.2) and internodal explants (51.9). Internodal explants survived sterilant treatment better than nodal explants. For shoot regeneration, MS (Murashige and Skoog medium) medium supplemented with indole butyric acid (IBA) (0.25 mg L^{-1}), benzyl adenine (BA) (4 mg L^{-1}) proved best for both nodal and internodal explants. The maximum number of shoots (9.7 explant^{-1}) greater shoot length (3.9 cm) was obtained from the internodal explants on MS medium supplemented with BA (4 mg L^{-1}) + IBA (0.25 mg L^{-1}). MS medium supplemented with IBA (1 mg L^{-1}) and BA (2 mg L^{-1}) showed 100% rooting percentage as well as highest number and length of roots (15.7 shoot^{-1}, 3.7 cm, respectively). The internodal explants showed better response than nodal explant. Rooted plantlets were then transferred to perforated plastic pots containing sterilized mixture of sand and soil (1:1) ratio, and grown in greenhouse. The survival percentage after 4 weeks in greenhouse was recorded as 62.3%.

Key words: Chinar, *Platanus orientalis*, tissue culture, micropropagation, *in vitro* propagation

INTRODUCTION

Chinar (*Platanus orientalis* L.) is a tree species belonging to the family Platanaceae. It is the only species of Platanaceae found in India and its growth is confined to Jammu & Kashmir state (Kozgar and Khan, 2011). It can attain a height of about 30 m and a girth upto 12 m. The bark is greyish and leaves are borne alternately on stem, deeply 5-7 lobed 12-20 cm in length, and palmate or maple-like with long stalk. Petiole is quite long and length ranges between 3 and 8 cm. Flowers are dense spherical heads, monoecious and unisexual. Fruits are sphere-shaped, compound, and 2 to 6 of them are found on a long leafstalk. Presumably chinar originated in geological times from divergence involving the intercontinental disjunction and other barriers (Sert *et al.*, 2008; Pilotti *et al.*, 2009). It is found naturally in the forest lands of Turkey and is widely used in landscape design (urban open green spaces, parks, arboreta, water fronts, industrial areas, shade bearer and street tree), and there are numerous examples of these being protected as natural monumental trees in Turkey (Zencirkiran, 2010; Zencirkiran and Erken, 2012). It spread wide across a region of cool climate with sufficient water. The chinar trees have spread to Asia, especially the Western Himalayan

region of India, perhaps from Greece. Chinar has become an integral part of Kashmiri culture and a heritage tree that has survived for ages. Kashmir houses Asia's largest and oldest chinar tree which is 700-years-old, located at Chattergam (Budgam) with a circumference of 31.85 m and height of 14.78 m (Wadoo, 2007).

Chinar, a multipurpose tree, is of immense aesthetic importance owing to its beauty and magnificence. Its leaves and bark are used as medicine, the wood is used for making delicate furniture items and the twigs and roots are used for making dyes (Sharma, 2008). Jammu and Kashmir is ecologically fragile and ranks 20th on environment sustainability index (ESI) with ESI score of 40.0 which indicates greater pressure on its ecosystem, higher pollution and vulnerability to environmental predicaments (*http://www.greenindiastandards.com/jammu-kashmir.php?stateid =14*). Chinar population in India is declining due to illegal felling and needs immediate efforts for its conservation (*https://www.kashmirmonitor.in/Details/123741/chinar-the-dying-heritage*). The number of chinar trees in J&K state has reduced to over half - from 42,000 in 1970 to less than 27,000 (Kozgar and Khan, 2011).

Chinar is propagated by generative and vegetative methods. Propagation through seeds (after stratification) is the first option, however, the rate of germination is poor (30-40%) [Anonymous, 2003; Hartman *et al.,* 2011]. Propagation by stem cutting is the second option; however, rooting potential is affected by the factors like genotype, type of cutting (basal median), position of cutting-donor shoots on mother plant, cutting stem diameter, humidity and temperature of rooting medium, time of cutting collection, etc. (Grolli *et al.,* 2005; Dirr and Heuser, 2006; Khosrojerdi *et al.,* 2006). These limitations can be overcome through development of *in vitro* propagation protocol. However, the availability of information about the micropropagation of *Platanus* species is very scanty (Guoqiang *et al*., 2003). Most of the studies pertain to *P. acerifolia* (London plane tree), a hybrid of *Platanus orientalis* (oriental plane) and *P. occidentalis* (American sycamore) [Gjuleva and Atanasov, 1994; Liu *et al*., 2002; Liu and Bao, 2003]. Perusal of literature reveals that there is only one report on micropropagation of *P. orientalis* through use of leaf explant and no protocol is available while using node or internodes as explant. This is not only a limitation for propagation of this plant, but also a major bottleneck in its genetic improvement using plant tissue culture methods. The present study was, therefore, aimed at to standardize an *in vitro* micropropagation protocol for chinar (*P. orientalis*).

MATERIALS AND METHODS

Explant source

The present study was conducted in the Centre for Plant Biotechnology, SKUAST-Kashmir, Shalimar (J&K). *Platanus orientalis* trees (of more than 300 years age) growing at the campus were chosen as mother trees for explant collection. The first and second nodal and internodal segments were collected from the tip of disease free juvenile branches of plus trees. The succulent and actively growing nodes were chosen for the purpose while nodes older than 40 days were hard. Explants were washed with running tap water 2-3 times followed by 2-3 rinses with double distilled water. These were then surface sterilized by mercuric chloride (0.1-0.3%) for different time durations (1-10 min) followed by washing of explants with autoclaved water. Each treatment constituted of 60 explants and was repeated twice. Mortality percentage of explants, contamination percentage, and survival percentage were recorded for each treatment.

Culture conditions

All chemicals and reagents were procured from HiMedia (India) while borosilicate glassware was procured from Borosil Glassworks Ltd. (India). The sterilized explants were cultured on ium (Murashige and Skoog, 1962) supplemented with B_5 vitamins, 3% sucrose and 0.8% agar agar.

Different concentrations and combinations of auxins and cytokinins were used for plant regeneration (Table 2). For every combination, 10 test tubes with 1 explant each were inoculated and the experiment was repeated twice. The pH of medium was adjusted to 5.8 prior to adding 0.8% agar. The cultures were incubated at 25±2˚C in sterilized room with a 16/8h light/dark regime, 75% relative humidity and a light intensity of 3500 lux. The *in vitro* developed plantlets were removed from the agar medium and washed gently to record the observations. For shoot regeneration, the parameters recorded were shoot regeneration percentage, shoot length (cm) and number of shoots regenerated explant^{-1} (Husaini and Abdin, 2007). For rhizogenesis, the parameters studied were root regeneration percentage, root length (cm) and number of roots regenerated shoot^{-1} (Shah *et al.*, 2013). Each parameter was recorded on at least three biological replicates.

Statistical analysis

A completely randomized design was followed for the experiment. The data recorded for different parameters of surface sterilization, shoot regeneration, rhizogenesis was subjected to analysis of variance (Cochran and Cox, 1963).

RESULTS AND DISCUSSION

Surface sterilization of explants

P. orientalis explants (nodal and internodal segments) were subjected to 8 different sterilization regimes followed by culturing on MS basal medium. The effect of various sterilization regimes on explants survival, mortality and contamination percentages was highly significant (Table 1). The highest mean survival of 46.0% was observed in explants treated with 0.1% $HgCl_2$ for 10 min, followed by 40.0% in the explants treated with 0.1% $HgCl_2$ for 5 min. The response of internodal explants was significantly better (36.0%) than nodal explants (30.0%). Maximum percentage of surviving explants (51.9%) was when internodal explants were treated with 0.1% $HgCl_2$ for 10 min.

Maximum mean mortality of 44.8% was observed in explants treated with 0.3% $HgCl_2$ for 3 min followed by 40.3% in explants treated with 0.3% $HgCl_2$ for 2 min. The percent mortality of internodal explants was significantly lower (33.7%) than nodal explants (38.3%). The least mortality of 21.5% was observed in internodal explants treated with 0.1% $HgCl_2$ for 10 min. Maximum mean contamination of 34.1% was observed in explants treated with 0.3% $HgCl_2$ for 3 min. The lowest mean contamination of 26% was observed in explants treated with 0.1% $HgCl_2$ for 10 min. The contamination of internodal explants was significantly lower (30.6%) than nodal explants (31.7%). The maximum contamination of 35.9% was noticed in nodal explants treated with 0.3% $HgCl_2$ for 3 min and minimum contamination of 23.2% in internodal explants treated with 0.1% $HgCl_2$ for 1 min. The results are in close conformity with those obtained in *P. acerifolia* micropropagation through mesophyll protoplast culture (Liu *et al*., 2002). They reported that the treatment of explants with 0.1% $HgCl_2$ for 10 min was effective for surface sterilization with maximum explant survival. Liu and Bao (2003) who worked on micro-propagation of *P. acerifolia* through adventitious shoot regeneration from *in vitro* cultured leaves found that the treatment of explants with 0.1% $HgCl_2$ for 10 min was the most effective surface sterilization procedure for maximum survival of explants with minimum tissue injury.

Shoot regeneration

Eighteen combinations of benzyl adenine, indole butyric acid, naphthalene acetic acid (BA + IBA or BA + NAA) in MS medium were assessed to evaluate their effects on shoot regeneration percent, number of shoots explant^{-1} and shoot length of nodal and intermodal shoot regeneration. Shoot regeneration response of internodal explants was significantly better than the nodal explants, which showed that the morphogenetic response of different explants, even from the same plant may

Table 1: Effect of concentration and duration of mercuric chloride sterilization of nodal and internodal explants of *Platanus orientalis* on explant survival, mortality and contamination

$HgCl_2$ treatment conc.& duration	Survival (%)			Mortality (%)			Contamination (%)		
	Nodal segments	Internodal segments	Mean	Nodal segments	Internodal segments	Mean	Nodal segments	Internodal segments	Mean
0.1% for 1 min.	30.8 (5.74)	37.3 (6.10)	34.0 (5.83)^{c}	36.5 (6.04)	39.5 (6.28)	38.0 (6.16)^{b}	32.7 (5.71)	23.2 (4.81)	28.0 (5.28)^{c}
0.1% for 2 min.	34.6 (5.88)	34.2 (5.84)	34.4 (5.86)^{bc}	32.7 (5.71)	31.5 (5.61)	32.1 (5.66)^{c}	32.7 (5.71)	34.4 (5.86)	33.6 (5.78)^{ab}
0.1% for 3 min.	26.9 (5.18)	34.6 (5.88)	30.7 (5.54)^{cd}	43.5 (6.59)	33.1 (5.74)	38.3 (6.18)^{b}	29.6 (5.44)	32.3 (5.68)	30.9 (5.56)^{b}
0.1% for 5min.	35.9 (5.98)	44.1 (6.63)	40.0 (6.32)^{ab}	31.6 (5.61)	23.3 (4.82)	27.5 (5.23)^{d}	32.6 (5.70)	32.7 (5.71)	32.7 (5.71)^{ab}
0.2% for 3 min.	24.9 (4.99)	29.7 (5.44)	27.3 (5.22)^{d}	41.3 (6.42)	39.3 (6.27)	40.3 (6.34)^{ab}	33.7 (5.80)	31.0 (5.56)	32.4 (5.68)^{ab}
0.3% for 2 min.	27.9 (5.28)	30.3 (5.50)	29.1 (5.39)^{cd}	41.1 (6.41)	37.6 (6.12)	39.4 (6.27)^{b}	31.0 (5.56)	32.1 (5.66)	31.6 (5.61)^{ab}
0.3% for 3 min.	18.9 (4.35)	25.6 (5.06)	22.3 (4.72)^{e}	45.2 (6.72)	44.3 (6.65)	44.8 (6.68)^{a}	35.9 (5.98)	32.4 (5.69)	34.1 (5.84)^{a}
0.1% for 10 min.	40.2 (6.34)	51.9 (7.20)	46.0 (6.78)^{a}	34.4 (5.86)	21.5 (4.63)	27.9 (5.28)^{d}	25.4 (5.03)	26.7 (5.16)	26.0 (5.10)^{c}
Mean	30.0 (5.47)^{B}	36.0 (5.99)^{A}		38.3 (6.18)^{A}	33.7 (5.80)^{B}		31.7 (5.63)^{A}	30.6 (5.52)^{B}	
$CD_{0.05}$									
Sterilant		0.49			0.37			0.24	
Explant		0.34			0.28			0.09	
Sterilant x explant		ns			ns			0.34	

Values in the parenthesis are square root transformed

vary. The variation in regeneration potential of different explants of same plants is well documented in many other plant species (Husaini *et al.* 2011). The interaction effect of different media and explants on shoot regeneration was significant with highest shoot regeneration (100%) observed in internodal explants cultured on MS medium supplemented with BA (4.00 mg L^{-1}) and IBA (0.25 mg L^{-1}). Least shoot regeneration of 13.2% was noticed in nodal segment explants cultured on MS medium supplemented with 1 mg BA L^{-1} + 0.5 mg IBA L^{-1}. The interaction effect of different media and explants on number of shoots $explant^{-1}$ was significant with maximum number of shoots (9.7 $explant^{-1}$) in internodal explants cultured on MS medium supplemented with BA (4.00 mg L^{-1}) + IBA (0.25 mg L^{-1}). Least number of shoots (2.7 $explant^{-1}$) was obtained from the nodal segment explants cultured on MS medium supplemented with BA (1 mg L^{-1}) + IBA (0.25/0.5 mg L^{-1}). The interaction effect of different media and explants on length of shoots was significant. Maximum shoot length of 3.9 cm was observed in internodal explants cultured on MS medium supplemented with BA (4.00 mg L^{-1}) + IBA (0.25 mg L^{-1}) and minimum shoot length of 1.1 cm in nodal segment explants cultured on MS medium supplemented with BA (1 mg L^{-1}) + IBA (0.5 mg L^{-1}). The overall best shoot regeneration medium was MS medium supplemented with BA (4.0 mg L^{-1}) and IBA (0.25 mg L^{-1}) which showed maximum shoot regeneration percentage (72.8%), number of shoots $explant^{-1}$ (7.5) and shoot length (3.0 cm) [Table 2]. The results are in consonance with Liu *et al.* (2002) who found IBA (0.25 mg L^{-1}) + BA (4.0 mg L^{-1}) best for shoot proliferation, number of shoots and maximum shoot length. Likewise, Liu and Bao (2003) reported maximum shoot proliferation percentage on 0.25 mg L^{-1} IBA and 4 mg L^{-1} BA.

Rhizogenesis

Shoots were rooted simultaneously on the same medium used for shoot regeneration, hence it is not necessary to subculture the shoots on separate rooting medium. Simultaneous development of

Table 2: Shoot regeneration from nodal and internodal explants of *Platanus orientalis* on MS medium supplemented with auxins and cytokinin, after 4 weeks

Growth regulators (mg L^{-1})			Shoot regeneration (%)		Mean	shoots explant^{-1} (No.)		Mean	Shoot length (cm)		Mean
NAA	IBA	BA	Nodal segments	Internodal segments		Nodal segments	Internodal segments		Nodal segments	Internodal segments	
0	0.25	1	13.4 (3.79)	23.4 (4.93)	18.4 (4.44)^{f}	3.7	4.7	4.2^{de}	1.4	2.0	1.7^{d}
0	0.5	1	13.2 (3.75)	20.0 (4.56)	16.6 (4.15)^{r}	2.7	4.3	3.5^{e}	1.1	1.4	1.3e
0	0.25	2	14.8 (3.97)	31.1 (5.66)	23.0 (4.81)^{e}	2.7	4.3	3.5^{e}	1.3	1.9	1.6^{d}
0	0.5	2	14.1 (3.88)	33.3 (5.85)	23.7 (5.01)^{e}	4.0	5.3	4.7^{cd}	1.4	1.8	1.6^{d}
0	1	2	14.8 (3.97)	33.3 (5.85)	24.1 (5.06)^{e}	4.0	5.3	4.7^{cd}	1.1	2.1	1.6 ^{d}
0	0.25	4	45.7 (6.82)	100 (10.05)	72.8 (7.79)^{a}	5.3	9.7	7.5^{a}	2.2	3.9	3.0^{a}
0	0.5	4	29.7 (5.54)	66.6 (8.21)	48.2 (6.73)^{b}	5.0	7.3	6.2 ^{b}	2.0	2.4	2.2^{b}
0	1	4	19.2 (4.49)	35.5 (6.03)	27.3 (5.11)^{e}	4.0	7.3	5.7^{bc}	1.9	2.0	1.9^{c}
0.25	0	1	20.7 (4.65)	29.7 (5.54)	25.2 (5.09)^{e}	4.0	6.7	5.3^{bc}	1.2	2.2	1.7^{d}
0.25	0	4	16.8 (4.21)	57.1 (7.61)	36.9 (6.01)^{c}	4.7	7.3	5.6^{b}	1.6	2.3	1.9c
0.5	0	4	25.0 (5.08)	50.0 (7.14)	37.5 (6.10)^{c}	3.3	7.3	5.3^{bc}	1.2	2.3	1.7^{d}
1	0	4	25.0 (5.08)	33.3 (5.85)	29.6 (5.51)^{d}	4.3	7.3	5.8^{b}	1.8	2.1	1.9^{c}
Mean			21.0 (4.59)^{B}	42.8 (6.44)^{B}		4.0^{B}	6.4^{A}		1.5^{B}	2.2^{A}	
$CD_{0.05}$											
Medium				0.30			1.06			0.15	
Explant				0.52			2.15			0.32	
Medium x Explant				0.83			ns			0.48	

Values in the parenthesis are square root transformed

shoots and roots, without transfer into new rooting medium, has been reported in other plant species (Husaini *et al.*, 2008). The number of roots with their length and regeneration per cent was calculated on the same culture media (Table 3). The interaction effect of different media and explants on root regeneration was significant. Highest root regeneration of 100% was observed

Table 3: Rhizogenesis of shoots of *Platanus orientalis* on MS medium supplemented with auxins and cytokinin, after 4 weeks

Growth regulators (mg L^{-1})			Root regeneration (%)		Mean	No. of roots/shoot		Mean	Root length (cm)		Mean
NAA	IBA	BA	Nodal segments	Internodal segments		Nodal segments	Internodal segments		Nodal segments	Internodal segments	
0	0.25	1	96.2 (9.85)	99.8 (10.03)	98.0 (9.94)^{ab}	4.7	7.3	6.0^{cd}	2.1	3.2	2.7^{b}
0	0.5	1	98.3 (9.96)	100 (10.05)	99.2 (10.00)^{ab}	9.3	12.0	10.7^{b}	2.1	3.2	2.7^{ab}
0	0.25	2	93.1 (9.69)	99.6 (10.03)	96.3 (9.86)^{cd}	4.0	7.3	5.7 ^{cd}	2.0	3.2	2.6^{b}
0	0.5	2	94.7 (9.77)	98.7 (9.98)	96.7 (9.87)^{cd}	4.0	7.3	5.7^{cd}	2.1	3.1	2.6^{b}
0	1	2	100 (10.05)	100 (10.05)	100 (10.05)^{a}	10.0	15.7	12.8^{a}	2.2	3.7	3.0^{a}
0	0.25	4	82.7 (9.14)	96.3 (9.86)	89.5 (9.50)^{e}	2.7	4.3	3.5^{e}	1.3	1.9	1.6^{d}
0	0.5	4	79.3 (8.96)	83.3 (9.28)	81.3 (9.12)^{f}	2.7	4.3	3.5^{e}	1.9	1.5	1.7^{cd}
0	1	4	91.4 (9.61)	98.6 (9.97)	95.0 (9.79)^{cd}	3.3	7.3	5.3^{d}	1.9	2.0	2.0^{c}
0.25	0	1	95.7 (9.83)	99.2 (10.01)	97.5 (9.92)^{bc}	4.3	9.7	6.7^{c}	2.2	3.4	2.8^{ab}
0.25	0	4	88.2 (9.44)	99.1 (10.20)	93.6 (9.82)^{cd}	4.0	6.0	5.0^{de}	2.0	1.8	1.9^{cd}
0.5	0	4	95.1 (9.80)	96.7 (9.88)	95.9 (9.84)^{cd}	3.7	4.7	4.2^{de}	2.0	1.8	1.9^{c}
1	0	4	92.6 (9.67)	96.8 (9.81)	94.7 (9.74)^{d}	3.3	7.3	5.3^{de}	2.0	3.0	2.5^{b}
Mean			92.3 (9.64)^{B}	97.3 (9.91)^{A}		4.7^{B}	7.7^{A}		2.0^{B}	2.7^{A}	
$CD_{0.05}$											
Medium				0.13			1.54			0.32	
Explant				0.20			2.13			0.51	
Medium x Explant				0.33			3.68			0.83	

Values in the parenthesis are square root transformed

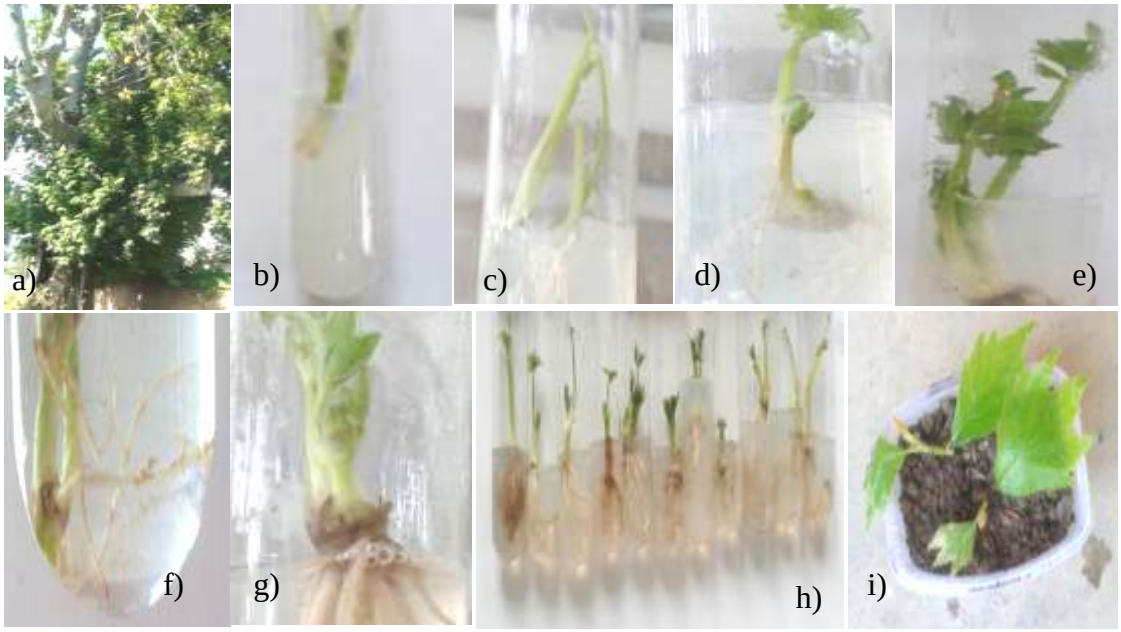

Fig. 1. *In vitro* propagation of chinar (*Platanus orientalis* L.): a) source of explants, the mother plant; b) explant on MS medium; c) shoot initiation; d) shoot elongation e) multiple shoot formation; f) root initiation; g) multiple root formation; h) complete plantlets; i) hardening in pots

when both nodal and internodal explants were cultured on MS medium supplemented with 2.0 mg L^{-1} BA + 1.0 mg L^{-1} IBA. The lowest root regeneration of 79.3% was obtained from nodal segment explants cultured on MS medium supplemented with 4.0 BA mg L^{-1} + 0.5 mg IBA L^{-1}. The interaction effect of different media and explants on number of roots per shoot showed highest number of roots 15.7 $shoot^{-1}$ when internodal explants were cultured on MS medium supplemented with BA (2.0 mg L^{-1}) + IBA (1.0 mg L^{-1}). Lowest number of roots 2.7 $shoot^{-1}$ was obtained from nodal segment explants cultured on MS medium supplemented with either 4.0 mg BA L^{-1} + 0.25 IBA mg L^{-1} or 4.0 BA mg L^{-1} + 0.5 mg IBA L^{-1}. The interaction effect of different media and explants on length of roots was significant. Maximum root length of 3.7 cm was observed in internodal explants cultured on MS medium supplemented with 2.0 BA mg L^{-1} + 1 mg IBA L^{-1} and a minimum of 1.3 cm from nodal segment explants cultured on MS medium supplemented with 4.0 BA mg L^{-1}) + 0.25 IBA mg L^{-1}. The overall best root regeneration medium was MS medium supplemented with 2.0 mg BA L^{-1} +1.0 mg IBA L^{-1} which showed maximum root regeneration percentage (100%), number of roots $shoot^{-1}$ (10), and root length (3 cm) [Table 3]. Reports of Gjuleva and Atanasov (1994) and Zencirkiran and Erken (2012) support our finding as they too obtained rooting with MS medium supplemented with IBA, BA, NAA from 0-4 mg L^{-1}. Liu *et al.* (2002) reported that 1.0 mg IBA L^{-1} + 2.0 BA mg L^{-1} was best for root proliferation, number of roots and highest root length while Guoqiang *et al.* (2003) reported that 1/2 MS + 0.1-0.5 mg IBA L^{-1} in medium was good for root induction. The complete *in vitro* protocol for regeneration of *P. orientalis* plants was developed which takes only 4 months to develop disease free plantlets of Chinar from these explants (Fig. 1).

Conclusions: Chinar (*Platanus orientalis* L.) is the only species of family Platanaceae found in India and has economic and cultural importance. The study developed first protocol for *in vitro* propagation of chinar by using nodes and internodes as explant materials. Various sterilization treatments yielded aseptic cultures but sterilization with 0.1% $HgCl_2$ for 10 min yielded maximum percentage of aseptic cultures and highest percentage of surviving explants. The survival response of internodal explants was significantly higher than that of nodal explants. The constituents of

medium especially growth regulators significantly influenced the regeneration potential of explants. MS medium with B_5 vitamins supplemented with IBA (0.25 mg L^{-1}) or BA (4 mg L^{-1}) was best for shoot regeneration; while MS medium supplemented with IBA (1 mg L^{-1}) or BA (2 mg L^{-1}) was most suitable for rhizogenesis. The shooting as well as rooting response of internodal explants was significantly higher than nodal explants. The study shall be helpful in designing a comprehensive commercial tissue culture programme for chinar.

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