The Bioscan 11(3): 1551-1554, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

DE NOVO ORGANOGENESIS IN *GREWIA OPTIVA*-AN IMPORTANT FODDER TREE

S. K. JHA[1]*, R. N. SEHGAL[1] AND KAMLESH KANWAR[2]
[1]Department of Tree Improvement and Genetic Resources, College of Forestry,
[2]Department of Biotechnology, College of Horticulture,
Univeristy of Agricultural Forestry, Solan, H.P-173 230, INDIA
e-mail: sumanfort@gmail.com

KEYWORDS
Grewia optiva
hypocotyl
Cotyledon
microshoots

Received on :
01.01.2016

Accepted on :
17.07.2016

***Corresponding author**

ABSTRACT

In present investigation, callus through regeneration obtained from cotyledon and hypocotyls explants in *Grewia optiva*. The seeds were germinated *in vitro* and cotyledon and hypocotyl were used as explant. Cotyledon showed better response for callogenesis with 64.49 per cent callus formation in MS media supplemented with 2.00 mg l^{-1} NAA+ 1.00 mgl^{-1}BAP. The best treatment for shoot bud induction for callus was 3.00 mg l^{-1} + 0.10 mg l^{-1}NAA which produced 64.49 per cent shoot buds in cotyledonary callus. Regeneration potential of callus decreased after second subculture. Rooting of microshoots obtained in media supplemented with 2.00 mgl^{-1} IBA, which showed maximum per cent rooting (70.97%). When rooted shoots further transferred to pot 52.68 per cent survived.

INTRODUCTION

Grewia optiva Drummond belonging to family Tiliaceae is one of the most important fodder tree of north eastern and central Himalayas (Brandis *et al*., 1972). It provides green leaf fodder in lean periods especially during the winter season, when there is no other source of green fodder available. The leaves are highly nutritious, and fairly rich in protein and mineral nutrients (Khosla *et al*., 1992; Negi 1977). Its fodder can play an important role in improving the nutrition of livestock in the hills where the poverty of the farmers neither allows them to purchase concentrated feeds nor their small holdings allow them to resort to cultivation of nutritious leguminous fodder/ forage crops (Sharma *et al*., 2000; Swamy *et al*., 2002).

The inherent capacity of plant cell to give rise to whole plant, a capacity which is often obtained even after a cell has undergone a final differentiation in the plant body is described as 'totipotency' from time to time (Cuenca *et al*., 2000). For a differentiated cell to express its totipotency it first undergoes dedifferentiation (callusing) followed by redifferentiation (Bhojwani and Razdan 1986). The ability to redifferentiate or regenerate efficiently from cell and callus culture is a pre-requisite for the application of many biotechnological approaches including regeneration, growth of large scale cultures, *in vitro* selection strategies and genetic engineering techniques, aimed at genetic improvement of plants (Jiang *et al*., 2015; Pandey *et al*., 2013; Ribas *et al*., 2011; Stevens and Pijut 2014; Zhang *et al*., 2014). The present work is first report on indirect organogenesis in *G. optiva*.

MATERIALS AND METHODS

In vitro seedlings were raised from excised seed of *Grewia optiva*. Completely ripened fruits were collected. The collected seeds were washed under running tap water for half an hour and soaked in water for overnight. The seeds were separated from the flesh and washed thoroughly with distilled water. Before, *in vitro* inoculation the seeds were immersed in Bavistin (0.20%) for three minutes followed by washing with double distilled water thrice, and then treated with 0.10 per cent mercuric chloride solution for two minutes followed by three to four thorough washings with autoclaved distilled water and excised seed were inoculated on basal MS medium. The cultures were incubated at 25±2°C under 16 hours photoperiod. Well-developed seedlings used as the source for cotyledon and hypocotyls explants. Cotyledon and hypocotyls segments were excised from ten days old *in vitro* geminated seedlings and cultured on MS supplemented with different constructions of NAA (1.00, 2.00, 3.00, 5.00 mg1^{-1}) either alone or in combination with BAP (0.10, 0.50, 1.00, 2.00 mg 1^{-1}).The cultures were maintained under 16 hours photoperiod for four weeks. The observations were recorded for i) per cent callus induction measured as per cent explants inducing callus, ii) morphological characters of callus such as growth, colour and type. Different callus types were categorized as I) friable- liable to crumble, ii) compact firmly bounded. With 30 explants in each treatment the experiment was repeated thrice.

For shoot induction, small callus pieces were cut from the

cotyledon as well as hypocotyls derived calli and subcultured on the medium with different concentration of BAP (0.50, 1.00, 2.00 and 3.00 mg1^{-1}) and NAA (0.01, 0.10, 0.50 and 1.00 mgl^{-1}) under 16 hours photoperiod to promote callus proliferation and observe shoot bud induction. The observations for i) per cent shoot bud induction quantified as per cent callus pieces showing shoot bud induction and ii) average number of shoots per callus piece were recorded after four weeks of culture. With 30 explants in each treatment the experiment was repeated thrice.

For *in vitro* rooting on MS basal medium the microshoots were transferred to culture tubes containing solid MS medium supplemented with different concentration of NAA (0.50,1.00,2.00,3.00 and 5.00 mgl^{-1}) and IBA (0.50,1.00,2.00,3.00 and 5.00 mgl^{-1}) and cultures were incubated in culture room. Observations were recorded for i) percentage of root induction was quantified as per cent inoculated microshoots inducing roots, ii) average root length-measured as average length of randomly selected three roots per plantlet and iii) average number of roots per microshoot. The last three observations were recorded after seven weeks. With 30 microshoots in each treatment the experiment was repeated thrice.

After six weeks of incubation on MS rooting medium the roots of well established plantlets were washed thoroughly to remove the sticking medium. The plantlets were dipped in 0.01 per cent carbendazim (Bavistin) solution for 20-25 minutes and transferred to potting mixture comprised of stratified sand and soil in the ratio of 3:1 so that soil was in the lower strata and sand in the upper portion of the pot. For initial 15 days the potted plants were covered with jam bottles to maintain the humidity. Acclimatization to external environmental conditions was done by removing the jam bottles to reduce humidity gradually. After seven weeks well established plantlets rooted on MS medium were transferred directly to pots containing the same potting mixture as plants were nourished by adding 10 ml of 1/10 MS basal medium (devoid of sucrose) on alternate days. After four weeks the observations were recorded for percent survival of plantlets upon transfer to pots.

All the data analyzed for ANOVA for completely randomized design and means were compared using critical difference at 95% confidence interval.

RESULTS AND DISCUSSION

The cotyledon and hypocotyl explants were inoculated on solid MS medium supplemented with varying concentrations of NAA alone or in combination with different concentrations of BAP for four weeks to observe the effect of explant and best treatment of growth regulator combination suitable for callus induction. The callus initiated after one week from the cut ends of both types of explants and the whole surface of explants was covered with callus within four weeks of culture.

Perusal of Table 1 indicates that the excellent proliferation rate for cotyledon explant was observed in treatment with NAA 2.0 mg l^{-1} + 1.0 mg l^{-1} BAP and NAA 3.0 mg/l + 1.0 mg/l BAP. The callus derived from both the treatments was compact and green with good regeneration potential. These treatments were also found to be good for hypocotyl explants, which showed good proliferation rate in the presence of these combinations. But the cotyledonary callus had comparative higher proliferation rate than the hypocotyls callus. Maximum callus induction for both the explants was observed in media supplemented with 3.0mg/l NAA + 1.0 mg/l BAP (with mean of 71.51%). This treatment was statistically significant from all

Table1: Effect of different concentration of NAA and BAP on cotyledonary and hypocotyls explant for callus induction, proliferation rate, type, colour of callus

Growth regulator mg/l		cotyledon				hypocotyl			
NAA	BAP	Percent response	proliferation	type	colour	Percent response	proliferation	type	colour
0	0	-	-	-	-	-	-	-	-
1	0	-	-	-	-	-	-	-	-
1	0.1	7.22±0.4	+	c	b	-	-	-	-
1	0.5	27.52±1.6	+	c	b	5.36±0.3	+	f	b
1	1	23.69±1.4	+ +	f	yg	7.59±0.4	+	f	yg
1	2	27.26±1.6	+ +	f	yg	5.29±0.3	+	f	yg
2	0	14.82±0.9	+	f	b	7.19±0.4	+	f	b
2	0.1	36.69±2.1	+	f	b	24.35±1.4	+	f	b
2	0.5	26.32±1.5	+ +	c	yg	26.56±1.5	+	c	yg
2	1	64.59±3.7	+ + + +	c	g	22.67±1.3	+ + + +	c	g
2	2	15.67±0.9	+ +	c	g	8.81±0.5	+	c	g
3	0	12.49±0.7	+ +	f	b	6.36±0.4	+	f	b
3	0.1	42.16±2.4	+ +	f	b	28.36±1.6	+	f	b
3	0.5	67.23±3.9	+ + +	f	g	32.11±1.9	+ +	c	yg
3	1	86.56±5.0	+ + + +	c	g	56.46±3.3	+ + +	c	yg
3	2	31.91±1.8	+ +	c	g	10.22±0.6	+ +	c	g
5	0	11.63±0.7	+ +	f	b	7.67±0.4	+	f	b
5	0.1	15.24±0.9	+ +	c	b	4.99±0.3	+ +	f	b
5	0.5	51.69±3.0	+ + +	c	b	37.64±2.2	+ +	f	b
5	1	58.48±3.4	+ + +	c	yg	36.25±2.1	+ +	c	b
5	2	23.13±1.3	+	c	b	16.98±1.0	+	c	yg
$CD_{0.05}$		3.39				3.26			

f-friable, c-compact, g-green,b-brown, yg-yellowish green; + poor, + + good, + + + very good, + + + + excellent

Table2 : Effect of BAP and NAA on shoot bud induction and number of shoot per shot bud from cotyledon and hypocotyls derived callus

Growth regulator mg/l		cotyledon		hypocotyl	
BAP	NAA	% calli with shoot	Number of shoot bud	% calli with shoot	Number of shoot bud
0	0	-	-	-	-
0	0.01	-	-	-	-
0	0.1	-	-	-	-
0	0.5	-	-	-	-
0.5	0	-	-	-	-
0.5	0.01	7.18±0.4	0.78±0.1	-	-
0.5	0.1	7.5±0.4	0.95±0.1	-	-
0.5	0.5	-	-	-	-
1	0	15.63±0.9	1.12±0.1	-	-
1	0.01	33.33±1.9	2.25±0.1	12.36±0.7	0.85±0.1
1	0.1	43.56±2.5	3.5±0.2	25.29±1.5	2.41±0.1
1	0.5	16.64±1.0	1.19±0.1	6.67±0.4	0.68±0.1
2	0	25.67±1.5	1.83±0.1	16.85±1.0	1.58±0.2
2	0.01	46.91±2.7	2.58±0.1	24.24±1.4	2.52±0.1
2	0.1	57.63±3.3	4.36±0.3	27.18±1.6	2.18±0.1
2	0.5	37.89±2.2	2.37±0.1	12.39±0.7	1.17±0.2
3	0	19.71±1.1	1.45±0.1	6.64±0.4	0.78±0.0
3	0.01	57.33±3.3	5.77±0.3	36.83±2.1	3.71±0.2
3	0.1	64.19±3.7	5.93±0.3	43.64±2.5	4.06±0.2
3	0.5	44.83±2.6	2.77±0.2	28.33±1.6	3.1±0.2
$CD_{0.05}$		3.49	0.45	2.76	0.32

Table3: Effect of IBA and NAA on per cent root induction and number of roots per microshoot produced from callus

Growth regulators	Concentrations (mg/l)	% rooting	Number of roots/shoot
	0.5	33.26±1.9	3.73±0.2
	1	52.9±3.1	4.5±0.3
IBA	2	70.97±4.1	6.53±0.4
	3	63.22±3.7	3.43±0.2
	5	50.32±2.9	4.34±0.3
	0.5	41.77±2.4	4.23±0.2
	1	21.66±1.3	5.07±0.3
NAA	2	14.91±0.9	2.3±0.1
	3	16.09±0.9	1.57±0.1
	5	9.82±0.6	1.7±0.1
$CD_{0.05}$		2.36	0.32

other treatments. Higher concentration of NAA with combination of moderate concentration of BAP (0.5 and 1.0 mg/l) gave better result as compared to the treatments with lower concentration of NAA or NAA in combination with higher ratio of BAP. Higher concentration of NAA turned callus brown with lower regeneration potential. BAP was essential for the induction of shoot buds. Absence of NAA affected the shoot bud induction potential of BAP. Higher concentration of BAP in combination of lower concentration of NAA gave better results. The best result for shoot bud induction from cotyledonary callus was 3.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA, with 64.19 per cent shoot bud induction for cotyledonary callus and 43.64 per cent for hypocotyls derived callus . Although BAP 3.0 mg/l + NAA 0.01 mg/l and BAP 2.0 mg/l + NAA 0.10 mg/l also showed higher value with 57.33 and 57.63 per cent for shoot bud induction, respectively. Number of shoot buds was again best for the same treatment with highest value of 5.93 for cotyledonary callus and 4.06 for hypocotyls callus (Table 2). Similar higher regeneration potential of cotyledon as compare to hypocotyls explants has been reported by (Mohamed *et al.*, 2013; Pawar *et al.*, 2013; Rashid *et al.*, 2012)

Table 3 shows the effect of IBA and NAA on *in vitro* rooting of callus derived microshoots. It clearly shows the superiority of IBA over the NAA for root induction of *in vitro* derived microshoots. The maximum rooting was observed in media supplemented with 2 mg l^{-1} IBA but as the concentration of IBA was raised over 2 mg/l, significant decrease was observed, whereas IBA at 1.00 mg l^{-1} showed maximum number of root per microshoot. Similar result has been found by different authors in different species (Jani *et al.*, 2015; Kansara *et al.*, 2013; Nagar *et al.*, 2015a; Nagar *et al.,* 2015b). Well rooted microshoots were transferred to pot after six weeks. Survival in the pot was 52.68 per cent.

REFERENCES

Bhojwani, S. S., Razdan, M. K. 1986. *Plant tissue culture: theory and practice*. Elsevier,

Brandis, D., Stewart, J. L., Fitch, W. H. 1972. *Illustrations of the forest flora of North-West and Central India*. Bishen Singh Mahendra Pal Singh,

Cuenca, B., Ballester, A., Vieitez, A. 2000. In vitro adventitious bud regeneration from internode segments of beech.Plant Cell, Tissue and Organ Culture. **60:** 213-220

Jani, J. N., Jha, S. K., Nagar, D. S. 2015. Root explant produces multiple shoot from pericycle in *Psoralea corylifolia*–a leprosy destroyer medicinal plant.Industrial Crops and Products. **67:**324-329

Jiang, Q. et al. 2015. Optimization of the conditions for *Casuarina cunninghamiana* Miq. genetic transformation mediated by *Agrobacterium tumefaciens*.Plant Cell, Tissue and Organ Culture (PCTOC). **121:** 195-204

Kansara, R., Jha, S., JHA, S. K., Mahatma, M. 2013. An efficient protocol for in vitro mass propagation of Fusarium wilt resistant castor (*Ricinus communis* L.) parental line Skp-84 through apical meristem.Bioscan. **8:** 403

Khosla, P., Toky, O., Bisht, R., Hamidullah, S. 1992. Leaf dynamics and protein content of six important fodder trees of the western Himalaya. Agroforestry systems. **19:**109-118

Mohamed, A. N., Ismail, M. R., Kadir, M. A., Saud, H. M. 2013. In vitro performances of hypocotyl and cotyledon explants of tomato cultivars under sodium chloride stress. *African J. Biotechnology*. **10:**8757-8764

Nagar, D. S., Jha, S. K., Jani, J.2015a. Direct adventitious shoot bud formation on hypocotyls explants in *Millettia pinnata* (L.) Panigrahi-a biodiesel producing medicinal tree species. *Physiology and Molecular Biology of Plants*. **21:**287-292

Nagar, D. S., Jha, S. K., Jani, J. N., Chauhan, R. S. 2015b. Adenine sulphate and $AgNO_3$ Promote In vitro Shoot Proliferation in *Millettia pinnata* (L.) Panigrahi-a Biodiesel Producing Tree.Vegetos-An *International J. Plant Research*. **28:**10-14

Negi, S. 1977. Fodder trees in Himachal Pradesh. Indian Forester. **103:**616-622

Pandey, S., Mishra, A., Patel, M. K., Jha, B. 2013. An efficient method for Agrobacterium-mediated genetic transformation and plant regeneration in cumin (*Cuminum cyminum* L.). *Applied biochemistry and biotechnology*. **171:**1-9

Pawar, B., Jadhav, A., Kale, A., Chimote, V., Pawar, S. 2013. *In vitro* Plant Regeneration in Brinjal (*Solanum melongena* L.) using cotyledon, hypocotyl and root Explants.Vegetos-*An International J. Plant Research*. **26:**312-317

Rashid, R., Bhat, J., Bhat, Z., Dar, W., Shafi, W. 2012. Callus Formation and Organogenesis of Tomato (*Solanum lycopersicum* L.).*Vegetos-An International J. Plant Research*. **25:**243-248

Ribas, A. F. et al. 2011. Agrobacterium-mediated genetic transformation of *Coffea arabica* (L.) is greatly enhanced by using established embryogenic callus cultures.*BMC plant biology*. **11:**92

Sharma, R., Singh, B., Bhat, T. 2000. Nitrogen solubility, protein fractions, tannins and in sacco dry matter digestibility of tree fodders of Shiwalik range. *Indian J. Animal Nutrition*. **17:**1-7

Stevens, M. E., Pijut, P. M. 2014. Agrobacterium-mediated genetic transformation and plant regeneration of the hardwood tree species *Fraxinus profunda*. *Plant cell reports*. **33:**861-870

Swamy, S., Puri, S., Singh, A. 2002. Effect of auxins (IBA and NAA) and season on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacacia* and *Grewia optiva*. *New Forests*. **23:**143-157

Zhang, D., Jiang, L., Luo, Y., Qin, R. 2014. A Genetic Transformation System for *Rosa multiflora* Thunb. var. cathayensis Rehd. et Wils through Callus Induction. *Agricultural Science and Technology*. **15:**1