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Short Communication

SOMATIC EMBRYOGENESIS IN WALNUT (*Juglans regia* L.)

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In Walnut (*Juglans regia* L) cultivars viz., 'Netar Akhrot' and 'Govind', somatic embryos were produced on embryonic axis and cotyledonary tissue explants cultured on DKW medium supplemented with 2mg/l kinetin, 1mg/l BA and 0.01 mg/l IBA for 3 weeks. In the present investigation, apparently normal bipolar somatic embryos have been obtained. However, since the explant source is immature embryo, the culture represents varying genotypes. The difference could be seen from differential response of explants to culture condition. Attempts were made to regenerate plants from somatic embryos.

Key words : Walnut, DKW medium, somatic embryogenesis

In India, walnut is mostly grown as scattered trees and not in the form of well maintained orchards. Many areas of Jammu and Kashmir, Kangra (H.P.) and hills of Uttar Pradesh are famous for walnut production. The Jammu and Kashmir is the principal walnut growing state having monopoly in production of export quality nuts (Dar, 1987; Qureshi and Dalal, 1985). In Himachal Pradesh, the area under cultivation was 5459 hac, in 1994-95 (Anon., 1995). Almost all parts of the plant are utilized in one way or the other but the fruit and timber have been put to maximum use. The fruit has excellent flavour and is mainly consumed as a dry fruit. Walnut is rich in proteins, fats and minerals. However, the cultivation of this crop has remained neglected on account of non-availability of better genotypes and due to its long bearing habit. Conventional methods of vegetative propagation are relatively slow and often difficult. The use of micropropagation is possible (McGranahan *et al.*, 1987), however, many problems, like ageing and juvenility (Jay-Allemand *et al.*, 1987), contaminants, rate and quality of rooting and soil transfer are still remain constraints for mass propagation. The possibility of inducing somatic embryogenesis for obtaining plants has been investigated (Tulecke and McGranahan, 1985; Tulecke *et al.*, 1988). In the present investigation, attempts have been made for somatic embryogenesis from immature embryos in *Juglans regia*.

Nuts from open pollinated crop of walnut cultivars, viz., 'Netar Akhrot' and 'Govind' were collected from the Orchards, Department of Fruit Breeding, University of Horticulture and Forestry, Solan (H.P.) at an interval of four weeks after full bloom. They were surface sterilized with 1-1.5% (v/v) sodium hypochlorite for 15-20 min and washed with sterile water 3-4 times and cracked under a laminar flow with the help of vice. The embryonic axes and cotyledonary explants were removed using aseptic procedure and inoculated into Driver and Kuniyuki walnut (DKW) medium (1984) supplemented with

different concentrations of 1-2 mg/l BA, 2 mg/l kinetin, 0.01-0.05 mg/l IBA, 250 mg/l glutamine and 500 mg/l kanamycin alone or in combination. The cultures were incubated in dark at 25 ± 2°C and were transferred to the basal medium after three weeks to induce somatic embryos. They were subsequently maintained on the same medium by repeated transfers at an interval of two weeks.

Table 1 shows the sampling time and the type of fruit used. There is a specific time between the anthesis and fruit maturity when somatic embryogenesis was possible. The nuts collected in June i.e., 12 weeks after pollination, were capable of forming somatic embryos. Tulecke and McGranahan (1985) reported optimum time for obtaining somatic embryos from the walnut 6-11 weeks after pollination. Long *et al.* (1995) reported that in Eastern black walnut nuts collected 16 weeks after anthesis formed adventitious shoots on cotyledonary explants on medium containing 2, 4-D. There was no callus initiation on cotyledonary tissue and embryonic axis on DKW medium supplemented with IBA or kinetin. However, on DKW medium with 2 mg/l kinetin, 1 mg/l BA and 0.01 mg/l IBA, the callus was induced which was nodular, friable creamish in colour (Plate 1a). A light brown, friable and nodular callus was formed when kanamycin (500 mg/l) was added to the medium (Plate 1b). There was no callusing on basal medium.

Table 1. Sampling time and type of fruit used (after full bloom)

Sampling time (month)	Type of fruit
April (4 weeks)	Wndocarp soft, non-lignified endosperm liquid and the cotyledons were not visible. Fruits can be cut easily with knife.
May (8 weeks)	Endocarp soft, slightly lignified, endosperm gelatinous and cotyledons beginning to fill the seed cavity. Fruits can cut with knife.
June (12 weeks)	Endocarp hard,lignified. Cotyledons completely fill the seed cavity with fully developed embryonic axis no visible endosperm. Fruits can be cracked under aspectic condition with vice.

In the present investigation, somatic embryos were produced on the embryonic axis and cotyledonary tissue explants cultured on DKW medium supplemented with 2 mg/l kinetin, 1 mg/l BA and 0.01 mg/l IBA for 3 weeks and transferred to the basal medium subsequently and incubated in dark (Plate 1c and d). Tulecke and McGranahan (1985) reported somatic embryogenesis from cotyledonary explants in walnut using 4.4 μM BA, 93 μ M kinetin and 0.05 μM IBA. However, Neuman *et al.* (1993)

Table 2. Effect of growth regulators on somatic embryogenesis in walnut

DKW medium with growth regulators (mg/l)				Number of explants showing somatic embryogenesis in cultivars			
				Netar Khrot		Govind	
				Embryonic axis	Cotyledon	Embryonic axis	Cotyledon
Kinetin	BA	IBA	Kanamycin				
-	-	-	-	-	-	-	-
-	-	-	500	-	-	-	-
2	1	0.01	-	3.33 (27.75)	9.33 (31.10)	2.33 (19.41)	8.00 (26.66)
2	1	0.01	500	7.66 (63.83)	20.67 (68.90)	5.33 (44.46)	18.00 (66.00)
CD 0.05				0.769	0.768	0.768	0.941

Figures within parentheses represent percentage value; Number of explants cultured: Cotyledonary tissue 30; Embryonic axis 12

did not obtain embryogenesis from *J. regia* following Tulecke's approach which shows differential response of the genotypes. Aly *et al.* (1992) could obtain somatic embryos by using DKW supplemented with 4.6 µM zeatin, 0.45 µM TDZ and 17 µM IAA. Long *et al.* (1995) used Woody Plant Medium (WPM) to produce somatic embryos from immature cotyledon explants of Eastern black walnut. It is noticed that only 26.6-30 per cent cotyledonary tissue and 19-27 per cent embryonic axis induced somatic embryos in both the cultivars (Table 2). However, the number of explants producing somatic embryos increased with the addition to Kanamycin in the medium. Aly *et al.* (1992) used 100 uM silver nitrate and 500 mg/l cefotaxime to obtained somatic embryos from *J. regia* and reported that antibiotics stimulated walnut somatic embryo production.

Somatic embryos, in walnut were produced from dark brown coloured callus formed on the explants cultured in DKW with various additives. Comu (1989) also reported clusters of somatic embryos from dense callus in black walnut hybrid. However, Tulecke and McGranahan (1985) reported somatic embryos in *J. hindsii* and *J. regia* directly from the explants without an intervening callus. Neuman *et al.* (1993) reported in *J. nigro* that somatic embryos formed both directly from original cotyledon explants and indirectly via callus and probably, there was either differences among walnut genotypes regarding direct and indirect somatic embryogenesis or similar pathways were followed which could not be sufficiently characterized histologically. In the present investigation, apparently normal bipolar somatic embryos have been obtained from two cultivars of walnut. However, since, the explant source is the immature embryo which is the result of open pollination, the culture might represent varying genotypes. This difference could be seen from differential response of explants to culture conditions. Attempts are being made to regenerate plants from somatic embryos.

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