Callus Cultures from *Castanea sativa* Mill.

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ABSTRACT

Callus cultures were established from excised embryo, hypocotyls, stem, root, cotyledon and leaf explants of *Castanea sativa* Mill. using MS (x ½) basal medium (1962) fortified with various concentrations and combinations of phytohormons. Compact nodular callus was obtained after 6-8 weeks. MS (x½) basal medium fortified with BAP either alone or in combination with NAA has been found to be effective in callus induction and proliferation in various explants used. Basal medium failed to favour callus induction in all the cases hence presence of phytohormones viz. BAP alone or in combination with NAA was found as a pre-requisite for dedifferentiation of callus in various explants of *C. sativa* Mill.

Keywords: Callus cultures, *Castanea sativa* Mill., phytohormones

Abbreviations: MS (x ½): Murashige and Skoog; BAP: 6-Benzylaminopurine; NAA: Napthalene acetic acid

INTRODUCTION

Chestnut (*C. sativa* Mill.) also called as sweet chestnut (ver. Name *Punjayb gour*) belongs to family fagaceae and grows into beautiful tall trees attaining height of 25-35 mts. The tree is found with less abundance in the valley of Kashmir. The nuts develop in autumn and are protected by a very prickly shell containing beautiful dark nuts. The nuts are reported to be very nutritious. Raffinose, stachyose and sucrose are the major sugars of the chestnut seeds (Dey, 1981; c.f. Jindal and Karkara, 1991)

Chestnuts are mainly propagated by seeds but the established cultivars are raised by vegetative methods. The conventional methods of propagation of chestnuts are very slow and cumbersome and this has resulted in reduction of germplasm, frequency of occurrence of the plant in the valley so there arises a need to incorporate the unconventional method for its quick multiplication. The *in vitro* technique is considered superior to conventional methods of propagation because of quick propagation rate of plants in relatively shorter period of time and irrespective of season.

Several workers have considered cultures of embryonic axis, mature and juvenile material for shoot multiplication and plantlet regeneration of *C. sativa* (Vieitez and Vieiez, 1980; Vieitez *et al.*, 1983; Biondi *et al.*, 1981; Mecphecters *et al.*, 1980; Rodriguez, 1982; Chevre *et al.*, 1983; Chauvin and Saulesses, 1988). Till date no attempt has been made elsewhere in the state to initiate tissue culture studies in the plant for propagation and improvement though various techniques of *in vitro* culture. Our earlier communication has reported multiple shoot induction, their elongation through embryo culture. The aim of the present work was to initiate and establish callus cultures from various organs of *in vitro* germinated seedlings and embryos. An attempt was therefore made for raising callus cultures from different excised explants of chestnut for future research work using callus cultures for induction of somatic embryogenesis and somoclonal variations. Present communication reports the induction and establishment of callus cultures from various explants of chestnut.

MATERIAL AND METHODS

Nuts of *Castanea sativa* Mill. were collected from horticulture garden, Theed, Harwan (Distt. Srinagar) in autumn. Before storage nuts were given 0.2% $HgCl_2$ treatment for 10-15 minutes followed by triple rinse with distilled water. This was done in order to avoid fungal contamination during storage. The seeds were finally dried and stored in polyethylene bags (30-40cm) with 15-20 pinholes at 4°C in refrigerator. The nuts were chilled for minimum period of 25-30 days. Before using nuts were soaked in filtered water for 24 hrs. The embryos were dissected out from the surrounding cotyledons under laminar air flow. The excised embryos were sterilized with $HgCl_2$ 0.1% solution for 8-10 minutes followed by triple rinsing with autoclaved double distilled water. The sterilized embryos were then inoculated on MS medium (1962) with and without various growth regulators. MS (x ½) strength was used throughout the experiments. Cotyledon slices were used as explants after sterilant 0.2% $HgCl_2$ was used for 20 minutes. Rest of the procedure for media preparation, pH adjustment, autoclaving and incubation of cultures is same as reported in our earlier communications (Kamili *et al.*, 2001; Bashir *et al.*, 2005). The excised embryo explant was used for *in vitro* germination and seedling formation. Various explants viz. whole embryos, hypocotyls segments, stem segments, root segments and leaf segments excised from *in vitro* seedlings were used for raising the callus.

RESULTS

Different morphogenetic responses of various explants of *Castanea sativa* on MS medium fortified with different concentrations and combinations of phytohormones are summarized in the Table 1.

Excised embryos

Excised and sterilized embryos from 1 month chilled seeds of *C.sativa* when cultured on MS basal medium showed germination response but no callus was reported. However, when MS basal medium was fortified with various phytohormonal concentrations, callus formation was recorded. Moderate callusing was obtained on MS + BAP (7.5 μM) and very high callusing on MS + BAP (15 μM) on hypocotylar region of embryo. (Fig. 1a). The callus continued to prolifer even after subculturing on BAP (15μM) +NAA (5μM). (Fig 1b)

Hypocotyl Segment

1-2 cm long hypocotyls segments obtained from *in vitro* raised seedlings when cultured on basal medium showed no response. However, on MS+BAP (10μM) the explants produced compact whitish nodular callus after 6-8 weeks (Fig. 2) which turned brownish after 8 weeks without further growth. Very high callusing was obtained on MS (x ½) + BAP (22 μM) with 80% response.

Stem segment

Basal medium favoured no response while as MS basal medium supplemented with phytohormones revealed moderate whitish callus formation on MS + BAP (0.60 μM) and BAP (4μM). (Fig.3).

Root segments

No response on *in vitro* raised root segments was obtained on MS basal medium but degree of callus formation was high when medium was enriched with BAP (10 μM)+ NAA (5 μM).(Fig. 4)

Cotyledon

Cotyledon slices when cultured on MS basal medium showed no response however, on MS + BAP (10 μM) + 0.60 NAA moderate callus formation was achieved. (Fig.5)

Table1. Morphogenetic response in various explants of *C. sativa* under the influence of various phytohormonal concentrations and combinations

	Medium	Nature of Response [a]	Degree of callus formation	%of response
Excised embryos	MS (x ½)basal	No callus induction	--	-
	MS (x ½) + BAP (7.5µM)	Whitish callus formation at root pole.	++	50
	MS (x ½) + BAP (10µM) + NAA (0.60µM)	Whitish callus formation at root base	++	60
	MS (x½)+BAP (15µM)	Whitish nodular callus formation at hypocotyls region	+++	70
Hypocotyl segments	MS (x ½)basal	No response	--	-
	MS (x ½) + BAP (10µM)	Nodular compact greenish callus formation	++	70
	MS (x ½) + BAP (22µM)	Nodular compact greenish callus formation	+++	80
Stem segment	MS (x ½)basal	No response	--	-
	MS (x½)+BAP (0.60µM)	Greenish compact callus formation	++	40
	MS (x ½) + BAP(4µM)	Greenish compact callus formation	++	50
Root Segment	MS (x ½)basal	No response	--	-
	MS (x ½) + BAP (5µM)	Greenish callus formation	+	60
	MS (x ½) + BAP(10µM)+ NAA (5µM)	Greenish compact callus formation	+++	70
Coty ledon Segment	MS (x ½)basal	Turned greenish, no dedifferentiation	--	-
	MS (x ½) + BAP (10µM) + 0.60 NAA	Compact nodular greenish callus formation	++	70
	MS (x ½) + BAP(4.4µM) + 2-4D (4.6µM)	No response	--	-
Leaf Segment	MS (x ½)basal	No response	--	-
	MS (x ½) + BAP (5µM)+ NAA (2.5µM)	Compact light greenish nodular callus formation	++	70
	MS (x ½) + BAP (6.2µM)	Compact light greenish nodular callus formation	++	50

[a] 10 replicates/ treatment; data scored after 8 weeks (-) no callus, + low, ++ moderate, +++ High

Leaf segments

Leaf segments obtained from *in vitro* born shoots again showed no signs of growth on basal medium. However, if grown on medium augmented with BAP (6.2 μM) it resulted in compact nodular non-regenerating callus. (Fig.6). The response was similar when BAP 5μM +NAA (2.5μM) were supplied to the medium even after subculturing on same medium (Fig.7).

DISCUSSION

Jacquoit (1947, 1950, 1953, 1968, 1969) for the first time reported callus formation from cambial tissue of adult trees of *Castanea vesca.* Borrod (1971 a,b) reported callus from sprouts of *C.sativa* on various phytohormonal regimes. However, in present studies nodular callus response was obtained using either BAP alone or with NAA in various explants viz. excised embryo, hypocotyls segments, stem segments, root segments, cotyledon and leaf segments of *C. sativa.*

Sanjose (1983) using hypocotyl segments reported callus formation on H+IBA or NAA (0.1mg/l); likewise in present studies using hypocotyl segments, compact nodular callus formation resulted on MS medium supplemented with BAP (10μM) and BAP (22μM).

Establishment of callus tissue from cotyledon fragments was achieved on various combinations of auxin by Vieitez *et al.* (1975,1978 a, b) on MS+IBA (1mg)+BAP(0.5) mg./l and H+N+2,4-D (1mg)+K or BAP (0.5) ml. or CM 12%. Root regeneration was achieved from callus of the same explant on H+N+IBA (1-5)mg./l+K(0.1)mg./l. (Vieitez *et al.*, 1978 a,b). Recently *invitro* organognsis of *C. sativa* Mill was reported by Giovannelli *et al*, (2004) using cotlyedon as explants. However, in present studies nodular compact callus was obtained on BAP 10iM +NAA 0.60 iM but with no differentiation into adventitious roots or shoots.

The present investigation proved that the callus cultures can be successfully obtained from the various explants of *C. sativa* but presence of phytohormones (BAP or BAP+NAA) was found essential for this response. The *in vitro* system is a potential way of getting genetic variability through regeneration of callus cultures. The regenerated plants may assume importance for genetic improvement when evaluated for somoclonal variation.

Hence the callus raised from various explants provide a scope for future studies in relation to induction of organogenesis in callus cultures for somoclonal variations. Callus raised by various methods also provide a platform for induction of somatic embryogenesis as reported in many woody plants e.g. *Picea abies* (Becwar *et al.*,1987), *Picea omorika* (Budimir and Vujicic, 1992), *Juglans regia* (Tulecke and Mc Granahan, 1985), *Pinus radiate* (Chandler and Young, 1995), *Pinus strobus* (Finer *et al.*, 1989) etc. Thus present results on raising callus cultures from *C.sativa* can prove fruitful for such future studies.

ISSN 0972-5407


Fig. 1a


Fig. 1b


Fig.2


Fig. 3


Fig. 4


Fig. 5


Fig. 6


Fig. 7

Figures (1-7). Callus cultures of *C.sativa*

Fig 1a. Whitish nodular callus formation of excised embryo on MS (x ½) + BA 15 iM (after 4 weeks)
Fig. 1b. Callus formation of subcultured embryo callus on MS(x½)+BAP 15μM+NAA 5μM (after 6 weeks)
Fig.2. Green callus formation on MS(x½)+ BAP10 μM (after 8 weeks)
Fig.3. Moderate green compact callus formation on MS (x ½) + BAP 0.60 iM (after 6 weeks)
Fig.4. High degree of green compact callus on MS(x½)+ BAP 10 μM+NAA 5μM (after 8 weeks)
Fig.5. Green callus formation from cotyledon on MS(x½)+ BAP 10μM+NAA 0.60μM (after 8 weeks)
Fig.6. Compact, nodular callus formation from leaf segments on MS + BAP 6.2 iM (after 6 weeks)
Fig.7. Green callus formation from subcultured leaf callus on MS (x½)+BAP 5μM+NAA 2.5μM (after 8 weeks)

ACKNOWLEDGMENT

The authors wish to thank Director, CORD for providing facilities to carry out these investigations

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