

IN VITRO REGENERATION OF MONOEMBRYONIC MANGO CULTIVARS

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ABSTRACT

Different explants of mango cultivars were explored for *in vitro* plantlet regeneration on Murashige and Skoog (MS) and Gamborg (B5) medium. Only zygotic embryos of Chaunsa cultivar initiated callus formation and successful plant regeneration with highest percentage on MS medium supplemented with 2,4-D 1mgL⁻¹. Calli were also observed from zygotic embryos of cv. Anwar Rataul but could not regenerate into plantlets. Nucellus and shoot tip explants of both cultivars showed no callus induction because of excessive phenolic exudation from explant in the medium. The plantlets generated through embryo culture were transplanted to green house after acclimatization. Further studies are suggested, however, to optimize somatic embryogenesis from nucellar explants of mango for clonal propagation and biotechnology applications.

Keywords: Mango, embryo culture, nucellus, shoot tip, callus induction, *in vitro*, plant biotechnology,

INTRODUCTION

The mango (*Mangifera indica* L.), family Anacardiaceae is amongst the most important tropical fruits of the world. It is also called as king of the fruits (Purseglove, 1972). Mango has been cultivated for thousands of years in India (Mukherjee, 1953; Kostermans and Bompard, 1993) and its cultivation is as old as Indian civilization (DeCandolle, 1884). Its development and culture in the sub-continent is mainly contributed by the Mughal Emperors especially Akbar who planted Lakh Bagh, amateur gardeners, nurserymen and farmers by means of selection and subsequent cloning. Now, it is an integral part of history and culture of Indo-Pak subcontinent. Pakistani mango cultivars are monoembryonic in nature. Most of the mango industry throughout the world depends on the use of the chance seedlings maintained by different means of asexual propagation. The chance seedlings are continuous source of variation for selection due to highly heterozygous nature of the plant. Propagation by cuttings is not highly successful due to lack of proper rooting.

Conventional breeding has only limited potential in improvement of cultivars because of long life cycle and allotetraploid nature of the crop (Mathews *et al.*, 1992). Use of *in vitro* techniques for clonal propagation of woody plant species has become a well established commercial practice. These techniques offer advantages of rapid clonal multiplication of disease and pathogen free plants of better quality and yield. *In vitro* regeneration systems are available for mango that with some refinement could be used for clonal propagation of rootstocks and dwarf scion cultivars, medium term germplasm conservation and genetic transformation of mango. *In vitro* techniques used for the regeneration of mango include somatic embryogenesis (Litz *et al.*, 1982; 1984; 1989; Litz, 1984; Dewald *et al.*, 1989a,b); cotyledon, embryo and shoot tip culture (Rao, 1981; Maheshwari and Rangaswamy, 1958) had been described. The study was initiated with the objective to develop a micropropagation protocol for the regeneration of disease and pathogen free mango plants of local commercial cultivars.

MATERIALS AND METHODS

Explant Sources and Sterilization

Mango cultivars Chaunsa and Anwar Rataul were explored for *in vitro* propagation. The plant material (immature fruit and shoot tips) was collected from the fruit plant nursery of the Institute. The fruitlets were washed with tap water, surface sterilized by immersion in 70% (v/v) ethanol for ten minutes followed by 3-5 washings with sterile double distilled water (ddH₂O). The fruit-lets were then dipped in 1% (v/v) sodium hypochlorite (NaOCl) solution plus two drops of tween 20 as surfactant and the excessive sterilant was removed by thorough rinsing with ddH₂O. Shoot tips (4-5 cm long) of both mango cvs. were surface disinfested in 2% (v/v) sodium hypochlorite solution plus two drops of tween 20 for ten min. and rinsed 3-5 times with sterile ddH₂O.

Media Formulations

a) **Embryo Culture:** Embryos of mango (*Mangifera indica* L.) cultivars Chaunsa and Anwar Rataul were cultured on Murashige and Skoog, (1962) medium supplemented with 60g/L-1 sucrose, 400 mg/L-1 glutamine, 100 mg/L-1 ascorbic acid, 0.5 g/L-1 Charcoal (to minimize the exudation of phenolic compounds), 1.75 mg/L-1 gelrite (solidifying agent) and modified with different levels of 2,4-D (1-3 mg/L-1) for callus induction and subsequent plant regeneration.

b) **Nucellus Culture:** For nucellus culture the B5 medium (Gamborg *et al.*, 1968) was used as the basal medium supplemented with 30 g/L-1 sucrose, 0.3 g/L-1 each casein hydrolysate (CH) and glutamine, 0.1 g/L-1 ascorbic acid and 1.5 g/L-1 gelrite and modified with different levels of 2,4-D (1-3 mg/L-1) for callus induction and subsequent plant regeneration. Charcoal and polyvinyl pyrrolidone (PVP) were added separately in each combination at 0.5 and 0.25 g per liter to minimize the exudation of phenolic substances from explant in the medium.

c) **Shoot Tip Culture:** The B5 medium was modified with 30 g/L-1 sucrose, 0.3 g/L-1 each casein hydrolysate (CH) and glutamine, 0.1 g/L-1 ascorbic acid, 1.5 g/L-1 gelrite, 0.5 g/L-1 charcoal and modified with IAA, IBA and NAA (0.5 mg/L-1, separately) and Kin and BA (0.5, 0.2 and 5.0 mg/L-1, separately).

Explant excision, Culture Procedure and Growth Conditions: Ovules were removed from mango fruit-lets by dissecting them longitudinally under sterile conditions. Embryos and nucellus were removed from plump ovules under dissecting microscope (Litz *et al.*, 1982). Embryos, nucellus and shoot tips (3-4 mm in size) were placed in their respective medium. Medium pH was adjusted to 5.7. The 10 ml of the medium was poured in each test tube, capped with plastic sheet and placed in an autoclave for 20 min. at 121°C under 15psi pressure. The cultures were maintained at 25 ± 1 °C with 16 h photoperiod provided by cool white fluorescent lights.

RESULTS AND DISCUSSION

EMBRYO CULTURE

Phenolic Exudation (%)

Frequent sub-culturing of the embryos of both cultivars of mango viz. Chaunsa and Anwar Rataul was performed to minimize the exudation of the phenolic compounds from the embryo explants. Though the browning was not very effectively reduced by the subculturing, however, the browning percentage was minimum during the second subculture of embryos of both cultivars on MS medium supplemented with 0.5 g/L-1 activated charcoal to check the phenolic exudation from explants and 2,4-D for callus induction (Fig. 2a). The cultivar Anwar Rataul depicted more exudation of the phenolic compounds even after the third sub-culture compared to cv. Chaunsa. The embryos showed growth initially in both the cultivars but after second week of culture majority of the embryos of Anwar Rataul ceased their growth and eventually died. The results are supported by the findings of John *et al.*, (1985) who also found browning difficult to control in tissues containing high concentrations of phenolic compounds, however, the metabolic process of the polyphenolases stimulated by tissue injury can be minimized thorough subculturing the explants on fresh medium. Our results are in line with those of Amin (1994) who also reported that embryos turned brown to black after fourth week and died while germinated embryos showed radical growth upto 8th week of culture. During sub-culturing Chaunsa showed sudden increase in mortality during and after the second sub-culture while cv. Anwar Rataul showed gradual rise in mortality (%) which was much higher than the death rate of embryos of Chaunsa (Fig. 2b).

Embryo Germination and Plant Development (%)

The embryos of mango cultivars Chaunsa and Anwar Rataul germinated successfully and developed in to plants on MS medium supplemented with various levels of 2,4-D (Fig. 1B). The highest percentage of germination was observed on minimum level of 2,4-D and medium devoid growth hormones (control) and germination percentage decreased with increase in level of 2,4-D. The findings are in line with Baleriola *et al.*, (1982), Amin (1994) and Litz *et al.* (1982, 1989, 1998) who observed precocious germination of the embryos and plant let development on MS medium devoid growth hormones.

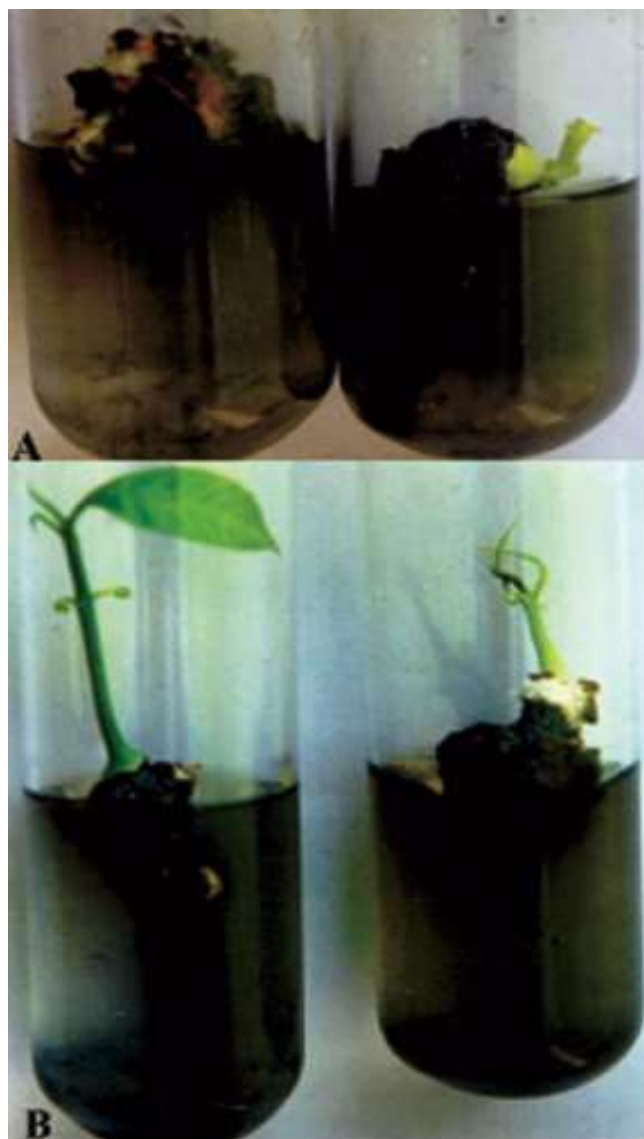


Fig. 1. *In vitro* propagation in Mango cultivar Chaunsa from embryo explant A) Callus induction and shoot regeneration B) Plantlet development.

Table 1. Plantlet development from embryo culture of mango cv. Chaunsa.

Treatments MS+2,4-D (mg/l)	Shoot induction	(%) Rooting (%)	Means
0	46.66 b	46.66 b	46.66 b
1	53.33 a	46.66 b	49.95 a
2	33.33 c	46.66 b	39.95 b
3	20.00 d	13.33 d	16.65 c

Means showing similar letters are statistically non-significant.

Table 1. Plantlet development from embryo culture of mango cv. Chaunsa.

Treatments MS+2,4-D (mg/l)	Callus induction (%) Anwar Rataul	Chaunsa	Treatment means
0	53.33 b	80.25 ab	66.79 ab
1	46.66 bc	86.66 a	66.66 ab
2	53.33 b	93.33 a	73.33 a
3	33.33 c	86.66 a	59.99 b
Cultivar Means	46.66 b	86.72 a	

Means showing similar letters are statistically non-significant.

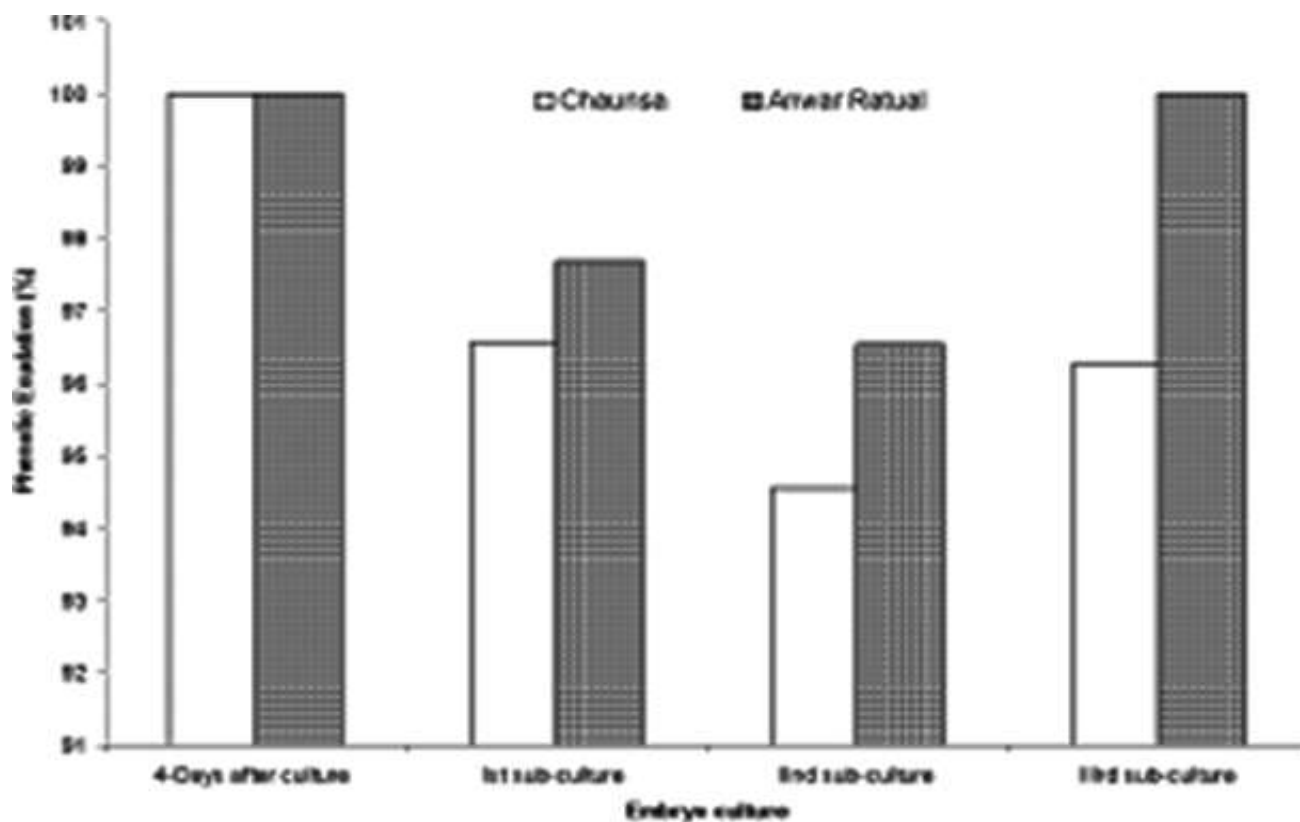


Fig.2a. Effect of weekly subculturing on browning (%) of embryo explants in mango cultivars.

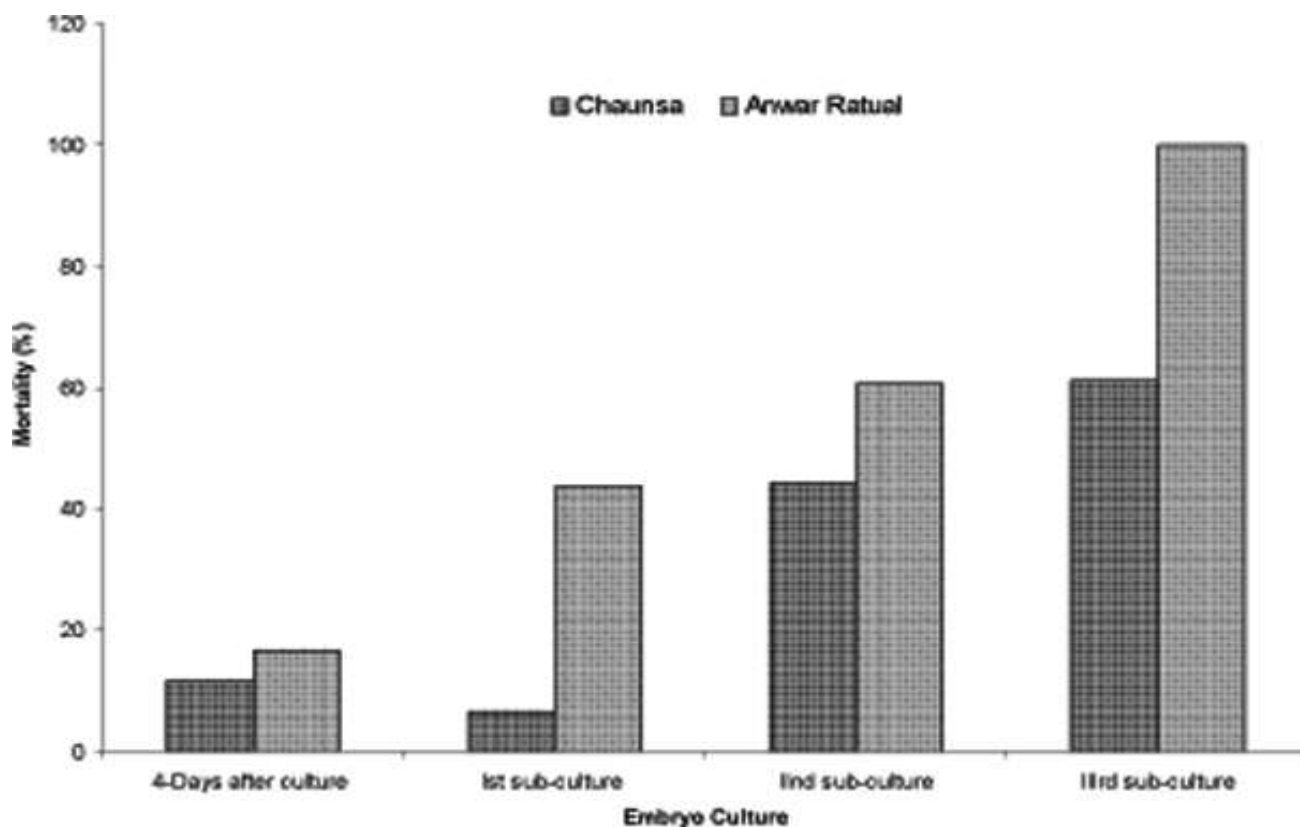


Fig.2b. Effect of weekly subculturing on mortality (%) of embryo explants in mango cultivars.

Callus Induction (%): Both cultivars, treatments and their interaction showed significant results for callus induction on the basal ends of embryo germinated in to plants. Mango cultivar Chaunsa showed significantly more callus induction (%) as compared to Anwar Rataul. Supplement of 2,4-D at 2 mgL⁻¹ yielded highest percentage of callus while further increment of growth hormone showed decline in callogenesis (Fig. 1A) The findings of Litz and Knight (1983) are harmonious to our results as they also obtained callus at low levels of 2,4-D. The results are also in agreement with Litz and Jaiswal, (1991) and Litz (1984, 1989m 1998). The developed plantlets were shifted in the pots for further development and acclimatization. There was little or no success obtained regarding nucellus and shoot tip cultures due to excessive phenolic exudation from both the explants in spite of supplementation of charcoal and PVP in B5 medium and frequent sub-culturing. The findings correspond with Iqbal (unpublished data) and Awan (unpublished data). Further studies are, however, suggested to explore the regeneration from these two explants and control of browning.

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