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High frequency callus initiation, somatic embryogenesis and plantlet regeneration in *Carica papaya* L. cv. COORG HONEYDEW

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Two month old stem explants of *Carica papaya* L. cv. Coorg Honeydew showed 80 per cent callus initiation on Murashige-Skoog (MS) nutrient medium supplemented with 3.0 µM of 2,4-dichloro phenoxyacetic acid (2,4-D). Treatment with phytohormones like Kinetin (Kin) or Benzyl adenine (BA) (@ 0.2 to 2.0 mg l^{-1}) were found to have no role with regard to callus initiation. However, these initiating calli when subcultured on MS + 2,4-D (3.0 µM) + Kin (0.5 mg l^{-1}) showed a two-fold growth by proliferation within 21 days after the date of sub-culture. During this period, 30 per cent of the callus tissue underwent necrosis. Thereafter, the best of 70 per cent friable, healthy calli were recultured on MS + 2,4-D (3.0 µM) + Napthalene acetic acid (NAA, 2.0 mg l^{-1}) + Kin (0.5 mg l^{-1}), also supplemented with casein (50 mg l^{-1}). This combination for reculture resulted in vigorous callus growth on fresh weight basis. Best somatic ernbryogenesis was achieved when callus tissue so obtained was further recultured in MS + NAA (1.0 mg l^{-1}) + Kin (0.5 mg l^{-1}) + Gibberelic acid (GA_3 1.0 mg l^{-1}) + L- Ascorbic and (Asc, 50 mg l^{-1}) alongwith glycine (1.0 mg l^{-1}) + thiamine (Thia, 1.0 mg l^{-1}) as adjuvants. The pH of such culture media was maintained at 5.7, incubated under a 16/8-hr light/dark cycle at 25°±1°C in the culture room. This protocol resulted in 80 per cent somatic embryogenesis out of which about 20 per cent yielded regenerants. The plantlets were carefully transferred to half-strength MS medium for further growth and hardening.

Key words: *Carica papaya* callus, Somatic embryogenesis, Regeneration, Tissue culture.

INTRODUCTION

PAPAYAS have been cultivated from seeds by the conventional methods. As such, considerable variation usually occurs in the commercial plantings. At this juncture, tissue culture technology has proved to be useful tool in selecting certain traits of horticultural, plant production and protection disciplines. It has been observed that clonal propagation methods such as grafting and root cuttings are impractical, especially for large scale production of plants. Tissue culture applications by way of micro-propagation can thus produce many true-to-type papaya seedlings or seedlings with special characteristics in a short period of time (Burikam *et al*., 1988).

Moreover, the cultivation of papayas in our Udaipur district of Rajasthan, has been constantly threatened by the severity of viral diseases, namely the papaya mosaic virus (PMV) and papaya ring-spot virus (PRSV). Besides, these two viruses, papaya leaf curl (PLC) has also been observed to be quite severe and of wide occurrence. Hence, the use of meristern cultures has been suggested by some workers (Agnihotri, 1994, Litz., 1984). At this time, meristem cultures are of little significance because of widespread distribution of alternate hosts and of vectors for most papaya viruses. Somatic embryogenesis by tissue culture can be chosen for micropropagation of certain selected agronomic, horticultural or breeding traits as worked out by many scientists. (Pandey and Rajeevan, 1988; Arora *et al.*, 1978; Mehdi Ali and Hogan, 1976; Rajeevan and Pandey, 1983; Yie and Liaw, 1979 and Litz, 1984). Therefore, in the, present study, we have chosen to produce the somatic embryoids and evolve an efficient method for *in vitro* plantlet regeneration of *C. papaya* L. cv. Coorg Honeydew. The somaclones shall be useful in fulfilling the above said objectives. Later, progress in this endeavour may enable us to obtain disease resistant lines that can be mass propagated.

MATERIALS AND METHODS

Two month old *C. papaya* L. cv. Coorg Honeydew were obtained from the farm of Department of Horticulture, Rajasthan College of Agriculture, Udaipur. Stem-derived discs 2-3 mm were sectioned and immediately surface sterilized first in a 0.1 per cent $HgCl_2$ solution for 120 seconds followed by treatment with 70 per cent alcohol for another 60 seconds. The explants were then repeatedly washed with sterile distilled water and quickly transferred to flasks containing MS basal medium fortified with 2,4-D (3.0 µM) set to a pH of 5.7 in 20 replicates. Initial culture batches were placed under fluorescent lamps (3000 lux intensity) at 25±1°C on a 16/8-hr light/dark cycle. Callus obtained were found to be best initiated after 21 days of initial culture that were further subcultured on MS + 2,4-D (3.0 µM) + Kin (0.5 mg l^{-1}) . Later, after three weeks of incubation the best proliferated calli were recultured on MS + NAA (2.0 mg l^{-1}) + Kin (0.5 mg l^{-1}) + GA_3 (1.0 mg l^{-1})+ L-ascorbic acid (Asc, 50 mg l^{-1}) + glycine (1.0 mg l^{-1}) + thiamine (1.0 mg l^{-1}) at pH of 5.7.

After two weeks of incubation under similar conditions of culturing, somatic embryogenesis had taken place showing 20 per cent regeneration. All stages thus far were photographed (Figure 1, 2, 3, 4). The healthy plantlets were further transferred onto half-strength MS medium for growth and hardening.

RESULTS AND DISCUSSION

Somatic cells may regenerate an entire plant via somatic embryogenesis or organogenesis *in vitro*. Differentiation of organs depends upon the exogenous supply of growth substances, particularly auxins and cytokinins in the culture medium. The regeneration of plants from explants has proved to be a valuable source of somaclonal variation (Larkin and Scowcroft, 1981; Tran Thanh Van, 1981 and Lee and Phillips, 1988). In our experiments on callus initiation, 80% callusing was obtained on MS + 2,4-D (3.0 µM) (Figure-1). Upon subculturing this initiated calli after about three weeks, the MS + 2,4 D (3.0 µM) + Kin (0.5 mg l^{-1}) combination at pH of 5.7 was found optimal. After 21 days of this subculture stage, about 70 per cent of the calli were found healthy, creamy in texture and friable (See Figure-2). About 30% callus in this lot was found to turn necrotic

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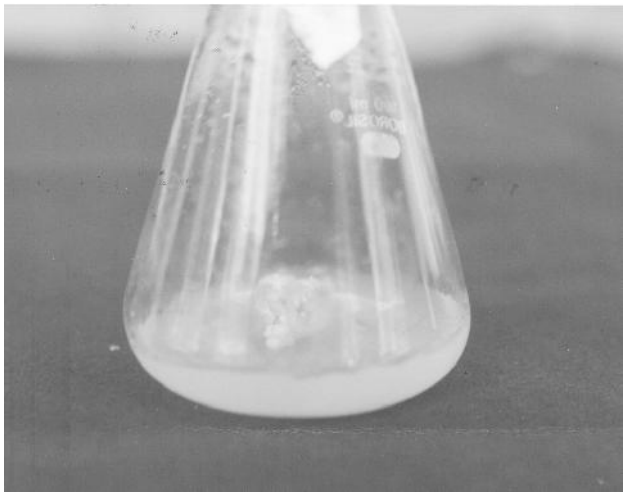

Fig. 1 : Callus initiated from two month old stem derived disc (1-2mm) on MS+2,4-D 3uM + $CaCl_3$ 500mg/l at pH 5.7

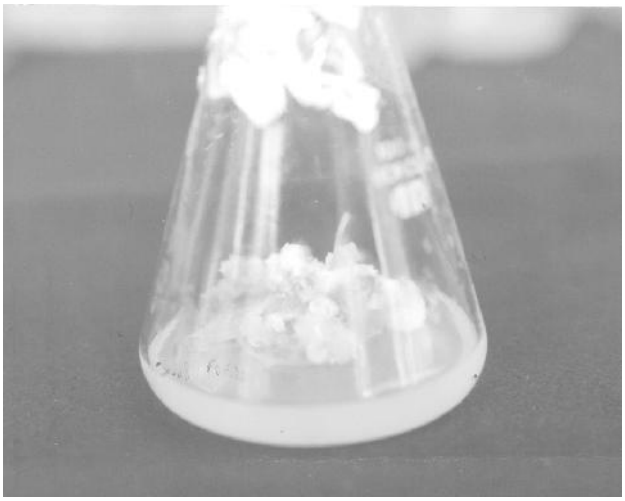

Fig. 3: Best organogenesis and somatic embryogenesis of a month old subcultured callus on MS + N.A.A. 1 mg./1 + Kinetin 0.5 mg/1 also fortified with GA_3 1mg/l + L-Ascorbic acid 50mg/l + Glycine 1.0 mg/l + Thiamine 1 mg/l (pH 5.7)

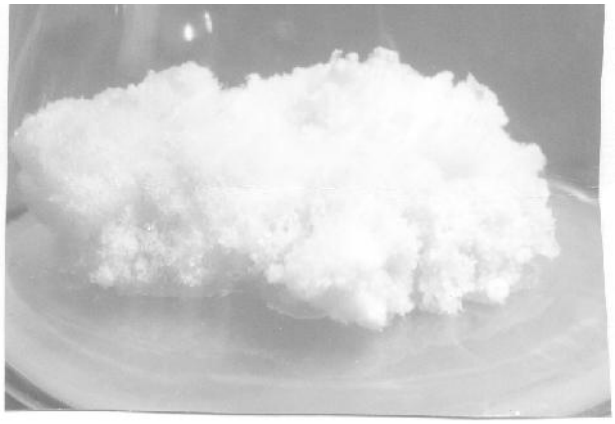

Fig. 2: Two month old subcultured friable soft callus 21 dys after subculture on MS+2,4 D 3u M + Kinetin 0.5mg/l + $CaCl_3$ 500mg/l at pH 5.7

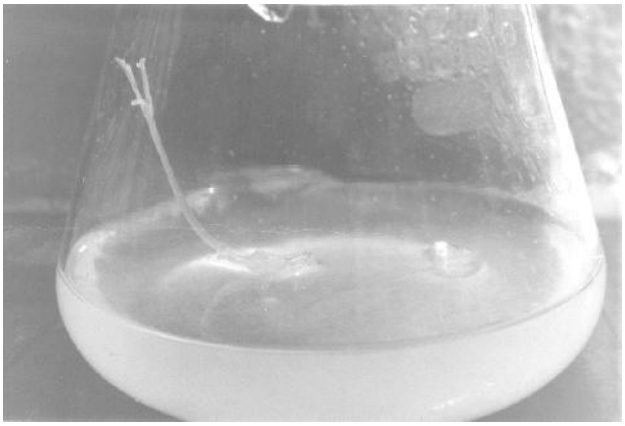

Fig. 4 : Regenerated neonate Plantlet of *Carica papaya variety* Coorg Honeydew.

which must have occurred due to exudation of phenolic and other secondary metabolites characteristic of callus proliferation. Further, these proliferated, friable callus lines were then selected and subcultured/recultured on MS medium fortified with 2,4-D (3.0 µM) + NAA (1.0 mg l^{-1}) + Kin (0.5 mg l^{-1}) + L-asc (50 mg l^{-1}) and glycine (1.0 mg l^{-1} + Thia (1.0 mg l^{-1}) at a pH of 5.7 in 20 replicates. After 21 days incubation out of these proliferated calli, about 20% somatic embryos had emerged showing organogenesis (Figure-3). These somatic embryos were further subcultured/recultured on the same medium (containing 2,4-D, NAA, Kin, Asc, Gly and Thia) under similar culture conditions for complete plantlet regeneration (Figure-4). Therefore, these plantlets were taken onto half-strength MS medium for hardening. The method reveals a novel approach by simple somaclonal variation without opting for genetically modified plants, thus obtaining virus-free papaya plants. The protocol will be used for micropropagation of papaya.

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