Role of Polyethylene Glycol in Maturation and Germination of Transformed Somatic Embryos of Papaya (*Carica papaya* L.)

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Abstract

In vitro regeneration and transformation protocol has been standardized in papaya world over. However, recovery of transformants remains very low. One of the reasons for low out put in transformation is lack of synchronized maturity and germination of transformed embryos. We report here very efficient maturation and germination of transformed papaya embryos by fortifying 45 mg/L polyethylene glycol in conversion medium. Around 28.23% embryos regenerated in to normal plantlets.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most widely growing fruit crops in the tropics and subtropics of the world. It is rich source of vitamin A, C and papain. The genus *Carica* comprises 21 species (Purseglove, 1968) but only *Carica papaya* has economic importance. Papaya production has been threatened by a potyvirus which causes *Papaya ringspot virus* disease. It is a deadly disease and spread through out papaya growing areas. *Papaya leaf curl virus* (Geminivirus) disease is also very severe at nursery stage and it is confined to Northern part of India (Saxena et al., 1998). Papaya is the first transgenic fruit crop that has been developed and commercialized in US (Cai et al., 1999; Fitch et al., 1992, 1993; Gonsalves, 1998). Transgenic technology heavily depends on efficient and repeatable regeneration system for transfer of gene of interest. Somatic embryogenesis is the preferred pathway of in vitro papaya regeneration (Fitch and Manshardt, 1990). After transformation, embryos are selected on kanamycin fortified medium for obtaining transformed plantlets. Transformation efficiency in papaya has been very low (1.42%) through both the methods viz., *Agrobacterium* and ballistic mediated transformation (Fitch et al., 1992, 1993). Lack of synchronized maturity and germination in papaya leads to even lower transformation efficiency. Therefore, it is imperative to achieve higher level of maturity of transplanted somatic embryos for higher plant conversion ratio. We have investigated role of poly ethylene glycol for synchronized maturation and germination of transformed somatic embryos of papaya cv. Pusa Delicious.

MATERIALS AND METHODS

The study was conducted at Biotechnology Laboratory, Central Institute for Subtropical Horticulture, Lucknow. Somatic embryos of papaya cv. Pusa Delicious were developed as per the protocol described by Mishra et al. (2007). Younger (12-week-old) embryogenic tissue, including a slimy yellow brown substance squashed or spread over a filter paper (Whattman) placed on the induction medium (1/2 MS + 10 mg/L 2,4-D + 400 mg/L L-glutamine + 8 gm/L agar) for three weeks. Numerous somatic embryos regenerated from this tissue forming loose layer of somatic embryos. Hundred somatic embryos of similar size and age were used for each bombardment. *Cp* gene mobilized in pBINAR121 binary vector driven by 35S promoter and NOS terminator was utilized for the purpose of genetic transformation. *E. coli* containing gene construct (developed at IARI, New Delhi) was grown overnight in LB (10 g/L trypton, 5 g/L yeast extract and 10 g/L NaCl) medium containing 50 mg kanamycin. Plasmid was isolated using alakaline

lysis method (Maniatis et al., 1982). Microprojectile suspension containing 50 µl tungsten in an eppendorf tube, having 50 µl plasmid DNA (1 µg/µl), 50 µl 2.5M $CaCl_2$ and 20 µl filter sterilized 0.1 M spermidine was prepared. Gene gun (Gene Pro He-2000) kept in laminar airflow was sterilized with UV overnight. The helium gas pressure was kept at 12 kg/cm^2 and tissue was kept at 9 cm distance from the micro holder on sliding tray. Four µl plasmid suspension was loaded to the micro-holder. Each plate bombarded at least twice. Bombarded cultures were transferred on the MS medium devoid of any hormone and kept at 27°C in the dark for a week. In order to bring maturity in transformed somatic embryos, all the embryos were transferred to MS medium containing osmoticum such as polyethylene glycol, mannitol and sorbitol (15, 30, and 45 mg/L) along with 75 mg/L kanamycin, 0.5 mg/L BAP, 20% sucrose and 0.8% agar. Media was autoclaved at 121°C on 20 psi for 20 min. pH of the media was kept at 5.8. The cultures were incubated under 16/8 h light (4000 lux) and dark cycle at 25±2ºC with 50% RH. The data pertaining to maturation, conversion and germination was recorded periodically.

RESULTS AND DISCUSSION

It is evident from data (Fig. 2) that out of three osmoticum used to dehydrate embryos, polyethylene glycol was found to be most effective in converting maximum globular embryos to cotyledonary embryos at 45 mg/L. This treatment helped in maximum conversion of embryos in to microshoots (81 shoots/culture) and a total of 28.33% embryos regenerated in to normal plantlets (Fig. 1). Maximum mortality occurs during kanamycin selection. Mannitol and sorbitol did influenced conversion of embryos to cotyledonary stage. However, only very few plants could be regenerated with mannitol and sorbitol. Polyethylene glycol significantly not only influenced conversion but subsequent germination of plants as well. Similar results were obtained by Li et al. (1997) who investigated that polyethylene glycol (5-7.5%) consistently produced stage 2 to stage 3 somatic embryos in Lobolly pine. None of the embryos matured without polyethylene glycol. Li et al. (1998) found that polyethylene glycol (6%) along with maltose 4% resulted in higher embryo maturation efficiency in Loblly pine. Around 100 cotyledonary embryos were produced from 1 gm of embryonic tissue. Attree et al. (1995) reported that polyethylene glycol 7.5% triggered conversion of embryos of white spruce to plantlets at frequency of 76 to 84%. Moisture content of embryos drastically reduced from 96 to 47%. Igasaki et al. (2003) reported phytosulfokine along with polyethylene glycol had dramatic stimulating effect on formation of somatic embryo of *Cryptonesia japonica*. The resulting embryos germinated with synchronous sprouting. Development of embryos to heart and torpedo stage in *Dianthus* was achieved in liquid medium (MS) incorporated with polyethylene glycol (6000) at 2.5%. In *Citrus microcarpa* (Rangaswamy, 1958), the nucellus derived embryoids in cultures have exhibited better maturation and subsequent germination upon culturing on White (1963) medium supplemented with casein hydrolysate (400 mg/L). The effect of an osmoticum, polyethylene glycol, was examined using embryogenic cells of *Pinus densiflora* on somatic embryo production. In the basal medium containing 30 µM abscissic acid and 6% maltose, the quality of the embryos formed was poor even though somatic embryos were produced. The addition of polyethylene glycol with molecular weight of 4000 or 8000 significantly enhanced the development of both the quality and quantity of somatic embryos. Furthermore, higher levels of a constant osmotic pressure with polyethylene glycol 8000 in a range from about 300 to 450 mmol/kg could remarkably enhance the morphogenesis of somatic embryos and their number of embryos produced. A higher stable osmotic pressure with an appropriate molecular weight of polyethylene glycol is a key factor for the production of good quality somatic embryos in *P. densiflora* (Mariko et al., 2006). Embryogenic culture was initiated from mature zygotic embryos of *Panax ginseng*. Multiple somatic embryos formed and proliferated on Murashige and Skoog (1962) medium supplemented with 2,4-dichlorophenoxyacetic acid (2.26 µM) and kinetin (0.046 µM). Mature as well as immature somatic embryos grew into plantlets lacking roots on the same media. Histomorphological analysis of somatic embryos treated with abscisic acid (ABA) and

polyethylene glycol (4000) showed a slight improvement in the root meristem organization of torpedo-stage embryos (embryos were more compact and their cells exhibited a lower degree of vacuolation). Shoot regeneration of non-treated somatic embryos was 31% while that of somatic embryos treated with polyethylene glycol (4000) and ABA was 70%. Moreover, 75% of plants regenerated from PEG and ABA treated embryos formed roots while plants from non-treated embryos did not form roots. Somatic embryos inoculated on MS medium (1962) fortified with PEG (45 mg/L) converted easily on to cotyledonary stage in about four week time. Cotyledonary embryos were further transferred on MS medium fortified with BAP 0.5 mg/L + casein hydrolysate 100 mg/L + sucrose 20 gm/L. Higher exposure (>4 weeks) of somatic embryos in polyethylene glycol fortified medium has been found detrimental. Therefore, it is concluded that polyethylene glycol (4000) at 45 mg/L helped in maturation and subsequent germination of transformed somatic embryos of papaya (Fig. 1).

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Figures

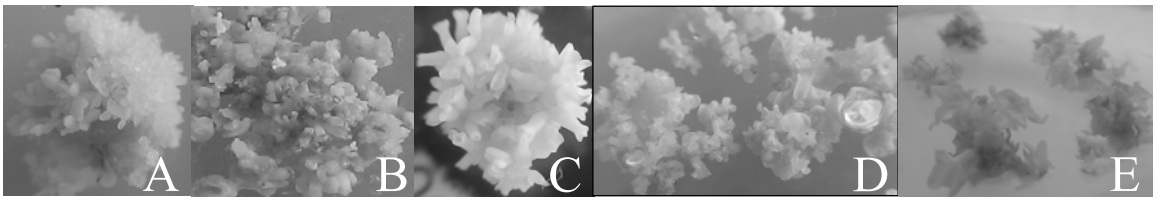

Fig. 1. Influence of polyethylene glycol on maturation of somatic embryos. (a) Healthy somatic embryos of papaya; (b) Bombarded globular embryos; c) Conversion of globular embryo into cotylednary embryos; (d) Somatic embryo germination; and (e) Shoot regeneration.

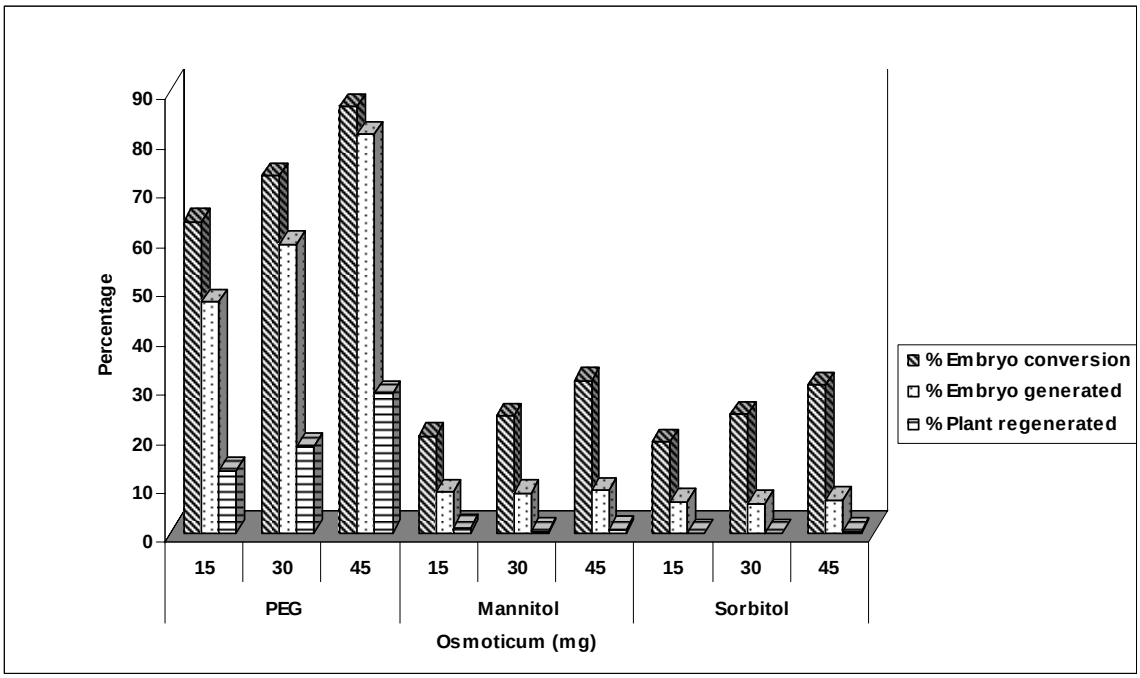

Fig. 2. Effect of osmoticum on maturation and germination of transformed somatic embryos.