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BRIEF COMMUNICATION

Influence of boron on somatic embryogenesis in papaya

N. RENUKDAS, M.L. MOHAN, S.S. KHUSPE and S.K. RAWAL*

Plant Tissue Culture Division, National Chemical Laboratory, Pune 411008, India

Abstract

Influence of boron on somatic embryogenesis in papaya (*Carica papaya* L.) cv. Honey Dew was investigated. Immature zygotic embryos were grown in the induction medium containing Murashige and Skoog basal salts, with B_5 vitamins, picloram (1 mg dm^{-3}) or 2,4-dichlorophenoxy acetic acid (2 mg dm^{-3}) and different concentrations of boric acid (30 to 500 mg dm^{-3}). Maximum somatic embryo initiation was observed at 62 mg dm^{-3} boric acid irrespective of the growth regulator used. The cotyledonary stage somatic embryos were germinated on MS basal medium devoid of growth regulators. The regenerated plantlets were hardened under greenhouse conditions and transferred to field.

Additional key words: *Carica papaya*, 2,4-dichlorophenoxyacetic acid, picloram.

Papaya (*Carica papaya* L.) is an important fruit crop, widely grown in the tropical and the subtropical areas of the world for its fruit and as a source for proteolytic enzymes papain and chymopapain (Medora *et al.* 1979). *In vitro* regeneration of papaya through organogenesis has been achieved using a variety of explants (Rajeevan and Pandey 1986, Winner 1988, Reuveni *et al.* 1990, Hossain *et al.* 1993). Regeneration through somatic embryogenesis in papaya has also been reported earlier (Chen *et al.* 1987, Fitch 1990, 1993, Bhattacharya *et al.* 2002).

Boron, a micronutrient used in the plant tissue culture media is supplied as boric acid. It plays an important role in phenylpropanoid metabolism and lignin biosynthesis. Boron has been shown earlier to influence somatic embryogenesis in rice (Sahasrabudhe *et al.* 1999). The present study reports on the influence of boron on induction of somatic embryogenesis in papaya.

Immature fruits of papaya (*Carica papaya* L.) cv. Honey Dew were washed with running tap water for 1 h and then immersed for 10 min in 1 % solution of a commercial disinfectant containing chlorohexidine gluconate and *Cetrimide* (*Savlon*™, *Johnson and Johnson*, Hyderabad, India). The fruits were rinsed with water and then with 70 % ethanol for 20 - 30 s. The fruits were next washed with sterile distilled water in a laminar airflow cabinet. The surface sterilized fruits were cut and the seeds collected. Immature zygotic embryos were excised from the seeds and used as the explant.

Induction medium consisting of modified Murashige and Skoog (1962; MS) salts, B_5 (Gamborg *et al.* 1968) vitamins and 30 g dm^{-3} sucrose was supplemented either with picloram (1 mg dm^{-3}) or 2,4-dichlorophenoxyacetic acid (2,4-D; 2 mg dm^{-3}) and different concentrations of boric acid (30 to 500 mg dm^{-3}). The pH of the media was adjusted to 5.8 before the addition of 7.5 g dm^{-3} agar and autoclaving at 1.5 kg cm^{-2} and 121 °C for 20 min. Ten immature zygotic embryos were cultured in each Petri dish (55 × 15 mm) containing induction media. Thirty explants were used per treatment and the experiments repeated thrice. MS basal medium with 6.2 mg dm^{-3} boric acid and supplemented with either picloram (1 mg dm^{-3}) or 2,4-D (2 mg dm^{-3}) served as the control. Cultures were incubated at 25 ± 2 °C under irradiance of 38 μmol m^{-2} s^{-1}. Effect of different media on the induction of embryogenesis from each explant was statistically analyzed by *ANOVA* and Student's *t*-test (Snedecor *et al.* 1967, Sokal *et al.* 1973, Chandel 1993). The efficiency of somatic embryogenesis was defined as the percentage of explants forming somatic embryos with reference to all the concentrations of boric acid.

Fully germinated torpedo and cotyledonary stage

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Abbreviations: 2,4-D - 2,4-dichlorophenoxyacetic acid; picloram - 4-amino-3,5,6-trichlorcpicolinic acid.

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* Corresponding author; fax: (+91) 020 5893438, e-mail: rawal@dalton.ncl.res.in

embryos were transferred to growth regulator-free MS basal medium for further elongation of shoots and roots. Regenerated plantlets were dipped in 0.5 % *Bavistin* (*BASF*, Bombay, India) for 5 min and then washed with running water, transferred to pots containing autoclaved soil and sand mixture (1:2) and kept for hardening under diffused light conditions. Hardened plants were transferred to field 3 - 4 weeks after hardening.

For scanning electron microscopy (SEM) samples were fixed in 2 % glutaraldehyde (*Sigma*, St. Louis, USA) for 48 h at room temperature and then rinsed with phosphate buffer pH 7.2 (NaH_2PO_4 27.6 g dm^{-3} and Na_2HPO_4 28.4 g dm^{-3}). The samples were dehydrated by stepwise passage through ethanol series, dried in a *Bio-Rad E3000* critical point drying apparatus (*VG Microtech*, Oxford, UK) on SEM stub and automatic sputter-coated with gold-palladium (20 - 30 nm) (*Bio-Rad*

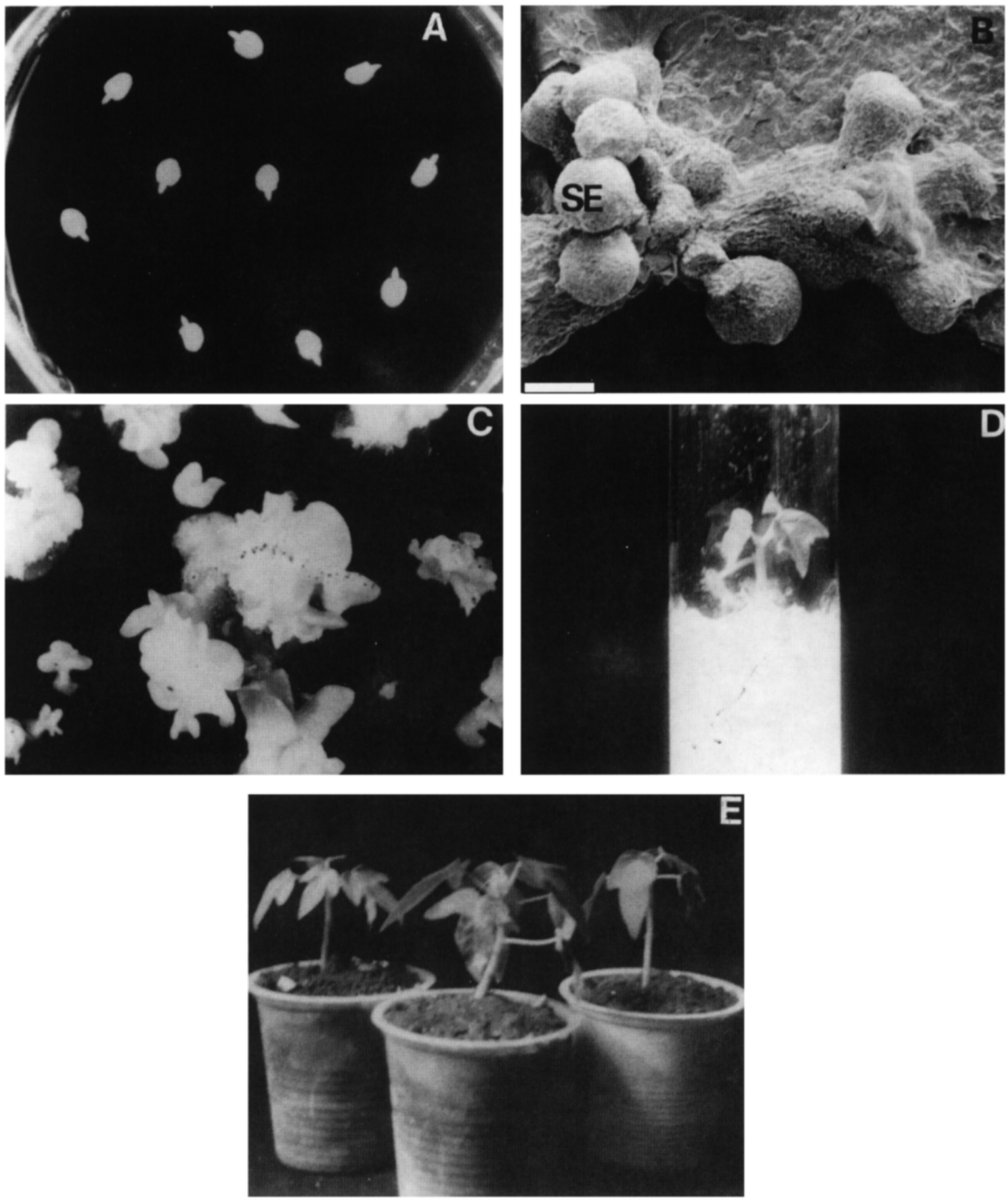

Fig. 1. Effect of boron on the somatic embryo induction in papaya: *A* - immature zygotic embryo used as explant; *B* - scanning electron micrograph of globular somatic embryos (*bar* = 400 μm); *C* - somatic embryos showed rosette structures; *D* - plantlet formation from the somatic embryo; *E* - plantlets after hardening (SE - somatic embryos).

E5200). The samples were examined under a *Stereoscan S120* (Cambridge, England) scanning electron microscope.

Immature zygotic embryo explants of papaya have the ability to differentiate somatic embryos in response to the nutrition factors present in the media. These explants inoculated in different induction media (Fig. 1*A*) elicited different response. In the present study the morphogenetic response varied with the concentration of boric acid in the medium (Table 1). Induction of somatic embryos was seen from the meristematic region (Fig. 1*B*) of the zygotic embryo explant. Globular embryo formation was observed after 6 - 8 weeks of incubation on the induction medium. Highest percentage of embryo induction was observed upon incorporation of either picloram (96.67 %) or 2,4-D (90.0 %) in the culture medium containing 62 mg dm^{-3} boric acid. A drastic reduction in the percentage induction of somatic embryogenesis occurred when boric acid concentration in the medium was increased to 100 mg dm^{-3}. Further increase of boric acid concentration in the medium completely inhibited induction of somatic embryos from the zygotic embryo explants. Morphological and histological observations of the explants revealed embryo formation in presence of boric acid concentrations of up to 100 mg dm^{-3}. No somatic embryogenesis was not observed when the boric acid concentration in the medium was increased above 100 mg dm^{-3}.

The best response was obtained when the medium was supplemented with 62 mg dm^{-3} boric acid and significantly higher somatic embryognsis was obtained irrespective of growth regulators used in the medium. Embryogenesis in the form of rosette structure was achieved from the meristematic regions of the explant (Fig. 1*C*). It exhibited short span of initial stages and then switched over to cotyledonary stage. Formation of embryos was continuous even after 6 - 8 months of culture. Cotyledonary structures obtained were morphologically normal. In control all stages of somatic embryo development were observed. Normal structures had well-developed shoot and root meristems. Somatic embryos germinated and grew into whole plantlets (Fig. 1*D*). These germinated plantlets were hardened in greenhouse under controlled conditions (Fig. 1*E*).

Table 1. Effect of different concentrations of boric acid [mg dm^{-3}] on somatic embryogenesis (% of explants forming somatic embryos) induced on MS medium with 1 mg dm^{-3} picloram or 2 mg dm^{-3} 2,4-D (means ± SD; * - significantly higher than control at 0.05 probability; concentration of boric acid higher than 200 mg dm^{-3} completely inhibited somatic embryogenesis).

H_3BO_3	Picloram	2,4-D
Control	83.33 ± 5.77	73.33 ± 5.77
30	86.67 ± 5.77	76.66 ± 5.77
62	96.67 ± 5.77*	90.00 ± 0.00*
100	13.33 ± 5.77	10.00 ± 10.00
150	6.67 ± 5.77	3.33 ± 5.77
180	3.33 ± 5.77	3.33 ± 5.77
200	3.33 ± 5.77	0.00 ± 0.00

Similar results were obtained during pine somatic embryogenesis induction (Huang and Li 1994) where it was found that boric acid requirement is specific for embryogenesis. This is in contrast to monocot embryogenesis (Sahastrabudhe *et al.* 1999) where the embryogenesis increased with the increasing concentration of boric acid in the medium.

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