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ORIGINAL ARTICLE

Somatic embryogenesis and plant regeneration of *Capsicum baccatum* L.

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Abstract A plant regeneration protocol via somatic embryogenesis was achieved in cotyledon and leaf explants of *Capsicum baccatum*, when cultured on MS medium supplemented with various concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D, 0.5–5.0 mg l^{-1}) in combination with Kinetin (Kn, 0.5 mg l^{-1}) and 3% sucrose. Various stages were observed during the development of somatic embryos, including globular, heart, and torpedo-stages. Torpedo stage embryos were separated from the explants and subcultured on medium supplemented with various concentrations of different plant growth regulators for maturation. Maximum percentage (55%) of somatic embryo germination and plantlet formation was found at 1.0 mg l^{-1} BA. Finally, about 68% of plantlets were successfully established under field conditions. The regenerated plants were morphologically normal, fertile and able to set viable seeds.

1. Introduction

Chilli pepper (*Capsicum* spp.) is one of the important vegetables and spice crops around the world. The genus Capsicum comprises about 30 species, of which only five species *Capsicum annuum* L., *C. frutescens* Mill., *Capsicum baccatum* L., *C. chinense* Jacq., *C. pubescens* Ruiz and Pavon have been domesticated and currently cultivated [1]. *Capsicum* species are used widely in foods, drugs, and cosmetics based on their nutritional value, flavor, aroma, texture, pungency, and color, while some are cultivated as ornamental plants due to their bright glossy fruits of various colors, shape, and sizes [2]. *C. baccatum* L. is a cultivated type of pepper grown primarily in Central and South America, which has been studied by several groups for its *in vitro* regeneration capability [3,4]. Successful plant regeneration has been reported in *C. baccatum* from different explants, such as cotyledon, hypocotyl, leaf, and shoot tip explants [5–9], and from callus derived from both cotyledon and hypocotyl explants [10] or half seed explants [11] via multiple shoot induction.

In the case of *C. baccatum*, it is essential to establish a reliable and reproducible regeneration system that will allow the

Abbreviations: BA, 6-benzyl adenine; 2,4-D, 2,4-dichlorophenoxyacetic acid; GA_3, Gibberellic acid; Kn, kinetin; MS, Murashige and Skoog (1962) medium; TDZ, thidiazuron; 2,4,5-T, 2,4,5-trichlorophenoxy acetic acid; Picloram, 4-amino-3,5,6-tri chloropicolinic acid; NAA, α-naphthalene acetic acid; IAA, indole-3-acetic acid.

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incorporation of desirable genes through genetic transformation. Somatic embryogenesis is the only efficient way to regenerate complete plants from individual cells, and with this system, the risk of undesirable chimerical plants is minimal. Successful plant regeneration through direct or indirect somatic embryogenesis was reported in *C. annuum* L. and *C. chinense* Jacq. using diverse explants, such as intact seedling explants [12], cotyledonary leaves [13], cotyledons, hypocotyls [13–15], stem segments and shoot tips [16], fully expanded leaf explants [17] and hypocotyl explants [18]. The development of protocols to establish embryogenic cultures using more readily available material viz. seedling and mature explants would alleviate many of the problems to improve this crop through plant biotechnology methods [4]. In the present communication, we report reproducible plant regeneration protocol via somatic embryogenesis in *C. baccatum* that will be useful for the genetic improvement of this species.

2. Materials and methods

2.1. Plant material

Seeds of *C. baccatum* PI 260434 were obtained from Regional Plant Introduction Station, Griffin, USA, through National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Seeds of *C. baccatum* soaked for 24 h in sterile distilled water, were surface sterilized with 0.1% $HgCl_2$ for 3–5 min, rinsed several changes of sterile distilled water, and germinated aseptically on MS [19] (Murashige and Skoog 1962) basal medium. Cotyledon, hypocotyls (3-week-old), and leaf (6-week-old) explants were obtained from axenic seedlings and cultured on various media. Single explant was placed in each tube and the explants were placed as the abaxial side of explants in contact with medium.

2.2. Culture media and conditions

All media used in the present study were MS basal medium supplemented with Indole 3-acetic acid (IAA), α-naphthalene acetic acid (NAA), 4-amino-3,5,6-trichloropicolinic acid. (Picloram), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4, 5-trichlorophenoxy acetic acid (2,4,5-T) (0.5–5.0 mg l^{-1}) alone or in combination with either 6-benzyl adenine (BA) or Kinetin (Kn) (0.5 or 1.0 mg l^{-1}) and various concentrations of sucrose (1.0–10.0%). The pH of the media were adjusted to 5.8, gelled with 0.8% Difco-bacto agar before autoclaving at 121 °C and 103.4 kPa for 15–20 min. All the cultures were incubated under 16 h photoperiod with cool white fluorescent lights (40–60 μmol m^{-2} s^{-2}) at 25 ± 1 °C.

2.3. Somatic embryo induction

The MS basal media supplemented with various concentrations and combinations of 2,4-D (0.5–5.0 mg l^{-1}) and Kinetin (0.5 mg l^{-1}) were used for the induction of somatic embryogenesis for cotyledon (3 week-old) and leaf (6 week-old) explants.

2.4. Maturation

Torpedo stage (3–5 mm in length) embryos were separated from the explants and transferred to MS medium supplemented with Abscisic acid (ABA) (0.1, 0.5, 1.0 and 2.0 mg l^{-1}), Gibberellic acid (GA_3) (0.1, 0.5, 1.0 and 2.0 mg l^{-1}), Kn (0.1, 0.5, 1.0 and 2.0 mg l^{-1}), and BA (0.1, 0.5, 1.0 and 2.0 mg l^{-1}) individually for maturation (cotyledonary stage). Somatic embryos (Torpedo stage) were transferred to growth regulator-free MS basal medium to evaluate the effect of the above growth regulators on maturation of somatic embryos from torpedo-stage stage to cotyledonary stage.

2.5. Conversion into plantlets

Cotyledonary stage embryos were separated and transferred to MS medium supplemented with Abscisic acid (ABA) (0.1, 0.5, 1.0 and 2.0 mg l^{-1}), Gibberellic acid (GA_3) (0.1, 0.5, 1.0 and 2.0 mg l^{-1}) Kn (0.1, 0.5, 1.0 and 2.0 mg l^{-1}), and BA (0.1, 0.5, 1.0 and 2.0 mg l^{-1}) individually for conversion into plantlets (germination). Cotyledonary stage somatic embryos were transferred to growth regulator-free MS basal medium to evaluate the effect of the above growth regulators on germination and conversion into plantlets.

2.6. Acclimatization and transfer of plantlets to soil

The embryo-derived plantlets measuring ~5 cm height were removed from agar medium and transferred to liquid medium containing MS basal salts for 8–10 d to harden the root system. Plants were subsequently transferred to autoclaved soil mixture containing coarse sand: soil: manure (1:2:1) and covered with polythene bags. The plantlets were irrigated with tap water as and when required. These plantlets were gradually acclimatized with an increase in the temperature from 25 to 28 °C and decrease in relative humidity from 80% to 30% for 7–10 d in a control growth chamber. After 4 weeks, the plants were transferred to earthen pots containing garden soil mixed with organic manure. When the plantlets showed signs of establishment in pots with the appearance of new leaves, the polythene bags were removed gradually for acclimatization to field conditions.

2.7. Cytological procedure

Chromosome counts were carried out for randomly selected plants regenerated from somatic embryos. Root tips of 5 plants were pretreated with 0.0002 M 8-hydroxyquinoline at 18–20 °C for 3 h, then fixed in ethanol:glacial acetic acid (3:1). They were dipped in 1 N HCl and 2% aceto-orcein (9:1) for about 2 h and then squashed with a drop of 45% acetic acid. Chromosomes were counted in 2–5 well-spread cells of each plant [20].

2.8. Data analysis

A minimum of 20 explants were cultured for each treatment. All the experiments were repeated thrice and the standard deviation and standard error were calculated. Data pertaining to the percentage of embryogenesis and mean number of embryos per culture were statically analyzed using a completely randomized block design and means were evaluated the level of significance using Duncan's new multiple range test. Among the treatments, the average figures followed by similar letter are not significantly different at the $P < 0.05\%$ level.