SOMATIC EMBRYOGENESIS OF AN EARLY COTTON CULTIVAR

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ABSTRACT - Somatic embryogenesis in cotton has been previously reported in a limited number of genotypes, predominantly Coker cultivars. Before tissue culture techniques are widely applied to cotton improvement programs, regeneration must be possible for a broad range of genotypes. The aim of the present work was to develop a method to obtain somatic embryos of an early cotton cultivar (Gossypium hirsutum L. race latifolium Hutch cv. CNPA Precoce 2) and their subsequent development. Callus induction was attempted with cotyledon and hypocotyl explants. These explants were cultured on MS medium supplemented with five concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and N6-(2-isopentenyl)-adenine (2iP) either alone or in combination. Based on callus appearance (light brown and granular), four different growth regulator combinations were selected for further callus development. Callus was subcultured on 2.45 μM 2iP and subsequently transferred to 0.45 and 22.50 μM 2,4-D. Somatic embryos of different sizes and shapes subsequently appeared on MS medium supplemented with 2 g L-1 glutamine and no growth regulators. Plantlets were developed from those embryoids. Plant regeneration through somatic embryogenesis is achieved for the first time in the early cultivar CNPA Precoce 2.

Index terms: callus, somatic embryos, plant regeneration.

INTRODUCTION

Somatic embryogenesis in cotton (Gossypium hirsutum L.) has been previously reported (Davidonis & Hamilton, 1983; Gawel et al., 1986; Trolinder & Goodin, 1987, 1988a, 1988b; Finer, 1988; Voo et al., 1991), but only in a limited number of genotypes, predominantly Coker cultivars. Before tissue culture techniques are widely applied to cotton improvement programs, regeneration must be possible for a broad range of genotypes. The aim of the present work was to develop a method to obtain somatic embryos of an early cotton cultivar (Gossypium hirsutum L. race latifolium Hutch cv. CNPA Precoce 2) and their subsequent development. Callus induction was attempted with cotyledon and hypocotyl explants. These explants were cultured on MS medium supplemented with five concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and N6-(2-isopentenyl)-adenine (2iP) either alone or in combination. Based on callus appearance (light brown and granular), four different growth regulator combinations were selected for further callus development. Callus was subcultured on 2.45 μM 2iP and subsequently transferred to 0.45 and 22.50 μM 2,4-D. Somatic embryos of different sizes and shapes subsequently appeared on MS medium supplemented with 2 g L-1 glutamine and no growth regulators. Plantlets were developed from those embryoids. Plant regeneration through somatic embryogenesis is achieved for the first time in the early cultivar CNPA Precoce 2.
of genotypes, predominantly Coker cultivars. Before tissue culture techniques are widely applied to cotton improvement programs, regeneration must be possible for a broad range of genotypes. Embryogenic and non-embryogenic plants have been crossed, showing that embryogenesis is a heritable trait, but further research must be carried out to understand the genetic model (Gawel & Robacker, 1990a).

Early cotton cultivars are of great interest due to their avoidance of plagues such as boll weevil (*Anthonomus grandis* Boh.), which attacks cotton flower buds and capsules, specially in North and South America. The development of tissue culture techniques for those cultivars would be of great importance for cotton breeding programs.

The objective of the present work was to establish a method to obtain somatic embryos of an early cotton cultivar, CNPA Precoce 2, and their subsequent growth into plants.

**MATERIAL AND METHODS**

**Explant source**

Cotton (*Gossypium hirsutum* L. race *latifolium* Hutch) seeds of cultivar CNPA Precoce 2 were obtained from the "Centro Nacional de Pesquisa de Algodão-Embrapa", Campina Grande, Brazil. Acid delinted seeds were rinsed in running water 15 min, soaked 15 min in 1% (w/v) NaOCl with one drop of “Tween 20” per 100 mL, and rinsed again three times in sterile distilled water. Disinfested seeds were transferred to test tubes containing 15 mL MS medium (Murashige & Skoog, 1962) solidified with 7 g L⁻¹ Difco Bacto-agar. pH was adjusted to 5.7-5.8 before autoclaving. Test tubes were incubated at 25°C with a 16 h light/8 h darkness photoperiod (50 µmol m⁻² s⁻¹).

**Callus induction**

Four days after germination, cotyledon (3x3 mm) or hypocotyl (4 mm long) explants were excised and each one cultured in a Petri dish (6 cm diameter). Each dish contained 10 mL semi-solid MS medium with 30 g L⁻¹ glucose, and 1.6 g L⁻¹ gelrite (Sigma Phytage™) as the gelling agent, adding 0.75 g L⁻¹ MgCl₂ to the medium. This gelling agent was used in all subsequent media. Basal medium was supplemented with all possible combinations of 0.00, 0.45, 2.25, 4.50, 22.50 and 45.00 µM 2,4-dichlorophenoxyacetic acid (2,4-D) with 0.00, 0.49, 2.45, 4.90, 24.50 and 49.00 µM N⁶-(2-isopentenyl)-adenine (2iP). The pH was adjusted to 5.7-5.8 before autoclaving. Ten replicates were used for each treatment and type of explant. Cultures were incubated at 30°C with a 16 h light/8 h darkness photoperiod (50 µmol m⁻² s⁻¹). These conditions were maintained in further steps.

**Callus proliferation**

Four weeks after initiation, callus induction was evaluated. Calli which did not look necrotic or dark brown were transferred (removing from the original explant) to media which consisted of MS salts, B₅ vitamins (Gamborg et al., 1968), 30 g L⁻¹ sucrose instead of glucose, and supplemented with the same growth regulator combinations. The selected calli were transferred twice to the same medium at four-week intervals. Calli from four different growth regulator combinations were selected because of their light brown colour and partially friable appearance to attempt embryogenesis. This type of callus has been reported in the literature to be precursor of embryogenic cultures in some cotton cultivars (Gawel et al., 1986; Schoemaker et al., 1986; Finer, 1988). Portions of these calli were cultured onto MS medium with the same salts and vitamins as in the previous step, but with 20 g L⁻¹ sucrose and 0.45 µM 2,4-D. Four weeks later calli were transferred to higher 2,4-D concentration (22.5 µM).

**Embryogenesis induction**

After four more weeks, calli (1 g on each Petri dish) were subcultured onto basal medium (MS salts, B₅ vitamins and 20 g L⁻¹ sucrose) alone or supplemented with 2 g L⁻¹ glutamine, or with 2 g L⁻¹ glutamine + +1.9 g L⁻¹ KNO₃.

**Embryoid conversion**

Four weeks later embryoids were observed on some cultures and they were transferred to media consisting of half strength MS salts and B₅ vitamins supplemented with: 1) 20 g L⁻¹ sucrose + 0.2 mg L⁻¹ GA₃; 2) 10 g L⁻¹ sucrose + +0.1 mg L⁻¹ GA₃; or 3) 10 g L⁻¹ sucrose + 3.8 g L⁻¹ KNO₃ (1/2 MS without NH₄NO₃).

**RESULTS AND DISCUSSION**

Different basal media and growth regulator concentrations have been used for callus induction in *Gossypium hirsutum*. The basal medium used in...
this work has been employed in several studies
(Trolinder & Goodin, 1987; Finer, 1988; Trolinder
& Xhixian, 1989; Voo et al., 1991). The growth
regulators were chosen after preliminary tests for
the cultivar CNPA Precoce 2. As 2,4-D and,
especially, 2iP concentrations increased, callus
colour became darker and more necrotic portions
were observed.

Only 16 of the 36 tested media were subsequently
studied. The rest of the treatments were discarded
due to the dark brown colour of calli. Again, only
calli with the best appearance (granular, partially
friable, light brown) were subsequently subcultured.
This type of callus was obtained on 2.45 μM 2iP;
0.45 μM 2,4-D + 0.49 μM 2iP; 0.45 μM 2,4-D +
+ 2.45 μM 2iP; and 2.25 μM 2,4-D + 0.49 μM 2iP.

After a subculture period (four weeks) on
0.45 μM 2,4-D and then on 22.5 μM 2,4-D, calli
were transferred to the three different embryo
induction media. Embryos were observed only on
calli of hypocotyl origin, which had been induced
on 2.45 μM 2iP (Fig. 1A). Glutamine induced
somatic embryogenesis in CNPA Precoce 2, as
72 embryoids of different appearance and size were
excised from 60% of the callus cultures grown on
medium containing this amino acid (Fig. 1B).
Glutamine had shown to improve embryoid
development (Finer, 1988), although later studies
have indicated that the number of recoverable
embryoids was reduced (Voo et al., 1991).
Moreover, our study showed that the addition of
1.9 g L⁻¹ KNO₃ decreased the embryogenic response:
only three embryoids were observed on 20% of
the calli. However this salt appeared to have the opposite
effect on embryo conversion. Of the three media
studied, best results were obtained on medium
1/2 MS (without NH₄NO₃) + B₅ vitamins + 10 g L⁻¹
sucrose + 3.8 g L⁻¹ KNO₃. On that medium, 25% of
the embryoids developed into small plantlets
(Fig. 1C), while the addition of GA₃ without KNO₃
decreased the number of embryoids to 13%.
Similarly, Trolinder & Goodin (1988b) observed
higher percentages of embryoid development when
medium with no growth regulators and higher KNO₃
concentration was employed. However, in the
present study, some of the recovered plantlets
showed an abnormal morphology.

FIG. 1. A) Embryogenic callus of Gossypium hirsutum
cultivar CNPA Precoce 2. Callus was induced
on 2.45 μM 2iP, transferred three times to the
same medium and subsequently to 0.45 μM
2,4-D, to 22.5 μM 2,4-D and to a medium with
2 g L⁻¹ glutamine and without growth regu-
lators; B) Somatic embryoids excised from
callus of Fig. 1A (bar = 1 mm); C) Plantlet
developed from somatic embryos, eight weeks
after culture on 1/2 MS salts + B₅ vitamins +
+ 10 g L⁻¹ sucrose + 3.8 g L⁻¹ KNO₃.
Several studies have shown a great interaction between genotype and calus induction medium in the embryogenic response of cultures (Gawel et al., 1986; Trolinder & Xhixian, 1989; Gawel & Robacker, 1990a, 1990b). Protocols for somatic embryogenesis induction tested on several cultivars are being developed in laboratory. Further research is needed especially in the embryoid conversion and plant development stages, as early germination of embryos may be an important limiting factor.

CONCLUSIONS

1. Somatic embryogenesis was obtained for the first time in the early cultivar CNPA Precoce 2.

2. A method for indirect somatic embryogenesis is established for the cotton cultivar CNPA Precoce 2: calus is induced from hypocotyl on medium supplemented with 2.45 μM 2iP, transferred at four weeks intervals to 0.45 μM 2,4-D and afterwards to 22.5 μM 2,4-D; embryoids develop when calus is cultured on medium lacking growth regulators but with 2 g L⁻¹ glutamine.

3. Embryoids are germinated on medium 1/2 MS (without NH₄NO₃) + B₅ vitamins + 10 g L⁻¹ sucrose + + 3.8 g L⁻¹ KNO₃, and plantlets are obtained.

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REFERENCES


