Plant Cell Reports (1986) 5:457–459

Plant Cell Reports

Somatic embryogenesis from leaf and petiole callus cultures of *Gossypium hirsutum* L.*

Nick J. Gawel, Arelli P. Rao, and Carol D. Robacker

Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79409, USA

Received May 21, 1986 / Revised version received October 21, 1986 – Communicated by G. C. Phillips

Abstract

Leaf discs from four strains and petioles from six strains of Gossypium hirsutum were cultured on a variety of media. Callus formed from explants on all media, though embryogenesis was highly specific. Embryos formed from only three strain x media combinations. A small percentage of these embryos developed into plantlets. These results demonstrate that cotton plants can be obtained from leaf tissue explants.

Abbreviations:

BA, benzyladenine; 2,4-D, 2,4-dichlorophenoxyacetic acid; GA_3, gibberellic acid; Kn, Kinetin; NAA, 1-naphthalene acetic acid.

Introduction

A basic prerequisite for the application of cell culture techniques to crop improvement is the ability to regenerate plants from callus or suspension cultures. Embryogenesis and subsequent plant regeneration has been obtained in Gossypium hirsutum from callus produced from immature tissues, i.e., cotyledons (Davidonis and Hamilton, 1983), immature embryos (Rangan et al., 1984), immature embryos and hypocotyls (Mitten et al., 1984), and seedling hypocotyls (Trolinder and Goodin, 1985). Embryogenesis from mature tissues has been reported in the related species, G. klotzschianum (Finer and Smith, 1984). Genetic variability for callus and embryo formation has been identified among genotypes within strains and varieties of cotton (unpublished). Since very few explants can be derived from immature tissue of any given genotype (a particular embryo or seedling), many genotypes must be used for each tissue culture experiment, resulting in the introduction of genetic variability. Use of explants derived from mature tissue would reduce or eliminate this problem due to the larger amount of genetically identical tissue available. Furthermore, use of mature tissue would allow in vitro culture and plant regeneration from identified genotypes. This report describes procedures that permit embryogenesis from mature tissue.

Materials and Methods

Plant Growth Conditions:

Plants were grown in a greenhouse in 5 gal. pots in a 2:1:1 peat, perlite, vermiculite mixture. Greenhouse temperatures were 20-22° night/30-37°C day. Explants were collected from mature, flowering plants.

Explant source:

Leaf discs: Fully-expanded apical leaves from T141, T147, Acala SJ-1 and Coker 312 plants were used.

Petioles: Petioles were removed from leaves at the middle of the main stem from T1, T25, T169, Coker 312, Acala SJ-5 and Paymaster 303 plants.

Disinfection procedure:

Leaf discs: Leaves were rinsed four hours in running tap water, soaked thirty seconds in 95% ethanol and ten minutes in 10% Clorox bleach, and rinsed three times in sterile, distilled, deionized water. Leaf discs with at least one major vein were excised with a sterile #5 cork borer.

Petioles: Petioles were rinsed two to three hours in running tap water, soaked twenty seconds in 95% ethanol and twenty minutes in 10% Clorox bleach, and rinsed three times in sterile, distilled, deionized water.

Callus induction/embryogenesis media:

Leaf discs: All media consisted of Linsmaier and Skoog (LS) inorganic salts (1965) with the following modifications:

(1) 0.1 mg/l 2,4-D, 0.2 mg/l NAA, 0.1 mg/l GA_3, 0.4 mg/l thiamine
(2) 0.5 mg/l NAA, 1.0 mg/l Kn, 0.1 mg/l GA_3, 0.4 mg/l thiamine
(3) 0.1 mg/l 2,4-D, 0.1 mg/l BA, 0.1 mg/l GA_3, 0.4 mg/l thiamine

Petioles: All media consisted of Murashige and Skoog (MS) salts and vitamins (1962) with the following modifications:

(4) 4.0 mg/l NAA, 1.0 mg/l Kn , 100 mg/l inositol
(5) 4.0 mg/l NAA, 1.0 mg/l Kn , 10 mg/l thiamine, 100 mg/l inositol
(6) 0.1 mg/l 2,4-D, 0.1 mg/l Kn, 10 mg/l thiamine, 100 mg/l inositol

All media contained 30 g/l glucose and were solidified with 1.6 g/l Gelrite (Kelco, San Diego, CA) with 0.75 g/l $MgCl_2$ to aid in gelling. The pH was adjusted to 5.5 before autoclaving.

* College of Agricultural Sciences Publication Number T-4-193; this research was partially funded by the USDA-ARS Plant Stress and Water Conservation Research Program

Offprint requests to: N. J. Gawel, Department of Horticulture, University of Georgia, Experiment, GA 30212, USA

Culture protocol:

Leaf discs: Media were dispensed into 150 ml glass jars. Five jars were used for each variety-media combination; four leaf discs were placed in each jar.

Petioles: Media were dispensed into 8 dram glass vials. Three 1 cm sections were cut from each petiole, and each section was placed on one of the three media. Each variety-media combination was replicated 36 times.

All cultures were incubated at 31°C with a 16 hour day/8 hour night photoperiod. As embryos formed, they were isolated and subcultured onto MS basic medium (MS) modified with 30 g/l glucose, 100 mg/l inositol, 10 mg/l thiamine, 1.6 g/l Gelrite and 0.75 g/l $MgCl_2$ (Medium 7). The thiamine level was recommended by Trolinder (personal communication). Embryos with developed cotyledons and hypocotyls were transferred to 125 ml flasks with 20 ml of sterile deionized, distilled water and placed on a gyratory shaker (120 rpm). After approximately 48 hours, the embryos were returned to Medium 7 for further development. A small percentage developed normally and were transferred to sterile Pro-Gro 300S (Pro-Gro Industries, Elizabeth City, NC), moistened with liquid Medium 7. Eventually, the plants were transferred to the greenhouse.

Results and Discussion

All genotypes initiated callus within two weeks. On leaf disc explants, callus formed on the disc edges and from the midrib. The callus which formed from the edges of the leaf discs was bright green and white and very hard. This callus is not favorable for embryogenesis. The midrib callus was brown to grayish-white and friable; these are characteristics of embrypgenic callus. Such callus formed from several media x genotype combinations, but only the combination of Coker 312 on medium 2 actually produced embryos. Three of the twenty leaf discs produced embryos.

As the leaf midrib is an extension of the petiole, the suitability of petiole tissue for embryogenesis was investigated. Callus formed from all 6 strains on all 3 media and was characteristic of embryogenic callus. Again, the embryogenic response was genotype specific; only callus from T25 and Coker 312 explants produced embryos (Table 1). Embryo development is illustrated in Fig. 1.

Table 1

Percentage of embryogenic petiole calli

Strain	Media 4	Media 5	Media 6
T1	0	0	0
T25	29.6	12.5	23.3
T169	0	0	0
Acala SJ-5	0	0	0
Coker 312	22.2	41.6	6.5
Paymaster 303	0	0	0

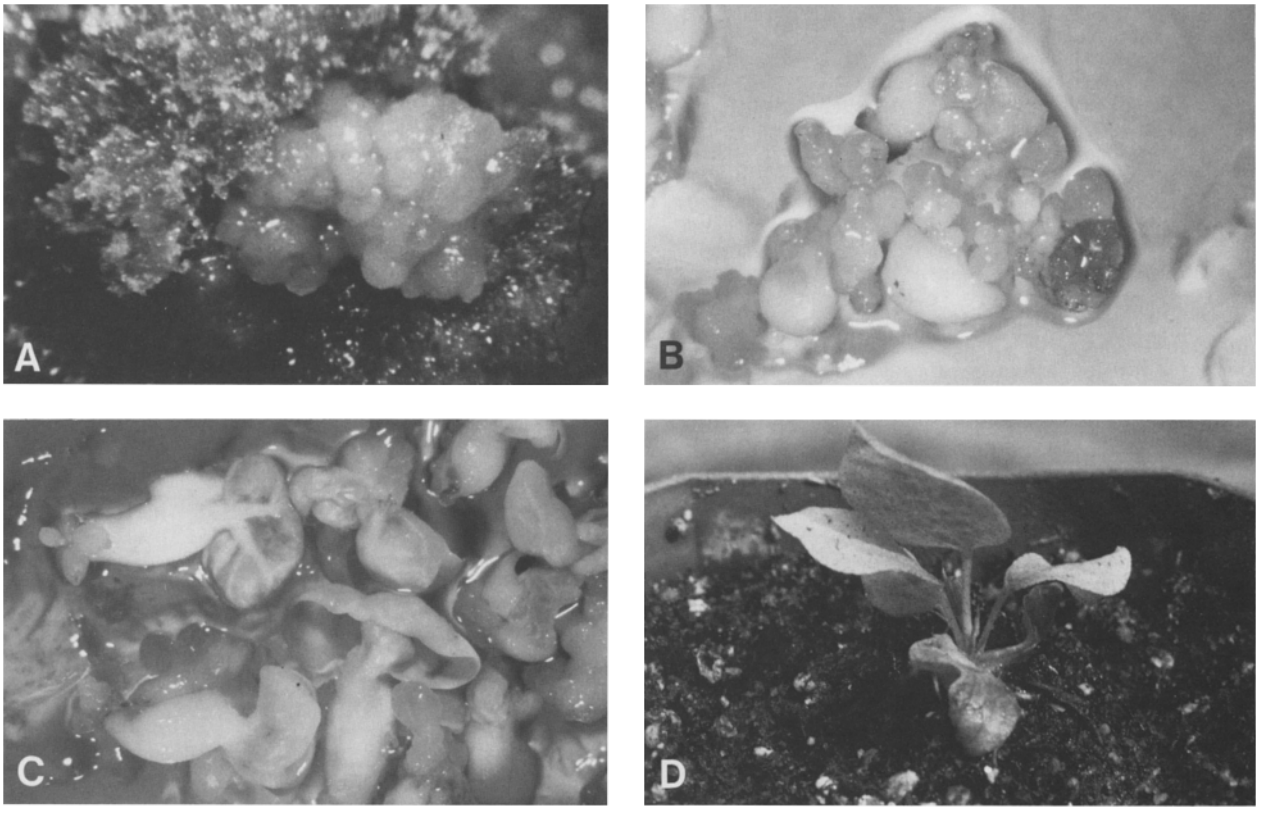

Fig. 1. G. hirsutum embryo development. A. Embryos differentiated from callus (11X). B. Further development of isolated embryos (9X). C. Germinated embryos with cotyledons and hypocotyls evident (7X). D. Plant regenerated from T25 embryo.

Clearly, these data indicate that embryogenesis in cotton is strongly affected by genotype. Coker is particularly embryogenic, forming embryos on several media and explant sources (Davidonis and Hamilton, 1983; Mitten et al., 1984; Trolinder and Goodin, 1985). Data obtained in our laboratory revealed that strain T25 is also highly embryogenic, exhibiting behavior similar to Coker. It is interesting to note that media used to obtain embryos from mature T25 tissue also supports embryogenesis from immature (hypocotyl) T25 explants (unpublished). Additional studies are being conducted to develop a medium to optimize embryogenesis from mature tissue.

References

Davidonis GH, Hamilton RH (1983) Plant Sci. Lett. 32:89-93.
Finer JJ, Smith RH (1984) Plant Cell Reports 3:41-43.
Linsmaier EM, Skoog F (1965) Physiol. Plant. 18:100-127.
Mitten DH, McLean BR, Stratton JR (1984) Amer. Soc. Agron. Abstr. 79.
Murashige T, Skoog F (1962) Physiol. Plant. 15:473-497.
Rangan TS, Zavala T, Ip A (1984) In Vitro 20:256.
Stewart JM, Hsu CL (1977) Planta 137:113-117.
Trolinder NL, USDA, Dept. of Biol. Sci., Texas Tech Univ., Lubbock, TX 79409.
Trolinder NL, Goodin JR (1985) Beltwide Cotton Production Res. Conf., Proc. p. 46.