Plant Cell Reports (1989) 8: 270-273

Plant Cell Reports

© Springer-Verlag 1989

Somatic embryogenesis and plantlet regeneration in Cornus florida

Robert N. Trigiano, Robert M. Beaty, and Joseph T. Dietrich

Department of Ornamental Horticulture and Landscape Design, P.O. Box 1071, University of Tennessee, Knoxville, TN 37901, USA

Received April 4, 1989/Revised version received June 14, 1989 - Communicated by J. M. Widholm

ABSTRACT

Somatic embryos were initiated from 12 to 15 weeks postanthesis (WPA) zygotic embryos of Cornus florida L. (flowering dogwood) cultured on Murashige-Skoog (MS) or Schenk and Hildebrandt (SH) medium amended with either 3 mg/L 2,4-D or 5 mg/L 2,4-D and 1 mg/L kinetin. White, opaque globular and early cotyledonary stage embryos were formed directly on detached cotyledons from 2 of the 5 trees sampled after 7 weeks of culture. Morphologically mature embryos developed after an additional 4 weeks incubation on medium without growth regulators; however, many of the embryos were fused in pairs along the entire length of the hypocotyl-radicle axis. Indirect embryogenesis was observed from callus cultures initiated from 9 to 15 WPA zygotic embryos. These cultures have continued to produce embryos for 16 months. Many of the embryos formed roots on germination medium, but only 12% formed plantlets and none developed past the first true leaf stage.

ABBREVIATIONS

2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine; NAA, 1-naphthaleneacetic acid; FPA, Formalin-propionic acid-ethanol (50%); WPA, weeks post-anthesis.

INTRODUCTION

Cornus florida L. (flowering dogwood) produces small inconspicuous flowers subtended by showy white, occasionally pink, bracts and is highly valued as an ornamental species in the United States. Native dogwoods are normally propagated from seeds and can exhibit a wide range of growth habits and bract characteristics. Cultivars that have been selected for either large or pigmented (red) bracts or variegated foliage are usually produced by grafting dormant buds onto native rootstock or occasionally by rooted cuttings. Establishment of plants using either of these propagation methods for cultivars can be difficult and yield unpredictable results. Presently, there are no reports of in vitro regeneration of flowering dogwood.

A propagation system that utilizes somatic embryogenesis has the potential to reliable and efficiently produce clones of dogwood cultivars. Furthermore, a tissue culture system may be helpful for selecting and regenerating plants resistant to dogwood anthracnose (Hibben and Daughtrey, 1988). This disease is destroying many wild populations and may become a limiting factor in nursery production of the species.

In this report, we describe somatic embryogenesis directly from immature zygotic embryos and indirectly from embryo derived callus of flowering dogwood.

MATERIALS AND METHODS

Five 20 to 30 year old dogwood trees growing on the University of Tennessee Agriculture campus, Knoxville, TN were used in this study. Four of the trees were of unknown origin (native); whereas, the remaining tree was the cultivar 'Cherokee Princess'. Developing fruits from each tree were collected at weekly intervals from 15 May to 1 September, 1987 or approximately 4 to 17 WPA. Drupes were surface sterilized in 50% (v/v) commercial bleach (2.6% NaOCl) containing 0.1% (w/v) Triton X-100 with constant agitation for 15 min and then rinsed three times with sterile distilled water. The fleshy mesocarp was removed with a scalpel and the stony endocarp at the micropylar end cracked with sterile, needle-nosed pliers. Developing ovules and/or immature embryos, depending on the sample date, were excised; when possible cotyledons were separated from the hypocotyl-radicle axis before culture. Explants were placed on a basal medium containing either SH (Schenk and Hildebrandt, 1972) or MS (Murashige and Skoog, 1962) salts supplemented with 100 mg myo-inositol, 1 mg thiamine, 30 g sucrose and 8 g/L Phytagar (Gibco). Treatments consisted of 4 concentrations of 2,4-D (1, 2, 3, or 5 mg/L) and two levels of kinetin (0 or 1 mg/L) arranged as a factorial in a randomized complete block design. The pH of all media was adjusted to 5.8± 0.1 before sterilization. A minimum of 24 explants, 1 ovule or embryo per 60 mm petri dish, were cultured every week from each tree. All cultures were incubated at 22C in the dark for 7 weeks, and then transferred to SH or MS basal medium without growth regulators. Cultures were incubated for 4 weeks at 24C with 25 $\mu Em^{-2}sec^{-1}$ of light provided by cool white fluorescent tubes for 16h/day.

Eleven weeks after initial culture, any somatic embryos that had formed directly were removed from the explants. At this time, explants and associated callus from all sampling times were transferred from medium lacking growth regulators through the protocol presented in Fig. 1. All cultures were incubated at 22C in the dark in this scheme.

Offprint requests to: R. N. Trigiano

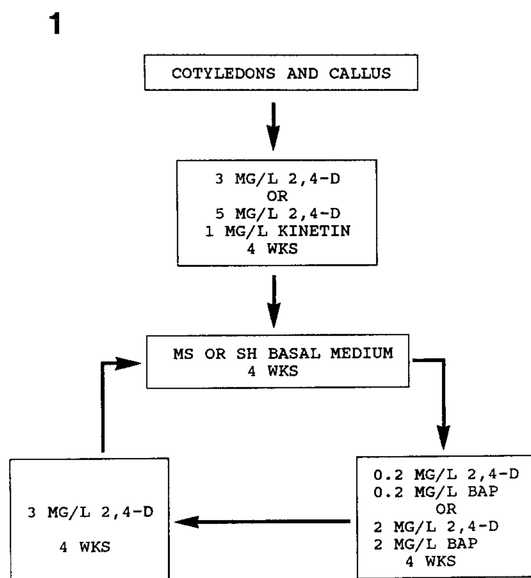


Figure 1. Protocol for formation and maintenance embryogenic cultures and induction of indirect somatic embryos of Cornus florida. Unless otherwise noted, basal medium was SH and cultures were incubated in the dark at 22C.

Embryo Germination

Morphologically mature somatic embryos derived from direct or indirect embryogenesis were transferred to GA7 boxes (Magenta Corp., Chicago, IL) containing 50 ml of SH medium without growth regulators and incubated at 24C in the light as described above.

Histology

Three to five developing ovules and/or zygotic embryos were collected at weekly intervals, fixed in FPA for a minimum of 48h, dehydrated and embedded in Paraplast Plus (Sherwood Medical, Saint Louis, MO). Ten µm-thick longitudinal sections were stained with safranin O, crystal violet and fast green (Johansen, 1940). Seven weeks after culture initiation, eight somatic embryos were excised, oriented for longitudinal sectioning, and immobilized in 0.8% low temperature gelling agarose (Trigiano et al., 1987). Additionally several explants with somatic embryos and indirectly formed embryos with adjacent callus were fixed and processed for histological investigations as described above.

RESULTS AND DISCUSSION

Most ovules and ovules containing globular stage embryos collected from all trees prior to 9 WPA produced callus on all media used in the study. However, prolific callus growth occurred on either MS or SH basal media supplemented with 3 mg/L 2,4-D. Callus was frequently initiated from integumentary tissue and was composed primarily of slow growing, elongated, vitreous cells. These cultures exhibited little morphogenetic potential except for occasional roots or root hairs observed on various media (see Fig. 1). Young expanding leaves, bracts, anthers and flower receptacles also produced callus on most media and likewise were not morphogenetic (data not shown). Callus formation from cambial explants of C. stolonifera Michx. was reported on MS medium supplemented with 5 mg/L NAA (Niki et al., 1978). Zygotic embryos explanted from 16 and 17 WPA fruits did not form somatic embryos and produced little callus.

Direct Embryogenesis

The ability to produce somatic embryos directly from cotyledons was restricted to two native trees and limited to the 12 to 15 WPA sampling period corresponding to when the zygotic embryo explants were 3.6 ± 0.2 to 5.3 ± 0.5 mm in length, respectively. Explants from the other trees, including the named cultivar, only produced callus during this period. Somatic embryogenesis has been reported to be highly dependent on genotype for some species (Chen et al., 1987; Hodges et al., 1986; Keyes et al., 1980; Hanning and Conger, 1982). Moreover, the developmental stage of the explant has been demonstrated to acutely influence the ability of explants to form somatic embryos (Williams and Maheswaran, 1986). Somatic embryogenesis in several tree species is strictly contingent on the ontological stage of the explant (Merkle et al., 1987; Trigiano et al., 1988; Tulecke and McGranahan, 1985).

Direct somatic embryogenesis occurred from explants cultured on either MS or SH media supplemented with 3 mg/L 2,4-D or 5 mg/L 2,4-D and 1 mg/L kinetin. Approximately 4% of the explants from each of the two native trees produced somatic embryos during the 12 to 15 WPA period. The frequency of somatic embryo formation was low; an average of 3.5 embryos were formed per explant.

Somatic embryos were initially observed 3 weeks after culture as regular mounds of tissue arising from only the cotyledons that had been excised from the hypocotyl-radicle axis. Embryo initiation and development were not synchronous; 7 weeks after culture, groups of globular and early cotyledonary stage embryos from individual explants could be identified (Fig. 2). Some of the globular

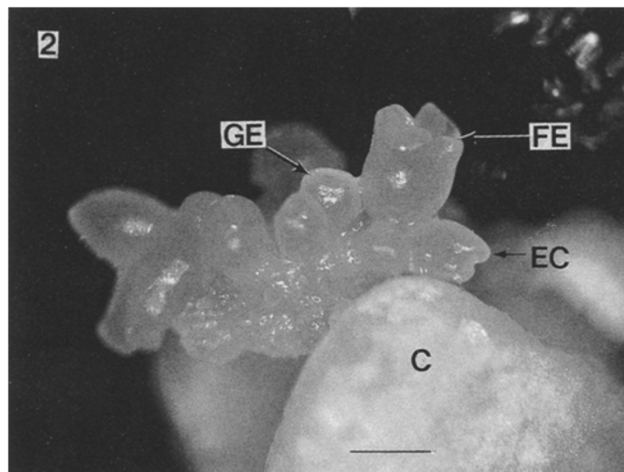


Figure 2. Somatic embryos formed directly from a Cornus florida cotyledon after 7 weeks in culture. EC= early cotyledonary stage embryo; GE= globular embryo; FE= fused embryos; C= zygotic embryo cotyledon. Bar= 2 mm.

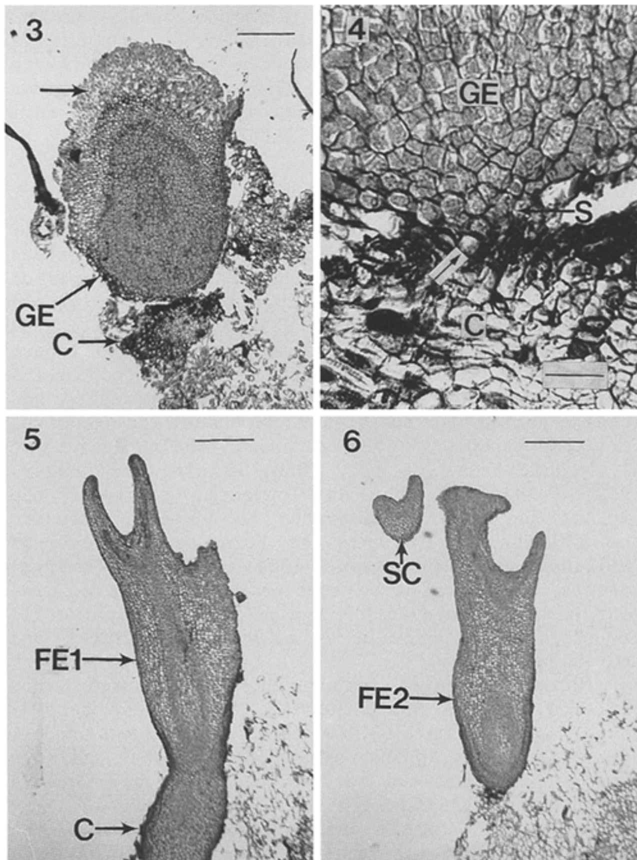
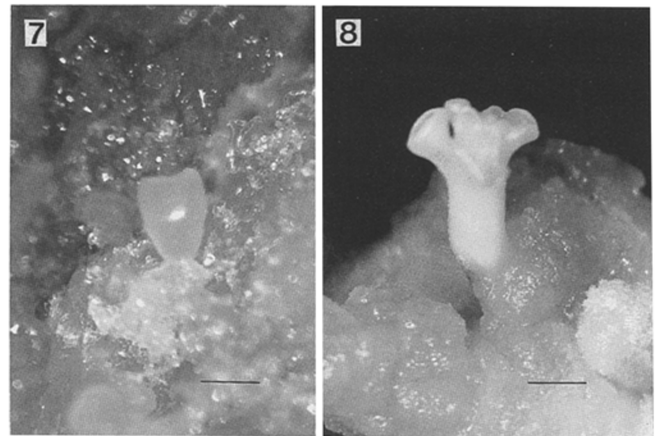


Figure 3 - 6. Histology of somatic embryos formed directly from a zygotic embryo cotyledon of Cornus florida. 3. Longitudinal section through a globular embryo (GE) and zygotic embryo cotyledon (C) shown in Figure 2. Note callus at the proximal end of the embryo (arrow). Bar= 265 µm. 4. Enlargement of the globular embryo (GE) and the suspensor (S) shown in Figure 2. The basal cell of the suspensor is indicated with an arrow C= zygotic embryo cotyledon. Bar= 20 µm. 5 and 6. Oblique longitudinal sections through the fused embryos shown in Figure 2 and designated as FE1 and FE2. C= zygotic embryo cotyledon; SC= somatic embryo FE1 cotyledon. Bars= 450 µm.

embryos were developmentally arrested and had callused at the proximal end (Fig. 3). Most of the embryos examined histologically possessed short, multiseriate suspensors that abruptly terminated in a single cell embedded in necrotic explant tissue (Fig. 4). This type of suspensor and the condition of the surrounding parent tissue suggests that direct somatic embryos were derived from a single cell (Haccius, 1978; Trigiano et al., 1989). Many of the embryos that had developed beyond the globular stage were fused in pairs along the entire length of the hypocotyl-radicle axis. Each embryo had a closed vascular system, a well-developed root meristem and a poorly organized shoot meristem (Figs. 5 and 6). Two fused complete embryos indicate that two growth centers had differentiated early, possibly in the early globular stage, and had developed independently (Ammirato, 1987). Maturation of the embryos was promoted by incubation in the light for 4 weeks on a medium lacking growth regulators; occasionally, a single embryo with well-developed cotyledons was formed. Embryos remained white during the light incubation period.

Indirect Embryogenesis

All cultures were transferred through the scheme illustrated in Fig. 1, but only 2.5% produced somatic embryos. Somatic embryos formed only from callus derived from 9 to 15 WPA zygotic embryos from the same two trees that produced direct embryos. Embryogenic calli varied from friable and milky white to watery and light brown. Somatic embryos were only initiated after incubation on medium that contained 3 mg/L 2,4-D (see Fig. 1) and globular through cordate stage embryos (Fig. 7) developed during this 4 week period. Further development of embryos occurred only after the embryogenic callis with embryos were transferred to a medium growth lacking regulators. Many of the embryos were fused in pairs similar to that described for those obtained through direct somatic embryogenesis (Fig. 8). There was no evidence of repetitive direct embryogenesis from cotyledons as was reported for Juglans cotyledon culture (Tulecke and McGranahan, 1985). Embryogenic calli and embryo production has been maintained for 16 months by transferring the cultures through the scheme depicted in Fig. 1.



Figures 7 and 8. Somatic embryos of Cornus florida formed from callus. 7. Cordate stage somatic embryo. Bar= 1.1 mm. 8. Fused somatic embryos formed from callus. Bar= 2.0 mm.

Embryo Germination

Cotyledons of somatic embryos placed on germination medium and incubated in the light became green within a month. Approximately 50% of the embryos placed on medium lacking growth regulators produced a primary root. In the cases where two fused embryos were incubated on germination medium, radicles usually emerged from both embryos, but shoot initiation was typically limited to one or the other embryo. Complete plantlets were formed from approximately 12% of the embryos. Dogwood seeds normally require a cold stratification treatment of 4C for 3 or 4 months to break dormancy. A chilling treatment may improve the conversion rate from somatic embryos. Cold treatments have been successfully used to increase plantlet formation rates from somatic embryos of Juglans (Tulecke and McGranahan, 1985) Vitis (Rajasekaran et al., 1982) and Eschscholzia (Kavathekar et al., 1977). Alternatively, conversion of embryos to plantlets may not be possible since their shoot meristems were poorly developed. In all cases, shoot development was limited to the first pair of true leaves

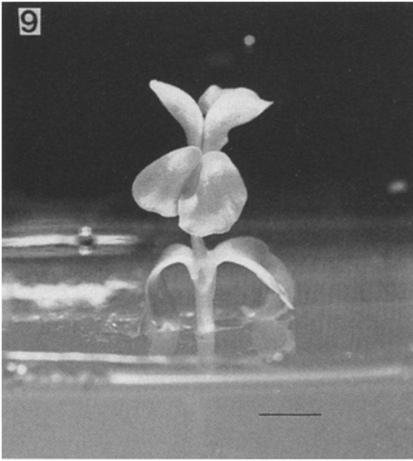


Figure 9. Cornus florida plantlet regenerated from a somatic embryo. Bar= 1.2 cm.

stage (Fig. 9) and no additional growth occurred. However, these leaves were similar in shape, venation pattern and arrangement as those formed on plantlets derived from zygotic embryos.

This study demonstrated that somatic embryogenesis from immature zygotic embryos can be achieved for C. florida. Although somatic embryos were formed only on explants from native trees, embryogenesis in economically important cultivars and increased regeneration rates may possibly be obtained by a combination of traditional breeding and in vitro selection techniques (Riemenschneider et al., 1987). If the rate of embryogenesis and regeneration can be enhanced, then this in vitro culture system may be useful for propagating flowering dogwood and evaluating genotypes for resistance to dogwood anthracnose.

REFERENCES

Ammirato PV (1987) In: Plant Tissue and Cell Culture (Green CD, Somers DA, Hackett WP, and Biesboer DD, eds.) AR Liss, Inc. pp 57-81.

Chen THH, Marowitch J, and Thompson BG (1987). Plant Cell Tissue and Organ Culture 8:73-81.

Haccius B (1978) Phytomorphology 28:74-81.

Hanning GE and Conger BV (1982) Theor. Appl. Genet. 63:155-159.

Hibben C and Daughtrey M (1988) Plant Disease 72:199-202.

Hodges TK, Kamo KK, Imbrie CW, and Becwar MR (1986). Biotechnology 4:219-223.

Johansen DA (1940) Plant Microtechnique. McGraw-Hill.

Kavathekar AK, Ganapathy GS, and Johri BM (1977) Z. Pflanzenphys. 81:358-363.

Keyes GB, Collins GB, and Taylor NL (1980) Theor. Appl. Genet. 58:265-271.

Merkle SA, Wetzstein HY, and Sommer HE (1987). HortScience 22:128-130.

Murashige T and Skoog F (1962) Physiol. Plant. 15:473-497.

Niki T. Yoshida S, and Sakai A (1978). Plant and Cell Physiol. 19:139-148.

Rajasekaran K, Vine J, and Mullins MG. (1982) Planta 154:139-144.

Riemenschneider DE, Haissig BE, and Bingham ET (1987) In: Genetic Manipulation of Woody Plants (Hanover JW and Keathley DE, eds.) Plenum Press pp 433-449.

Schenk RU and Hildebrandt AC (1972). Can. J. Bot. 50:199-204.

Trigiano RN, Beaty RM, and Graham ET (1988). Plant Cell Rep. 7:148-150.

Trigiano RN, Conger BV, and Songstad DD (1987). J. Plant Growth Regul. 6:133-146.

Trigiano RN, Gray DJ, Conger BV, McDaniel JK (1989). Bot. Gaz. 150:72-77.

Tulecke W and McGranahan G (1985) Plant Science 40:57-63.

Williams EG and Maheswaran G (1986) Ann. Bot. 57:443-462.