Plant regeneration and protoplast culture of *Browallia speciosa*

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**ABSTRACT**

Explants from hypcotyls and cotyledons of *Browallia speciosa* were shown to regenerate plantlets. Protoplasts were isolated from etiolated cotyledon material, and, although callus was readily obtained, plantlet regeneration was not observed using numerous hormone regimes.

**ABBREVIATIONS:**

- M - Mannitol
- 2,4-D - Dichlorophenoxyacetic acid
- NAA - Naphthalene-acetic acid
- BAP - Benzylaminopurine
- MS medium - Murashige and Skoog (1962) medium
- UM medium - Uchimiya and Murashige (1974) medium
- COT - cotyledon
- SH - shoot
- R - root

**KEY WORDS:** *Browallia speciosa*, callus induction, shoot regeneration, plantlets, protoplast isolation and culture.

**INTRODUCTION**

Plant regeneration both from explants and protoplasts has been reported for many members of the Solanaceae family (Lu et al. 1982). Within the genus *Browallia*, a neotropical genus containing annual herbaceous plants, plant regeneration from protoplasts has been observed from cell suspension protoplasts of *B. viscosa* (Power and Berry 1979).

Since this report, much attention was focused on other source material for protoplast isolation, with regard to high regeneration potential. Protoplasts isolated from cotyledons of green etiolated seedlings of various species have been shown to have high protoplast regeneration potential (Lu et al. 1982; Ahuja et al. 1983, Burger and Hackett 1982, Berry et al. 1982). Cotyledon-derived protoplasts, because of their special characteristics, could be used as one partner for various protoplast fusion systems.

The results of studies undertaken with seedling material of *Browallia speciosa* are described here.

**MATERIAL AND METHODS**

**Growth of seedlings**

Seeds of *Browallia speciosa* (Apmer Seeds Ltd., Leicester) were surface sterilized in 96% ethanol for 30 s followed by immersion in 10% commercial bleach solution (Domestos) and 5 washes in sterile tap water. Germination and subsequent growth of seedlings was on Murashige and Skoog (1962) medium lacking growth regulators but with 2% sucrose and 0.0% agar (pH 5.8).

Seedlings were maintained in 9 cm plastic petri dishes in the dark and 27°C for 12 days. Seedlings at the unfolded cotyledon stage were used in this study.

**Callus induction from explants**

Three types of explants were taken: cotyledon (COT), hypocotyl (SH) and root (R). Particular care was taken with cotyledon and hypocotyl to avoid meristematic regions.

Explants were cultured on multi-dishes with 5x5 replicates. Each replicate held 3.0 ml of medium. Eleven media regimes were tested. Initial assessments were carried out using Murashige and Skoog (1962) medium supplemented with the following plant growth hormone combination:

- MS + 2.0 mg/dm³ NAA + 0.5 mg/dm³ 6-BAP (MS6P)
- MS + 0.1 mg/dm³ NAA + 0.5 mg/dm³ 6-BAP (MSP)
- MS + 2.0 mg/dm³ IAA + 1.0 mg/dm³ 6-BAP (MSI)
- MS + 0.05 mg/dm³ NAA + 0.5 mg/dm³ 6-BAP (MSD)
- MS + 1.0 mg/dm³ zeatin (MSZ)
- MS + 2.0 mg/dm³ 2,4-D + 0.25 mg/dm³ kinetin (UM)

Cultures were maintained on the media mentioned above in mixture with UM medium in the proportion 1:1. For each combination of media 25 explants in two independent replications were used.

**Protoplast isolation and culture**

Cotyledons (approx. 5–7 mm long) were sliced transversely into 0.5–1.0 mm pieces and were plasmolysed for 4 hours in CWF 13M medium. The CWF salt solution consisted of: KH₂PO₄ -27.2 mg/dm³, KNO₃ -101 mg/dm³, CaCl₂.2H₂O-1480 mg/dm³, MgSO₄.7H₂O -264 mg/dm³, KI -0.16 mg/dm³, CuSO₄.5H₂O -0.025 mg/dm³ (pH 5.8) (Freason et al. 1973). Slices were incubated in 2.0 ml of an enzyme mixture in plastic petri dishes (3.0 cm Nunc), in the dark for 12-14 hours on a rotary shaker (20–30 cycles per min) at 25°C. The enzyme mixture consisted of 2.0% Rhozyme HP 150 (Robert & Hans, Ltd.), 4.0% Macelase P (Meiji Seik Kaisha, Ltd.) and 0.3% Macerozyme R10 (Kinki Yakult Manuf. Co. Ltd.) in CWF 13M, pH 5.8.

Protoplasts were released by gently squeezing and passing the tissue through a nylon sieve (45 μm pore size) and freed of cell debris by resuspension and centrifugation (100 g/10 min) in CWF 21S medium (CWFS salts plus 21% sucrose). Protoplasts were finally washed 3 times in CWF 13M medium and resuspended at a density 5 x 10⁶/ml of protoplast media: KP8 medium (Kao 1977), KP8 medium (Kao and Michayluk 1975), MSPI9M medium (MSPI medium used for explant culture...
Fig. 1. Callus regeneration by 12 day old seedling explant of Browallia speciosa; root (R), hypocotyl (SH) and cotyledon (COT) on UM medium after 4 weeks in culture.

Fig. 2. Callus regeneration by seedling explants on regeneration media mixed 1:1 with callus inducing UM medium.

Fig. 3. Shoot regeneration from seedling explants; cotyledon on MSD4 and MSZ and hypocotyl on MSD3, MSD4 and MSZ.

Fig. 4. Morphogenic response of cotyledons on different combinations of growth substances in MS medium.

Fig. 5. Regenerated plantlet in flowering stage on MSZ medium.

**RESULTS AND DISCUSSION**

**Seedling explant culture**

UM medium was used to induce friable callus from cotyledons (COT), hypocotyls (SH) and roots (R) of 12 day old seedlings (Fig. 1). In the case of the root and hypocotyl, callus induction occurred over the entire explant surface but cotyledon callus regenerated only on the cut surface. On the media combinations of UM medium with other media (see material and methods) explants regenerated less friable callus than that obtained on UM medium alone. No shoot differentiation was observed (Fig. 2).

Investigations carried out using different seedling explants from species of diverse families have indicated that cotyledon explants are capable of callus formation, rhizogenesis and in certain cases embryoid and bud formation (Blaydes 1966, Jelaska 1972, Rybczynski 1975).