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Plant regeneration through somatic embryogenesis in peanut (*Arachis hypogaea* L.)

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Abstract

Somatic embryos were induced from immature cotyledons and immature embryonal axis of *Arachis hypogaea* L. on L-6 basal medium supplemented with NAA, picloram or 2,4-D at 5 - 50 mg l^{-1}. Immature embryonal axis produced a higher number of somatic embryos in comparison with immature cotyledons. The highest number of responding cultures was produced on medium supplemented with NAA (50 mg l^{-1}), while the highest average number of somatic embryos per culture was produced on medium with 2,4-D (10 or 20 mg l^{-1}) and picloram (30 mg l^{-1}) from cotyledons. The somatic embryos developed into plants on basal medium supplemented with activated charcoal and about 100 plants were successfully transferred to the field.

Introduction

Organogenesis and multiple bud formation (Illingsworth 1968, Guy *et al.* 1978, Mroginski *et al.* 1981, Kartha *et al.* 1981, Sastri *et al.* 1982, Narasimhulu and Reddy 1983, Bhatia *et al.* 1985, Mhatre *et al.* 1985, Seitz *et al.* 1987, McKently *et al.* 1990, 1991) and recently somatic embryogenesis have been reported in *Arachis* (Hazra *et al.* 1989, Ozias-Akins 1989). The aim of presented communication, was the induction of somatic embryos from immature cotyledons and embryonal axis of *A. hypogaea* and the establishment of the embryo-derived plants in the field.

Materials and methods

Peanut (*Arachis hypogaea* L. cv. JLM-1) plants were grown in the Experimental Field Station and pods were collected 25-30 d after anthesis. They were surface-

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sterilized with 70 % ethanol and 0.1 % mercuric chloride (m/v). The embryonal axis was carefully excised from the cotyledon and de-embryonated cotyledons (10 - 12 mm) and embryonal axis were cultured separately.

Immature cotyledons of *A. hypogaea* were cut longitudinally into two halves and cultured on various media with the abaxial side in contact with the medium. The medium consisted of L-6 salts (Kumar *et al.* 1988), B_5 vitamins (Gamborg *et al.* 1968) 200 mg l^{-1} casein hydrolysate, 1 % sorbitol, 6 % saccharose and 0.6 % agar (*Hi-Media*, Bombay). The media were supplemented with α-naphthalene acetic acid (NAA, concentrations 10, 20, 30, 40, 50 mg l^{-1}), 2,4-dichlorophenoxyacetic acid (2,4-D, 5, 10, 20, 30 mg l^{-1}) or picloram (5, 10, 20, 30 mg l^{-1}). Immature embryonal axis was cultured on medium supplemented with 2,4-D (20 mg l^{-1}). The pH was adjusted to 5.8 prior to autoclaving. For the development of somatic embryos into plants, the embryos were excised and cultured on basal medium supplemented with activated charcoal (0.1 % and saccharose 3 %).

All cultures were incubated under continuous irradiance of 12.1 μmol m^{-2} s^{-1} and temperature of 25 ± 2 °C.

Results and discussion

Halved cotyledons when cultured on L-6 medium supplemented with 1 % sorbitol, 6 % sucrose, casein hydrolysate (200 mg l^{-1}) and NAA (10 or 20 mg l^{-1}), responded by producing a large number of thick roots from the abaxial side of the cotyledon within 10 - 15 d (Table 1). Subsequently, when the roots reached about 1/2 to 1 cm in length, clusters of green organized structures resembling somatic embryos were observed from the top portion of the root at the point of emergence from the cotyledons. These organized structures appeared to be directly originating from the root and were also firmly attached to the abaxial side of the cotyledon. In areas of cotyledon, where roots were not initiated, dark-green, organized structures unaccompanied by roots developed from the abaxial side. However, these embryo-like structures did not differentiate further. With increased concentrations of NAA (30, 40 and 50 mg l^{-1}) callussing was observed from the surface of cotyledon and clusters of highly organized somatic embryos differentiated from the adaxial side of the cotyledon (Fig. 1). Majority of these embryos originated from the nodal end of the cotyledon and occurred in clusters of 4 - 6 (Table 1). In media supplemented with 2,4-D (5, 10, 20 and 30 mg l^{-1}), clusters of somatic embryos (5 - 9) developed from the proximal part of the adaxial side of the cotyledon from both sides of the notch (Table 1). This was also accompanied by callussing. In media containing picloram (5 mg l^{-1}), callussing was observed from the surface of cotyledons without any sign of embryogenesis, however, at concentrations 10, 20, or 30 mg l^{-1} of picloram clusters of yellowish somatic embryos differentiated from the nodal region (Table 1). NAA at 50 mg l^{-1} produced the highest number of responding cultures, while the highest average number of somatic embryos per culture was obtained in media containing 2,4-D (10 and 20 mg l^{-1}) or picloram (30 mg l^{-1}) (Table 1).

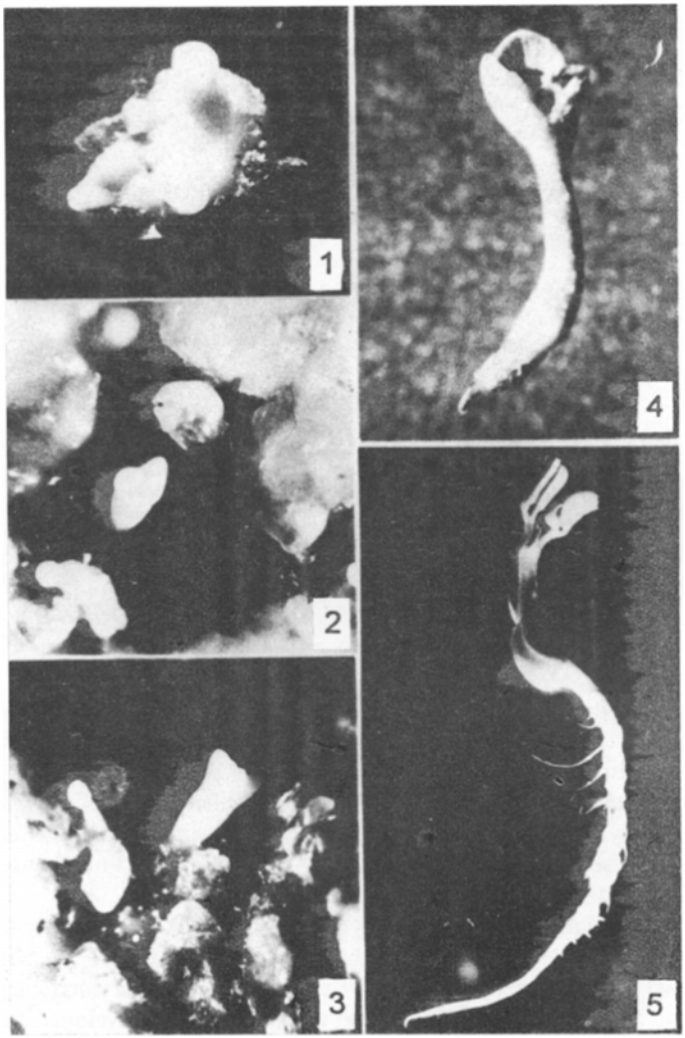
Table 1. Response of *A. hypogaea* L. cotyledons cultured on L-6 medium to addition of different phytohormones for 40 d. The values are means of two independent experiments.

Phytohormones [mg l^{-1}]	Response	Somatic embryos from adaxial side [%]	No. of somatic embryos per culture (mean ± S.E.)
NAA 10	Rooting and organised structures from abaxial side	0	0
NAA 20	Rooting and organised structures from abaxial side	0	0
NAA 30	Callussing and somatic embryos from adaxial side	8.5	6.0 ± 1.6
NAA 40	Callussing and somatic embryos from adaxial side	10.4	4.8 ± 1.0
NAA 50	Callussing and somatic embryos from adaxial side	84.4	4.3 ± 0.6
2,4-D 5	Callussing and somatie embryos from adaxial side	4.3	5.0 ± 1.0
2,4-D 10	Callussing and somatic embryos from adaxial side	22.2	9.3 ± 2.4
2,4-D 20	Callussing and somatic embryos from adaxial side	48.8	9.3 ± 1.0
2,4-D 30	Callussing and somatic embryos from adaxial side	31.5	4.9 ± 0.8
Picloram 5	Callussing	0	0
Picloram 10	Callussing and somatic embryos	8.2	4.8 ± 1.0
Picloram 20	Callussing and somatic embryos	21.0	5.1 ± 0.7
Picloram 30	Callussing and somatic embryos	21.6	10.5 ± 2.0

In cultures of immature embryonal axis on medium supplemented with 2,4-D (20 mg l^{-1}), about 90 % of the explants produced somatic embryos and the average number of somatic embryos per embryonal axis was about 28. Embryos resembled typical dicotyledonous zygotic embryos with two distinct cotyledons (Fig. 2 and 3). However, the cotyledons of somatic embryos were comparatively smaller and thinner in size in comparison with the cotyledons of zygotic embryos. In many instances, the cotyledons fused together and appeared tubular.

The embryos initiated from cotyledons in presence of NAA, 2,4-D or picloram and transferred to medium supplemented with activated charcoal (0.1 %) turned green and developed by elongation of hypocotyl revealing the folded cotyledon (Fig. 4). Later, the cotyledons unfolded (Fig. 5) and the shoot region developed further with the emergence of the first leaves. Only 10 % of the somatic embryos developed into complete plantlets. After about 4 - 6 weeks in this medium, the plantlets were transferred to paper cups (Fig. 6). About 100 plants were transplanted to paper cups and were subsequently transferred to field where they flowered and set seeds. The different characteristics of regenerated plants are being studied.

The present studies have demonstrated that it is possible to induce somatic embryogenesis and plant differentiation from cultured immature cotyledons and embryonal axis of *A. hypogaea*. The studies have also indicated that the embryonal axis is a better source for induction of somatic embryos. Immature embryos or



Figs. 1 to 5. Somatic embryogenesis from cultured cotyledons of *A. hypogaea*.
Fig. 1. Differentiation of clusters of somatic embryos from adaxial side of cotyledon after 3 weeks of culture.
Figs. 2, 3. Well developed somatic embryos with cotyledons.
Figs. 4, 5. Germinating somatic embryos.

immature cotyledons were reported to be the responsive explants for producing somatic embryos in *Glycine max* (Christianson *et al.* 1983, Lippmann and Lippmann 1984, Li 1985, Ghazi *et al.* 1986, Lazzeri *et al.* 1987, Komatsuda and Ohyama 1988), *Pisum sativum* (Kysley *et al.* 1984) and *Vicia narbonensis* (Pickardt *et al.* 1989). 2,4-D (Christianson *et al.* 1983, Lippmann and Lippmann 1984, Ghazi *et al.* 1986, Komatsuda and Ohyama 1988) and NAA (Lazzeri *et al.* 1987, Komatsuda and Ohyama 1988) are the most frequently used auxins for the induction of somatic embryogenesis in grain legumes. When 2,4-D and NAA were compared at similar comcentrations, 2,4-D produced higher number of embryos compared to NAA (Lazzeri *et al.* 1987). However, the morphology of the somatic embryos varied with the auxin present in the media, with NAA producing normal somatic embryos, while 2,4-D producing more aberrant embryos (Lazzeri *et al.* 1987).

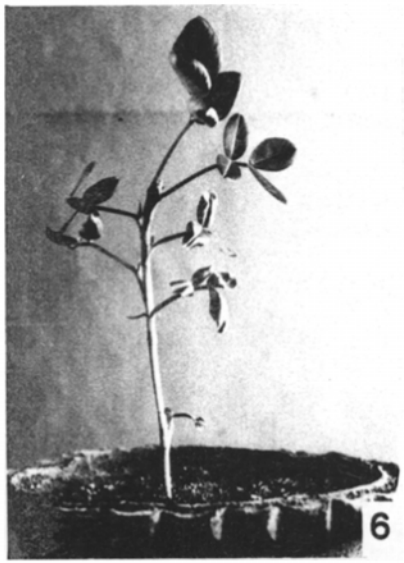


Fig. 6. A plant of *A. hypogaea* differentiated from somatic embryo growing in paper cup.

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