IN VITRO REGENERATION OF ARACHIS VILLOSULICARPA HOEHNE FROM COTYLEDON SEGMENTS, LEAVES AND CELL SUSPENSION[1]

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ABSTRACT - *In vitro* regeneration from immature leaves and cotyledon segments of *A. villosulicarpa* Hoehne was obtained on Murashige & Skoog medium (1962) plus 1.0 mg/l 1-naphthalene acetic acid (NAA) and 1.0 mg/l 6-benzylaminopurine (BAP). Cotyledon segments showed higher morphogenetic potential than immature leaves. Morphogenic calli could also be obtained from leaves of regenerated plants propagated *in vitro*. Cell suspension cultures were established from friable calli originated from leaf and cotyledon explants cultured on MS medium supplemented with 0.2 mg/l picloram and 1.0 mg/l BAP. Calli from liquid culture developed shoots on regeneration medium. Root differentiation of plantlets was achieved on liquid MS medium.

Index terms: immature leaves, NAA, morphogenetic potential, morphogenic calli, peanut ,tissue culture.

REGENERAÇÃO *IN VITRO* DE *ARACHIS VILLOSULICARPA* HOEHNE A PARTIR DE SEGMENTOS DE COTILÉDONE, FOLHAS E CÉLULAS EM SUSPENSÃO

RESUMO - A regeneração *in vitro* a partir de folhas imaturas e segmentos de cotilédone de *A. villosulicarpa* foi obtida em meio MS (Murashige & Skoog 1962) acrescido de 1,0 mg/l de ácido 1-naftaleno acético (ANA) e 1,0 mg/l de 6-benzil-aminopurina (BAP). Segmentos de cotilédone apresentaram um potencial regenerativo maior que o de folhas imaturas. Calos morfogênicos foram também obtidos de folhas de plantas propagadas *in vitro*. Foram estabelecidas culturas de células em suspensão em meio MS líquido acrescido de 0,4 mg/l de picloram a partir de calos friáveis originados de explantes de folha e cotilédone cultivados em meio MS suplementado com 0,2 mg/l de picloram e 1,0 mg/l de BAP. Calos desenvolvidos em cultura líquida originaram brotos, em meio de regeneração. O enraizamento de plântulas foi obtido em meio MS líquido.

Termos para indexação: folhas imaturas, ANA, potencial morfogenético, calos morfogênicos, células em suspensão, amendoim, cultura de tecidos.

INTRODUCTION

Arachis villosulicarpa is a diploid species (2n=20) of the Leguminosae family, section Extranervosae, only collected so far from indian villages in Western Mato Grosso and Southeastern Rondonia States, Brazil (Krapovickas et al. 1985). This species presents a high oil and tryptophan content and resistance to *Cercospora arachidicola* and *Cercosporidium personatum* (Smartt & Stalker 1982; Pittman et al. 1984).

Plantlets of *A. villosulicarpa* were regenerated from *in vitro* cultures initiated from immature and mature leaflets (Pittman et al. 1983, 1984; Johnson & Pitmann 1986). Regeneration from cell suspension cultures among wild species of *Arachis* has been reported for *A. paraguariensis* (Still et al. 1987). The establishment of cell culture systems has also been described for the cultivated peanut, but no regeneration has been achieved (Mroginski & Kartha 1984).

Here, we report *in vitro* regeneration of *A. villosulicarpa* plantlets from immature leaves, cotyledon segments, leaves from callus-derived

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plantlets and cell suspension cultures. Studies on *in vitro* culture systems for this species, including germplasm preservation, are of great interest. The establishment of *in vitro* regeneration systems is a requirement for somatic hybridization, which is an alternative to circumvent the sexual incompatibility barrier between *A. villosulicarpa* and the most commonly cultivated peanut, *A. hypogaea* (Rugma & Cocking 1985).

MATERIALS AND METHODS

Seeds of *A. villosulicarpa* HL490 (BRA-012343), provided by Dr. J.F.M. Valls, from CENARGEN/ EMBRAPA, Brasília, DF, Brazil, were washed with 0.1% Tween 80, surface sterilized with 2% sodium hypochloride for 15 minutes and rinsed five times with sterile distilled water. Seeds were then germinated in 250 ml glass jars on sterile cotton wool wetted with MS salts solution (Murashige & Skoog 1962).

After three days, immature leaves and cotyledons were excised. Cotyledons were sliced into segments of approximately 4 mm. Leaves from regenerated plantlets were excised after rooting or from shoots still attached to calli. Leaf and cotyledon explants were cultured in medium Al - MS salts with B5 vitamins (Gamborg et al. 1968), 0.7% agar, supplemented with 1.0 mg/l 1-naphthaleneacetic acid (NAA) and 1.0 mg/l 6-benzylaminopurine (BAP) (Mroginski et al. 1981). At 3-4 week intervals, calli were divided in pieces of approximately 1.0 cm^3 and transferred to the same medium. Cultures were kept at 25-28 °C, under fluorescent light (3000 lux) with a 16 h/day photoperiod.

Cell suspension cultures were initiated from friable calli obtained by culturing leaves and cotyledon segments in MS medium supplemented with 0.2 mg/l picloram and 1.0 mg/l BAP. Cultures were established from 100-150 mg calli inoculated in 20 ml liquid MS medium supplemented with 0.4 mg/l picloram. Cultures were transferred on a 20-30 day basis, by pippeting 10 ml into 40 ml of fresh medium in 250 ml Erlenmeyers flasks and kept on a gyratory shaker (120 rpm). Calli from 30-40 day-old cultures (3-5 mm diameter) were blot-dried on sterile filter paper and transferred to solid Al regeneration medium.

Regenerated shoots were excised and rooted on liquid MS medium, on paper bridges. After root development, plantlets were transferred to soil, acclimatized for one week and cultivated in greenhouse.

RESULTS AND DISCUSSION

The frequency of callus induction was 100% for cotyledon segments and 79% for immature leaves (Table 1). Leaf and cotyledon explants enlarged during the first week in culture and calli with buds were visible after ten days. Calli attained a diameter of 3-4 cm after four weeks and maintained this growth rate for 6 transfers, after which it started to decrease (data not shown). As shoot production was occurring simultaneously, a high number of individuals was regenerated per explant (Fig. 1). Regeneration was more effective from cotyledon calli than from calli of immature leaves (Table 1). Both leaf and cotyledon explants cultured in the presence of NAA and BAP formed hard, compact green calli. However, in the presence of picloram and BAP, explants formed friable calli which did not develop shoots (data not shown).

Both cotyledon and leaf explants showed a high morphogenetic potential as compared to the same explants of the cultivated peanut, *A. hypogaea* (Mroginski et al. 1981). Moreover, all cotyledon segments of *A. villosulicarpa* showed the same apparent regenerative capacity, independently of their original location, indicating a complete totipotency of this organ. In contrast, regeneration in *A. hypogaea* cotyledons occurs only from proximal segments (Illingworth 1974; Narasimhulu & Reddy 1983; Atreya et al. 1984; Mansur 1992).

In vitro regeneration from leaf explants of *A. villosulicarpa* has been studied by Pittman et al. (1984). These authors cultured explants from 6-10 day-old seedlings on the culture medium described by Mroginaski et al. (1981) and obtained lower regeneration rates than the ones observed in the present study. This difference could be due to

TABLE 1. Regeneration frequency of immature leaves and cotyledon explants of *A. villosulicarpa*.

Explant	Number of explants	Callus formation (%)	Plants/ callus[a]
Immature leaflets	24	79	8
Coyledon segments	40	100	14

[a] average number of shoots per callus.

FIG. 1. Shoot regeneration from callus grown in Al medium (MS medium plus 1.0 mg/l NAA and 1.0 mg/l BAP), after 4 weeks culture (x1.5).

the developmental stage of the leaflets used as explants or to differences between the genotypes used.

Friable calli that were obtained from leaf and cotyledon explants cultured on MS medium supplemented with 0.2 mg/l picloram and 1.0 mg/l BAP were used to initiate cell suspension cultures. Established cultures consisted of small compact cells forming microcalli (Fig. 2). Calli (3-5 mm) formed after 30-40 days of culture were transferred to Al medium and plants were regenerated 6-8 weeks after transfer.

Our results show that tissue culture of cotyledons, in addition to immature leaves, can be used as an alternative for multiplication of *A. villosulicarpa*. Regeneration from cell suspension cultures confirms the high morphogenetic potential of *.A. villosulicarpa* and may also be regarded as a potential source for protoplast isolation and somatic hybridization experiments.

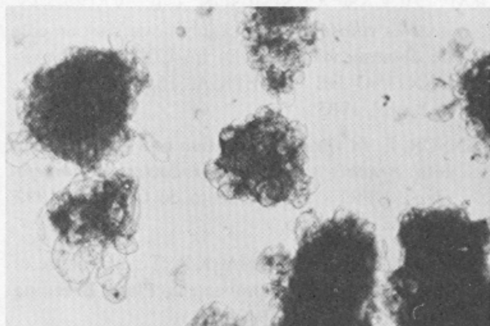

FIG. 2. Microcalli from cell suspension culture (X30).

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REFERENCES

ATREYA, C.D.; RAO, I.P.; SUBRAHMANYAM, N.C. *In vitro* regeneration of peanut (*Arachis hypogaea* L.) plantlets from embryo axis and cotyledon segments. **Plant Science Letters,** v.34, p.379-383, 1984.

GAMBORG, O.L.; MILLER, R.A.; OJIMA, K. Nutrient requirements of suspension cultures of soybean root cells. **Experimental Cell Research,** v.50, p.151-158, 1968.

ILLINGWORTH, J.E. Peanut plants from single de-embryonated cotyledons or cotyledonary fragments. **HortScience,** v.9, p.426, 1974.

JOHNSON, B.B.; PITTMAN, R.N. Factors affecting in vitro differentiation of explants from mature leaves of *Arachis villosulicarpa* Hoehne. **In vitro cellular & Developmental Biology,** v.22, p.713-715, 1986.

KRAPOVICKAS, A.; SIMPSON, C.E.; VALLS, J.F. *Arachis villosulicarpa* (Leguminosae), un proceso de domesticación aislado. In: CONGRESO ARGENTINO DE GENÉTICA, 16, 1985. **Anais...** [S.l.:s.n.], 1985.

MANSUR, E. ***In vitro* regeneration and gene transfer into *Arachis* spp. via *Agrobacterium tumefaciens*.** UFRJ - Departamento de Genética, 1992. Tese de Doutorado.

MROGINSKI, L.A.; KARTHA, K.K. Tissue culture of legumes for crop improvement. **Plant Breeding Review,** v.2, p.215-264, 1984.

MROGINSKI, L.A.; KARTHA, K.K.; SHYLUK, J.P. Regeneration of peanut (*Arachis hypogaea* L.) plantlets by *in vitro* culture of immature leaves. **Canadian Journal of Botany,** v.59, p.826-830, 1981.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum,** v.15, p.473-497, 1962.

NARASIMHULU, S.B.; REDDY, G.M. Plantlet regeneration from different callus cultures of *Arachis hypogaea* L. **Plant Science Letters,** v.31, p.157-163, 1983.

PITTMAN, R.N.; BANKS, D.J.; KIRBY, J.S.; RICHARDSON, P.E. *In vitro* culture of immature peanut (*Arachis* spp.) leaves: morphogenesis and plantlet regeneration. **Peanut Science,** v.10, p.21-25, 1983.

PITTMAN, R.N.; JOHNSON, B.B.; BANKS, D.J. *In vitro* differentiation of a wild peanut, *Arachis villosulicarpa*. **Peanut Science,** v.11, p.24-27, 1984.

RUGMA, E.E.; COCKING, E.C. The development of somatic hybridization techniques for groundnut improvement. In: INTERNATIONAL WORKSHOP ON CYTOGENETICS OF ARACHIS, 1985, Patancheru. **Proceedings...** Patancheru, India: ICRISAT, 1985. p.167-174.

SMARTT, J.; STALKER, H.T. Speciation and cytogenetics in *Arachis*. In: PATTEE, H.E.; YOUNG, C.T., (Eds.). **Peanut Science and Technology,** [s.l.]: American Peanut Research and Education Society, 1982. p.21-49.

STILL, P.E.; PLATA, M.I.; CAMPBELL, R.J.; BUENO, L.C.; CHICHESTER, E.A.; NIBLETT, C.L. Regeneration of fertile *Arachis paraguariensis* plants from callus and suspension cultures. **Plant Cell, Tissue and Organ Culture,** v.9, p.37-43, 1987