BIOLOGIA PLANTARUM 45 (3): 353-357, 2002

Germplasm preservation of wild *Arachis* species through culture of shoot apices and axillary buds from *in vitro* plants

R.F. GAGLIARDI*, G.P. PACHECO*, J.F.M. VALLS** and E. MANSUR*,[1]

*Laboratório de Micropropagação e Transformação de Plantas, UERJ, Rio de Janeiro, Brazil** *EMBRAPA/CENARGEN, Brasília, Brazil***

Abstract

A study was conducted to evaluate *in vitro* techniques for germplasm preservation of wild species of *Arachis*. Nodal segments excised from *in vitro*-grown plants of *A. retusa*, *A. macedoi* and *A. burchellii* were used to examine the effects of explant position and age of the donor plant. Explants were excised from plants maintained in culture for 30, 60, 90 or 180 d, numbered I - V from top to bottom and cultured on MS medium supplemented with 2.7 μM NAA or different BAP concentrations (0, 4.4, 13.2 and 22 μM). The age of the donor plant has not influenced the responses of the four genotypes studied. In contrast, shoot regeneration ability was significantly affected by the original explant position, decreasing from top to bottom. In media supplemented with different BAP concentrations, multishoot formation was induced from apical segments at low frequencies (10 - 20 %) and segments of all positions originated calluses at the explant basis after 30 d of culture. The culture of nodal segments in the presence of 2.7 μM NAA as the sole growth regulator is recommended for the multiplication of *in vitro* collections of wild groundnut species in order to avoid callusing and adventitious shoot formation.

Additional key words: clonal multiplication, groundnut, *in vitro* regeneration, nodal explants, peanut.

Introduction

Wild species of *Arachis* are important gene sources for the improvement of groundnut and some of them are restricted to the west central region of Brazil (Valls *et al.* 1985, Krapovickas and Gregory 1994). There is great interest in the preservation of these species but groundnut conservation through seed banks requires constant germplasm renewal, since the seeds display a sub-orthodox behavior due to their high lipid contents and thin seed coat, which result in short viability (Vásquez-Yanes and Aréchiga 1996). *In vitro* germplasm collections have the advantages of requiring reduced space, facilitating germplasm exchange and reducing the risk of losses caused by biotic and abiotic factors (Towill 1988, Villalobos *et al.* 1991).

The methods based on the activation of pre-formed meristems (shoot tips and axillary buds), which retain the potential to recover true-to-type plants, are desirable for *in vitro* conservation programs. The growth potential of these explants is affected by a number of factors, including seasonal effects, age of the donor plants, genotype, medium composition and explant position on the stem (Bonnier and van Tuyl 1997, Pattnaik and Chand 1997, Kim *et al.* 1997, Ajithkumar and Seeni 1998, Kaur *et al.* 1998, Lin *et al.* 1998, Quraishi and Mishra 1998).

Several wild groundnut species have been successfully regenerated from seed explants including cotyledons, leaflets and embryo axes (Pittman *et al.* 1984, Still *et al.* 1987, Mansur *et al.* 1993, Dunbar *et al.* 1993, Rani and Reddy 1996, Rey *et al.* 2000), but studies on the

Received 28 March 2001, *accepted* 15 September 2001.

Abbreviations: NAA - 1-naphthaleneacetic acid, BAP - 6-benzylaminopurine.

Acknowledgments: This work was supported by the Common Fund for Commodities (project "Preservation of Wild Species of *Arachis* in South America", coordinated by EMBRAPA/CENARGEN) and Research Support Foundation of Rio de Janeiro - Faperj. Fellowships were received from the National Council for Scientific and Technological Development - CNPq. The authors thank Dr. A. Caldeira de Araujo for the statistical analysis, Dr. C. Magioli for help with the photographs and Mrs. Telma Rocha for technical assistance.

[1]Author for correspondence; fax: (+55) 212 5877377; e-mail: mansur@uerj.br

morphogenetic potential of nodal segments from *in vitro* plants are limited. In a previous work, we described a method to recover plants from embryo axes and cotyledons of non-viable seeds of species from Section *Extranervosae* (Gagliardi *et al.* 2000). The objectives of the present study were to determine the effects of explant position and age of the donor plant as well as of NAA and BAP in plant regeneration from shoot apices and axillary buds derived of primary cultures of *A. retusa, A. macedoi* and *A. burchellii* as means of medium-term maintenance and for germplasm multiplication and distribution.

Materials and methods

Shoot apices and nodal segments (0.5 cm long) were excised from *in vitro*-grown plants derived from cotyledons and embryo axes cultures of *A. retusa* V 9950, *A. macedoi* V 13472 and *A. burchellii* V 14119 and V 7863 (Gagliardi *et al.* 2000).

The media used consisted of Murashige and Skoog (1962) salts and vitamins, supplemented with 3 % (m/v) sucrose and 2.7 µM 1-naphthaleneacetic acid (NAA) (Gagliardi *et al.* 2000) or different 6-benzylaminopurine (BAP) concentrations (0, 4.4, 13.2 and 22 µM) and were solidified with 0.7 % agar. The pH was adjusted to 5.8 before autoclaving for 15 minutes at 121 °C.

In vitro plants after 30, 60, 90 or 180 d of culture were used as explants. Nodal segments were excised and numbered I - V from top to bottom. Cultures were maintained in a growth chamber at 28 ± 2 °C with 16-h photoperiod and irradiance of 46 µmol m^{-2} s^{-1} provided by cool-white and *Gro-lux* fluorescent lamps (3:1). Experiments were evaluated after 60 d of culture.

Ten replicates were taken in each treatment and each experiment was repeated three times. Data of regeneration rates were analyzed by *ANOVA* one-way and Tukey-Kramer comparisons test (0.05 % significance level) using the software *Graph-pad Instat.*

Results and discussion

Nodal segments excised from *in vitro* plants maintained for 30, 60, 90 or 180 d on MS medium without growth regulators and pooled independently of their position, originated whole plants upon culture on MS medium supplemented with 2.7 µM NAA. No significant influence of the age of the donor plant ($P > 0.05$) was observed in the responses of the four genotypes studied (Fig. 1). In contrast, bud regeneration ability was significantly affected by explant position on the stem, decreasing from top to bottom. Shoot apices showed regeneration frequencies of 100 %, regardless of the age of the donor plant, while basal explants displayed lower frequencies (Fig. 2). Similar results were found in *Dianthus*, *Geranium*, and *Brassica* (Lin *et al.* 1998). The influence of bud position was also observed in bamboo, both *in vitro* and in field-grown plants (Ramanayake and Yakandawala 1997). The differential morphogenetic potential of *in vitro* responses of buds along the stem can be a consequence of apical dominance (Le Bris *et al.* 1998). Alternatively, this can be explained by factors intrinsic to the buds and related to growth regulators, including endogenous levels, cellular sensitivity and differences in receptor affinity (Kim *et al.* 1997).

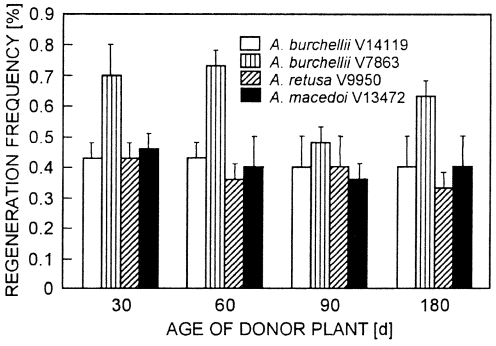

Fig. 1. Influence of the age of the donor plant on the regeneration frequencies from nodal segments of wild species of *Arachis*. Nodal segments were obtained from *in vitro* plants with 30, 60, 90 and 180 d of culture on MS medium supplemented with 2.7 µM NAA. Data were recorded after 60 d of culture. Means ± SE, $n = 10$.

The production of multiple shoots from nodal segments was also studied with the objective of enhancing the rate of multiplication, as previously reported for cassava (Konan *et al.* 1997). In preliminary studies with independently pooled segments of *A. burchellii* (V14119), the presence of NAA in combination with BAP resulted in very low frequencies of bud development. (data not shown). In this work, the response of nodal segments of *A. retusa* and two accessions of *A. burchellii* was studied in the presence of different BAP concentrations (0, 4.4, 13.2, 22 µM). Multishoot formation was induced from apical segments at frequencies between 10 and 20 %, in response to 4.4 and 13.2 µM BAP. After 30 d of culture, segments of all positions also originated organogenic calli at the explant base (Table 1). Hence, shoots arising after callus formation were not considered as derived from preexisting meristems because it was difficult to distinguish their adventitious origin.

Although the induction of higher rates of shoot proliferation was desirable, adventitious shoots may have an increased frequency of clonal variation. Thus, taking into account the requirement of clonal fidelity, we

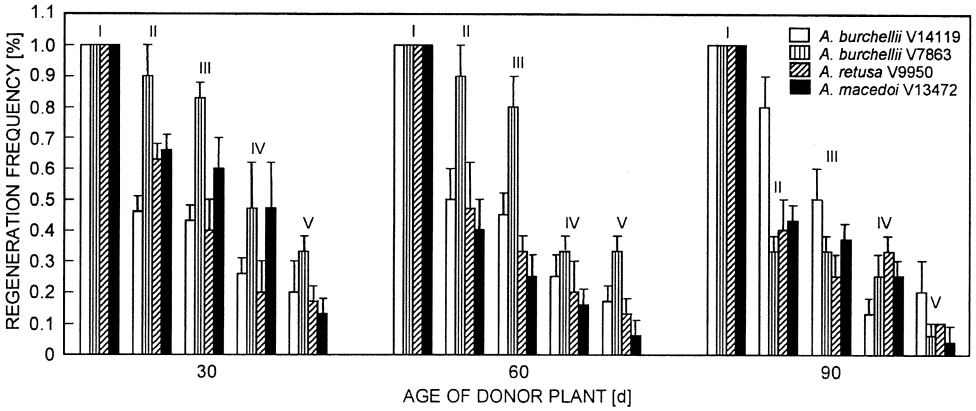

Fig. 2. Influence of the original position of nodal segments (I - V) and the age of the donor plant on the regeneration frequencies from nodal segments cultured on MS medium supplemented with 2.7 μM NAA. Data were recorded after 60 d of culture. Means ± SE, $n = 10$.

Table 1. Effect of different BAP concentrations and explant original position (I, II, III, IV, V) on regeneration percentage and number of shoots per explant. Ten nodal segments were used for each condition and data were recorded after 60 d of culture. Means ± SE. Means within each column followed by the same superscript are not significantly different at $P = 0.05$ %.

Species and accession	BAP [μM]	Regeneration [%] I	II	III	IV	V
A. retusa	0	0.80 ± 0.10^{a}	0.70 ± 0.2^{a}	0.50 ± 0.08^{ab}	0.60 ± 0.1^{ab}	0.30 ± 0.08^{b}
V 9950	4.4	$0.90 \pm 0,03^{a}$	0.60 ± 0.1^{ab}	0.90 ± 0.06^{a}	0.60 ± 0.03^{ab}	0.40 ± 0.03^{ab}
	13.2	0.80 ± 0.03^{a}	0.30 ± 0.03^{b}	0.50 ± 0.03^{ab}	0.40 ± 0.03^{ab}	0.30 ± 0.03^{b}
	22.0	0.70 ± 0.10^{a}	0.30 ± 0.03^{b}	0.40 ± 0.03^{ab}	0.30 ± 0.03^{b}	0.20 ± 0.03^{bc}
A. burchellii	0	0.96 ± 0.03^{a}	0.80 ± 0.03^{a}	0.50 ± 0.03^{b}	0.40 ± 0.03^{b}	0.30 ± 0.03^{b}
V 14119	4.4	0.96 ± 0.03^{a}	0.83 ± 0.03^{a}	0.56 ± 0.03^{ab}	0.36 ± 0.03^{b}	0.30 ± 0.1^{b}
	13.2	0.93 ± 0.03^{a}	0.60 ± 0.05^{ab}	0.26 ± 0.08^{b}	0.36 ± 0.03^{b}	0.13 ± 0.03^{bc}
	22.0	0.83 ± 0.03^{a}	0.60 ± 0.20^{ab}	0.26 ± 0.08^{b}	0.23 ± 0.06^{b}	0.10 ± 0.05^{bc}
A. burchellii	0	0.63 ± 0.13^{ab}	0.46 ± 0.06^{ab}	0.30 ± 0.10^{a}	0.36 ± 0.03^{a}	0.20 ± 0.05^{b}
V 7863	4.4	0.63 ± 0.06^{ab}	0.76 ± 0.03^{a}	0.86 ± 0.03^{a}	0.96 ± 0.03^{a}	0.93 ± 0.03^{a}
	13.2	0.73 ± 0.03^{a}	0.56 ± 0.17^{ab}	0.80 ± 0.05^{a}	0.56 ± 0.06^{ab}	0.90 ± 0.05^{a}
	22.0	0.93 ± 0.03^{a}	0.36 ± 0.03^{b}	0.53 ± 0.03^{ab}	0.46 ± 0.03^{ab}	0.16 ± 0.06^{bc}

Accession	BAP [μM]	Number of shoots per explant I	II	III	IV	V
A. retusa	0	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
V 9950	4.4	2.3 ± 1.4	3.4 ± 0.4	3.5 ± 0.5	4.3 ± 0.6	3.3 ± 0.8
	13.2	1.9 ± 1.2	3.4 ± 0.5	3.0 ± 0.3	3.6 ± 0.5	3.3 ± 0.5
	22.0	1.4 ± 0.8	1.8 ± 0.3	2.6 ± 0.4	2.5 ± 0.4	1.6 ± 0.3
A. burchellii	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
V 14119	4.4	5.3 ± 0.5	5.6 ± 0.5	7.6 ± 0.9	4.2 ± 0.8	5.4 ± 1.4
	13.2	4.2 ± 0.5	5.0 ± 1.0	4.3 ± 1.9	3.7 ± 0.5	1.5 ± 0.5
	22.0	3.2 ± 0.6	2.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5
A. burchellii	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
V 7863	4.4	2.4 ± 0.7	3.0 ± 0.6	1.5 ± 0.2	3.0 ± 0.5	1.4 ± 0.3
	13.2	1.4 ± 0.2	1.5 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
	22.0	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.2

consider that culture of nodal segments in the presence of 2.7 μM NAA as described here and in our previous work (Gagliardi *et al.* 2000), is the most adequate for the multiplication of *in vitro* collections of wild groundnut, species allowing for germplasm maintenance and distribution. This method also results in the development of whole plants from nodal segments (Fig. 3), which is in contrast to many other culture systems based on this explant type, that require additional operations for rooting and elongation (Patnaik and Chand 1997, Ramanayake and Yakandawala 1997, Ajithkumar and Seeni 1998, Quraishi and Mishra 1998). This aspect is beneficial for preservation, as it results in less manipulation and use of growth regulators, which may cause contamination or induce somaclonal variation. Although optimization of shoot proliferation for other genotypes will require additional studies, it is most likely that the same protocol will be useful for other *Arachis* species.

Fig. 3. Whole plants obtained from nodal segments after 40 d of culture in MS supplemented with 2.7 μM NAA. *A*) *A. retusa* V9950, *B*) *A. burchellii* V14119. *Bar* = 1 cm.

References

Ajithkumar, D., Seeni, S.: Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. - Plant Cell Rep. **17**: 422-426, 1998.

Bonnier, F.J.M., Van Tuyl, J.M.: Long term *in vitro* storage of lily: effects of temperature and concentration of nutrients and sucrose. - Plant Cell Tissue Organ Cult. **49**: 81-87, 1997.

Dunbar, K.B., Pittman, R.N., Morris, J.B.: *In vitro* culture of embryonic axes from *Arachis* species for germplasm recovery. - J. Seed Technol. **17**: 1-8 1993.

Gagliardi, R.F.G., Pacheco, G.P., Coculilo, S.P., Valls, J.F.M., Mansur, E.: *In vitro* regeneration from seeds explants of wild groundnut species (genus *Arachis*, section *Extranervosae*). - Biodiversity Conserv. **9**: 943-951, 2000.

Kaur, L., Verma, B., Kant, U.: Plants obtained from the Khair tree (*Acacia catechu* Willd.) using mature nodal segments. - Plant Cell Rep. **17**: 427-429, 1998.

Kim, M.-S., Schumann, C.M., Klopfenstein, N.B.: Effects of thidiazuron and benzyladenine on axillary shoot proliferation of three green ash (*Fraxinus pennsylvanica* Marsh.) clones. - Plant Cell Tissue Organ Cult. **48**: 45-52, 1997.

Konan, N.K., Schöpke, C., Cárcamo, R., Beachy, R.M., Fauquet, C.: An efficient mass propagation system for cassava (*Manihot esculenta* Crantz) based on nodal explants and axillary bud-derived meristems. - Plant Cell Rep. **16**: 444-449, 1997.

Krapovickas, A., Gregory, W.C.: Taxonomia del género *Arachis* (Leguminosae). - Bonplandia **8**: 1-186, 1994.

Le Bris, M., Champeroux, A., Bearez, P., Page Dgivry, L.: Basipetal gradient of axillary bud inhibition along a rose (*Rosa hybrida* L.) stem: Growth potential of primary buds and their two most basal secondary buds as affected by position and age. - Ann. Bot. **81**: 303-309, 1998.

Lin, H.-S., De Jeu, M.J., Jacobsen, E.: Formation of shoots from leaf axils of *Alstroemeria*: The effect of the position on the stem. - Plant Cell Tissue Organ Cult. **52**: 165-169, 1998.

Mansur, E., Lacorte, C., Rabello, A.C.G., Cordeiro, A.R.: *In vitro* regeneration of *Arachis villosulicarpa* Hoehne from cotyledon segments, leaves and cell suspension. - Pesq. agropec. bras. **28**: 1143-1146, 1993.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Pattnaik, S.K., Chand, P.K.: Rapid clonal propagation of three mulberries, *Morus cathayana* Hemsl., *M. lhou* Kois. and *M. serrata* Roxb., through *in vitro* culture of apical shoot buds and nodal explants from mature trees. - Plant Cell Rep. **16**: 503-508, 1997.

Pittman, R.N., Johnson, B.B., Banks, D.J.: *In vitro* differentiation of a wild peanut, *Arachis villosulicarpa* Hoehne. - Peanut Sci. **11**: 24-27, 1984.

Quraishi, A., Mishra, S.K.: Micropropagation of nodal explants from adult trees of *Cleistanthus collinus*. - Plant Cell Rep. **17**: 430-433, 1998.

Ramanayake, S.M.S.D., Yakandawala, K.: Micropropagation of the giant bamboo (*Dendrocalamus giganteus* Munro) from nodal explants of field grown culms. - Plant Sci. **129**: 213-223, 1997.

Rani, A.S., Reddy, G..M.: Multiple shoot regeneration from de-embryonated cotyledons of cultivated and wild species of *Arachis*.. - J. Genet. Breed. **50**: 351-355, 1996.

Rey , H.Y., Scocchi, A.M., Gonzalez, A.M., Mroginski, L.A.: Plant regeneration in *A. pintoi* (Leguminosae) through leaf culture. - Plant Cell. Rep. **19**: 856-862, 2000.

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 Organ Cult. **9**:37-43, 1987.

Towill, L.E.: Genetic considerations for germplasm preservation of clonal materials. - HortScience **23**: 91-95, 1988.

Valls, J.F.M., Rao, V.R., Simpson, C.E., Krapovickas, A.: Current status of collection and conservation of South American groundnut germplasm with emphasis on wild species of *Arachis*. - In: Proceeding of an International Workshop on Cytogenetics of *Arachis*. Pp. 15-35. ICRISAT Center, Patancheru 1985.

Vásquez-Yanes, C., Aréchiga, M.R.: *Ex situ* conservation of tropical rain forest seed: problems and perspectives. - Interscіencia **21**: 293-298, 1996.

Villalobos, V.M., Ferreira, P., Mora, A.: The use of biotechnology in the conservation of tropical germplasm. - Biotechnol. Adv. **9**: 197-215, 1991.