Notas Científicas

Influence of substrates and in vitro preconditioning treatments on ex vitro acclimatization of *Arachis retusa*

Georgia Pacheco[1], Rachel Fátima Gagliardi[1], Mariana Buturini Cogliatti[1], Harlen Barreira Manhães[1], Leonardo Alves Carneiro[1], José Francisco Montenegro Valls[2] and Elisabeth Mansur[1]

[1]Universidade do Estado do Rio de Janeiro, Lab. de Micropropagação e Transformação de Plantas, Rua São Francisco Xavier, nº 524, Maracanã, CEP 20550-013 Rio de Janeiro, RJ, Brazil. E-mail: mansur@uerj.br [2]Embrapa Recursos Genéticos e Biotecnologia, Caixa Postal 02372, CEP 70770-900 Brasília, DF, Brazil. E-mail: valls@cenargen.embrapa.br

Abstract – The objective of this work was to evaluate the influence of substrate and preconditioning treatments on the acclimatization of in vitro plants of *Arachis retusa*. Plants were transferred to Plantmax or sand, and fertilized with Hoagland's nutrient solution. Plants maintained in sand, with or without fertilizer, showed the highest survival rates. In order to evaluate the influence of in vitro preconditioning treatments, stem segments were cultured on MS medium supplemented with different sucrose concentrations. The highest survival and developmental rates were observed in plants from two accessions cultured on MS supplemented with 1.5% and 3% sucrose. Flowering and fruit production were observed after five months.

Index terms: *Arachis*, groundnut, in vitro conservation, micropropagation.

Influência de substratos e de pré-tratamentos in vitro na aclimatização ex vitro de *Arachis retusa*

Resumo – O objetivo deste trabalho foi avaliar a influência de diferentes substratos e pré-tratamentos in vitro, na aclimatização de plantas in vitro de *Arachis retusa*. As plantas foram transferidas para Plantmax ou areia e adubadas com solução de Hoagland. Plantas mantidas em areia, com adubação ou sem adubação, apresentaram maiores taxas de sobrevivência. Para avaliação da influência de pré-tratamentos in vitro, segmentos de caule foram cultivados em meio MS suplementado com diferentes concentrações de sacarose. As maiores taxas de sobrevivência e desenvolvimento foram observadas em plantas cultivadas em sacarose a 1,5% e 3%. Depois de cinco meses, foram observadas a floração e a produção de frutos.

Termos para indexação: *Arachis*, amendoim, conservação in vitro, micropropagação.

Wild species of *Arachis* are important gene sources for the improvement of groundnut (*Arachis hypogaea* L.), due to their extensive genetic variability. These species are endemic to five South American countries, and most of them are currently restricted to areas endangered by intensive environmental disturbance and human actions (Jarvis et al., 2003). *Arachis retusa* Krapov., W.C. Greg. & Valls (Section *Extranervosae*) is restricted to the West Central Region of Brazil, and 66.5% of its potential habitat is under agricultural land use (Jarvis et al., 2003). Hence, urgent actions are needed towards its conservation.

In vitro preservation methodologies require the previous establishment of micropropagation systems (Gagliardi et al., 2000, 2002, 2003). These systems must include the recovery of whole plants from the preserved material and the efficient acclimatization of plants to ex vitro conditions. However, the adaptation of in vitro grown plants to the ex vitro environment can be a limiting step because of their anatomical, morphological and physiological features, which are different from those presented by greenhouse or field plants (Van Huylenbroeck & Debergh, 1996).

Different approaches have been employed, in order to improve survival rates after transplanting. The type of substrate play an important role on survival and plant development during the acclimatization process, since it provides different pH, aeration and water retention ability, and can also affect adventitious rooting (Hoffmann et al., 2001). Modifications in the later stages of micropropagation have also been extensively investigated, including sucrose pretreatments, reduced humidity and variations in light intensity, air exchanges and CO_2 concentrations in the culture vessels (Shim et al., 2003).

This work was directed towards the establishment of an acclimatization protocol for *A. retusa*, evaluating the influence of different substrates and in vitro preconditioning treatments, with different sucrose concentrations, on the viability after transplantation of in vitro-grown plants, as part of a conservation and breeding program.

Seeds of two accessions of *A. retusa* (V 9950 and V 12939) were provided by Embrapa Recursos Genéticos e Biotecnologia seed bank, Brasília, DF. In vitro shoots of both accessions were obtained by culturing embryonic axes and cotyledons according to Gagliardi et al. (2000). To investigate the influence of in vitro preconditioning treatments, stem segments of *A. retusa* from both accessions were transferred to MS medium supplemented with 0, 1.5, 3 or 6% sucrose, four weeks before transplanting. The basal medium for all experiments consisted of MS salts and vitamins supplemented with 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. The pH of all media was adjusted to 5.8, before autoclaving for 15 minutes at 121ºC. Cultures were maintained at 28±2°C, under 16 hour light regime and 46 μmol m^{-2} s^{-1} irradiance provided by cool-white fluorescent lamps.

In vitro-grown plants (4.5 cm height and 2 nodes) were taken out of culture tubes and washed several times with distilled water to remove traces of medium on root surfaces. Plants were then transferred to test tubes (20 cm x 25 mm) completely covered with PVC film, containing distilled water (10 mL), and maintained at room temperature (27±2°C) for two weeks before transplanting. Humidity was decreased by gradually exposing plants to growth cabinet conditions by perforating the PVC film. Thereafter, these plants were transferred to tubes containing Hoagland's nutrient solution (Hoagland & Arnon, 1938) with half-salt concentration, and after one week they were transplanted to commercial plastic containers (37.5x22.5x6.5 cm) filled with substrate (5 dm^3). Two types of substrate (Plantmax and sand) were evaluated, in association to Hoagland's nutrient solution, using plants from accession V 9950. Plants were maintained at greenhouse conditions (30±2°C under 50% of shading), and were watered (10 mL) twice a week. The pH of all substrates was evaluated before and 30 days after transplanting. Ten replicates were used, and experiments were repeated twice. Statistical analysis was performed by ANOVA one-way and Tukey-Kramer comparisons test (0.05% significance level) using the software Graph-pad Instat.

Plants from accession V 9950 transplanted to sand showed the highest survival rates, when compared to those transplanted to Plantmax. The addition of Hoagland's nutrient solution to both substrates was not effective in improving plant survival. Lowest survival rates were observed in plants transplanted to Plantmax and fertilized with Hoagland's nutrient solution (Figure 1). These results were probably related to the natural occurrence of *A. retusa* in sandy and dry soils from the semi-arid region of the Northeast of Brazil (Krapovickas & Gregory, 1994). The pH of all substrates was about 5 at transplanting, with a small increase after 30 days. A slight decrease in plant survival was observed in response to all substrates, with subsequent stabilization after two weeks ex vitro. Plants transplanted to sand (especially without fertilizer) showed the highest heights and number of nodes and leaves after four weeks ex vitro (Figure 1). This behavior could be attributed to the water loss during the first days of the acclimatization process (Shim et al., 2003).

Plants from the two accessions were cultured on MS supplemented with different sucrose concentrations four weeks before transplanting. During the first two weeks, survival decreased in all treatments in plants from accession V 9950, whereas no differences were

observed in the survival rates of plants from accession V 12939 (Figure 2). However, after an additional two-week period, plants from accession V9950, cultured on sugar-free medium or on medium supplemented with 6% sucrose, displayed the lowest survival rates. The highest survival rates in plants from both accessions were observed following culture on MS medium supplemented with 1.5 and 3% sucrose 30 days after transfer to ex vitro conditions (Figure 2). Preconditioning treatments in these media also resulted in higher heights and number of nodes and leaves (Figure 2).

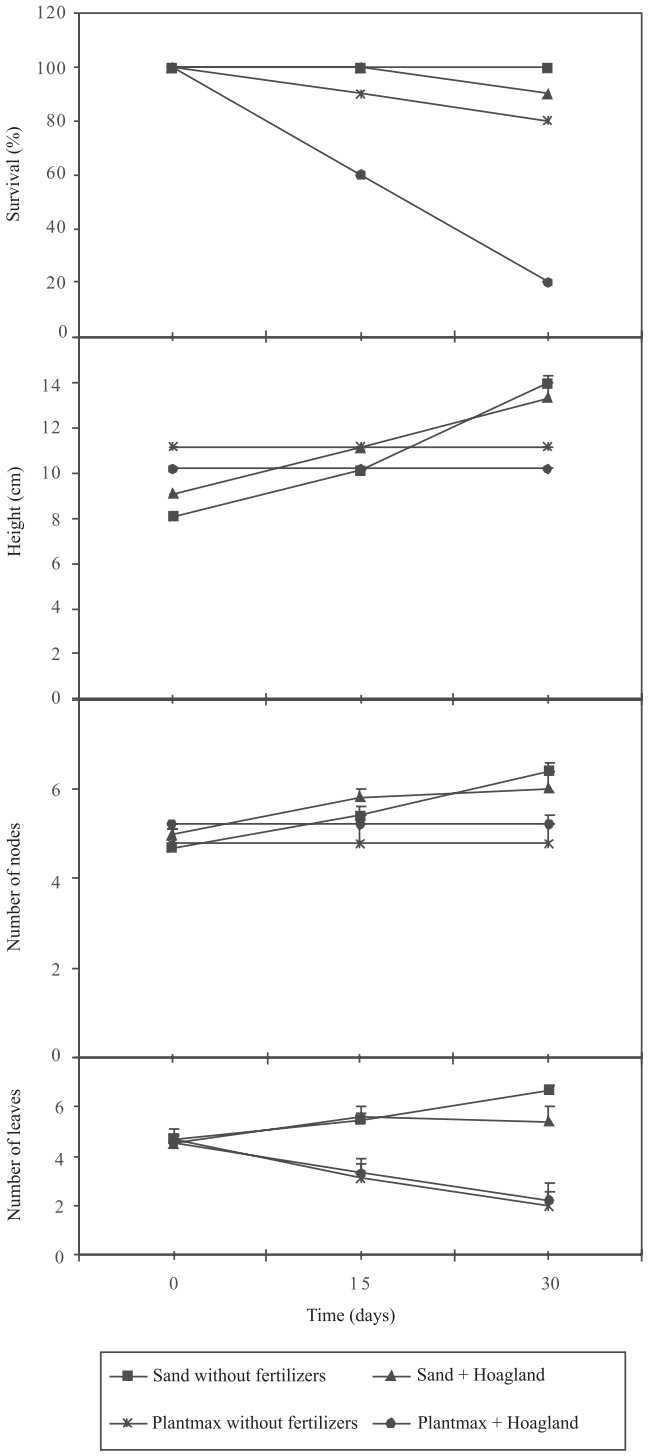

Figure 1. Influence of substrates and fertilizer on the acclimatization process of plants of *A. retusa* V 9950.

Van Huylenbroeck & Debergh (1996) demonstrated that high sucrose levels induce high survival rates after ex vitro transfer. This exogenous supply of high sucrose concentrations leads to the accumulation of starch as storage reserve, which is used to overcome the stress during the first days of adaptation to ex vitro environment. Low survival rates displayed by plants of *A. retusa* from accession V 9950, cultured on MS supplemented with high sucrose concentration (6%), 30 days after transplantation, indicated that the effect of sucrose modification on ex vitro plant performance is also influenced by genotypic differences. Shoots from accession V 9950 that were preconditioned on sugar-free medium did not produce roots during in vitro culture, and were unable to survive at ex vitro conditions, probably due to lack of replenishing water that was lost due to malfunctioning stomata (Klerk, 2002).

Plants transferred to sand were monitored during a post-acclimatization period of five months, and produced new shoots at a frequency of 50%, after three months ex vitro. Growth and new leaf formation were clearly influenced by the growing environment fluctuations, as plant height increase and leaf production were only observed after dry periods and temperatures between 30 and 38ºC. Flowering and fruit production also occurred following these conditions.

Results indicate that the acclimatization process of micropropagated plants of *A. retusa* can be achieved by submitting in vitro plants to a suitable hardening method of gradual exposure to lower relative humidity conditions, during ex vitro transfer, and using sand as substrate without any fertilizer. Moreover, no preconditioning treatments regarding sucrose concentration are necessary in order to improve plant performance at ex vitro conditions, since plant survival and development was highest at standard MS sucrose

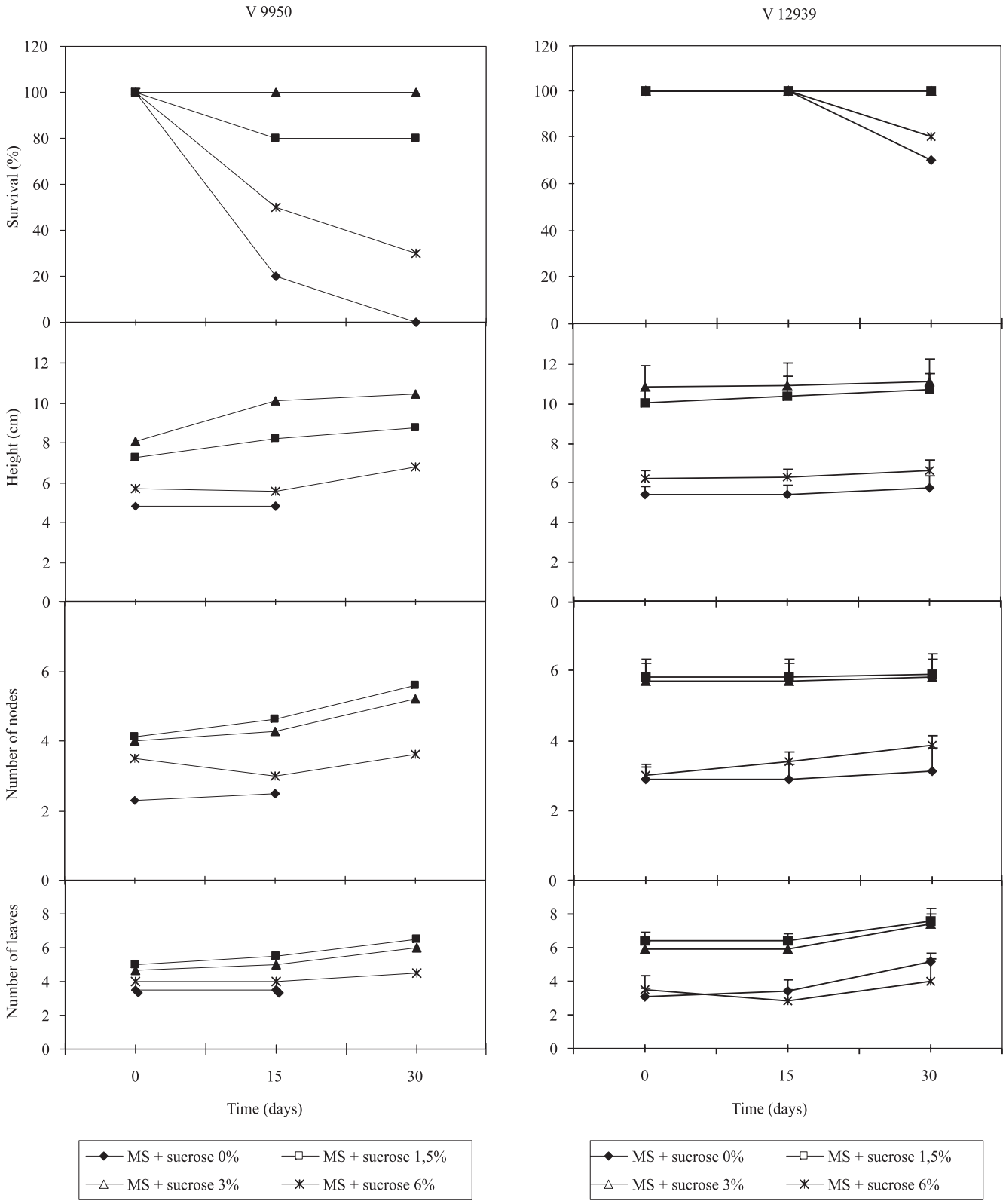

Figure 2. Influence of in vitro preconditioning, with different sucrose concentrations on the acclimatization process of *A. retusa* (V 9950 and V 12939), during 30 days ex vitro.

concentration (3%). This procedure might be adopted for the acclimatization of in vitro preserved plants from other *Arachis* species occurring in similar habitats.

Acknowledgements

To Faperj, for awarding a research fellowship to Georgia Pacheco; to CNPq, for research fellowships to Elisabeth Mansur and José Francisco Montenegro Valls.

References

GAGLIARDI, R.F.; PACHECO, G.P.; CARNEIRO, L.A.; VALLS, J.F.M.; VIEIRA, M.L.C.; MANSUR, E. Cryopreservation of *Arachis* species by vitrification of in vitro - grown shoot apices and genetic stability of recovered plants. **Cryo-Letters**, v.24, p.103-110, 2003.

GAGLIARDI, R.F.; PACHECO, G.P.; COCULILO, S.P.; VALLS, J.F.M.; MANSUR, E. In vitro plant regeneration from seed explants of wild groundnut species (Genus *Arachis*, Section *Extranervosae*). **Biodiversity and Conservation**, v.9, p.943-951, 2000.

GAGLIARDI, R.F.; PACHECO, G.P.; VALLS, J.F.M.; MANSUR, E. Germplasm preservation of wild *Arachis* species through culture of shoot apices and axillary buds from in vitro plants. **Biologia Plantarum**, v.45, p.353-357, 2002.

HOAGLAND, D.R.; ARNON, D.I. **The water culture method for growing plants without soil**. Berkeley: University of California Agricultural Experiment Station, 1938. 34p.

HOFFMANN, A.; PASQUAL, M.; CHALFUN, N.N.J.; FRÁGUAS, C.B. Effect of substrates on acclimatization of micropropagated "Marukaido" apple rootstock plantlets. **Ciências Agrotécnicas**, v.25, p.462-467, 2001.

JARVIS, A.; FERGURSON, M.A.; WILLIAMS, D.E.; GUARINO, L.; JONES, P.G.; STALKER, H.T.; VALLS, J.F.M.; PITTMAN, R.N.; SIMPSON, C.E.; BRAMEL, P. Biogeography of wild *Arachis*: assessing conservation status and setting future priorities. **Crop Science**, v.43, p.1100-1108, 2003.

KLERK, G.J. de. Rooting of microcuttings: theory and practice. **In vitro Cellular and Developmental Biology – Plant**, v.38, p.415-422, 2002.

KRAPOVICKAS, A.; GREGORY, W.C. Taxonomía del género *Arachis* (Leguminosae). **Bonplandia**, v.8, p.1-186, 1994.

SHIM, S.W.; HAHN, E.J.; PAEK, K.Y. In vitro and ex vitro growth of grapevine rootstock "5BB" as influenced by number of air exchanges and the presence or absence of sucrose in culture media. **Plant Cell, Tissue and Organ Culture**, v.75, p.57-62, 2003.

VAN HUYLENBROECK, J.M.; DEBERGH, P.C. Impact of sugar concentration in vitro on photosynthesis and carbon metabolism during ex vitro acclimatization of *Spathiphyllum* plantlets. **Physiologia Plantarum**, v.96, p.298-304, 1996.

Received on February 3, 2005 and accepted on September 20, 2005