International Journal of Agricultural Technology 2020 Vol. 16(5):1175-1184
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

In vitro propagation from nodal segments of *Arachis glabrata* cultivar Florigraze

Pholjad, A.[1], Pongtongkam, P.[2], Arananant, J.[3] and Poeaim, A.[1*]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok. Thailand; [2]128/13 Moo5 Tambon Sano Loi, Bang Bua Thong, Nonthaburi, 11110. Thailand; [3]Feed and Forage Analysis Section. Animal Nautrition Division, Department of Livestock Development, Pathumthani Province. Thailand.

Pholjad, A., Pongtongkam, P., Arananant, J. and Poeaim, A. (2020). *In vitro* propagation from nodal segments of *Arachis glabrata* cultivar Florigraze. International Journal of Agricultural Technology 16(5):1175-1184.

Abstract *Arachis glabrata* Benth. of rhizome perennial peanut is used as a forage plants with higher nutrient value. The nodal segments were used to propagate *in vitro* by 0.2% of $HgCl_2$ plus antibiotic and cefotaxime and preservative for plant tissue culture media active (PPM) at 15 min. It was the most effective procedure for disinfecting plant parts (survival of 73.33%). Shoot induction used to the nodal segments of *A. glabrata* cultivar Florigraze were cultured on MS medium combination with plant growth regulators in cytokinin group at different concentrations 0.5, 0.75, 1, 2, 3 and 5 mg L^{-1} of 6-benzylaminopurine (BAP), N6-furfuryladenine (Kn), *meta*-Topolin (*m*T) and Thidiazuron (TDZ). The maximum growth rate was 66.67% on 5 mg L^{-1} of *m*T was the highest shoot length of 18.43 mm after 30 days. Root induction was achieved on MS medium supplemented with 2 mg L^{-1} of α-Naphthaleneacetic acid (NAA) and 0.2% Activated Charcoal (AC) after 8-12 days cultured when compared with MS medium without plant growth regulators at the basal condition. Thereafter, the plantlets were transferred into the soil for acclimatization. It is the first report using the nodal segment *in vitro* propagation.

Keywords: Activated Charcoal, *Arachis glabrata* cultivar Florigraze, Nodal segment, Shoot induction

Introduction

Arachis glabrata Benth. belongs to Fabaceae, the common name is rhizome perennial peanut. *Arachis* was originated from the aeschynomene tribe of 80 species from Paraguay and growing throughout South America. (Krapovickas and Gregory, 1994; Valls and Simpson, 2005). Perennial peanut had initially known in Brazil since 1936, it is both single and multi-season plants. The characterization of this plant is semi-vertical and horizontal stems. This kind of peanuts are well-known as *A. hypogaea*, and it has been produced as vegetable oil and as a forage crop (Fernandez and Krapovickas, 1994;

[*] **Corresponding Author:** Poeaim, A.; **Email:** anurug.po@kmitl.ac.th, anurug@hotmail.com

Penaloza and Valls, 2005). However, there are other species of *Arachis* instead of *A. glabrata* for example, *A. correntina*, *A. pintoi*, and *A. repens* that can display a great possibility as new agricultural selections, and it was very important plant genetic raw materials for the cultivated eatable peanut development (Pacheco *et al.*, 2009). Currently, the breeds used in foreign Florigraze varieties grown in Florida (USA) (French *et al*., 1994). It would consist of sections beneath 32 north latitude (Florida-Georgia situation line) having a long and lukewarm growing season (Rouse *et al*., 2004). In Australia, *A. glabrata* has been evaluated as a high-quality feed plant and can grow and expand throughout the grass fields of the grassland grown in the summer. Florigraze was released in 1978 for use in pastureland and along the roadside. Florigraze also resembles to be appropriate for landscape. *A. glabrata* crops as forage plants with higher nutrient value and growing in acidic (pH~4.5) and alkaline salt (pH~8.5) resistant soils. It grows in the sandy clay soil with well drainage. In addition, it can be used to ground cover on soils and ornamental plants. Even during the dry season and the leaves on the soil surface is dry to death. The rhizome is alive and continually grows up to the new season. Moreover, it can withstand flooding in a short time and will grow well at temperatures above 20 ℃, and it will stop growing during the winter. In addition, rhizome perennial peanut has been established to be resistant to the various types of pests and diseases (Angelici *et al*., 2008).

Because of the perennial peanut species have many underground rhizomes root and it can survive well in the drought situation. Also, it can immobilize the function of microorganisms that nitrogen uses in the atmosphere. Ammonia is converted to protein. Therefore, leguminous plants have high protein. It is considered as a source of animal protein and has a positive impact on the economy. Natural growth can be associated with local grass growth without any additional fertilizer. So, it can help farmers in reducing the amount of fertilizer they applied (Rouse and Mullahey, 1997). However, the information from the geographic area in the past propagation of perennial peanut species decreased significantly. The impact caused by human actions. As a result, the conservation of this plant species is needed.

The problems found in *A. glabrata* are pest risk, drought, and fire danger. Even though there are flowering significantly but found that this type of peanut that does not produce seeds and the disadvantage of seed viability happens even underneath enough preservation conditions (Dunbar *et al*., 1993). Nevertheless, natural propagation is required to use only rhizomes. Therefore, the perennial peanut must use plant tissue culture techniques to assist the species to produce a lot of *A. glabrata* and disease-free plant species that help a suitable distribution to farmers in the future (Rouse *et al*., 2004).

Previous research was rarely in *A. glabrata*. Most reports were discussed on *A. pintoi* (Rey *et al.*, 1999; Rey *et al.*, 2003) and *A. hypogaea* (Li *et al.*, 1994). Although, previous reports showed that propagation of *A. glabrata* can be only induced calli from the leaflet, that has not been propagated by the nodal segment. The objective was to investigate a new completely from the nodal segment of the *A. glabrata* cultivar florigraze.

Materials and methods

Mature plants of *A.glabrata* cultivar Florigraze were received from the Thai Department of Livestock Development Fodder in Pathum Thani Province. The selected nodal segments (~20 mm long segments of a stalk with an axillary bud) were completely washed with clean water. Then, the surface of plant parts were sterilized with 70% (v/v) ethanol for 1 min, followed by different concentrations of mercuric chloride solution at containing 0.18, 0.2 and 0.2% (w/v) plus antibiotic (antibiotic Antimycotic Solution [100X]; Sigma), cefotaxime (Nida Pharma Inc) and PPM (Preservative for Plant Tissue Culture Media Active, Plant Cell Technology) containing 1 ml L^{-1} (v/v), mixed three drops of Tween 20 for 15, 15 and 20 min respectively, and followed by antibiotic, cefotaxime and PPM (v/v) at concentration which was equal to the above and added three drops of Tween 20 for 10 min. Finally, it washed three times to nodal explants with sterile distilled water for 5 min. Explants were cultured on MS medium (Murashige and Skoog, 1962) in solidified (2.6 g L^{-1} Gellan Gum Powder: Biotech) with 30 g L^{-1} sucrose and without of plant growth regulators. The above conditions, the explants were effectively in condition disinfected utilize to induction of shoots from nodal explants and cultured on solid consisted of MS medium in addition to plant growth regulators of cytokinin group. 6-benzlyaminopurine (BAP), N6-furfuryladenine (Kn), *meta*-Topolin (*m*T) and Thidiazuron (TDZ) in different concentrations 0.5, 0.75, 1, 2, 3 and 5 mg L^{-1} and without plant growth regulators. The media was adjusted to pH 5.8 bottoms with culture media were autoclaved at 15 psi and 121 ℃ for 15 min. the nodal explants cultures were incubated in standard conditions at 25±2 ℃ 8 hr. under photoperiodism, subcultured nodal explants every 4-6 weeks, and observed the shoot development for 4 weeks.

Completely shoot explants of *A. glabrata* cultivar florigraze *in vitro* were transferred to MS medium supplemented with 0.5, 1 and 2 mg L^{-1} of α-Naphthaleneacetic acid (NAA) or without plant growth regulators and 0.2% Activated Charcoal (AC), the incubated under the same conditions for rooting after 30 days. After that, the plantlets were transferred in pots containing a mixture of soil at the ratio of sterile and perlite (2:1), followed by covering with

a plastic bag. The plantlets were cultured under the same conditions for 2 weeks and acclimatized plants were transferred to the greenhouse.

The experimental design was used conducted in a completely randomized design for nodal inducted in vitro establishment. Three duplicates of 15 explants. We used analysis of variance (ANOVA) and previous verification of the normal distribution of data. The significance of mean differences was determined using the Duncan multiple comparison test ($p \leq 0.05$).

Results

Surface sterilization of explants

The nodal explants from natural were disinfected with $HgCl_2$ in the different concentrations and disinfection time listed in Table 1 and combined with antibiotic cefotaxime and PPM containing 1 ml L^{-1} (v/v). The result showed that was effective in preventing contamination and different survival for explants. Therefore, 0.2% $HgCl_2$ resulted to 73.33% survival, whereas 0.18% $HgCl_2$ resulted to 40% survival *in vitro* culture (Table 1). The 15 min exposure strongly revealed plants and shoots were developed. The plants were not contaminated but eventually turned brown and died at 20 min (Figure 1D). On the other hand, the tissue had a normal color but was contaminated with endophytes, which was inside the vascular bundle of the explant at 0.18% $HgCl_2$ (Figure 1A-B).

Table 1. Effects of different concentrations of Mercury Chloride ($HgCl_2$) and culture duration of the nodal explants of *A. glabrata* cultivar Florigraze on MS medium supplemented with 30 g L^{-1} sucrose

$HgCl_2$ Concentration	Disinfection time (min)	Survival[1] (%)	Contamination[2] (%)	Color of Explants	Growth type
0.18%	15	40	60	Greenish brown	Morbidness
0.20%	15	73.33	26.67	Greenish brown	Strong
0.20%	20	73.33	26.67	Brown	Dead

[1] /Survival was observed after 30 days of culture;
[2]/Contamination was observed after 30 days of culture.

In vitro shoot induction

The nodal segments of *A. glabrata* cultivar Florigraze were completely cultured in basal MS medium supplemented with plant growth regulators in the cytokinin group. 33-60% of *m*T showed the highest growth rate. However, the

highest occurrence percentage of shoot development was 66.67% on 5 mg L^{-1} of *m*T (Table 2). Which the appearance of the shoot explant was the maximum length to 18.43 mm, It resulted in the stem and leaflets of the Florigraze became strong and had a green color basal on it (Figure 2B-C). The overall appearance was more completed than the shoot induced from other cytokinins (BAP or TDZ) at the same concentration level. The growth of shoots from the nodal segment of Florigraze cultured with 1 mg L^{-1} of Kn was effective in induction up to 60% after 30 days of culture. On the other hand, when cultured in basal MS medium supplemented with another cytokinin (BAP or TDZ), the percentage of shoots was lower than mT in some concentrations. Also found that when cultured with 0.5 mg L^{-1} of TDZ was not influenced the growth of shoots from the node of *A. glabrata* (cultivar Florigaze). Although in some concentrations of TDZ caused the growth of shoots, but there was a low amount of rate. There was also a lot of calli that caused the growth of the shoots to become incompleted and unhealthy.

Figure 1. Nodal explant of *A. glabrata* cultivar Florigraze through sterilization different concentrations of $HgCl_2$ and duration after 30 days cultured (A), 0.18% $HgCl_2$, 15 min (B). Characteristics of endophytic contamination in the vascular bundle of the explant, 0.20% of $HgCl_2$ at 15 min (C) and 0.20% $HgCl_2$, 20 min (D)

Table 2. Effects of BAP Kn *m*T and TDZ on shoot induction from *A. glabrata* cultivar Florigraze nodal explants

Plant Growth Regulators (mg L^{-1})		Percentage of shoot (%)	Average number of length shoot[1,2,3] (mm)
Control	0	20.00	4.32 ± 0.46^{efg}
	0.5	20.00	5.54 ± 0.48^{cdef}
	0.75	33.33	9.01 ± 1.40^{bc}
BAP	1	40.00	4.49 ± 0.08^{efg}
	2	26.67	5.77 ± 1.17^{cdef}
	3	26.67	5.62 ± 0.43^{cdef}
	5	33.33	5.28 ± 0.19^{defg}
	0.5	33.33	4.64 ± 0.39^{efg}
	0.75	40.00	6.32 ± 0.60^{cdef}
Kn	1	60.00	9.89 ± 1.80^{b}
.	2	20.00	3.02 ± 0.24^{fg}
	3	26.67	5.44 ± 0.55^{cdef}
	5	33.33	5.61 ± 0.55^{cdef}
	0.5	33.33	5.56 ± 0.34^{cdef}
	0.75	40.00	3.82 ± 0.29^{fg}
	1	46.67	5.54 ± 0.14^{cdef}
***m*T**	2	26.67	3.78 ± 0.10^{fg}
	3	33.33	6.22 ± 0.04^{cdef}
	5	66.67	18.43 ± 0.77^{a}
	0.5	0	0 ± 0
	0.75	13.33	4.88 ± 0.58^{efgh}
TDZ	1	6.67	1.83 ± 1.83^{hi}
	2	13.33	4.64 ± 2.49^{fgh}
	3	26.67	7.98 ± 1.65^{bcde}
	5	40.00	8.34 ± 0.40^{bcd}

1/Cultured on MS medium supplemented with 30 g L^{-1} sucrose after 30 days.
[2]/Each value represents the mean ± SD of three repeats per treatment.
[3]/The data were statistically analyzed using Duncan's multiple range test (DMRT). In the same column, significant differences according to significant differences at the $p \leq 0.05$ level areindicated by different letters.

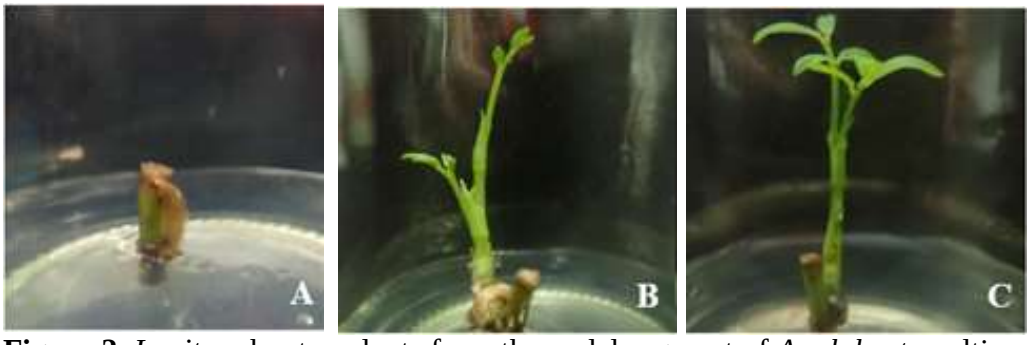

Figure 2. *In vitro* shoot explants from the nodal segment of *A. glabrata* cultivar Florigraze cultured on MS medium and without plant growth regulators(A), Shoot growth from nodal segments cultured on 5 mg L^{-1} of *m*T after 30 days (B-C)

Rooting induction

Shoot that grew *in vitro* was completely (Figure 3A) transferred to culture on MS medium, supplemented with 0.2% AC, combined with NAA at various concentrations and, compared without plant growth regulators. The root efficiency was induced maximum to 50% within 8-12 days after cultivation in MS medium supplemented with 0.2% AC with 2 mg L^{-1} of NAA. However, the medium without growth regulators (only 0.2% AC) was 50% of root emerging. In the same way, NAA increased the number and length of the root after 40 days in culture (~ 3.75 roots per shoot), resulting in a stem that appeared complete and strong (Figure 3B), when compared with the media were not supplemented with plant growth regulators. The acclimatization of plantlets grew normally in natural conditions (Figure 3D).

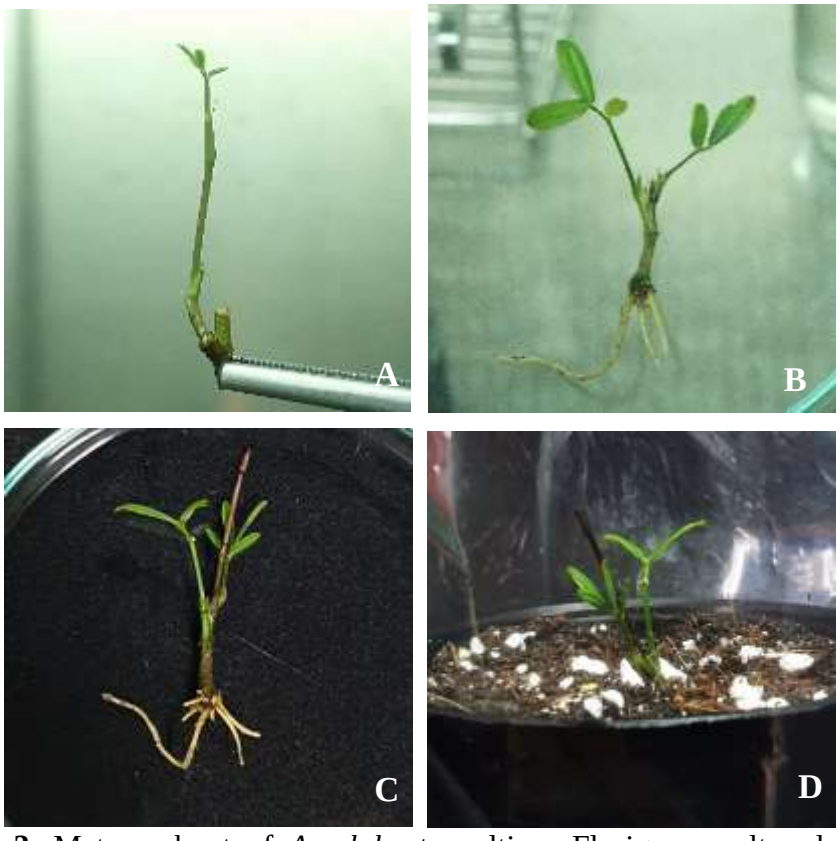

Figure 3. Mature shoot of *A. glabrata* cultivar Florigraze cultured on MS medium combined with 5 mg L^{-1} (A), Plantlets were growing on 2 mg L^{-1} NAA for root induction after 18 days (B) and 40 days (C), Plantlets completely *in vitro* were transferred to soil and acclimatized to *ex vitro* conditions (D)

Table 3. Effects of NAA concentration on rooting induction *in vitro* cultured on MS medium supplemented with 30 g L^{-1} sucrose and 0.2% AC

NAA	Percentage of root/[1] (%)	Average number of roots per shoot/[1]
0	50	1.25
0.5	50	0.50
1	25	0.25
2	50	3.75

1/Cultured on MS medium supplemented with 30 g L^{-1} sucrose after 40 days.

Discussion

Arachis glabrata cultivar Florigraze nodal segments, which are passed through the sterilization method, excised from natural and maintain cultivation by using different concentrations of $HgCl_2$ and times. The result found that 0.2% $HgCl_2$ at 15 min, the explants had the highest survival rate was 73.33% and its can be grown up completely. In contrast, to increase the time for sterilization at 20 min, the explant had no contamination and the nodal segments were brown, can not grow and died, the explants were decreased growth rate (Rey and Mroginski, 2003). Whereas, 0.18% $HgCl_2$ showed the segments grew completely and the explants showed strong green. Compton and Koch. (2001) and Jimenez *et al*. (2006) found that has contamination of endophytes in a vascular bundle of *A. glabrata* cultivar Florigraze. Therefore, added to the antibiotic, cefotaxime, and PPM helped eliminate microorganisms. Earlier reports of *A. glabrata* reported that the unsuccessful used sodium hypochlorite (NaOCl) in sterilizing for shoot tip and nodal segments, caused microbial contamination (bacteria or fungi) maximum to 100% (Dolce *et al*., 2017) as same as *A. pintoi* (Rey and Mroginski, 2003). The selection from natural or greenhouse explants, concentrations of chemicals and different times, are very necessary of sterilization (Cassells, 2001). For prediction, reducing chemical concentration and duration of time in the disinfecting process would have an affected on the in vitro of Arachis species. However, using PPM may increase the concentration of substances which could help to reduce the contamination of microbial without affecting the growth of plants.

The nodal segments of *A. glabrata* cultivar Florigraze cultured *in vitro* on MS medium supplemented with 5 mg L^{-1} of *m*T had the maximum of growth rate of 66.67% and the highest was 18.43 mm. When compared with cultured in other types of cytokinin growth regulators (BAP Kn or TDZ), found that the growth of shoots in BAP and *m*T was well similar. However, the shoot segments of Florigraze cultured in mT were easier to induce roots. (Werbrouck *et al*., 1996; Holub *et al*., 1998). Secondly, it was found that the cultured parts in Kn with 1 mg L^{-1} of Kn had the growth rate of the shoots as high as 60% and the average length was 8.89 mm. Nevertheless, the Florigraze's part cultured in MS medium which supplemented with TDZ found that the percentage of growth of the shoots was decreased and became callus when cultured in low

concentration of TDZ. Meanwhile, the percentage of shoots growth was increased when cultured in TDZ with a higher concentration level. Therefore, this research requires a high concentration of TDZ to induce the development of the shoot. This was consistent with previous research which showed the growth of *A.glabrata* leaflets with a percentage of 58.35% when cultured in supplemented with 6 mg L^{-1} of TDZ. (Dolce *et al*., 2017). On the contrary, there has been no previous research that successfully in propagation by using the fragments of *A. glabrata* cultivar florigraze.

After the *in vitro* shoots were induced root shoots by culturing in MS medium supplemented containing 2 mg L^{-1} of NAA with 0.2% AC, it can induce root growth up to 50% when cultured within 8-12 days. Consistent with other research found that NAA can induce roots in *A. correntina* (Rey *et al*., 2000; Rey *et al*., 2009), *A. hypogaea* (Mroginski *et al.*, 2004) and *A. pintoi* (Akasaka *et al.*, 2000). Although, Dolce *et al*. (2017) found that root induction of *A. glabrata* was cultured only on media added 0.2% AC without any plant growth regulators after cultured for 10-15 days. In the same way, our research found that when cultured in 0.2% AC, roots will occur after 40 days. However, root induction cultured in MS formula supplemented with 2 mg L^{-1} of NAA had the longest root than other concentrations. This is because of a large amount of porosity of activated charcoal and it absorbed various substances released from plants (Thomas, 2008). For acclimatization of *A. glabrata* cultivar Florigraze was successful because of the stability of cultivation area and humidity in the root system of plants.

Acknowledgements

The rhizome peanut *A. glabrata* cultivar Florigraze was provided by Feed and Forage Analysis Section. Animal Nautrition Division. Department of Livestock Development. Pathumthani Province. Thailand. The author would like to offer particular thanks to King Mongkut's Institute of Technology Ladkrabang responsible for scholarship to carry out our research program References.

References

Akasaka, Y. A., Daimon, H. B. and Mii, M. (2000). Improved plant regeneration from cultured leaf segments in peanut (*Arachis hypogaea* L.) by limited exposure to thidiazuron. Plant science, 156:169-175.

Angelici, C. M. L. C. D., Gimenes, M. A., Hoshino, A. A., Lopes, C. R., Palmieri, D. A., Valls, J. F. M. and Nobile, P. M. (2008). Genetic diversity in section Rhizomatosae of the genus *Arachis* (Fabaceae) based on microsatellite markers. Genetics and Molecular Biology, 31:79-88.

Cassells, A. C. (2001). Contamination and its impact in tissue culture. *In Vitro* Culture and Horticultural Breeding. Acta Horticulturae, 353-359.

Compton, M. E. and Koch, J. M. (2001). Influence of plant preservative mixture (PPM) TM on adventitious organogenesis in melon, petunia, and tobacco. *In Vitro* Cellular and Developmental Biology-Plant, 37:259-261.

Dolce, N. R., Faloci, M. M. and Gonzalez, A. M. (2017). *In vitro* plant regeneration and cryopreservation of *Arachis glabrata* (Fabaceae) using leaflet explants. *In Vitro* Cellular and Developmental Biology–Plant, 54:133-144.

Dunbar, K. B., Pittman, R. N. and Morris, J. B. (1993). *In vitro* culture of embryonic axes from *Arachis* species for germplasm recovery. Journal of Seed Technology, 17:1-7.

Fernandez, A. and Krapovickas, A. (1994). Cromosomas y evolución en *Arachis* (Leguminosae). Bonplandia, 8:187-220.

French, E. C., Prine, G. M., Ocumpaugh, W. R. and Rice, R. W. (1994). Regional experience with forage *Arachis* in the United States. In: Kerridge PC, Hardy B (eds) Biology and agronomy of forage Arachis. CIAT, Cali, pp. 167-184.

Holub, J., Hanus, J., Hanke, D. E. and Strnad, M. (1998). Biological activity of cytokinins derived from Ortho- and *Meta*-Hydroxybenzyladenine. Plant Growth regulation, 26:109-115.

Jimenez, V. M., Castillo, J., Tavares, E., Guevara, E. and Montiel, M. (2006). *In vitro* propagation of the neotropical giant bamboo, *Guadua angustifolia* Kunth, through axillary shoot proliferation. Plant Cell, Tissue and Organ Culture, 86:389-395.

Krapovickas, A. and Gregory, W. C. (1994). Taxonomía del género *Arachis* (*Leguminosae*). Bonplandia Revista Del Instituto De Botanica Del Nordeste, Corrientes, pp. 1-186.

Li, Z., Jarret, R. L., Pittman, R. N. and Demsk, J. W. (1994). Shoot organogenesis from cultured seed explants of peanut (*Arachis hypogaea* L.) using thidiazuron. *In Vitro* Cellular & Developmental Biology – Plant, 30:187-19.

Mroginski, E., Rey, H. Y. and Gonzalez, A. M. (2004). Thidiazuron promotes *in vitro* plant regeneration of *Arachis correntina* (Leguminosae) via organogenesis. Journal of Plant Growth Regulation, 23:129-134.

Murashige, T. and Skoog, F. (1962). A Revised medium for growth and bioassays with Tobacco tissue. Physiologia Plantarum, 15:473-797.

Pacheco, G., Gagliardi, R. F., Valls, J. F. M. and Mansur, E. (2009). Micropropagation and *in vitro* conservation of wild *Arachis* species. Plant Cell, Tissue and Organ Culture, 99:239-249.

Penaloza, A. and Valls, JFM. (2005). Chromosome number and satellited chromosome morphology of eleven species of *Arachis* (Leguminosae). Bonplandia, 14:65-72.

Rey, H. and Mroginski, L. (2009). Cryopreservation of *Arachis pintoi* (Leguminosae) seeds. Seed Science and Technology, 37:202-205.

Rey, H. Y. and Mroginski, L. A. (2003). Regeneration of plants from apical meristem tips and nodal segments of *Arachis pintoi*. Peanut Peanut Science, 30:75-79.

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

Rouse, R. E., Miavitz, E. M. and Roka, F. M. (2004). Guide to using rhizomal perennial peanut in the urban landscape. Guide to Using Rhizomal Perennial Peanut in the Urban Landscape, pp. 1-8.

Rouse, R. E. and Mullahey, J. J. (1997). Perennial peanut ground cover in citrus orchard row middles and discussion of potential environmental benefits. Proceedings of the Florida State Horticultural Society, 110:79-82.

Thomas, T. D. (2008). The role of activated charcoal in plant tissue culture. Biotechnology Advances, 26:618-631.

Valls, J. F. M. and Simpson, C. E. (2005). New species of *Arachis* L. (Leguminosae) from Brazil, Paraguay and Bolivia. Bonplandia Revista Del Instituto De Botanica Del Nordeste, 14:35-64.

Werbrouck, S. P. O., Strnad, M., Onckelen, H. A. V. and Debergh, P. C. (1996). *Meta*-topolin, an alternative to benzyladenine in tissue culture. An International Journal for Plant Biology, 98:291-297.

(Received: 25 August 2019, accepted: 30 August 2020)