Journal of Agricultural Technology 2015 Vol. 11(8): 2481-2488
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Callus Induction and Cell Suspension Cultures of Rhizome Peanut (*Arachis glabrata*) Cultivars: Arbrook

Anurug Poeaim[1*], Supattra Poeaim[1], Pradit Pongtongkam[2] and Jantakarn Arananant [3]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang , Bangkok. 10520. Thailand.
[2] Thepstri Rajabhat University, 321 Naraimaharat Road, Tambon Talaychubsorn, Amphur Muang, Lopburi, 15000, Thailand**.**
[3]Feed and Forage Analysis Section. Animal Nautrition Division. Department of Livestock Development. Pathumthani Province. 12000. Thailand.

Poeaim A., Poeaim S., Pongtongkam P. and Arananant J. (2015). Callus Induction and Cell Suspension Cultures of Rhizome Peanut (Arachis glabrata) Cultivars: Arbrook. Journal of Agricultural Technology 11(8):2481-2488.

Callus induction from leaflet explants of rhizome peanut (*Arachis glabrata*) cultivar: Arbrook were cultured on solid MS media (Murashige and Skoog 1962) supplemented with plant growth regulator 0.5, 1, 3 and 5 mg/l 2,4-D, 30 g/l sucrose and 2.6 g/l phytagel. The leaflet explants were cultured in the dark condition for 42 days. The results concluded that, The highest frequency of callus induction was formed leaflet at 62.5 percent at 3 mg/l 2,4-D. Callus amount of 0.15 g fresh weight were cultured in liquid MS medium supplemented with 3 mg/l 2,4-D and 30 g/l sucrose, for 30 days, with shaking speed at 120 rpm. Cell growth was determined by measuring the fresh weight and dry weight of the cell. The results showed that fresh weight and dry weight of cell suspension with the best growth for 24 days at 0.4396 g/10 and 0.0324 g/10 ml respectively. Cell suspension has grown rapidly during the period of 6-24 days. Viability of suspension cells were determined by the method of fluorescein diacetate for 30 days. Suspension cultures were life of cells green fluorescence for living cells. This work has developed an optimized protocol for plant breeding.

Keywords: rhizome peanut, callus, suspension, *Arachis glabrata,* Arbrook

Introduction

Arachis glabrata, commonly called ornamental peanut grass and perennial peanut, is a native forage crop of Brazil that grows in the United States for erosion control and as an ornamental, perennial groundcover. A number of recent reports describe somatic embryogenesis in peanut using a variety of different explants, including leaves (Baker and Wetzstein., 1992; Chengalrayan *et al*., 1994) mature embryo derived leaflets (Baker *et al*.,1995)

***Corresponding auther:** Anurug Poeaim; **Email:** anurug@hotmail.com

seedling-derived leaflets (Muruganantham *et al.,* 2010) cotyledons with or without embryos, epicotyls, leaflets (Cucco and Jaume., 2000) and mature epicotyl explants (Little and Magbanua., 2000). Some have great forage value. Therefore, interest in tissue culture of legume has increased considerably. Plant regeneration is one of basic requirements for employing cell, tissue and organ culture in plant breeding. This protocol opens new biotechnological strategies to transfer economically important genes to this important crop species.

The objective of this study was therefore to optimize the concentration of 2,4-D for callus induction from leaflet and growth curve of suspension culture of rhizome peanut *(Arachis glabrata*) cultivar: Arbrook

Materials and methods

Callus induction

Leaflet explants of rhizome peanut (*Arachis glabrata*) cultivar: Arbrook used in regeneration were obtained from Feed and Forage Analysis Section. Animal Nutrition Division. Department of Livestock Development. Pathumthani Province. The leaflets were washed by running of water for 5 min, followed by 20% sodium hypochlorite and 2-3 drops of tween20 for 15 min. The leaflets were rinsed thoroughly 3 times with sterile water and dried on sterile filter paper. Then, the leaflets were cut size 0.5x0.5 mm and culture on MS medium (Murashige and Skoog 1962) supplemented with various concentrations 0.5, 1, 3 and 5 mg/l 2,4-D, 30 g/l sucrose, pH adjusted to 5.8 before adding 2.6 g/l phytagel prior to autoclaving at 121ºC for 20 minutes and cultured in the dark at temperature of 25±2°C. Subculturing was done every 4 weeks. The percentage of responsive explants and the number of callus per explant were evaluated for each accession.

Suspension culture

Callus from leaflets (fresh weight at 0.15 mg) were transferred to bottle containing 10 ml of liquid MS medium supplemented with the best of 2,4-D, 30 g/l sucrose. They were placed on a rotary shaker (120 rpm) and incubated at 25±2 °C under white fluorescent light (16-h photoperiod). Study on growth curve at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days for fresh and dry weight by Poeaim (2550).

Statistical evaluation

Data on callus induction were analysed using the SPSS 17. Statistical package statistically significant analysis of variance (ANOVA) was further analysed using least significant difference Turkey's Test at P value of 0.05.

Results and Discussion

The leaflets were cultured on different concentrations of 2, 4- D (0.5, 1, 3 and 5 mg/l), 30 g/l sucrose, pH adjusted to 5.8 before adding 2.6 g/l phytagel. The leaflets tissues were observed in most of the media within 28 days. Callus developed from side of the margins of the explants cultured on media of 2,4-D. Number of callus induction and percentage of callus induction were showed in (Table 1, Fig.1 A and 1 B and Fig 2). Within 42 days of inoculation in media supplement with 3 mg/l 2,4-D, leaflet explants formed 62.5% callus. The percentage of callus induction at 5 mg/l 2,4-D and 1 mg/l 2,4-D was 30 and 12.5 respectively. Morphological variation of the induced callus was observed and they were categorized as callus. Fresh weight callus were could be induced in MS medium with 3 mg/l 2,4-D for 30 days. (Table 2). The results showed that MS medium with 3 mg/l 2,4-D could induce the highest percentage of callus per explant. Highest frequency of 87% was observed for embryogenic callus induction at the concentration of 3 mg/l 2,4-D (Iqbal *et al*., 2001). Explants treated with higher auxin concentrations (5 mg/l) 2,4-D, turned brown after 42 days. Ali *et al*. (2011) report that the low concentrations of 2,4-D induced normal embryogenesis, while the use of higher concentrations of 2,4-D (6.0-15.0 mg/l) not only decreased the number of embryos but delayed the process of their formation.

Callus from leaflets (fresh weight at 0.15 mg) were transferred to bottle containing 10 ml of liquid MS medium supplemented with 3 mg/l of 2,4-D, 30 g/l sucrose (Fig 4 A and Fig 4 B). The growth curve indicated four typical phase (lag phase, log phase, stationary phases and death phase). The lag phage of growth curve found from initial day to end of 9 days of culture where amount of cell was increased slowly in the suspension culture. In log phase, growth the cell growth drastically increased from 9 to end of 24 days. The stationary phase from 24 to end of 27 days. The cell suspension with the best growth for 24 days to be fresh weight (0.4396 g) and dry weight (0.0324 g) per 10 ml. The last phase was death phase in after 24-30 days (Table 2, Fig 3 A and Fig 3 B). Duration of log phage is important for the high production of cell and biosynthesis of cell compounds. These suspension culture have multi-celled proembryogenic structure of aggregate (Fig 4 C and Fig 4 D) and still viable

after fluorescein diacitate staining. After 30 days of culture, cell suspension observed under light microscope (Fig 4 E). Viability of suspension cells were determined by the method of fluorescein diacetate for 30 days. Suspension cell were life of cells green fluorescence for living cells (Fig 4 F). Steward *et al*. (1999) found that the esterase activity level per viable cell constituted of numerous enzymes depends on cell viability but is independent of cellular metabolism.

Table 1 Effect concentration of 2,4-D on callus induction from leaflet explants of rhizome peanut *(Arachis glabrata*) cultivar: Arbrook for 42 days.

Concentration of 2,4-D (mg/l)	Number of leaflet inoculation	Callus induction for 42 days	% callus induction for 42 days
0.5	20	0^{c}	0
1	20	2.5^{c}	12.5
3	20	12.5^{a}	62.5
5	20	6^{b}	30

Means followed by different characters showed the significant different at P ≤0.05 according to HSD Turkey's test.

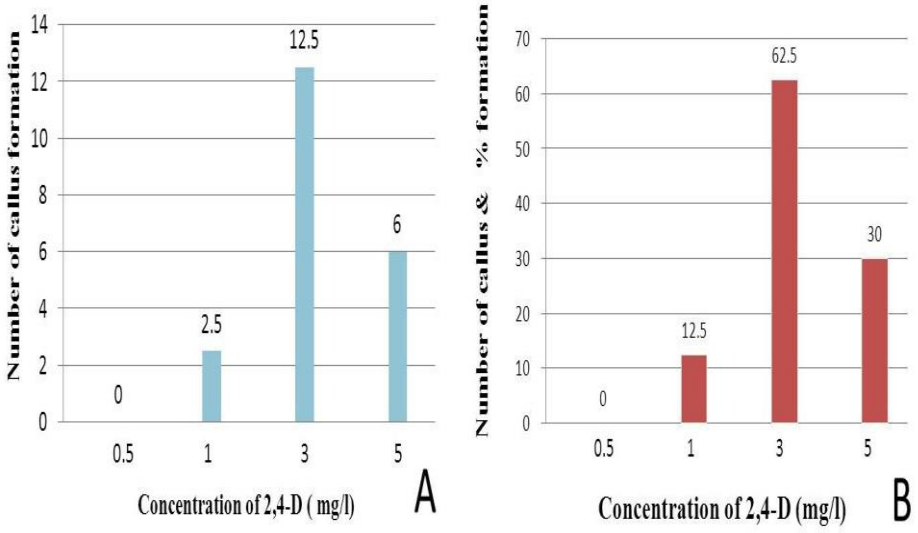

Figure 1 Number of callus induction and percentage of leaflet explants of rhizome peanut (*Arachis glabrata*) cultivar: Arbrook for 42 days.

A. Number of callus induction on concentration of 0.5, 1, 3, 5 mg/l 2,4-D

B. Percentage of callus induction on concentration of 0.5, 1, 3, 5 mg/l 2,4-D

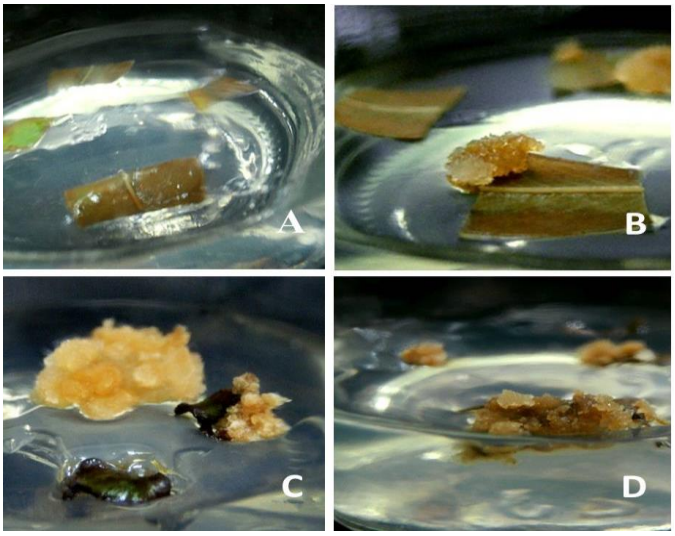

Figure 2 Leaflets were cultured on induction MS medium supplement with different concentration of 2,4-D for 42 days
A. 0.5 mg/l 2,4-D B. 1 mg/l 2,4-D C. 3 mg/l 2,4-D D. 5 mg/l 2,4-D

Table 2 The measurement of fresh and dry weight for suspension of rhizome peanut (*Arachis glabrata*) cultivar: Arbrook in liquid MS medium supplemented with 3 mg/l 2,4-D for 30 days.

Days	**Fresh weight (g/10 ml)**	**Dry weight (g/10 ml)**
0	0.2836	0.0057
3	0.3038	0.0068
6	0.3103	0.0084
9	0.3350	0.0104
12	0.3281	0.0164
15	0.3532	0.0199
18	0.3825	0.0202
21	0.3885	0.0248
24	0.4396	0.0324
27	0.3396	0.0269
30	0.3287	0.0260

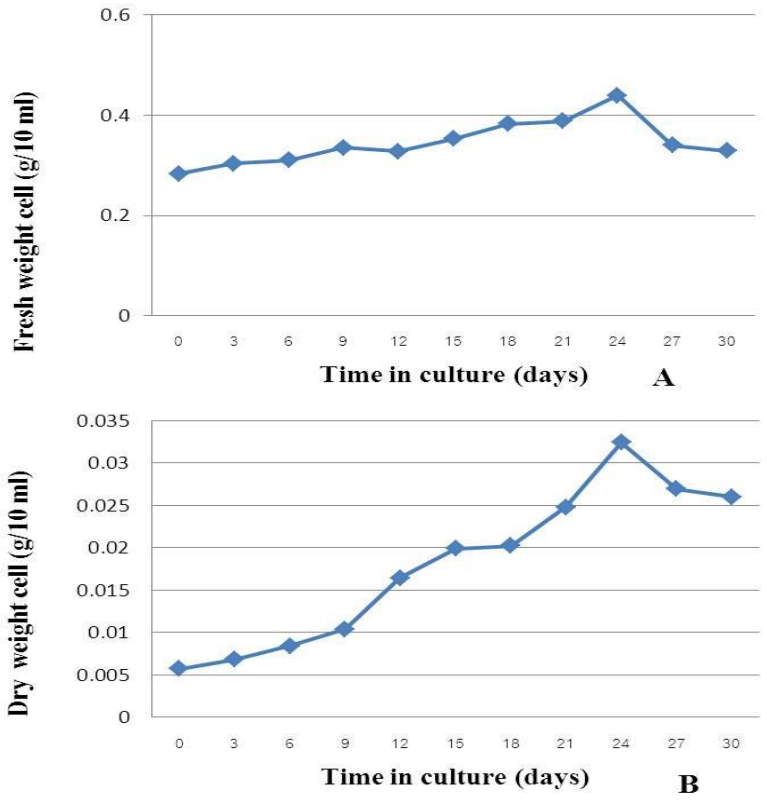

Figure 3 Growth curve of suspension culture cell of rhizome peanut *(Arachis glabrata*) cultivar: Arbrook culture in liquid MS medium supplemented with 3 mg/l 2,4-D for 30 days.
A. Fresh weight B. Dry weight

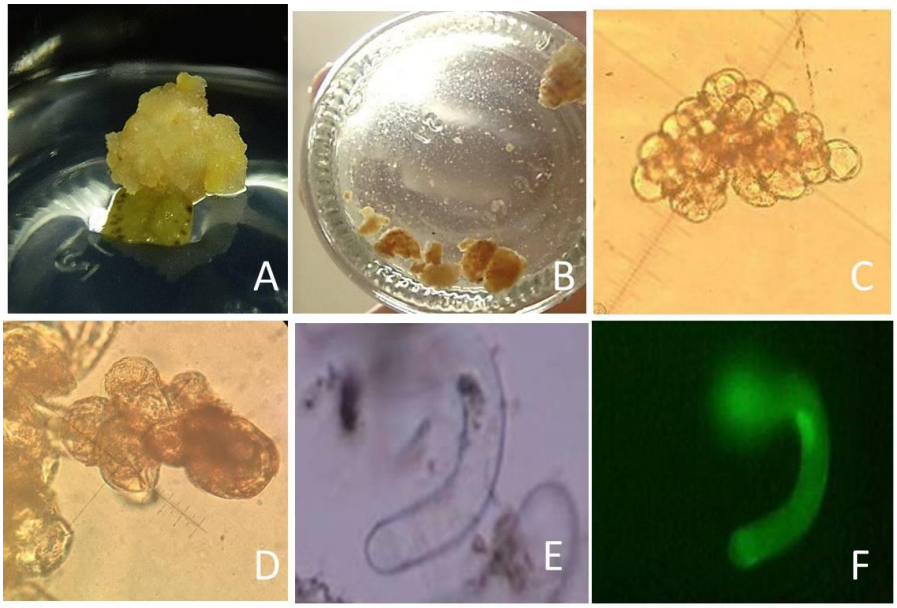

Figure 4 A. Callus were culture on MS medium supplemented with 3 mg/l 2,4-D.
B. Suspension cell were used to inoculate in 10 ml liquid MS medium.
C-D. Cells suspension scanning from the bright field microscope has multi-celled proembryogenic structure of aggregate for 7 days.
E. Cells suspension scanning from the bright field microscope for 30 days.
F. Viability of suspension cells was determined by fluorescein diacetate at 30 days.

Conclusion

Callus induction from leaflet explants of rhizome peanut (*Arachis glabrata*) cultivar: Arbrook. The best highest callus at 62.5 % were induced on MS medium containing 3 mg/l 2,4-D. Cell growth was determined by measuring the dry weight of cell suspension with the best growth for 24 days to be 0.0324 g per 10 ml. Cell suspension has grown rapidly during the period of 6-24 days. Suspension cultures were life of cells green fluorescence for living cells.

Acknowledgement

This research was supported by grant s from Research Fund of King Mongkut's Institute of Technology Ladkrabang.

The rhizome peanut (*Arachis glabrata*) cultivar: Arbrook was provided by Feed and Forage Analysis Section. Animal Nutrition Division. Department of Livestock Development. Pathumthani Province.

References

Ali, D. J., Sharma, M. M. and Batra A. (2011). Somatic embryogenesis and plant regeneration of *Arachis hypogaea* L. (Leguminosae) through mature embryo derived leaflet culture. International Journal of Biotechnology 4 (8) 637-643.

Baker, C.M. and Wetzstein, H. (1992). Somatic embryogenesis and plant regeneration from leaflets of peanut, *Arachis hypogaea*. Plant Cell Rep 11: 71-75.

Baker, C.M., Durham, R.E., Austin B. A., Parrott, W.A. and Wetzstein, H. (1995). High frequency somatic embryogenesis in peanut (*Arachis hypogaea* L.) using mature, dry seed. Plant Cell Rep 15: 38- 42.

Chengalarayan, K., Sathaye, S.S. and Hazra, S. (1994). Somatic embryo derived leaflets of peanut (*Arachis hypogaea* L.). Plant Cell Rep 13: 578-581,

Cucco, M.F. and Jaume, D.R. (2000). Protocol for regeneration *in vitro* of *Arachis hypogaea* L. J. Biotechnology 3:154-160.

Gohari, A.A. and Niyaki, A.N. (2010). Effects of iron and nitrogen fertilizers on yield and yield components of peanut (*Arachis hypogaea* L.) in Astaneh Ashrafiyeh, Iran. American-Eurasian. J Agric Environ Sci 9: 256–262.

Iqbal, M.M., Nazir, F., Iqbal, J., Tehrim, S. and Zafar, Y. (2011). *In vitro* micropropagation of Peanut. Agric. Biol 13: 811–814.

Little, E.L. and Magbanua, Z.V. (2000). Protocol for repetitive somatic embryogenesis from mature peanut epicotyls. Plant Cell Rep 19: 351-357.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497.

Muruganantham, M., Amutha, S. and Ganapathi A. (2010). Somatic embryo productions by liquid shake culture of embryogenic calluses in *Vigna mungo* (L.) Hepper. *In Vitro* Cell Dev Biol Plant 46:34–40.

Poeaim, A. (2550). Plant Biotechnology. VJ printing. Bangkok.159 p.

Steward, N., Martin, R., Engasser, J, M. and Goergen, J, L. (1999). A new methodology for plant cell viability assessment using intracellular esterase activity. Plant Cell Rep19: 171-176.