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POTENTIAL OF PRETREATED EXPLANTS OF PEANUT (*Arachıs hypogeae* LINN.) TO MİCROPROPAGATION UNDER *IN VITRO* CONDITIONS

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Peanut (*Arachis hypogeae* Linn.). zygotic embryos (ZE), plumular apices (PA) and embryonic Axis (EA) of cv. NC-7 were pretreated for 15 days on Murashige and Skoog (MS) medium enriched with 25 mg.L^{-1} 6-Benzylaminopurine (BA). Pretreated explants were then post treated on semi solid agar gelled MS medium possesses BA concentrations ranged 0.25 to 3.0 mg.L^{-1} for a period of 8 weeks. Callus and shoot induction was registered on all explants irrespective of BA pre-and post treatments. The highest shoot counts per explant ranged 2.67-4.43 (ZE), 2.33-4.11 (PA) and 1.87-3.33 (EA). Average shoot length ranged 1.75-3.62 cm (ZE), 1.45-3.70 cm (PA) and 2.10-2.60 cm (EA). Successful rooting (100%) of shoots regenerated were achieved on agar gelled MS medium provided with Indole-3-butyric acid (IBA) at the rate of 1.0 mg.L^{-1}. Successful adaptation of rooted plantlets were done by transferring them to pots filled with vermiculite. This study highlighted the high potential of micropropagation of three different explants in peanut biotechnology.
Keywords: In vitro, Embryonic axis, peanut, pretreatment**,** plumular apices, zygotic embryos.

INTRODUCTION

Peanut (*Arachis hypogeae* Linn) is recognized and economically significant legume of subtropical and warm regions (Venkatachalam and Jayabalan, 1997; Hasan *et al.,* 2013). It is used as an edible oil seed crop (Tuberoso *et al.,* 2007) alongwith used in making of highly important industrial products and biological compounds (Sanders *et al.,* 2000; Morris *et al.,* 2004; Blomhoff *et al.,* 2008). Peanut is playing an important role against human malnutrition due to rich source of protein, amino acids (Pelto and Armar-Klemesu, 2015) carbohydtrates and lipids (Settalıri *et al.,* 2012) which are essential for normal metabolism (Seal *et al.,* 2007) of human body. The peanut consumption is variable among countries and regions which include raw or roasted peanuts or peanut by-products (Campos-Mondragón et al., 2009). A large amount of peanut in Turkey is also used as snack like USA, which accounts $\leq$ 25% of peanut consumption (He *et al.,* 2005) in some cases. The plant plays important role in agriculture due to its use in crop rotation, cover crop, intercrop with other economic crops and as forage legume crops for making hay (Hill, 2002; Langat *et al.,* 2006; Balkcom *et al.,* 2007).

Developement of new peanut cultivars against biotic or abiotic stresses are of immense importance for breeders (Venkatachalam and Kavipriya, 2012). Biotechnological tools like tissue cultre and moleculer biology provides an opportunity to peanut breeders to breed the plants in a short time (Rey *et al.,* 2000).

Peanut like other legumes is considered as recalcitrant plant due to its response under in vitro conditions (Heatley and Smith, 1996) which may lead to low regeneration frequency and non-repeatable results. Although, researchers communicated researches which claims the establishment of protocols for peanut. But majority of these protocols are either very complex or offer low regeneration frequency (Matand and Prakash, 2007; Tiwari and Tuli, 2009). The above mentioned problems demand new regeneration protocol for the application of biotechnological tools for improving and/or developing elite cultivars of peanut. The present research was accomplished to compare and induce *in vitro* high shoot regeneration after pre and post treatments of three different peanut explants.

MATERIALS AND METHODS

Peanut seeds (NC-7 cv) were secured from Western Mediterranean Agricultural Research Institute (BATEM) Antalya, Turkey. The shells were removed by hand before sterilization and seeds were subjected to concentrated commercial bleach (5 % NaOCl -Ace, Turkey) for a time span of 30 min on magnetic stirrer. After this, rinsing of seeds for 3 × 5 min with distilled sterilized water was performed to minimize or eliminating the NaOCl traces. After the completion of sterilization process, seeds were carefully separated into two parts (Figure 1a) and mature ZE were detached and pretreatment with BA (25 mg.L^{-1}) for a period of 15 days on agar gelled MS (Murashige and Skoog, 1962) medium was performed.

Three contrasting explants; (i) pretreated mature ZE (Fig 1b), (ii) pretreated PA (Fig Fig 1b) and (iii) pretreated EA explants (Fig 1b) were used for regeneration *in vitro*. The MS culture medium contained sucrose (3% w/v) and agar (0.65% w/v) gelled MS regeneration medium. The post treatment medium was comprised of diverse BA concentrations (0.25, 0.50, 1.0, 2.0 and 3.0 $mg.L^{-1}$). The data regarding callus induction (%), shoot regeneration (%), shoot counts and shoot length (cm) were tabulated after 8 weeks of posttreatment. Subsequently, shoots regenerated *in vitro* were detached from explants aseptically and rooted by using 1.0 $mg.L^{-1}$ IBA for a culture time of 6 weeks. Plastic bags filled with homogenized humid vermiculite were used for acclimatization of rooted plants in growth room (Day et al 2017).

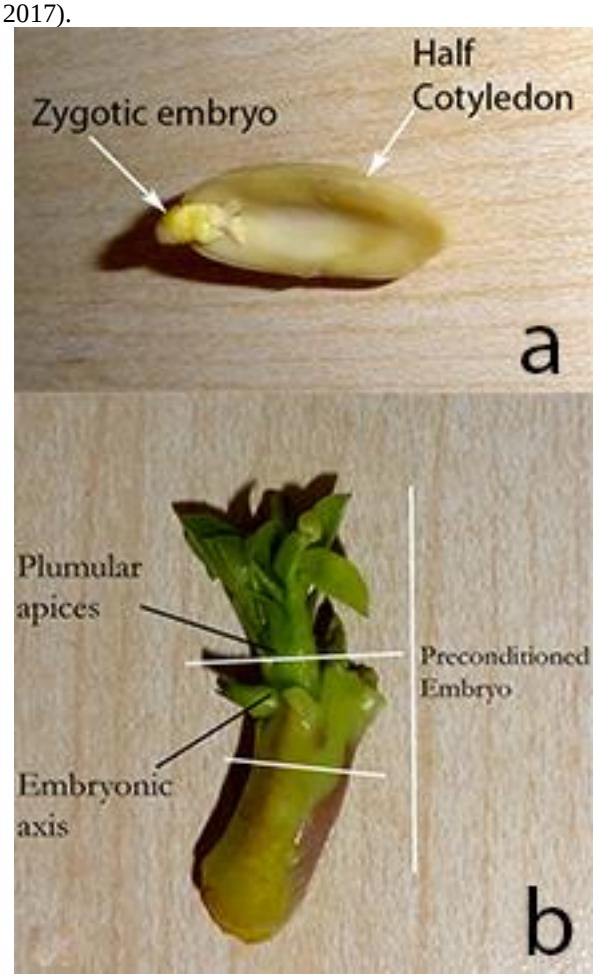

Figure 1. Explants used for in vitro regeneration (a) zygotic embryo (b) pretreated zygotic embryo with plumular apices and embryonic axis.

The basal medium used for both regeneration and rooting experiments was prepared by using MS medium, sucrose (30 g/L) and agar (6.5 g/L). The pH was automated to 5.6 - 5.8 ahead to autoclaving (104 kPa 120 °C temperature, for 20 min). White Light Emitting diodes (LEDs) having 16 h light photoperiod were used for incubation of all cultures provided with temperature of 24±2°C. Three replicates having 8 explants were used for shoot regeneration.

The experimental data tabulated was tested to Statistical Analysis by using One Way ANOVA (analysis of variance) by applying SPSS21 for Windows (SPSS Inc. Chicago, IL, USA). For comparison of means, Least significance Difference (LSD) or Duncan's Multiple Range Test (DMRT) at *p0.01* level of significance was used. The data presented in percentages (%) were arcsine (√X) transformed (Snedecor and Cocharan, 1967) before subjecting them to ANOVA and DMRT

RESULTS

The results of the study shows potential of three different pre-and posttreated explants (ZE, PA and EA) for *in vitro* regeneration. Availability of BA concentrations had clear impact ($p>0.01$) on shoot counts and shoot length but no significant impact was noted on callus induction (%) and shoot regeneration (%). Callus initiaton started within 10 days of culture on the basal part of EA and PA explants. Callus induction on ZE explants started from the radicle part that was evident after 3 weeks of post treatment. All explants induced shoots within 8-10 d. Both callus and shoot regeneration was documented 100% after 8 weeks of post treatment. Therefore, the data pertaining to these were not subjected to statistical analysis. However, the data pertaining to shoot counts and the shoot length were tested for statistical analysis.

Table 1. Efficacy of different BA concentrations on in vitro regeneration Potential of peanut (*Arachis hypogeae* LINN.)

Explant	Callogenesis (%)	Shoot regeneration (%)	Shoot counts	Shoot length (cm)
ZE	100ns	100ns	3.46a	2.44b
PA	100	100	2.99b	2.75a
EA	100	100	2.66b	2.38b

Means followed by different letters in single columns are statistically significantly (LDS test; $P<0.01$)

Results revealed the clear role of pretreated explants on in vitro regeneration of peanut. Explants tested in this study resulted in 100% callus induction and shoot regeneration (%). Shoot counts and shoot length were statistically significant from different explants ($p>0.01$). Shoot length and shoot counts ranged 2.66-3.46 and 2.38-2.75 cm respectively. The highest shoot counts (3.46) were achieved from ZE explants that was followed by 2.99 for PA and 2.66 for EA explants. Contrarily, longest shoots were achieved

from PA (2.75 cm) explants followed by 2.44 cm for ZE and 2.38 cm for EA explants (Table 1).

The results referring to the shoot counts openly indicated the carry over effects of BA pretreatment and post treatment concentrations on the regeneration potential of the explants. Provision of BA concentrations in the posttreatment medium regulated the in vitro regeneration of peanut. Although callus induction and shoot regeneration was recorded 100%, shoot counts and shoot length were statistically significant ($p>0.01$). Shoot counts and shoot length in response to different BA concentrations ranged 2.44-3.66 and 1.94-2.93 cm respectively. Maximum shoot counts (3.66) and shoot length (2.93 cm) were attained from medium with 0.50 mg/l BA. Contrarily, lowest shoot count (2.44) and shorter shoots (1.94 cm) were tested on medium having 3.0 $mg.L^{-1}$ BA (Table 2). Provision of higher concentration of BA above 1.0 $mg.L^{-1}$ was detrimental and resulted in reduction in shoot counts and shoot numbers.

Table 2. Efficacy of different BA concentrations on in vitro regeneration Potential of peanut (*Arachis hypogeae* LINN.)

BA	Callogenesis (%)	Shoot regeneration (%)	Shoot counts	Shoot length (cm)
0.25	100ns	100ns	2.76b	2.85a
0.50	100	100	3.66a	2.93a
1.00	100	100	3.48a	2.52b
2.00	100	100	2.83b	2.39b
3.00	100	100	2.44b	1.94c

Means followed by different letters in single columns are statistically sigificantl (DMRT test; P<0.01)

Interactive effect of explant × BA was statistically insignificant for callus induction and shoot regeneration (%) due to 100%. However, shoot counts and shoot length showed variable effects to explant × BA combination. All explants required different concentration of BA for regenerating highest shoot counts per explants and shoot length. The shoot counts ranged 2.67-4.43 (ZE - Fig 2a,b,c), 2.33-4.11 (PA - Fig 3a,b) and 1.87-3.33 (EA - Fig 4a,b) explants. Whereas, shoot length exhibited the clear impact of BA and type of explant but in general, longer shoots were achieved irrespective of pretreatment with 25 mg/l BA followed by post treatment with BA. Average shoot length ranged 1.75-3.62 cm (ZE), 1.72-3.70 cm (PA) and 2.11-2.60 cm (EA) on post treatment medium (Table 3). The ZE had the longest shoots of 3.62 cm on post treatment medium (0.50 $mg.L^{-1}$ BA). Whereas, the longest shoots with average length of from 3.70 cm or 2.60 cm were achieved from PA and EA explant respectively when post treated with BA at the rate of 0.25 $mg.L^{-1}$ (Table 2).

The highest shoots counts from ZE were documented as 4.43 from 0.50 $mg.L^{-1}$ BA and 4.67 form 1.0 $mg.L^{-1}$ BA, which were statistically similar (Table 3). Similarly, availability of 0.50 $mg.L^{-1}$ BA in the culture medium also generated longer shoots of 3.62 cm. PA explants regenerated highest shoots count of 4.11 (0.50 $mg.L^{-1}$ BA) but longer shoots were attained from 0.25 $mg.L^{-1}$ BA. Whereas, EA explants regenerated highest shoots counts of 3.33 when post treated on 1.0 $mg.L^{-1}$ or 2.0 $mg.L^{-1}$ BA (Table 1) and longest shoots (2.60 cm) were attained from medium having 0.25 $mg.L^{-1}$ BA (Table 3).

Table 3. Efficacy of explant × BA on in vitro regeneration Potential of peanut (*Arachis hypogeae* LINN.)

Explants	BA (mg L^{-1})	Callus induction (%)	Shoot regeneration (%)	Shoot counts	Shoot length (cm)
ZE	0.25	100ns	100ns	2.87cde	2.25cde
	0.50	100	100	4.43a	3.62a
	1.00	100	100	4.67a	2.45cde
	2.00	100	100	2.67c-f	2.12def
	3.00	100	100	2.67c-f	1.75f
PA	0.25	100ns	100ns	3.55bc	3.70a
	0.50	100	100	4.11ab	3.05b
	1.00	100	100	2.44def	2.72bc
	2.00	100	100	2.50def	2.58cde
	3.00	100	100	2.33ef	1.72f
EA	0.25	100ns	100ns	1.87f	2.60cd
	0.50	100	100	2.43def	2.11ef
	1.00	100	100	3.33bcd	2.40cde
	2.00	100	100	3.33bcd	2.46cde
	3.00	100	100	2.33ef	2.35cde

Means followed by different letters in single columns are statistically sigificantly (DMRT test; P<0.01)

Figure 2. *In vitro* shoot regeneration from different pretreated explants of peanut cv NC-7 after 8 weeks of culture (a) zygotic embryos-ZE (b) plumular apices-PA, (c) embryonic axis-EA

After successful *in vitro* regeneration from all explant, the shoots were excised aseptically and inoculated on rooting medium fortified with IBA at the concentration of 1.0 $mg.L^{-1}$. Induction of adventitious roots started within 10 days (Fig 5a) and the rooting reached 100% in 6 weeks period. Thereafter, these plantlets (Fig 5b) were acclimatized in pots filled with vermiculite and these pots were covered with transparent polyethylene bags for 15 days. These pots were kept in the growth room provided with ambient conditions (24±1 ºC temperature and 16h light photoperiod). Polyethylene bags were removed after 2 weeks and left

opened for acclimatization in open environment where plants showed high survival frequency.

Figure 5. ***In vitro*** **rooting and adaptation of peanut (a) root initiation after 10 days (b) rooted plantlets (c) acclimatized plants**

DISCUSSION

In vitro regeneration response of peanut is generally low and variable which make this plant recalcitrant like other legumes dependent on various factors (Rani ve Padmaja, 2005). Among these factors, application of plant growth regulators and explants are important factor which regulate the *in vitro* regeneration. Cytokinins are generally selected as singly for inducing shoots or combined with auxins. The study offers an unusually new technique in tissue culture using pre and post treatment of explants to induce shoot regeneration that has been rarely reported by the researchers in few plant species only (Brar *et al.,* 1999; Van Le *et al.,* 2002; Madhulatha *et al.,* 2004; Aasim *et al.,* 2013; Sağlam, 2012; Barpete *et al.,* 2014; Day *et al.,* 2017).

Pretreatment of ZE enables researchers to take PA and EA explants conveniently due to increased size of ZE (Aasim *et al.,* 2013). The other advantage of initial exposure of explants to higher cytokinins (pretreatement) is their rapid cell division which can be easily exploited to induce shoots from these dividing cells after post treatment. This pre and post treatment methodology has been successfully used for different legumes (Day *et al.,* 2017). Similarly, other studies have also emphasized the positive impacts of cytokinin pretreated explants for *Picea abies* (Von Arnold and Tillberg, 1987), banana (Madhulatha *et al.,* 2004), lentil (Aasim *et al.,* 2012) and dwarf chicklings (Saglam, 2012).

The results exhibited 100% callus and shoot regeneration frequency regardless of type of explant, pre or post treatments. Previous studies on pretreatment and post treatment using BA (Aasim *et al.,* 2009, 2013; Day *et al.,* 2017) or TDZ (Barpete *et al.,* 2014) have been reported. Low and varaible callus and shoot induction have also been reported for different explants pretreated with different cytokinins (Akasaka *et al.,* 2000; Sağlam 2012; Matand *et al.,* 2013).

In general, relatively low frequency of shoot regeneration is common for peanut in response to different protocols employed already (Sharma and Anjaiah, 2000; Shan *et al.,* 2009; Burns *et al.,* 2012; Venkatachalam and Kavipriya, 2012). Similarly, low shoot regeneration frequency in response to pretreatment of explants employed for peanut was also documented (Akasaka *et al.,* 2000; Matand *et al.,* 2013). These results are do not approve findings in this study which yielded 100% shoot regeneration. Previous studies on other legumes show agreement with these results, where shoot regeneration frequency of 100% after pretreatments was noted regardless of explant or culture treatment like cwopea (Aasim *et al.,* 2010), chickpea (Aasim *et al.,* 2013) or grasspea (Barpete *et al.,* 2014). These results suggested the positive bearings of pre-treatment of explants with BA.The results on shoot counts affirmed the significance of explant type and BA concentrations during post treatments. All explants generated maximum shoots on different concentration of post treated BA. Aasim *et al.* (2011) revealed the demand of different BA concentration for inducing maximum shoot counts on chickpea using mature ZE and EA explants (Aasim *et al.,* 2010). Application of higher concentration of BA in post treatment medium was harmful for explants for producing more number of shoots (Park et al. (2011).

Likewise shoots counts, variable impact in response to BA was noted on shoot length for all explants. ZE regenerated comparatively longer shoots (0.50 $mg.L^{-1}$ BA) in comparison to PA and EA explants, which required relatively low BA quantity of 0.25 $mg.L^{-1}$. The longest shoots originated at low concentration of post treated medium containing BA might be due to carry over effect of pretreatment dose of BA (Day *et al.,* 2017). Irrespective of pretreatment of explants with BA and post treatment, average longer shoots above 1.5 cm were originated which supports the previous findings in cowpea by using PA (Aasim *et al.,* 2009) or EA explants (Aasim *et al.,* 2010).

Auxins are genrally used for the root induction of *in vitro* propagated shoots. Hassan *et al.* (2013) presented the rooting of regenerated shoots of peanut relatively difficult under in vitro. In this study, 100 % rooting attained using 1.0 $mg.L^{-1}$ IBA confirmed the results of Day *et al.* (2017). Similarly, 100 % rooting of different legumes using pretreated explants have been documented by Aasim *et al.* (2009) for cowpea and Barpete *et al.* (2014) for grasspea. However, negative effects like low rooting frequency due to pretreatment has also been reported for other legumes like chickpea (Aasim *et al.,* 2011) or lentil (Aasim, 2012). In last step, successful adaptation of rooted peanut plantlets in the pots were performed which resulted in survival rate of ~ 80 % (Barpete *et al.,* 2014).

Conclusions**:** Development of new protocols for recalcitrant plants is of immense importance for the application of biotechnological tools in breeding. This study highlights successful use of pre-and posttreatment of explants with multiple shoot induction, improved rooting frequency and acclimatization of *in vitro* regenerated plantlets. The results emphasize positive role of in vitro pre and post treatments

that may find usage in future genetic transformation and breeding studies.

***Authors' contributions*:** This research is part of Master thesis by Hulya Ozkan. The hypothesis and execution of the idea was conceived by Muhammad Asim and Hulya Ozkan. Writting and statistical analysis was performed by Muhammad Aasim. The manuscript was controlled and approved by all authors prior to submission.

REFERENCES

Aasim, M. 2012. Micropropagation of lentil (*Lens culinaris Medik.)* using pulse treatment of immature plumular apices. Pak. J. Agric. Sci. 49:149-154.

Aasim, M., K.M. Khawar and S. Özcan. 2009. *In vitro* micropropagation from plumular apex of Turkish cowpea (*Vigna unguiculata* L.) Cultivar Akkiz. Sci. Horti. 122:468-471.

Aasim, M., K.M. Khawar and S. Özcan. 2010. Efficient *İn Vitro* propagation from pretreated embryonic axes of Turkish Cowpea (*Vigna unguiculata* L.) cultivar Akkiz. Arch. Biol. Sci. 62:1047-1052.

Aasim, M., S. Day, F. Rezai and M. Hajyzadeh. 2013. Multiple shoot regeneration of plumular apices of chickpea. Turk. J. Agric. For. 37:33-39.

Aasim, M., S. Day, F. Rezai, M. Hajyzadeh, S.T. Mahmud and S. Ozcan. 2011. *In vitro* shoot regeneration from preconditioned explants of chickpea (*Cicer arietinum L.*) Cultivar Gokce. Afr. J. Biotechnol. 10:2020-2023.

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

Balkcom, K.S., C.W. Wood, J.F. Adams and B. Meso. 2007. Suitability of peanut residue as a nitrogen source for a rye cover crop. Sci. Agric. 64:181-186.

Barpete, S., Aasım, M., Khawar, K.M. and S. Özcan. 2014. Preconditioning effect of cytokinins on *in vitro* multiplication of embryo the embryonic node of grass pea (*Lathyrus sativus* L.). Cultivar Gürbüz. Turk. J. Biol. 38:485-492.

Blomhoff, R., M.H. Carlsen, L.F. Andersen, D.R. Jr Jacobs. 2008. Health benefits of nuts: potential role of antioxidants. Br. J. Nutr. 99:447-448.

Brar, M.S., J.M. Al-Khayri, T.E. Morelock and E.J. Anderson. 1999. Genotypic response of cowpea *Vigna unguiculata* (L.) to *in vitro* regeneration from cotyledon explants. *İn vitro* Cell. Dev. Biol. 35:8-12.

Campos-Mondragón, M.G., A.M. Calderón De La Barca,A. Durán-Prado, L.C. Campos-Reyes,R.M. Oliart-Ros, J. Ortega-García, L.A. Medina-Juárez and O. Angulo. 2009. Nutritional composition of new peanut (*Arachis hypogaea* L.) cultivars. GRASAS ACEITES. 60:161-167.

Day, S., M. Aasim, N. Labar. 2017. Preconditioning of plumular apices explants of peanut (Arachis hypogeae) with 6-Benzylaminopurine. Paskistan journal of Agricultural Research, 54:255-260.

Hassan, M.U., Z. Akram, S. Ajmal, T. Mukhtar, S. Nasim, N. Shabbir and Y. Zafar. 2013. Hıghly efficıent *İn Vitro* root ınductıon ın peanut by mechanıcalstress method. J. Anim. Plant Sci. 23:425-429.

He, S., S. Fletcher and Rimal A. 2005. Snack Peanut Consumption: Type Preference and Consumption Manners. JFDRS. 36:79-85.

Heatley, M.E. and R.H. Smith. 1996. Whole plant regeneration from the shoot apex of *Arachis hypogaea* L. *İn vitro* Cell. Dev. Biol. 32:115-118.

Hill, G.M. 2002. Peanut by-products fed to cattle. Vet. Clin. North Am. Food Ani.m Pract. 18:295-315.

Langat, M.C., M.A. Okiror, J.P. Ouma and R.M. Gesimba. 2006. The effect of intercropping groundnut (*Arachis hypogea* L.) with sorghum (*Sorghum bicolor* L. Moench) on yield and cash income. Agricultura Tropica Et Subtropica. 39:87-90.

Madhulatha, P., M. Anbalagan, S. Jayachandran and N. Sakthivel. 2004. Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA). Plant Cell Tiss. Org. Cult. 76:189-191.

Matand, K. and C.S. Prakash. 2007. Evaluation of peanut genotypes for *in vitro* plant regeneration using thidiazuron. J. Biotechnol. 130:202-207.

Matand, K., N. Wu, H. Wu, E. Tucker and K. Love. 2013. More improved peanut (*Arachis Hypogaea L.)* protocol for direct shoot organogenesis in mature dry-cotyledonary and root tissues. J. Biotech. Res. 5:24-34.

Murashige, T. and E.A. Skoog. 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Park, W.T., Y.K. Kim, Y.S. Kim, N.I. Park, S.Y. Lee and S.U. Park. 2011. *In vitro* plant regeneration and micropropagation of *Liriope platyphylla*. POJ. 4:199-20.

Pelto, G.H. and Armar-Klemesu M. 2011. Balancing Nurturance, Cost and Time: Complementary Feeding in Accra, Ghana. Matern Child Nutr. 7: 66-81.

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

Sağlam, S. 2012. *In vitro* regeneration of Turkish dwarf chickling (*Lathyrus cicera* L.) from longitudinally sliced cotyledon node explants. J. Biotechnol. 161:15-16.

Sanders, T.H., R.W. McMichael Jr and K.W. Hendrix. 2000. Occurrence of resveratrol in edible peanuts. J. Agric. Food Chem. 48:1243-1246.

Seal, A. J., P.I. Creeke, F. Dibari, E. Cheung, E. Kyroussis, P. Semedo and T. Briel. 2007. Low and Deficient Niacin Status and Pellagra Are Endemic in Postwar Angola. Am J. Clin. Nutr. 85: 218-224.

Settaluri, V.S., C.V.K. Kandala, N. Puppala and J. Sundaram. 2012. Peanuts and their nutritional aspects—A Review. Food Nutr. Sci. 3:1644-1650.

Shan, L., G.Y. Tang, P.L. Xu, Z.J. Liu and Y.P. Bi. 2009. High efficiency *in vitro* plant regeneration from epicotyl explants of Chinese cultivars of peanut. *İn vitro* Cell. Dev. Biol. 45:525-531.

Sharma, K.K. and V. Anjaiah. 2000. An efficient method for the production of transgenic plants of peanut (*Arachis hypogaea* L.) through *Agrobacterium tumefaciens*-mediated genetic transformation. Plant Scientia. 159:7–19.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods. The Iowa State University Press, Iowa. USA.

Tiwari, S. and R. Tuli. 2009. Multiple shoot regeneration in seed-derived immature leaflet explants of peanut (*Arachis hypogea* L.). Sci. Horti. 121:223-227.

Tuberoso, C., A. Kowalczyk, E. Sarritzu and P. Cabras. 2007. Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. J. Food Chem. 103: 1494-1501.

Van Le, B.U.I., M.H.C. De Carvalho, Y. Zuily-Fodil, Y., Thi, A.T.P. and Van, K.T.T. 2002. Direct whole plant regeneration of cowpea (*Vigna unguiculata* (L.) from cotyledonary node thin cell layer explants. J. Plant Physiol. 59:1255-1258.

Venkatachalam, P. and N. Jayabalan. 1997. Effect of auxin and cytokinins on efficient plant regeneration and multiple-shoot formation from cotyledonary-node explants of groundnut (*Arachis hypogaea* L.) by *in vitro* culture technology. Appl. Biochem. Biotechnol. 67:237-247.

Venkatachalam, P. and V. Kavipriya. 2012. Efficient Method for *in vitro* plant regeneration from cotyledonary node explants of peanut (*Arachis hypogaea* L.). International Conference on Nuclear Energy, Environmental and Biological Sciences Bangkok, (Thailand).