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In Vitro Propagation of Groundnut (*Arachis hypogea* L.)

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Abstract

A protocol has been developed for micropropagation of *Arachis hypogea* L. under *in vitro* conditions. Nodal explants gave rise to multiple shoots when cultured on MS medium supplemented with different concentrations of BA (benzyladenine) with Kin (Kinetin) or GA3 (gibberellic acid). The highest response of shoot multiplication was obtained in MS containing 1.5 mg.l^{-1} BA and 0.5 mg.L^{-1} Kin. The regenerated shootlets were rooted on MS (Murashige and Skoog) basal medium with different concentrations of IBA (indolbutyric acid) and IAA (indol acetic acid). The highest response of rooting was achieved with IBA at 0.05 + IAA at 0.05 mg.L^{-1}. The maximum frequency of rooting and highest number of roots were produced on medium containing IBA 0.05 mg.L^{-1} and IAA 0.05 mg.L^{-1}. The plantlets, thus successfully established in soil.

Key words: *Arachis hypogea*, tissue culture, *in vitro*, plant regeneration.

Indroduction

Groundnut (*Arachis hypogea* L.) or peanut is an oil, food and fodder crop which plays an important role in the agricultural economies of countries of the semi-arid tropics. It contributes significantly to food security and alleviates poverty [1], and as a legume, improves soil fertility by fixing nitrogen and increases productivity for smallholder farmers of the semi-arid cereal croppingsystems [2]. It is one of the principle edible oil seed and protein rich leguminous crop [3], cultivated on over 20 million hectors in over 108 tropical and subtropical countries, with an annual yield of seeds estimated 28 million tons [4].

It is an annual herbaceous plant growing 30 to 50 cm (0.98 to 1.6 ft) tall. Peanuts are known by many other local names such as earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts is an oil, food and fodder crop which plays an important role in the agricultural economies of countries of the semi-arid tropics [5]. Micropropagation can be achieved by using different parts of the plant as the primary explant such as, the apical meristem, nodal bud, shoot buds,axillary buds, or through prodaction of somatic embryos, a process commonly known as somatic embryogenesis and aims at two things: production of large number of plantlets and propagation of the selected genotypes without inducing any genetic variation. A lot of research work has been done on nodel culture of several plants [6,7]. Asuccessful tissue culture protocol starts with effective explant sterilization. In some study a simple and fast protocol using commercial bleach (sodium hypochlorite, NaOCl) was evaluated for explant sterilization and *in vitro* establishment in comparison to mercuric chloride (HgCl2) which is mostly used in reported groundnut tissue culture studies [8]. Peanuts can be used like other legumes and grains to make a lactose-free milk-like beverage, peanut milk. Peanut plant tops are used for hay [9]. The protein cake (oilcake meal) residue from oil processing is used as an animal feed and as a soil fertilizer. Low grade peanuts are also widely sold as a garden bird feed [10].

The aim of this study is regenerate *Arachis hypogea* L.) through the establishment of in vitro propagation technique, It is necessary to device a rapid and efficient micropropagation

protocol for obtaining true ‑ to ‑ type regenerants without detriment to the survival of mother/donor plant and saving its populations from getting rarer in nature.

Material and Methods

The present study describes a protocol for rapid *in vitro* micropropagation of *Arachis hypogea* L. during the period from 2006 to 2008 through nodes explants (0.5–1.0 cm) obtained from a field in Al-Romadi, followed by soaking in mercuric chloride (HgCl2) for 5 minutes then by three times washing with sterilized distilled water. After that explants were emerged in a solution of sodium hypochlorite (chlorox) containing 3-5 drops of Tween- 20 for 20 minutes, then washed three times with sterilized distilled water [11] had been used for the establishment stage supplemented with 30 $g.L^{-1}$l sucrose and 6 $mg.L^{-1}$ agar, pH was adjusted at 5.7 before the addition of agar, and autoclaved at 121° C at the pressure of 1.5 kg\cm2 for 20 minutes. Several combinations of growth regulators were tested, including BA (1.0, 1.5, 2.0 $mg.L^{-1}$) and Kin (0.1,0.5,1.0 $mg.L^{-1}$) or GA3(0.05, 0.1, 0.5 $mg.L^{-1}$). Subcultures were done every 21 days interval. Nodal segments from the proliferated shoots were subcultured again for further multiple shoot induction.

The individual shoots were cultured on MS medium supplemented with different concentrations of IBA and IAA. For each treatment, 32 explants were used. Every experiment was repeated at least three times. The cultures were maintained in a culture room at 26±3℃ and were exposed to continuous fluorescent light for 16 h per day**.** The rooted plantlets transferred to perforated plastic pots.

Results and discussion

In vitro culture technique had been used for rapid plant propagation, tissue culture technology may help to conserve rare and endangered important plants [12,13]. Several factors such as choice of explants, culture environments, plant growth regulators act synergistically in determining the proper induction [14,15].

In this experiment, the effects of explants and different concentrations and combinations of BA and Kin or GA3 on shoot proliferation of **(***Arachis hypogea* L.) were studied. Nodal explants were used in this experiment and showed highest response in MS medium fortified with different concentrations of BA with Kin or GA3. Within five weeks of culture multiple shoots emerged directly from the explants. The variance analysis of the nodes explants cultures showed that the effects of the treatments with different combinations of BA and Kin were significant on shoot induction (Fig. 1). The rate of the response in medium MS with BA and Kin were greater than those observed in the medium supplemented with BA and GA3 (Fig. 2). At the combination 1.5 $mg.L^{-1}$ BA+0.5 $mg.L^{-1}$ Kin recorded that the highest rate of response 90.22%, Where as BA Added with GA3 at the concentration 1.5 $mg.L^{-1}$BA with 0.1 $mg.L^{-1}$ GA3 recorded 72.49 which was the highest rate of response for shoot intiation [16]. Similar results had already been reported in strawberry [17]. Also the result was in consistent with the findings of in papaya [18], as well as in *Eucalyptus grandis* [19]. New shoot development from nodel explants was observed within three weeks of culture and more shoots were found to develop during subcultures [20].

Formation of roots took place within three weeks of transfer to root induction media, therfore the regenerated shoots were then subcultured on MS medium supplemented with different levels of IBA and IAA in order to allow root formation (Fig. 3). Root development varied with combinations of IBA and IAA on root initiation were significant. The highest percentage of rooting 93.47 was achieved with IBA at 0.05 $mg.L^{-1}$ + IAA at 0.05 $mg.L^{-1}$ The result showed that, IBA at 0.05 $mg.l^{-1}$ and IAA at 0.05 $mg.L^{-1}$gave the highest root length which was 3.8 cm. A significant differences was recorded on the number of roots due to the effect of IBA and IAA [21]. Rooted plantlets were taken out from culture tubes and washed

thoroughly with tap water to remove the culture medium from the roots. Washed plantlets were sprayed with fungicide and planted to normal and sterilized soil in polybags (Fig. 4). After 7 days plantlets were planted in soil. The protocol reported here is reproducible; it has a potential for allowing a large scale micropropagation of this important and new plant in Iraq.

Conclusion

The present experiments have shown that it is possible to induce shoot differentiation and complete plantlet development from nodal explants of *Arachis hypogea* (L.). Among hormone concentrations, BA with Kin is best for shoot induction and multiplication and IBA at 0.05 $mg.L^{-1}$ + IAA at 0.05 $mg.L^{-1}$ are best for rooting.

References

1. Reddy, T. and Anbumozhi, V.(2003). Physiological responses of groundnut (*Arachis hypogaea* L.) to drought stress and its amelioration: acritical review. Plant Growth Reg., 41:75-88.

2.Giller, K. ; Cadish, G. and Palm, C. (2002). "The North-South divide! Organic wastes or resources for nutrient management" Agrom., 22 :703- 709.

3.Collino, D.; Dardanelli, L. ; Sereno, R. and Racca, W. (2001). Physiological responses of argentine peanut varieties to water stress. Light interception, radiation use efficiency and portioning assimilates. Field Crops Res. 70(3): 177-184.

4.FAO . (2007). Quarterly Bulletin of Statistics. FAO, 8(3).

5.Townsend, D. and Guest, E. (1974). Flora of Iraq. Robert Maclehose & Company Limited , Glasgow.

6.Sanjaya, S. and Rai, V. (2005). Micropropagation of *Pseudoxytenanthera stockssi*. In Vitro Cell. Dev Biol. Plant, 41: 333-337.

7.Siddique, M. and Jahan, A. (2006). Rapid multiplication of *Nyctanthes arbor* – tritis L. through *in vitro* axillary shoot proliferation. World J. Agri. Sci. 2:188-192.

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

9.Roja, R. and Padmaja, G. (2000). "A Protocol for high frequency plant conversion rom somatic embryos of peanut (*Arachis hypogaea.L*) cv", J. Plant Bio., 7(3) :187-193.

10.Roja, A. ; Venkateswarlu, P. ; Padmaja, G. ; Venkatesh, K. and Baburao, N. (2009). "Changes in protein composition and protein phosphorylation during somatic embryogenesis and plant regeneration in peanut (*Arachis hypogaea* L.)" *African J.* Bio., 8(15) :3472-3478.

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

12. Sagare, A. ; Lee, Y. ; Lin, T. ; Chen, C. and Tsay, H. (2000). Cytokinin-induced somatic embryogenesis and plant regeneration in Corydalis yanhusuo (Fumariaceae) _ a medicinal plant. Plant Sci, 16: 139-147

13.Shiau, Y. ; Chen, U. ; Yang, S. ; Sagare, A. and Tsay, H. (2002). Conservation of Anoectochilus formosanus, a medicinally important terrestrial orchid, by synchronizing flowering, hand-pollination, and in vitro culture of seeds. Bot. Bull. Acad. Sin., 43: 123-130

14.Chen, C. ; Chen, S. ; Sagare, A. and Tsay, H. (2001). Adventitious shoot regeneration from stem internode explants of Adenophora triphylla (Thunb.) A.DC. (Campanulaceae) _ an important medicinal herb. Bot. Bul. Acad. Sin., 42:1-7.

15.Florencia, M. (2000). Protocol for regeneration in vitro of *Arachis hypogaea* L. Electronic Journal of Biotechnology, 3:101-111.

16. Farhat, N.; Mohmood, H. ; Zahid, A. and Muhammad, M. (2011). *In vitro* regeneration of Pakistani peanut (*Arachis hypogaea* L.) varieties using de-embryonated coteledonary explants. African J. Bio., 10(43): 8599-8604.
17. Bhatt, I. and Dhar, U. (2000). "Micropropagation of Indian wild strawberry". Plant Cell, Tissue and Organ annum J. Culture, 60 :83-88.
18. Cononer, A. and Litz, R. (1978). "*In vitro* propagation of papaya". Hort. Sci., 13 :241-242

19. Teixetra, S. and. Silva, L. (1990). *In vitro* domestica and propagation of adults *Eucalyptus grandis* Hill Ex. Organ Maiden from epicormic shoots, VII Intl. Cong. On Plant, Tissue and Cell Cult., Amsterdam, :218-222.
20.Zaman, M.; Ashrafuzzaman, M.; Haque, M. and Luna, L. (2008). *"In vitro* clonal propagation of the neem tree(*Azadirachta indica* A. Juss.)" African J. Bio., 7(4) :386-391.
21.Pintu, B. Sharmi, S. and Sudhansu, S. (2007). Influence of genotype on *in vitro* multiplication potential of *Arachis hypogaea* L. Acta Bot. Croat. 66(1): 15-23.

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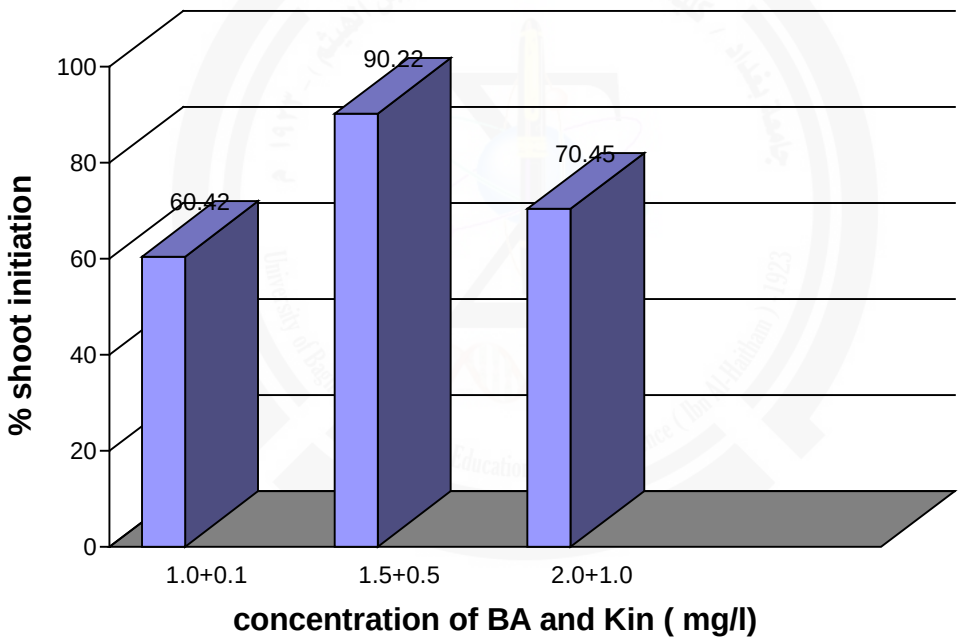
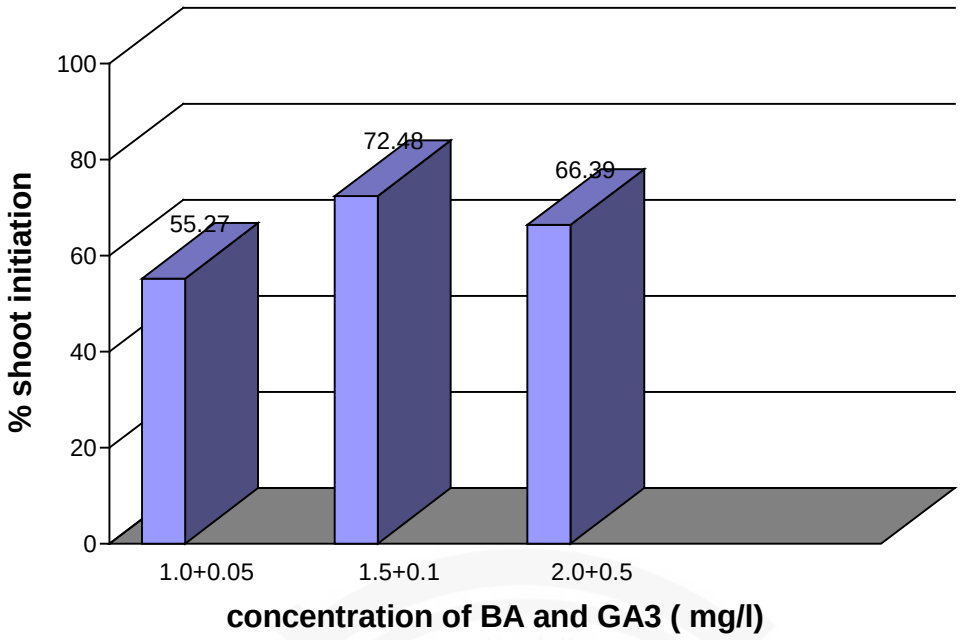
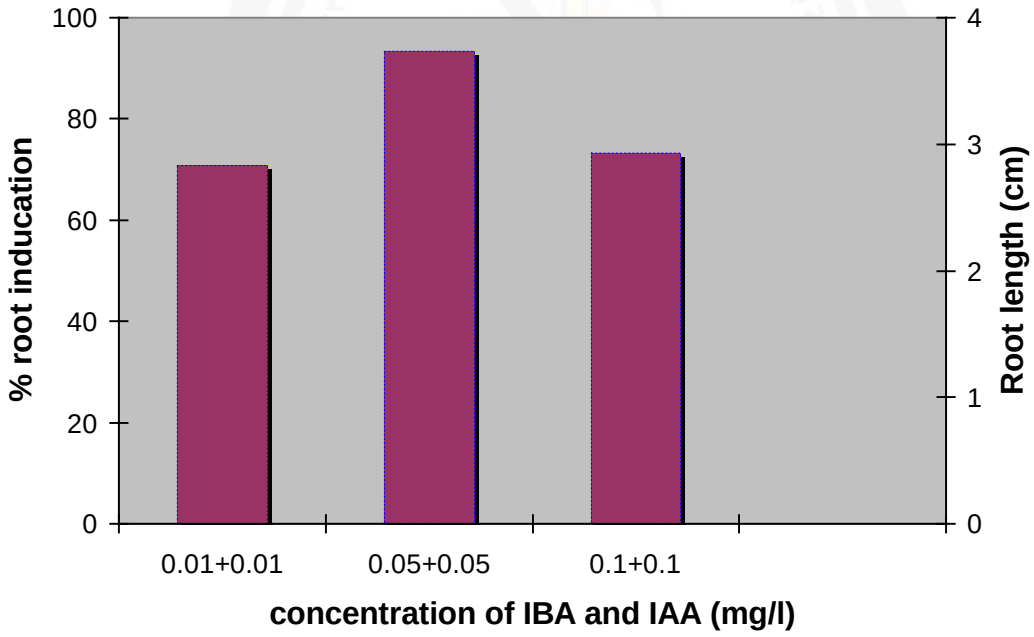


Fig(1): Effect of BA and Kin concentrations on shoot inducation of *Arachis hypogea*



Fig(2): Effect of BA and GA3 cocentrations on shoot induction of *Arachis hypogea*



Fig(3): Effects of different combinations of IBA and IAA on rooting of excised shoot in *Arachis hypogea*

A- B- C-

Fig(4): *In vitro* propagation of *Arachis hypogea* L.A- the nodes explants B- rooting of excised shoot C- acclimatized plantlet in pots

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الإكثار خارج الجسم الحي للفول السوداني *Arachis hypogea* L.

خنساء رشيد الجبوري
مركز بحوث ومتحف التاريخ الطبيعي/ جامعة بغداد
استلم البحث في : 1 تشرين الثاني 2011 قبل البحث في : 11 كانون الثاني 2012

الخلاصة

تم تطوير الإكثار الدقيق للـ *Arachis hypogea* L. خارج الجسم الحي عن طريق زراعة العقل على وسط MS المجهز بالهرمون النباتي BA و Kin او GA3 . وقد كانت أعلى استجابة لإكثار النموات في وسط يحوي 1.5 ملغم.لتر$^{-1}$ BA مع 0.5 ملغم.لتر$^{-1}$ ، Kin ثم جذرت النموات في وسط يحوي IBA مع IAA. وكانت أعلى النتائج لعملية التجذير واكبر عدد من الجذور المتكونه في وسط يحوي ملغم.لتر$^{-1}$ 0.05 IBA مع 0.05 ملغم.لتر$^{-1}$ IAA ثم نقلت النبيتات بصورة ناجحه للتربة.

الكلمات المفتاحية: *Arachis hypogea* ، زراعة الأنسجة ، خارج الجسم الحي ، إكثار النباتات