Callus Induction in *Arachis hypogaea L.*using Embryos and Effect of Plant Growth Regulators

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ABSTRACT

The objectives of this investigation were to develop mature plants from embryo callus of *Arachis hypogaea L. (variety K-6*) and effects of different plant growth regulators on embryo development. Embryos were cultured on full strength Murashige and Skoog (MS) supplemented with 2-4D (0.5 to 2mg/l) and combinations of NAA, BAP (0.5mg/l+1mg/l) showed the callus development. Multiple shoots were observed on 2-4D, Kinetin (2mg/l+1mg/l) combination 4-6 multiple shoots were obtained. Roots were developed in (2mg/l) 3-Indolebutyric acid (IBA) which showed thick rooting response in the medium.

Key Words: Embryo culture, 2, 4-D: 2, 4-Dichlorophenoxyacetic acid; MS: Murashige and Skoog salts medium. Kn-Kinetin, Tissue culture, IBA-Indole butyric acid.

Introduction: *Arachis hypogaea L.* is widely grown in the tropics and subtropics, being important to both small and large commercial producers. This belongs to the botanical family *Fabaceae* or *Leguminosae*, commonly known as the legume or pea family. High in Protein, Good source of Energy, Rich in Vitamins, Diabetic Friendly and prevents heart disease by lowering cholesterol levels and low-glycemic levels. Peanut (*Arachis hypogaea L.*) embryogenic callus was produced from the embryo of mature, seeds of cultivar K-7.Worked with Immature cotyledons (Ozias-Akins 1989, Ozias-Akins et al. 1992, Durham and Parrott 1992, Baker et al. 1994, immature embryo axes(Hazra et al. 1989, Ozias-Akins et al. 1992,Somatic embryogenesis in peanut (*Arachis hypogaea L.)* using a variety of different explants, including leaves (Bakerand Wetzstein 1992), RamdevReddy and Reddy 1993, Eapen et al. 1993), embryo axes collected at harvest (McKently 1991), and whole immature embryos (Sellars et al. 1990).

Material and Methods: Seeds of *Arachis hypogaea L (verity K-6)* were collected and placed under running tap water for 10 minutes to remove dust partials, then seeds were transferred to wash bottle containing 10-20 drops of Tween-20 and washed for 30min. Tween 20 is removed and washed three times with double distilled water. These seeds were transferred to new sterile (300ml capacity) wash bottle containing 300mg of Bavistine (fungicide) and washed for 8 minutes and rinsed with distilled water three times. After these seeds were washed with Sodium Hypo chloride (6%) and rinsed with distilled water three times. Embryos were separated from seed by using sterile forceps and scalpel.

Media preparation and Culture Conditions:

The nutrient medium used in all the experiments consisted of MS salts and vitamins with 3% (w/v) sucrose. The medium was solidified with 0.6% (w/v) bacteriological grade agar and the pH of the medium was adjusted to 5.8 before autoclaving at 1.06 Kg cm-2 pressure and 121°C temperature for 20 minutes. All the culture vials were placed in plant growth room at 25±2°C under 16/8 hr (light/dark) photoperiod with a light intensity of 50 µ mol m-2 s -1 supplied by cool white fluorescent lamps (2 tubes, 40 W, Philips, India) and 60±65% relative humidity.

Multiplication, Elongation of Shoots:

Embryos were inoculated on MS media which contain different concentration of 2,4-D (0.5 to2mg/l) and combination of NAA, BAP (0.5mg/l+1mg/l) results the soft white callus. This callus is subcultured with same media to increase the mass. Shoot length is very small with BAP and 2,4-D and in the presence of 2,4-D+Kn the shoot length is very good and we observed shoot length is moderate in the presence of only 2.4-D. Multiple shoots (4-6 shoots) are observed in 2,4-D+Kn(2mg/l+1mi/l). Micro shoots were transferred to multiple shoot

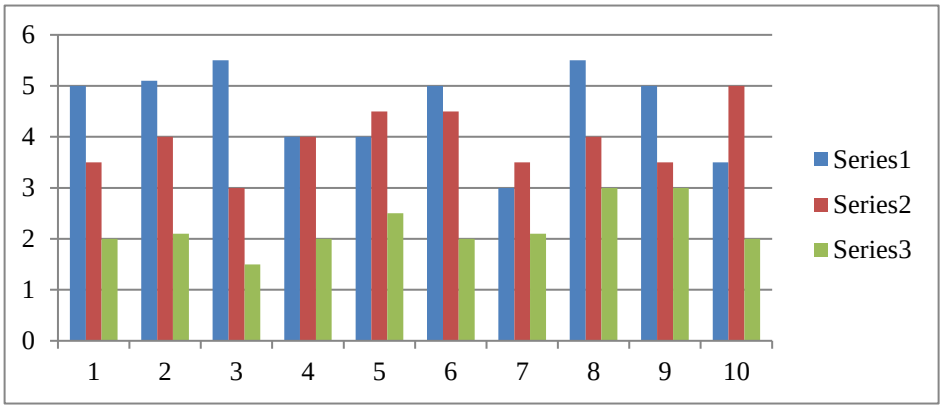
medium which contain 2,4-D+Kn (2mg/l+1mi/land adenine sulphate-0.8mg/l. These micro shoots were transferred to rooting media.

Rooting, Acclimatization and Data Analysis:
For root induction, in vitro raised micro shoot (3-5cm long) excised from shoot clusters and cultured on half strength basal medium supplemented with different concentration of Indole-3-Butyric Acid (IBA) (1.0-3.0mg/l). Plants were transferred to sterile soil moistened with sterile water and covered with transparent plastic glasses and kept at 24±1°C for 16hr photoperiod and 2500lux light intensity for 3 weeks. In all experiments, 10 explants were taken in each treatment, and each treatment was repeated three times.

Result and Discussion:
No callus induction was observed on the medium devoid of growth regulators, Embryogenic callus is obtained in NAA, BAP (0.5mg/l+1mg/l) and micro shoots were observed in the presence of BAP and 2,4-D and in the presence of 2,4-D+Kn the shoot length is very good and we observed shoot length is moderate in the presence of only 2.4-D. Multiple shoots (4-6 shoots) are observed in multiple shoot medium which contain 2,4-D+Kn(2mg/l+1mi/l and adenine sulphate-0.8mg/l). Rooting was good in half strength basal medium supplemented with different concentration of Indole-3 Butyric Acid (IBA) (2mg/l).Plant growth regulators regulate the dedifferentiation and redifferentiation of plant cells. They are known to particularly influence callus induction; a phase in which auxins play a major role by inducing callus proliferation and development (Paris et al., 2004).Well-developed rooted plants were taken out from paper glasses and transferred into polythene bags for hardening.

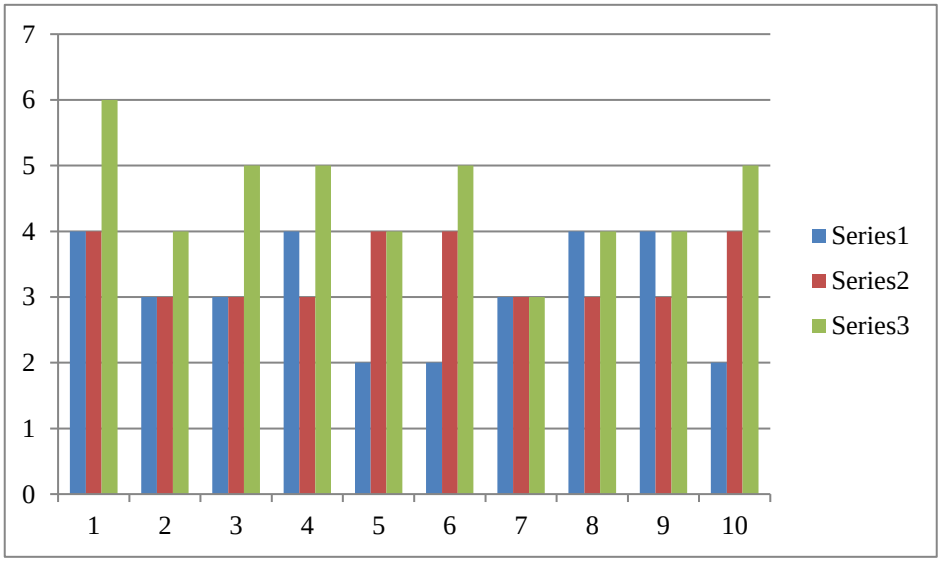
Conclusion:
These present experiments have shown that it is possible to get complete plantlets from embryogenic callus by using different concentrations of plant growth regulators.

Elongation with different Phyto Hormone Concentrations



Series 1: 2,4-D+Kn (2ml/1ml)
Series 2: 2,4-D+BAP(2ML/1ml)
Series 3: Kn+BAP (1ml+1ml/l)

Multiple shoots with different concentrations of Phyto Hormones



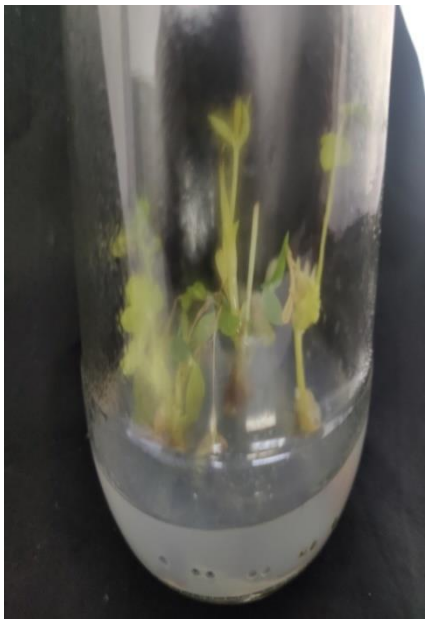
Series 1: 2,4D+NAA-2mg/l+1ml
Series 2: 2,4D+BAP+Adnine sulphate-2mg/l+0.8mg/l
Series 3: 2,4D+Kn+Adenine sulphate-2+1+0.8mg/l

A. EMBRYO INITIATION

B. CALLUS INDUCTION

C.SHOOT DEVELOPMENT

D. MULTIPLE SHOOTS



E. ROOTING

E.HARDENING

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