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MICROPROPAGATION OF *ARACHIS HYPOGAEA*

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Induction of embryogenesis and organogenesis was observed in one out of the three cultivars of *Arachis hypogaea* viz. *Arachis hypogaea* Cv. M-13, Cv. Chandra and Cv. Chitra. Embryo cultures gave large number of embryoids which could be induced to differentiate into plantlets and plants. MS+BAP+IAA (2.0+2.0 mg/1) resulted in shoot regeneration *Arachis hypogaea* Cv. M-13 BAP+IAA (1.0+2.0 mg/1) induced enhanced somatic embryogenesis. Plantlets thus produced could be developed into mature plants.

Keywords: *Arachis hypogaea;* Embryogenesis; Groundnut; Oil yielding plants; Organogenesis.

Introduction

Peanut is a leguminous oilseed crop and like all other legumes was considered as recalcitrant for several years. During recent years a large number of leguminous plants have been differentiated through somatic embryos viz. soyabean (*Glycine max*)[1], Clovers (*Trifolium* sp.)[2], Alfa alfa (*Medicago sativa*)[3], *Trifolium rubens*[4] and *Arachis hypogaea*[5,6].

Earlier investigation on *A. hypogaea* have indicated as it can develop functional photosynthetic system and undertake autotrophic growth[7-9]. However no differentiation could be induced in these autotrophic culture[9]. Present investigation was undertaken with an object to develop autotrophic culture which can be differentiated into plantlets and plants, besides to obtain micropropagation of *A. hypogaea.*

Materials and Method

Seeds of *A. hypogaea* Cv. M-13, Cv. Local, Cv. Chandra and Cv. Chitra were grown on water agar medium. Germinated seedlings were cut into pieces and different explants like leaf, coytledons and excised embryo were transferred aseptically on MS medium[10], containing different growth regulators. The pH of the medium was adjusted to 5.8 using 1 N HCl or NaOH prior to addition of 0.8% agar. Media was autoclaved to 120°C and 15 psi for 20 min.

Seeds of all four cultivars *A. hypogaea* Cv. M-13, Cv. Chandra, Cv. Chitra and Cv. local were treated in 25% H_2SO_4. The embryo was excised and sterlized in 0.1% $HgCl_2$ for two minutes. After three washings in sterlized distilled water, these sterlized embryos were transfered to water agar. The germinated seedlings were subsequently transferred to MS medium containing various growth regulators.

Different explants from nodal segment, stem, leaf, excised embryo from one month old plants grown under field conditions, were excised and sterlised in 0.1% $HgCl_2$ for two minutes and finally transferred to MS medium containing different growth regulators.

Cultures were incubated under cool fluorescent light (16 hours photoperiod, 32 u $E/M^2/S^2$) at 25 ± 2°C.

Observations were recorded periodically.

Results and Discussion

(i) *Explants from seedlings* : Leaf, cotyledon and excised embryo gave callus from margin on NAA+K (4.0+0.8 mg/1). This was much

Table-1.

Explant	BAP+NAA (2+2) (mg/1)	BAP+K (4+0.4) (mg/1)	BAP+NAA (4+2) (mg/1)	NAA+K (4+0.8) (mg/1)
Leaf	C +	C +	C +	C ++
Cotyledon	C	A	C +	C +
Excised embryo	S ++ + C ++	S ++ + C ++	C + + S ++	C +

more pronounced in combination of BAP+NAA (2.0 + 2.0 mg/1) excise embryo produced shoot as well as callusing on this combination (BAP+NAA 2.0 + 2.0 mg/1). However, BAP+K (4.0 + 0.4 mg/1) resulted in callus formation from leaf and cotyledons and shoot buds and cotyledons from embryo explants, resulted in callus formation but good differentiation. The leaf callus cultures when transfered to MS containing BAP+NAA (4+2 mg/1) resulted in profuse callusing after a period of 4 weeks (Fig. 1). Cotyledon and excised embryo cultures when subjected to the above medium exhibited poor results.

The shoots obtained from excised embryo were transferred to MS+GA_3 +BAP (0.5+3.0 mg/1) which caused elongation of shoots, while no response was observed in MS+BAP or MS without growth regulators.

Embryo culture: Embryos excised from Cv. M-13, Chandra and Chitra were grown on water agar medium containing 3% sucrose and 0.8% agar-agar. Although embryo from all the three cultivars germinated upon transfer to MS+BAP+NAA (2+2 mg/1) resulted in shoot formation in *A. hypogaea* Cv. M13. However no shoot formation was observed in *A. hypogaea* Cv. Chandra or Cv. Chitra.

Prolonged subculturing of differentiated shoots resulted in rooting from basal segments. Besides this callus formation was also observed (Fig. 2).

Mature explants: *Leaves:* Mature leaves from one month old plants of *A. hypogaea* Cv. M13 produced callus from margins and cut ends. No further differentiation was observed. Even if transferred on MS without growth regulators greening of callus without further differentiation was observed.

MS+NAA+Kinetin (10+0.8ppm) promoted formation of roots and callus from the leaves. No shooting was observed on this medium.

Nodal explants : Nodal explants from Cv. M13 when transferred to MS medium indicated shooting. Further elongation of shoots was recorded on the same medium. Rooting was not observed.

Direct embryogenesis : Embryo culture from Cv. M-13 produced shooting and rooting, besides callus production. The calus cultures upon transfer to MS medium containing BAP+NAA (1.0+2.00 ppm) resulted in induction of embryogenesis. Different stages of embryo formation from globular to torpedo to heart shaped embryos were recorded. These embryos differentiated into plantlets and plants (Fig.3).

Several factors influence morphogenetic potential of the cultivars and

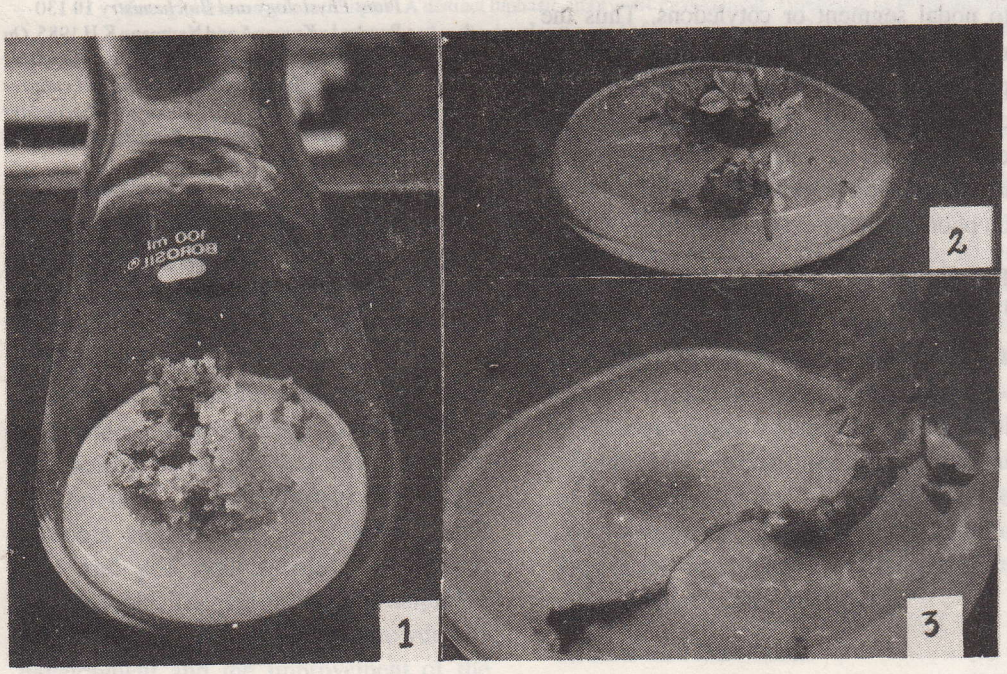

Fig. 1. Leaf culture showing profuse callusing.
Fig. 2. Embryo culture showing multiple shoot formation and Callus formation.
Fig. 3. Showing induction of embryogenesis and complete plant formation.

genetic factors probably played a major role. Among the three cultivars tried only *A. hypogaea* Cv. M13 could be induced to differentiate into plantlets. Probably genetic factors played a major role in determining the genetic potential.

Besides, plant parts also regulated morphogenetic response. Leaf & excised embryo gave better response in comparison to nodal segment or cotyledons. Thus the morphogenetic response of callus and excised plant parts was regulated by proper combination of growth regulator, the chlorophyllous callus cultures having morphogenetic potential will facilitate work on phoronjuthetic erzymes.

References

1. Lazzeri P A, Hildebrandt D F and Collins G B 1985, *Plant Mol. Biol. Rep* **3** 160
2. Maheshwari G and Williams E G 1984, *Ann. Bot* **54** 201
3. Sharp W R, Evans D A and Sondhahl M R 1982, *Application of somatic embryogenesis to crop improvement.* In: Frigiwara A. (ed). *Plant Tissue Culture* 1782. Marugen, Tokyo pp 759-762.
4. Cui D, Myers J R, Collins G B and Lazzeri P A 1988, *Plant Cell Tissue Organ Culture* **15** 33
5. Chengalrayan K, Sathye SS and Hazra S 1994, *Plant Cell Rep.* **13** 578
6. Hazra S, Sathaye SS and Mascarenhas A F 1989, *Biotechnology* **7** 949
7. Kumar A, Bender L and Neumann K H 1983, *J. Plant Physiology and Biochemistry* **10** 130
8. Bender L, Kumar A and Neumann K H 1985, *On the photosynthetic system and carbohydrate metabolism of carrot and Arachis cell cultures* In: Neumann K.H. (ed) *Primary and Secondary Metabolism of plant cell cultures* Springer Verlag Berlin Heidelberg, pp 24-42.
9. Neumann K H, Bender L, Kumar A and Megda Szegue 1982, *Photosynthesis and pathways of carbon in tissue cultures of Daucus and Arachis.* Proc. International Congress of Plant tissue and cell culture. Tokyo pp 251-252.
10. Murashige T and Skoog F 1962 *Physiol, Plant.* **75** 473