In vitro Multiplication and Conservation of Wild *Arachis* Germplasm

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With the receding genetic barriers among species because of biotechnological developments the possibilities of utilizing genes from wild alien species of a crop has become much higher. In groundnut, wild species of *Arachis* are potential sources of resistance to various pest and diseases and are being utilized in crop improvement programmes. Maintenance of germplasm of wild relative in groundnut is difficult because they produce either a few or no seeds (Stalker and Simpson, 1995). Sometimes poor seed viability under storage condition is a major problem in maintenance of an accession. Further, many species are perennials and as such the field gene banks are continuously exposed to the vagaries of environment including diseases. Micropropagation in these species will have the advantages of fast multiplication without genetic contamination, freedom from seed-borne diseases and ease in transportability and exchange. *In vitro* conservation of these propagules can be an alternative method of preservation of these germplasms. The pre-requisite for in *vitro* conservation is standardization of *in vitro* regeneration system without callus formation and standardization of culture media with prolonged sub-culturing intervals. Micropropagation in cultivated groundnut using various explants and a few wild species has been reported (Radhakrishnan *et al.* 2000, Rani and Reddy, 1996, Venkatachalam *et al.* 1999). Most of the regeneration studies with the wild species have been through indirect method from immature leaf and embryo axes and cotyledonary segments (Still *et al.*, 1987, Mansur *et al.*, 1993) which is believed to induce somaclonal variation and cannot be used for multiplication and conservation of germplasm lines. Recently Gagliardi *et al.* (2000) has reported direct regeneration from different explants of wild species of the section extranervosae to tackle the problem of seed viability under storage. The present study reports the *in vitro* regeneration potential of *Arachis* species through direct multiple shoot induction from de-embryonated cotyledons and protocols for slow growth *in vitro* conservation.

Sixteen accessions belonging to nine of *Arachis* species of the sections *Arachis*, and *erectoides* were studied. The procedure for surface sterilization of seeds and dissection of explants was followed as reported earlier (Radhakrishnan *et al.* 2000). The de-embryonated cotyledons cultured in MS medium with vitamins of B_5 medium. The basal medium was supplemented with one of the three level of BA *viz.*- 5 mg/l (MS1), 10 mg/l (MS2) and 15 mg/l (MS3). Magenta boxes were used as culture vessels. Fifty ml of culture media was dispensed in three boxes constituted each replication. Two explants were cultured in each box in two replications for each treatment. The number of responding cultures and number of shoots/explants were recorded after 30 days in culture under 4000 lux illumination and 26 ± 1°C. For further multiplication of shoots the portion of the explants bearing the shoots were dissected and cultured in the respective media at an interval of 21 days. The individual shoots were subcultured the basal medium for reducing the effect of cytokinin on to for 1 week and rooted in basal medium containing 1mg/l NAA and 1g/l activated charcoal.

Multiple shoots induced from the de-embryonated cotyledons of *A. monticola*, *A. duranensis*, *A. correntina* and *A. kempff-mercadoi* from the earlier experiment were used for *in vitro* conservation studies. Shoots were cultured on different media listed below:

1. MS+ 5mg/l BA + 30 g/l Sucrose
2. MS+ 5mg/l BA + 30 g/l Sucrose + 2 mg/l ABA
3. MS+ 5mg/l BA + 30 g/l Sucrose + 2% (w/v) Mannitol
4. MS+ 5mg/l BA + 30 g/l Sucrose + 2% (w/v) Mannitol + 2 mg/l ABA
5. MS+ 5mg/l BA + 30 g/l Sucrose + 4% (w/v) Mannitol
6. MS+ 5mg/l BA + 30 g/l Sucrose + 4% (w/v)Mannitol + 2 mg/l ABA

Culturing was done in test-tubes with 15 ml medium and incubated at 4000 lux illumination and 26 ± 0° C of 16 hrs photoperiod. Two to three shoots were

cultured in each of 20 tubes, which constitute one treatment. All experiments replicated twice. Shoots from three tubes/replication were harvested at an interval of 20 days and shoot length, fresh weight and dry weight were recorded until the shoots started drying.

Cotyledons were enlarged and turned green in culture and multiple shoots were mostly emerged from the proximal ends of the cotyledons without callus formation as reported earlier for cultivated species (Radhakrishnan *et al.* 2000). Though Gagliardi *et al.* (2000) reported the regeneration of shoots from several regions of the cotyledons, in our study callusing was associated with the shoot buds regenerating from the parts of the cotyledons other than the proximal ends. Significant variability was observed among the three media in multiple shoot induction (P=0.05). All the accessions produced multiple shoots in MS1 and the response ranged from 10% (ICG 8201 and ICG 8198) to 82% (ICG 8205). Only 11 accessions responded in MS 2 with response ranging from 17% (ICG 8208) to 68% (ICG 8918). In MS3 medium only 12 accessions responded with a range of response of only 11 to 50%. The variability among genotypes for response to induction was also significant (P=0.01). However, the interaction between genotypes and media combinations was not significant. Variation of shoots/responsive explant was significant among the genotypes but variation among three media was not significant. The interaction between the genotype and media was significant. The maximum number 10.5 shoots/explant was in *A. oteroi*. *A. oteroi* showed the maximum number of shoots/explant followed by *A. duranensis* and *A. kempff-mercadoi*. Though, the number of shoots in the induction phase is very low compared to the cultivated species of *A. hypogaea* the shoot-inducing portion of the explants on further subculturing produced approximately 1:20 multiplication in each subculture of three weeks interval. The requirement of growth hormone was also four times less in wild species compared to cultivated species. The shoots after hardening in the basal media, about 50% was induced rooting in the rooting medium with an average of 2 or 3 roots/shoot.

Among the six treatments tested for conservation, the control (without mannitol and ABA) had the maximum elongation of shoots followed by the treatment with 2 mg/l ABA. The variation in shoot growth was significant among media combinations and among genotypes (P= 0.01). The culture media supplemented with 2% mannitol alone could show uniformly retarded growth with healthy shoots. In some of the treatments, though the growth was retarded the shoots were not healthy and started wilting after 60 days from culture and would be unsuitable for maintaining the culture.

It is confirmed from the studies that the wild species of *Arachis* have the potential of *vitro* regeneration with a reasonable rate of multiplication on basal medium supplemented with 5 mg/l of BA and rooting could be obtained on MS medium supplemented with NAA and charcoal in the medium. The medium supplemented with 2% mannitol found suitable for prolonging the subculturing for more than 60 days. Hence, *in vitro* multiplication can be employed in multiplication exchange and storage of the valuable germplasm. However, the genetic integrity needs to be ascertained during prolonged storage as stress is induced for achieving slow growth.

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