*Indian J. Genet.*, 68(3): 261-264 (2008)

# Genotypic comparison for androgenic callogenesis and organogenesis among cultivated, wild and interspecific hybrid of groundnut

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(Received: December 2006; Revised: July 2008; Accepted: August 2008)



**Abstract**

**Anther or microspore culture is one of the efficient techniques for invitro plant regeneration and has been exploited in haploid breeding. Six groundnut cultivars, five wild *Arachis* species and four synthetic interspecific hybrids were studied for invitro androgenic callogenesis and regeneration. Androgenic callogenesis in groundnut starts four to five days after culturing in MS salts with vitamins of B5+12.362µM NAA+2.22µM BAP+87.64µM sucrose (m/v) and 0.6% agar (m/v) irrespective of ecotype. Callogenesis ranged from 21% to 72% among the genotypes studied. Cultivated genotypes were more responsive to callogenosis than wild species and interspeicific hybrids. Organogenesis induced in half the strength MS salts with vitamins of B5+6.66mM BAP+2.68µM NAA+2.89µM $GA_3$ +87.64µM sucrose (m/v) and 0.6% agar (m/v). Four cultivated genotypes *viz*., GG 2, J 11, JL 24 and TMV 2 induced shoot development and plants were regenerated from cultivars GG 2 and J 11. Regenerated plants were confirmed as 2n = 4x = 40 by root tip analysis indicating that regenerated plants were regenerated either from sporophytic tissue of microspore or spontaneous doubled haploid.**

**Key words:** Anther culture, microspore culture, groundnut, callus and regeneration

## Introduction

Anther or microspore culture is one of the efficient techniques for invitro plant regeneration and has long been utilized in haploid breeding [1-3]. However, the success is often hampered by the difficulty of inducing callus from gametophytic tissue of anther and inducing morphogenesis for different crops and cultivars [4, 5]. In groundnut, though several studies [6-11] have been reported on anther culture, responsive stage of microspore, media for callus induction and differentiation but regeneration of plants from anther calli remains the most difficult part. The primary objective of this study was to evaluate genotypic response for androgenic callogenesis and embryogenesis among cultivated, wild *Arachis* species and triploid interspecific genotypes.

## Materials and methods

The experiment was carried out using six cultivated groundnut genotypes, five wild *Arachis* species and four triploid interspecific hybrids (Table 1). Healthy immature (2-3 mm in length) flower buds, ready to bloom next morning were collected in tap water from field grown plants between 9-10 AM. Flower buds were then surface sterilized by agitating in 70% ethanol and freshly prepared 0.1% mercuric chloride solution for ten minutes each and washed thoroughly in sterilized distilled water for three to four times. Anthers of 0.5-1.0mm in length were dissected out from flower buds with help of sterilized needle under laminar flow and used as explants. All compounds and plant growth regulators used in the experiments were from Himedia laboratories Pvt. Ltd., Mumbai, India. The MS [13] salts with vitamins of B5 [14]+12.36µM NAA+2.22µM BAP+87.64µM sucrose (m/v) and 0.6% agar(m/v) for callogenesis and half the strength MS salts with vitamins of B5+6.66µM BAP+2.68µM NAA+2.89µM $GA_3$ +87.64µM sucrose(m/v) and 0.6% agar(m/v) for embryogenesis [15] were used through out the experiments. The medium was adjusted to pH 5.8 before autoclaving at 121$^{o}$C for15 minutes and cooled before use. Single anther was inoculated in 25 x 150mm test tubes (Borosil, Mumbai, India) containing 10cm$^3$ solidified media and then cultured at an irradiation of 30m molsm$^{-2}$s$^{-1}$ with 16 hours photoperiod and temperature of 25±2$^{o}$C. Experiments were carried out with 5 replicates and each experiment was repeated three times. Mean was calculated over 3 repeats of the experiment and analyzed statistically using CRBD [16].

**Table 1.** Cultivated, wild and interspecific hybrids of *Arachis* species

| Genotype | Type | Ploidy | Growth habit |
|---|---|---|---|
| CS 19 | Cultivated | 2n = 4x = 40 | Annual |
| GG 2 | Cultivated | 2n = 4x = 40 | Annual |
| J 11 | Cultivated | 2n = 4x = 40 | Annual |
| TAG 24 | Cultivated | 2n = 4x = 40 | Annual |
| TMV 2 | Cultivated | 2n = 4x = 40 | Annual |
| JL 24 | Cultivated | 2n = 4x = 40 | Annual |
| *A. pusilla* | Wild sp. | 2n = 2x = 20 | Perennial |
| *A. appresipilla* | Wild sp. | 2n = 2x = 20 | Perennial |
| *A. rigoni* | Wild sp. | 2n = 2x = 20 | Perennial |
| *A. glabrata* | Wild sp. | 2n = 4x = 40 | Perennial |
| *A. monticola* | Wild sp. | 2n = 4x = 40 | Annual |
| J11 x *A. kemf-mercadoi* | Interspecific $F_1$ hybrid | 2n = 3x = 30 | Perennial |
| J11 x *A. kret-mercadoi* | Interspecific $F_1$ hybrid | 2n = 3x = 30 | Perennial |
| J11 x *A. corre-ntina* | Interspecific $F_1$ hybrid | 2n = 3x = 30 | Perennial |
| J11 x *A. helodes* | Interspecific $F_1$ hybrid | 2n = 3x = 30 | Perennial |

## Results and discussion

Anthers of 15 genotypes were cultured in the MS medium for callogenesis. Anthers started callusing four to five days after culturing irrespective of ploidy level and genotype. The colour and structure of callus varied with genotypes and culture period (Table 2). Occasional root formation in the callus was observed after four weeks of culture. Calogenesis ranged from 21% to 72% among the genotypes (Table 2). Genotype TMV 2 recorded highest callogenesis (72%) followed by JL 24 (65%), *A. monticola* (60%) and *A. glabrata* (49%). Though TMV 2, JL 24, *A. monticola* and *A. glabrata* produced significantly higher callogenesis over the mean callogenesis of the population however, TMV 2, JL 24 and *A. monticola* produced more than 50% callogenesis. Hence, the medium can be effectively used for androgenic callogenesis of these 3 genotypes. Callogenesis was further compared based on domestication (cultivated, wild and hybrid) as well as ploidy of genotypes. Genotypes, TMV 2 (72%), *A. monticola* (60%) and J11 x *A. kretschmeri* (42%) recorded highest callogenesis among cultivars, wild species and hybrids respectively (Figs. 1 and 2). Parcent

**Table 2.** Callogenesis from anther of 15 genotypes

| Genotype | No. of anthers cultured | Calli induced (%) | Nature of callus |
|---|---|---|---|
| CS 19 | 150 | 36 | GF |
| GG 2 | 150 | 38 | DBF |
| J 11 | 150 | 38 | YGC |
| TAG 24 | 150 | 30 | GFY |
| TMV 2 | 150 | 72 | GC |
| JL 24 | 150 | 65 | YF |
| *A. pusilla* | 150 | 29 | GF |
| *A. appresipilla* | 150 | 39 | DBC |
| *A. rigoni* | 150 | 39 | DBF |
| *A. glabrata* | 150 | 49 | LBF |
| *A. monticola* | 150 | 60 | LB |
| J11 x *A. kemf-mercadoi* | 150 | 21 | YF |
| J11 x *A. kretschmeri* | 150 | 42 | NGC |
| J11 x *A. correntina* | 150 | 27 | DGC |
| J11 x *A. helodes* | 150 | 41 | GC |
| Mean | 150 | 41.73 | |
| SE ± | | 1.27 | |
| CD | | 2.64 | |

B-brown, C-compact, D-dark, F friable, G-green, L-light, N-nodular, Y-yellowish callus

callogenesis was more in cultivated (46.5%) than wild (43.6%) and hybrid (32.75%) groups. Callogenesis differed significantly between cultivated, wild and hybrid. Similarly, percent callogenesis in tetraploid, 2n = 4x = 40 (48.5%) was higher than triploid, 2n = 3x = 30 (35.6%) and diploid, 2n = 2x = 20 (32.7%) genotypes (Figs. 1 and 2). Callogenesis differed significantly between

**Table 3.** Induction of Anther Embryo and shoot induction in anther calli of cultivated genotypes

| Geno-type | No. of callus tested | Callus responded to organo-genesis(%) | Mean shoot development/ callus | Total plants regene-rated |
|---|---|---|---|---|
| GG 2 | 50 | 100 | 1.28 | 26 |
| TAG 24 | 50 | 20 | 0.22 | 0 |
| J 11 | 50 | 14 | 0.28 | 2 |
| TMV 2 | 50 | 6 | 0.12 | 0 |
| Mean | 50 | 35 | 0.47 | 7 |
| SE± | - | 1.35 | 0.12 | - |
| CD | - | 2.82 | 0.25 | - |

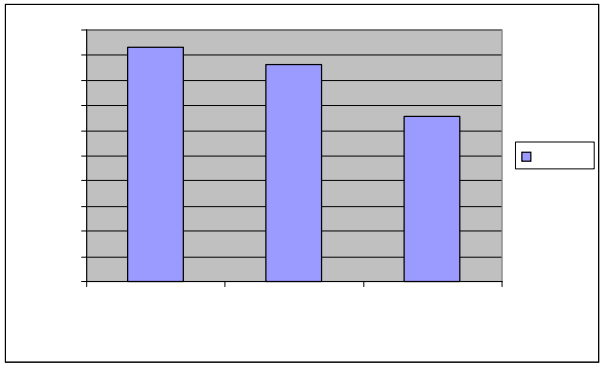

**Fig. 1. Mean callus induction in 3 group of genotypes**

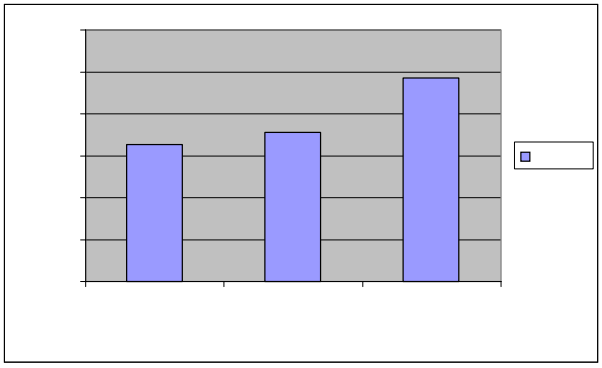

**Fig. 2. Mean callus induction in 3 ploidy levels**



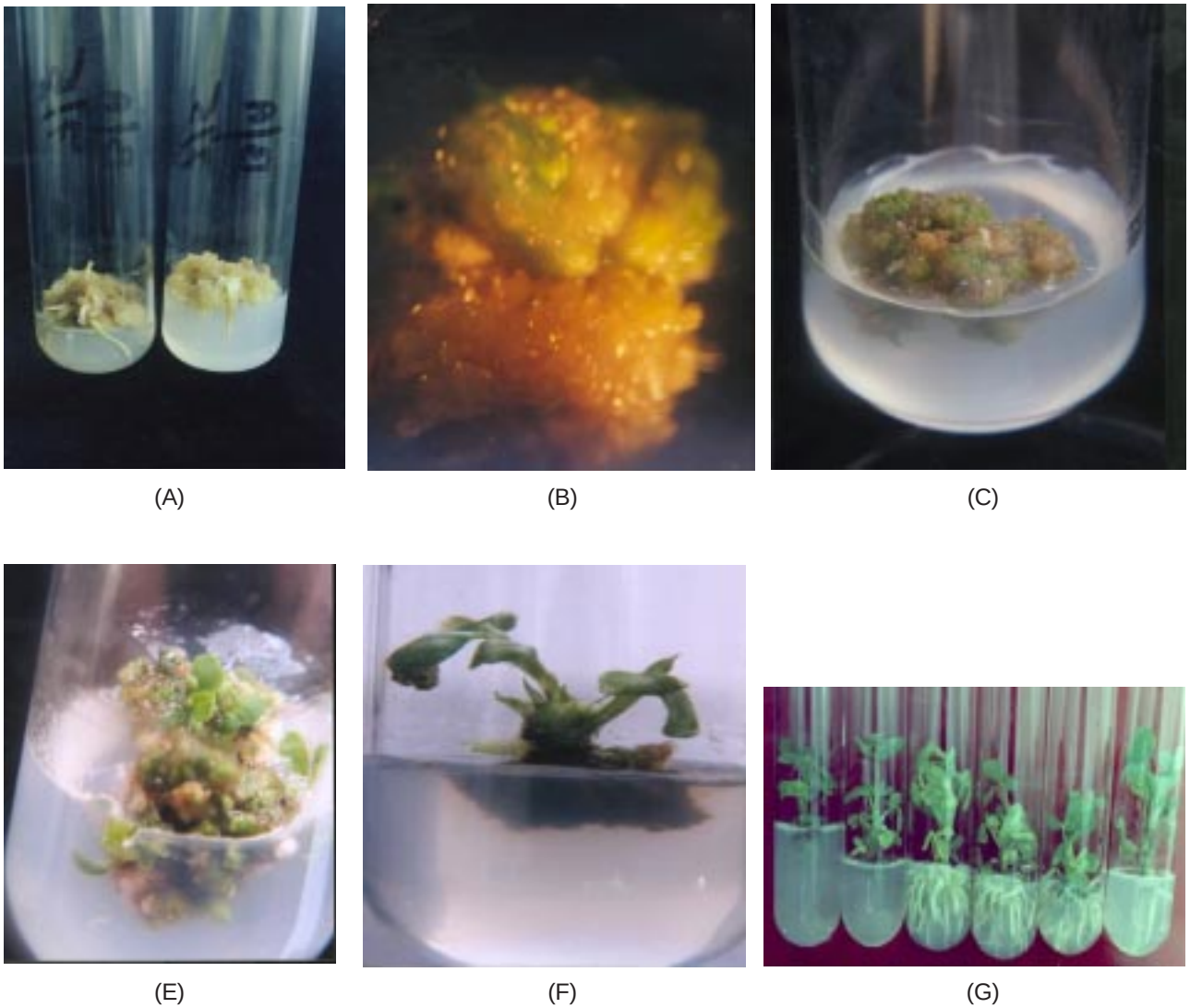


**Fig. 3. Regeneration of plantlets from anther derived callus of *Arachis hypogaea* L., A - Induce callus with root formation, B - Anther Embryo, C - Organogenesis from Anther Embrya, D - Young shoot tips, F - Growing shoots and G - Plantlets**

tetraploid, triploid and diploid groups. The medium tested [19] for callogenesis may efficiently be used for high frequency induction of anther calli in both cultivated and wild *Arachis* species while, may require refinement for triploid hybrids. The 2 week old calli of all genotypes under study were subcultured in differentiation medium [20]. The calli of GG 2, J 11, TAG 24 and TMV 2 started differentiating after three weeks of culture while, calli of hybrids and wild species did not differentiate even after six months of sub culturing in the same medium indicating that further refinement of medium is needed for regeneration of anther calli of wild species and interspecific hybrids. Regeneration of calli of GG 2, J 11, TAG 24 and TMV 2 varied from 6 to 100% (Table 3). The calli of GG 2 produced 100% shoot induction followed by TAG 24 (20%), J 11 (14%) and TMV 2 (6%). Organogenesis from calli differed significantly between genotypes. Mean shoot development/callus varied from 0.13 to 1.28. GG 2 produced maximum shoots/callus (1.28) followed by J 11 (0.28), TAG 24 (0.22) and TMV 2 (0.12%) after four weeks of culture (Table 3). GG 2 produced significantly higher mean shoot/callus (1.28) than other genotypes however, no significant difference was observed between TAG 24, J 11 and TMV 2. Plants generated successfully in both cultivars GG 2 and J 11. Twenty six and 2 plants of cv. GG 2 and J11, respectively were transferred to test tube containing Hogland's solution for hardening and maintained in culture room. Root tip analysis of all regenerated GG 2 and J11 plants showed 2n = 40 chromosomes indicating that anther derived plants were developed either from sporophytic tissue of microspore or spontaneous doubled haploids, needs confirmation.



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