Premature Precocious Hybrid Embryo Development in an Interspecific Derivative between *Arachis hypogaea* and *A. cardenasii*

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Arachis cardenasii is a wild species from section *Arachis* to which cultivated groundnut (*A. hypogaea*) belongs. There are fifteen accessions of *A. cardenasii* in the genebank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and all the accessions are natives of Santa Cruz state of Bolivia. The species in general has many desirable characters such as resistance to fungal foliar diseases (rust, late leaf spot, early leaf spot) and viral diseases (caused by tomato spotted wilt virus and peanut mottle virus). The accession ICG 11558 of *A. cardenasii* is the only source of resistance to peanut stripe virus (Prasad Rao et al. 1991).

Crosses between ICG 11558 and cultivated groundnut produced an average of 29% pods and 47% of the pods had small seeds which were physiologically immature. These pods did not germinate in vivo but when the embryos were dissected and cultured, they germinated in vitro. A total of 18 hybrid plants were obtained.

Pollen fertility in the F_1 hybrids ranged from 4 to 8%. Backcross between the hybrids and cultivated groundnut produced pegs in large numbers (68%) and 8% of the pegs formed pods. In one F_1 hybrid, backcross produced pegs but pod formation was not observed. Instead, when the pegs entered the soil, ovules germinated to form shoots. Well developed shoots emerged out of the soil. Pegs with shoots when placed on the surface of soil grew further and produced flowers. Flowers were tripped to encourage self-fertilization. Pegs were observed from these flowers too. Some of the pegs directly produced shoots instead of pods.

In a normal ovule, fertilization leads to the development of a pro-embryo (stage 1) having few cells. The pro-embryo becomes quiescent but the peg begins to grow by the activation of the intercalary meristem located at the base of the ovule (stage 2). The peg is positively geotropic and enters the soil after growing a few inches (stage 3) (Fig. 1a). Pod formation commences after the peg has entered the soil and then the pro-embryo (Fig. 1b) resumes cell division (stage 4) to form a mature seed.

In the pegs formed on the hybrid, growth of the embryo continued irrespective of peg growth. The embryos continued to grow and form shoots whether the peg was in the soil or not, foregoing the stages 3ñ4 (Fig. 1cñf). Shoots formed in the soil were white but when brought out of the soil these turned green. Many of these shoots set flowers and pegs. Microtomy of the pegs from hybrid plant showed the formation of multiple shoot buds at the peg tip instead of developing seeds. Some of the shoot buds had flower buds (Fig. 1g).

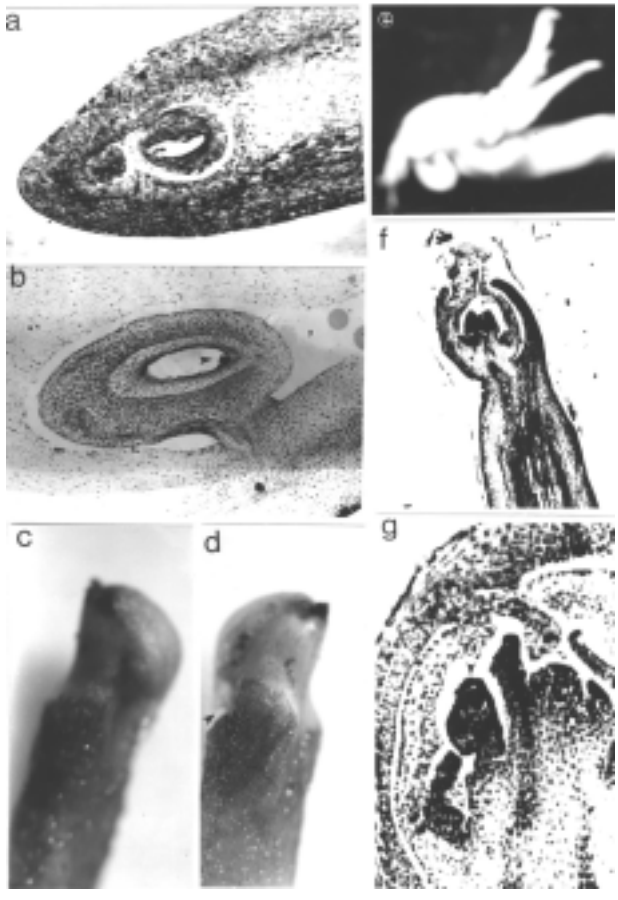

Figure 1. Precocious germination of ovules from the cross *Arachis hypogaea* × *A. cardenasii*: (a) Cross section of *A. hypogaea* peg tip at 15 days after pollination (DAP); (b) Cross section through a single ovule of *A. hypogaea* at 25 DAP (arrow points at the tiny embryo); (c) Swollen peg tip from the cross *A. hypogaea* × *A. cardenasii* at 20 DAP; (d) Swollen peg tip from the cross *A. hypogaea* × *A. cardenasii* at 22 DAP (arrow points at the shoot-like structures); (e) Well developed shoots emerged out of the peg from the cross *A. hypogaea* × *A. cardenasii*; (f) Cross section through the peg with shoots from the cross *A. hypogaea* × *A. cardenasii* at 25 DAP; (g) Close up of ëfí (arrow indicates presence of anther-like structures).

It will be of interest to check if precocious germination of hybrid pro-embryo is a heritable trait. Although such a phenomenon has been observed in interspecific crosses, only shoot buds were seen. But for the first time, it was observed that shoot buds produced flowers and pegs were formed. Sixty percent of the pegs formed shoots on this hybrid plant. The phenomenon of precocious germination of embryo can be exploited for rapid advancement of interspecific derivatives. Interspecific derivatives in general and those between *A. hypogaea* and *A. cardenasii* in particular take a minimum of 60 days for seed maturation and another 6 months for vegetative growth before the initiation of flowers. By the application of this method two generations can be obtained within 3 months thus completing the generation faster than ever achieved before.

Reference

Prasad Rao RDVJ, Reddy AS, Chakrabarty SK, Reddy DVR, Rao VR and **Moss JP.** 1991. Identification of peanut stripe virus resistance in wild *Arachis* germplasm. Peanut Science 18:1ñ2.

Screening Advanced Breeding Lines of Groundnut for Resistance to In Vitro Seed Colonization by *Aspergillus flavus*

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One of the serious food quality problems associated with groundnut (*Arachis hypogaea*) and its products is the aflatoxin contamination by *Aspergillus flavus* not only in the field but also during drying, storage and transit. Aflatoxin causes liver cancer in livestock as well as in human beings. Management of aflatoxin contamination requires both preventive and curative approaches throughout crop production during sowing, harvesting, processing and storage. Lack of single effective control measure further enhances the risk of aflatoxin contamination. Resistant variety is an essential component of any integrated disease management system and is considered as one of the viable and economic approaches to reduce aflatoxins in groundnut (Swindale 1989). This study was undertaken to screen a set of 30 advanced groundnut breeding lines (known to possess resistance to different stresses) for resistance to in vitro seed colonization by *A. flavus* (IVSCAF). Two entries TMV 2 (susceptible) and J 11 (resistant) were included as checks.

Sixty sound matured seeds (with intact seed coat) from each of the 30 entries were surface sterilized with 0.1% aqueous solution of mercuric chloride for 2 min and washed twice with sterilized distilled water. Seeds were uniformly wounded by pricking with a sterile needle to facilitate the invasion by *A. flavus* (isolate Af 11-4) spores. Seeds were placed in a sterilized petri dish (9 cm diameter) and spray inoculated with *A. flavus* spore suspension at 1×10^6 spores ml^{-1} and incubated at 25±1∞C under high humidity (>95% relative humidity) and in dark for 10 days. The experiment was conducted in two replications with 30 seeds per replication. Individual seeds were scored for surface colonization using 1ñ4 rating scale (Thakur et al. 2000) and the mean of two replications was expressed as colonization severity. Based on the reaction of genotypes to *A. flavus* seed colonization in the preliminary screening assay, nearly 67% of the genotypes showed susceptible reaction with high seed colonization comparable to the susceptible check TMV 2 (Table 1). Eleven genotypes showed low to moderate levels of seed colonization (<3) revealing their resistance to IVSCAF. Some genotypes show variable reactions in different assays (Bartz et al. 1978), necessitating repeated assays to select genotypes that show high degree of stable resistance.

Of the 11 genotypes, ten genotypes were assessed for the confirmation of resistance in four subsequent screening assays (Table 2). ICGV 87378 was not included in confirmation assays due to lack of seeds for testing. Six genotypes showed consistent reaction in all the four assays with seed colonization severity lower than or equal to J 11 (2.85). Among them, ICGV 86155 (1.20), ICGV 86699 (1.28) and ICGV 96266 (1.40) were significantly superior to the resistant check. ICGV 86699 also combines resistance to late leaf spot, rust, bud necrosis and iron chlorosis (Reddy et al. 1996). ICGV 96266 is resistant to rust and late leaf spot (Motagi 2001). ICGV 86155 is a Spanish bunch with high yield potential and fresh seed dormancy (Upadhyaya et al. 1997).

Genotypes ICGV 96262 (2.32), ICG 1697 (2.82) and R 9227 (2.90) were comparable to J 11 for resistance to IVSCAF. These genotypes are known to possess resistance to multiple stresses (Naidu 2002). All the three genotypes