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In vitro culture of peanut (*Arachis* sp.) pegs

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ABSTRACT

***In vitro* cultured peg tips of 3 selfed *Arachis hypogaea* (L.) cultivars, 2 wild peanut species *A. stenosperma* and *A. duranensis* and of cultivar Silihong pollinated with *A. glabrata* swelled and finally resulted in pods with viable seeds or precociously germinated, producing plantlets within 120-140 days after culture initiation. A success rate of 12.5-60% for selfed peanut and 0.4-5.2% for hybrid pegs was recorded. Totally 18 hybrid seeds and 6 precociously germinated hybrid plantlets were obtained. While most of the F_1 hybrids encountered various barriers leading to premature death, one F_1 plant grew to maturity and set seeds. The peg culture technique was also used to produce peanut mutants. Peg tips of selfed plants were irradiated with 0, 5, 10, 15, 20 Gy gamma rays prior to *in vitro* culture. All the M_1 plants which survived 5 Gy and 10 Gy treatments were identified as mutants. Peg culture technique may be useful in germplasm conservation and exchange, haploid production, mutation induction, genetic transformation and in studies on the development of peg.**

Key words: *Arachis sp.*, cross-incompatibility, gamma irradiation, mutations, peanut, peg culture.

INTRODUCTION

Due to its narrow gene base, cultivated peanut (*Arachis hypogaea* L.) is vulnerable to biotic and abiotic stresses. Fortunately, most of its wild relatives of the same genus have resistance to drought (French and Prine 1998; Murthy and Reddy 1993); pests (Lynch 1990; Murthy and Reddy 1993) and diseases (Murthy and Reddy 1993; Simpson 1995). Although pre-fertilization barriers exist in interspecific crosses, post-zygotic embryo abortion is the main problem (Pattee *et al.* 1988). Only after this cross-incompatibility is overcome, the wild species can be integrated into a varietal improvement programme. *In vitro* cultured ovules and embryos resulting from crosses involving several rhizomatous peanut species and *A. hypogaea* have routinely developed into hybrid plantlets (ICRISAT 1987; Wang and Shen 1991). Nevertheless, hybrid sterility is still a problem to be solved (Wang *et al.*, 1996). On the other hand, induction of mutations has proved to be a powerful tool in peanut germplasm enhancement (Feng *et al.* 1993; Murthy and Reddy 1993). Yet, in most cases, seeds have been irradiated, resulting in chimera production and complicated selection.

The rhizomatous peanut *A. glabrata* is believed to be the most resistant species in the *Arachis* genus for several diseases and pests (SN Nigam, personal communication). Since its first introduction into Florida, USA in 1936, no pests and diseases including nematodes have been identified that cause significant economic losses (French and Prine 1998). This species also shows good tolerance to drought and low soil fertility (French and Prine 1998). In this paper, we report progress in utilizing the incompatible species *A. glabrata* by means of peg culture and the preliminary results from culture of irradiated peg tips from *A. hypogaea*.

MATERIALS AND METHODS

Hybrid peg culture

In hybrid peg culture experiment, peg tips were collected from a peanut cultivar Silihong (Valencia type) pollinated with *A. glabrata* PI 262801(*Sect. Rhizomatosae*) and from selfed cultivars Silihong, Luhua 9 and Jiangban (both of Virginia type), and selfed wild species *A. duranensis* and *A. stenosperma* (both from *Sect. Arachis*) for comparison. The culture media made up of solidified MS or B5 basal salts and vitamins, 60-120 g L^{-1} sucrose and different combination of phytohormones (Table 1) were autoclaved at 1.2 kg cm^{-2} for 15 min. Six to ten days after artificial pollination, pegs were excised and sterilized in 75% ethyl alcohol for 30 sec, followed by 0.2% mercuric chloride for 7-8 min, after which they were rinsed 3 times in autoclaved distilled water under aseptic

conditions. Four peg tips were inoculated in each flask. The cultures were maintained in dark at 26-28°C in an artificial climatic chamber.

Irradiated peg tip culture

In a separate irradiation experiment, peg tips of 2 peanut cultivars, Baisha 1016 (Spanish type) and Luhua 10 (Virginia type), were treated with 0, 5, 10, 15, 25 Gy ^{60}Co gamma rays, and cultured on MS medium supplemented with 4 mg l^{-1} IAA and 4 mg l^{-1} GA. One hundred peg tips per variety per treatment were used. The peg tips were cultured under the same conditions as described for hybrid peg culture.

RESULTS

Hybrid peg culture

Elongation of pegs from the incompatible cross and from selfed peanut could be detected 15 days after culture. Pod formation commenced about 50 days after culture. In a few days afterwards, reticulation on pods gradually became clear, and pod color changed from white into yellow. The duration from culture initiation to harvest of test-tube seeds was 120-140 days.

Effect of media on success rate of culture

Totally 3,136 hybrid pegs were cultured on 17 media, among which 151 pegs (4.8%) swelled and 90 (2.9%) eventually formed pods. However, only 23 pods from 8 media (Table 1) contained seeds or precociously germinated plantlets. The success rate (ratio of the number of seeds and precociously germinated plantlets to the number of pegs cultured) of hybrid peg culture ranged from 0.4-5.2%, averaging 0.8%, as against 12.5-60.0% for selfed peanut cultivars and wild species (Table 1). Therefore, the hybrid pegs were much more difficult to culture than selfed ones.

The growth and development of F_1 hybrids

Hybrid peg culture resulted in a total of 18 seeds and 6 precociously germinated plantlets. The shoot apices of the precociously germinated plantlets stopped growth and their roots started to blacken. Even after removal of the blackened roots and production of induced adventitious roots, none of them survived 10-30 days after transfer to pots. Among the seeds, 7 had well-developed cotyledons but lacked radicles or plumules; 2 had only one cotyledon wrapping the plumules; 2 failed to germinate and only 7 seeds germinated normally and further developed into plantlets with normal appearance. However 6 of them died shortly after transfer to field.

The sole remaining F_1 plant grew slowly at the early stage, having tough branches, narrow leaflets similar to its male parent *A. glabrata*. The pollen grains were of different sizes. At the later stage, the growth became normal and the fertilization rate was high. The shape of the pods and seeds set on the F_1 plant were similar to those from selfed Silihong cultivar.

The plants grown form test-tube seeds of selfed Silihong and the seeds produced by these plants were identical with the cultivar, although some of the seeds germinated slowly.

The hybrid origin of the material has been further confirmed by character identification and leaf peroxidase PAGE (Shen and Wang 1992; Shen and Wang 1995).

Table 1. Rates of success of selfed and hybrid pegs cultured on different media.

Peg tip donor	Basal medium	Phyto-hormones, mg l^{-1} IAA	GA	BA	KN	Sucrose, %	Success rate, %
Silihong	MS	4	-	2	-	6	1.6
pollinated	MS	2	4	-	-	6	3.0
with	MS	4	-	-	2	6	1.1
A.glabrata	MS	4	2	-	-	6	1.1
	MS	2	-	2	-	12	0.4
	B5	2	2	-	-	6	5.2
	B5	2	-	2	-	6	0 5
	B5	2	2	2	-	6	1.1
Silihong	MS	4	-	2	-	6	28.6
	B5	4	2	-	-	6	50.0
Luhua 9	MS	2	-	2	-	6	20.8
	MS	4	-		-	6	60.0
Jiangban	MS	2	-	2	-	6	25.0
	MS	4	-	-	-	6	43.8
A stenosperma	MS	2	-	2	-	6	12.5
A. duranensis	MS	4	-	-	-	6	50.0

Table 2. Effect of irradiation on the response to peg culture in two peanut cultivars.

Dose of irradiation, Gy	Percentage of pegs elongated		Percentage of pegs swelled		Percentage of pods formed		Number of seeds/ plantlet harvested		Number of mutants identified	
	A'	B'	A	B	A	B	A	B	A	B
0	52	57	33	36	29	23	19	17	0	0
5	22	67	10	37	15	13	9	10	5	5
10	8	14	5	11	6	8	3	5	2	2
15	12	16	4	14	8	14	3	4	0	0
25	14	7	0	0	0	1	0	0	0	0

'A=Baisha 1016 B=Luhua 10

Irradiation experiment

Effect of irradiation on peg development

The results of *in vitro* culture of irradiated pegs are given in Table 2. The percentage of pegs elongated, swelled and the number of pods and seeds obtained dropped with the increase in irradiation dose. Pegs from Luhua 10 were less affected by irradiation than those from Baisha 1016.

Effect of irradiation on the development of M_1 plants

Irradiation treatment of the pegs had significant effects on the plants of M_1 generation. The survival rate of the M_1 plants decreased with the increase of irradiation dose. In Baisha 1016 it was 82.4% for 0 Gy, 71.4% for 5 Gy, and 66.7% for 10 Gy and in Luhua 10, 75.0%, 71.4% and 50% respectively. No plants survived the 15 and 25 Gy treatments. But all the plants which survived 5 and 10 Gy treatments were identified as mutants with mutations mainly in plant height, leaf size and colour, and pod shape. As high as 20% of the surviving M_1 plants derived from 5 Gy gamma ray treated Baisha 1016 pegs were sterile. No other sterile plants were detected.

Effect of irradiation on the selection of M_2 plants

Field selection of the M_2 plants indicated that a higher percentage of mutant plants (5.5%) could be selected despite the lower rate of survival of the plants for the 10 Gy treatment when compared with those in the 5 Gy treatment (4.0%). The average rate of selection for gamma ray treated Baisha 1016 was 7.2% and was only 2.4% for gamma ray treated Luhua 10.

DISCUSSION AND CONCLUSION

The results from peg culture of peanut demonstrated the effectiveness of the technique. Culture of pegs may have several advantages over culture of embryos or ovules. It is more convenient. No dissection and no subcultures are needed, thereby avoiding operations possibly damaging to embryos/ovules. Maternal tissue in pegs can absorb nutrients from media and cushion the effects of adversity, favoring embryo development. This technique has the potential in the rescue of embryos that abort in early development. In that case, frequently the ovules and embryos have already aborted before they reach a desired size, suitable for dissection and culture. There are at least 80 wild species within the genus *Arachis* (C.E. Simpson, personal communication), and most of them are incompatible with cultivated peanut. It is therefore possible to utilize these valuable genetic resources through peg culture.

Usually about 60 days are needed for the development of a mature peanut pod after pollination. In contrast, 120-140 days are needed from culture initiation to harvest in our experiment. Using selfed peanut pegs as explant to establish a model system and by studying roles of different hormones, the duration of culture may be shortened and the rate of success raised. The results from hybrid peg culture showed the difficulty in utilizing incompatible peanut species. Besides embryo abortion, the barriers also include the mortality of F_1 plants. Test-tube seeds can be obtained, but they cannot necessarily develop into healthy plants. Additional work is still needed to find solutions to this problem.

When seeds are irradiated, only a portion of the progenies will be mutants (Feng *et al.* 1993). But in the case of irradiated pegs, all the survivors were nothing but mutants and the rate of selection was relatively high. Further studies should be directed towards optimization of irradiation dose and culture conditions with the aim of producing more test-tube seeds and more viable plants.

In addition to the utility mentioned above, the peg culture technique may find its use in such areas as production of haploids (coupled with chemical treatment of peanut flower) and tranformants (using biolistic technology). It will also be useful in studies on the unusual developmental process of peanut pod.

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