CP 207

Utilization of Incompatible Species in *Arachis*: Sequential Hormone Applications

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Abstract

Barriers to interspecific crossability pose serious constraints to gene transfer by sexual means, and several methods are available to overcome these barriers.

Based on knowledge of the reproductive biology and interspecific incompatibility in the genus Arachis, *a range of techniques were tested. Experiments were also initiated to explore the utility of in vitro methods. In vitro pollinations, young ovule cultures, and young ovary cultures have been used to create hybrids from different incompatible crosses in different taxa.*

Applications of hormones to flowers induced peg and pod formation in incompatible interspecific crosses in the genus. Ovules in hormone-induced pods did not develop beyond a certain stage, so ovules and/or embryos from these pods were cultured to raise hybrids.

Résumé

*Utilisation des espèces incompatibles de l'*Arachis *: applications d'hormones séquentielles. Les barrières à l'aptitude aux croisements interspécifiques posent de sérieuses contraintes au transfert des gènes par voie sexuée. Or, plusieurs méthodes existent qui permettent de les surmonter.*

Tout un éventail de techniques, basées sur les connaissances de la biologie de la reproduction et de l'incompatibilité interspécifique dans le genre Arachis *ont été testées. Des essais ont également été entrepris pour étudier l'utilité des méthodes in vitro. Des pollinisations in vitro, des cultures de jeunes ovules et des cultures de jeunes ovaires ont été utilisées pour l'obtention d'hybrides à partir de différents croisements incompatibles dans des taxa différents.*

Des applications d'hormones aux fleurs induisent la formation de gynophores et de gousses dans des croisements interspécifiques incompatibles dans le genre. Les ovules dans les gousses induites par les hormones ne se développant pas au-delà d'un certain stade, il est nécessaire d'avoir recours à des cultures des ovules ou des embryons de ces gousses pour obtenir des hybrides.

Introduction

Interspecific incompatibility in angiosperms has been the subject of several investigations. The topic has recently grown in importance because of greater interest in utilization of germplasm with attributes desirable in the cultivated species.

Transfer of desirable characters between species is often difficult. The most common reason is the failure of either fertilization between the two gametes, or the development of the zygote. During the last three or four decades a number of ways have been found to tackle such problems in different taxa (Sastri 1984).

Many of the wild relatives of *Arachis hypogaea* have been identified as good sources of resistance to several diseases and pests which seriously reduce groundnut yields (Moss 1980, Subrahmanyam et al. 1985, Amin 1985). A few of these wild species are crossable with *A. hypogaea*, but most are not (Gregory and Gregory 1979). Although failure to obtain hybrids from interspecific crosses in the genus *Arachis* was known as early as 1938 (Hull and Carver 1938, Gregory 1946), no concerted attempts have been made to investigate the reasons for this, or to produce hybrids. In the single

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detailed report on embryology in the crosses *Arachis hypogaea* x *A. diogoi* and *A. hypogaea* x *A. glabrata*, Johansen and Smith (1956) found retardation and cessation of embryo and endosperm growth accompanied by hypertrophy of seedcoats and eventual embryo death without differentiation. Murty et al. (1981) observed that fertilization was delayed up to 48 hours after pollination in the incompatible cross, *A. hypogaea* x *A glabrata* and the seed aborted very early.

There are difficulties in following the known methods of overcoming barriers for production of hybrids in the genus *Arachis*.

The geocarpic habit of the genus is one constraint in any manipulation for sexual hybrid production of wide hybrids by sexual means. Unlike other taxa with aerial fruits, in this genus seed development has two phases. In the aerial phase, dominated by geotropic elongation of the gynophore, the proembryo formed after the first few divisions ceases to grow, and development is resumed only after the peg has entered the subterranean phase.

In such crosses so far attempted at ICRISAT and elsewhere, the peg aborted early in its aerial phase when ovules were very small and immature, and rescue by culture as suggested by Martin (1970) was difficult. Success with culture of such immature ovules has been limited to a very few taxa (Sastri et al. 1980); generally the culture requirements for younger ovules are more complex. Culture of very young embryos is more difficult because their dissection is difficult and time consuming. Hybridization by somatic methods is not warranted until sufficient information about isolation, culture, and fusion of protoplasts of groundnut and *Arachis* wild species is obtained. Such investigations have been recently initiated (Oelck et al. 1982, Rugman and Cocking 1985).

Sastri et al. (1982) and Sastri and Moss (1982) reported the production of hybrids by culture of embryos from some incompatible crosses with single hormone treatments. The hormone application was intended to delay peg degeneration but hormones not only prolonged peg survival, they also increased the numbers of pegs formed. However, the assumption that subsequent development would be normal was not realised. A few pods were formed, but these contained very immature ovules, from which very few embryos could be dissected. This report presents results on research to increase pod numbers and ovule sizes, to facilitate in vitro culture.

Materials and Methods

Five cultivars of *Arachis hypogaea* were grown in the screenhouse at ICRISAT Center. Emasculations and pollinations were performed as described by Sastri and Moss (1982). *A. hypogaea* cultivars were always used as female parents while the wild species were used as pollen donors (Tables 1 and 2).

Table 1. Production of pegs and pods in crosses of five cultivars of *A. hypogaea* with *Arachis* sp. PI 276233 following gibberellic acid application (87.5 mg/l).

Cultivar	Pollinations (no.)	Pegs/pollination (%)	Pods/pollination (%)	Pods/peg (%)
Robut 33-1	491	81.9	14.6	17.9
MK 374	707	77.4	32.7	42.2
M 13	54	87.3	1.9	[illegible]
TMV 2	423	76.6	20.6	26.9
Chico	103	81.7	11.6	14.1

To effect peg initiation and elongation, bases of incompatibly pollinated flowers were treated with 87.5 ppm gibberellic acid (GA) as described by Sastri and Moss (1982). In some crosses the gibberellin-induced pegs were subsequently treated with different concentrations of indole acetic acid (IAA) and/or kinetin 10, 15, 20 or 25 days after pollination to examine the effects, if any, of an auxin and/or kinetin on pod set, ovule size, and embryo development. Auxin and kinetin at various concentrations were separately incorporated into lanolin and the lanolin-hormone mixture was applied to the peg bases (proximal to the node).

About 25 to 30 flowers were pollinated on a plant during a period of three to four weeks. All the pods formed were harvested at least 30 days after the last treatment to the pegs obtained from the last pollination on each plant. Details on culture of immature ovules from these pods and culture of embryos are described by Nalini and Sastri (1985).

Results and Discussion

Effect of Gibberellin

Application of GA to the bases of incompatibly pollinated flowers was found to induce peg initiation and growth in a few intersectional crosses which were not earlier successful (Table 2). In

Table 2. Peg and pod production after gibberellin treatment in some intersectional crosses.

Cross	Pollinations (no.)	Pegs/pollination (%)	Pods/peg (%)
Section *Arachis* × Section *Triseminalae*			
A. duranensis (2n = 20) × *A. pusilla* (2n = 20)	33	79	39
A. hypogaea cv Robut 33-1 × *A. pusilla* (2n = 20)	78	46	0
Section *Arachis* × Section *Erectoides*			
A. hypogaea cv Robut 33-1 × *A. rigonii* (2n = 20)	45	64	13
A. hypogaea cv M 13 × *A. rigonii*	18	94	22
A. hypogaea cv TMV 2 × *A. rigonii*	43	86	26
Section *Arachis* × Section *Extranervosae*			
A. hypogaea cv Robut 33-1 × *A. villosulicarpa* (2n = 20)	39	59	3
A. hypogaea cv MK 374 × *A. villosulicarpa* (2n = 20)	9	89	11
Section *Extranervosae* × Section *Triseminalae*			
A. villosulicarpa (2n = 20) × *A. pusilla* (2n = 20)	24	54	46
Section *Arachis* × Section *Rhizomatosae*			
A. hypogaea cv Robut 33-1 × *Arachis* sp. Coll. 9649	82	44	6
A. hypogaea cv Robut 33-1 × *Arachis* sp. Coll. 9797	46	57	2
A. hypogaea cv Robut 33-1 × *Arachis* sp. Coll. 9806	26	62	0
A. hypogaea cv TMV 2 × *Arachis* sp. PI 276233	408	76	20
A. hypogaea cv TMV 2 × *Arachis* sp. Coll. 9649	11	73	0
A. hypogaea cv MK 374 × *Arachis* sp. PI 276233	648	68	32
A. hypogaea cv MK 374 × *Arachis* sp. Coll. 9649	26	42	15
A. hypogaea cv M 13 × *Arachis* sp. PI 276233	75	56	5
A. hypogaea cv Chico × *Arachis* sp. PI 276233	58	66	9
A. hypogaea cv Chico × *Arachis* sp. Coll. 9649	26	73	19

some crosses pods were also formed, notable among these were crosses involving *A. pusilla*, which belongs to a monotypic section and has not so far been crossed with any other species of the genus (Gregory and Gregory 1979).

Cultivar differences in gibberellin-aided crosses of *A. hypogaea* with *Arachis* sp. PI 276233 of section *Rhizomatosae*

Among the five cultivars used for crosses with three members of section *Rhizomatosae*, MK 374 produced most pods, although peg production did not differ between cultivars (Table 2).

Effect of IAA and kinetin on pod set, and ovule size in gibberellin-induced pegs

Pod set on gibberellin-treated incompatibly pollinated flowers in the cross *A. hypogaea* cv Robut 33-1 × *Arachis* sp. PI 276233 ranged from 0 to 36%. It varied from plant to plant and from season to season. On average about 15% of the flowers set pods. When IAA (four concentrations) was applied to the GA-induced pegs on different days after pollination, there was a varying response which appeared to be dependent more upon the day of application than on the concentration (Table 3). All concentrations applied on the 20th day after pollination increased pod set. Most IAA treatments increased ovule sizes, the largest ovules were from 100 ppm IAA on the 20th day (Table 4). Some of these treatments resulted in more, larger ovules for culture.

Kinetin on the 10th day, and at concentrations of 25 ppm, or more, reduced pod production. Some concentrations of kinetin did improve pod set to a maximum of 37.5% of the pegs formed (Table 3). Ovule sizes were also increased (Table 4). There were similar effects on ovule sizes in other cultivars of *A. hypogaea* crossed with the same male parent (Table 5).

The combinations of hormone treatments

Table 3. Pod production (% pods/peg) by *A. hypogaea* cv Robut 33-1 × *Arachis* sp PI 276233 after hormone treatments.

Hormones applied[1] (ppm)		Days after pollination 10	15	20	25
Control					
GA	17.9				
IAA	10	13.3	12.5	25.8	9.1
	25	22.2	16.0	20.0	13.9
	50	12.1	18.9	23.9	33.3
	100	30.8	18.0	20.0	40.0
Kn	1	16.7	18.4	7.0	7.1
	5	0.0	28.6	25.0	8.3
	10	0.0		14.7	37.5
	25	0.0	4.3	0.0	
	50	8.8		10.6	

1 GA = Gibberellic acid (87.5 ppm, aqueous) applied to bases of flowers soon after incompatible pollinations, followed by IAA or kinetin (Kn) at different concentrations in lanolin on different days after pollination

increased pod production and ovule size, but the treatments which are the best for maximum pod production may not be the best for obtaining the largest ovules (Tables 3 and 4). These ovules, however, have to be cultured for subsequent growth (Nalini and Sastri 1985).

Table 4. Ovule lengths (mm) in *A. hypogaea* cv Robut 33-1 × *Arachis* sp PI 276233 after hormone treatments.

Hormones applied[1]		Days after pollination 10	15	20	25
Control					
GA	2.1 mm				
IAA	10 ppm	2.8	2.1	2.9	2.0
	25	2.4	2.2	3.0	2.4
	50	2.5	2.6	2.3	2.5
	100	1.7	2.1	4.8	1.5
Kn	1 ppm		2.3	2.7	2.3
	5		2.1	2.8	2.0
	10			3.8	2.6
	50			3.0	

1. GA = Gibberellic acid (87.5 ppm; aqueous) applied to bases of flowers soon after incompatible pollinations, followed by IAA or kinetin (Kn) at different concentrations in lanolin on different days after pollination.

Table 5. Ovule length (mm) from pods obtained in three *A. hypogaea* cultivars crossed with *Arachis* sp PI 276233 with subsequent hormone treatments.

Hormone treatment[1] (ppm)		dap[2]	MK 374	TMV 2	M 13
Nil			1.6		
GA			2.6	2.3	2.8
GA, IAA	10	10		3.8	
GA, IAA	10	15	3.1		2.8
GA, IAA	25	10		2.5	
GA, IAA	25	15	2.0		
GA, IAA	50	15	2.4		
GA, IAA	100	15	2.8		

1 GA = Gibberellic acid (87.5 ppm aqueous) applied to bases of flowers soon after incompatible pollinations. IAA at different concentrations in lanolin applied to peg bases on different days after pollination

2 dap = days after pollination

Suggestions by Gregory (1946) for embryo culture and by Martin (1970) for ovule culture for hybrid production from incompatible crosses have not been taken up until the present study. In unaided pollinations a few pegs were obtained but the pegs, ovules, and embryos degenerated, and ovules and embryos were too immature to be successfully cultured before degeneration. Martin's (1970) success in culturing very young ovules from aerial gynophores could neither be repeated with the ovules obtained from these crosses, nor even with ovules from selfed pegs (Sastri et al. 1980). Peg production in crosses was insufficient to initiate in vitro experiments to culture ovules or embryos. Gibberellin treatment increased the number of pegs and delayed, if not prevented, their degeneration. The application of gibberellin followed by a further application of IAA or kinetin resulted in increased pod set and larger ovules.

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