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Effects of Growth Regulators on Incompatible Crosses in the Genus *Arachis* L.[1]

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ABSTRACT

Wild species in the genus *Arachis* L. have several desirable characters and are therefore of great interest for use in the genetic improvement of *A. hypogaea* L. but only a few closely related species have been used. There are many others which could not be used because they are incompatible with the cultivated species. This paper reports techniques to produce hybrids from incompatible crosses.

INTRODUCTION

Transfer of characters from a majority of the wild *Arachis* species to *Arachis hypogaea* is hindered to a great extent by reproductive isolation. Gregory and Gregory (1979) have classified the species, of which there are about 50, into seven sections, based on morphological similarities and cross compatibilities. Several of these wild species have been found to be resistant to many of the pathogens and pests affecting the cultivated species (Moss, 1980). The need for genetic control of groundnut diseases has been stressed very recently (Spielman, Burge, and Moss, 1979; Moss, 1980; Singh, Sastri, and Moss, 1980). Earlier attempts at interspecific hybridization in the genus *Arachis* have been largely for taxonomic and phylogenetic studies (Gregory and Gregory, 1979). Most attempts to utilize the wild species have been confined to species in the section ARACHIS, which are freely crossable with, and are closely related to, the cultivated species (Singh *et al.*, 1980). Though there are 42 possible intersectional combinations only in 11 have the intersectional hybrids been produced. Hybrids between *A. hypogaea* or *A. monticola*, both tetraploid species in the same series of section ARACHIS, and species in other sections are very rare. However, Raman (1973; 1976) did report one natural hybrid between *A. monticola* and a RHIZOMATOSAE species and an artificial hybrid between *A. hypogaea* and *A. glabrata*; but these have not been repeated, and the authenticity of these hybrids has been questioned (Gregory and Gregory, 1979; Stalker, 1980).

The tetraploid species in the section RHIZOMATOSAE are particularly interesting as potential parents in crosses with *A. hypogaea* or *A. monticola*; not only are they adapted to the same ploidy level as the cultivated species, but also have been recognized as potential sources of resistance to many pests and diseases, including rust (*Puccinia arachidis* Spegazzini) and mites (*Tetranychus tumidellus* Pritchard and Baker).

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This paper reports evidence for the nature of incompatibility, and the development of a practical method to produce hybrids between tetraploid species of section ARACHIS and species of section RHIZOMATOSAE.

MATERIALS AND METHODS

The taxa used in the present investigations are shown in Table 1.

Plants were grown in insect free conditions in a screenhouse at 25–30 °C and r.h. above 50%. Buds of female parents were emasculated in the afternoon one day before anthesis by pushing the wing petals apart and removing the anthers through a slit in the keel. The emasculated buds were pollinated between 0600 and 0800 hours the following day by depositing fresh pollen of the male parent on the stigma. The humidity was maintained at 70–80% during and for 1 h after pollination by overhead sprinkler irrigation.

TABLE 1

Parents	Species	Identity	Source
Female parents (Section ARACHIS)	*Arachis hypogaea* L. cv. Robut 33–1	ICG 799	Kadiri, A.P., India
	Arachis monticola Krap. *et* Rig.	P.I. 219824 /263393/298364 /298365/331338 /331339	NCSU, U.S.A.
Male parents (section RHIZOMATOSAE)	*A. glabrata* Benth.		TNAU, Coimbatore, India
	A. sp.	P.I. 276233	Reading Univ., U.K.
	A. sp.	P.I. 262848	Reading Univ., U.K.
	A. sp.	P.I. 262844	Reading Univ., U.K.
	A. sp.[a]		TNAU, Coimbatore, India

[a] Rhizomatous glabrous species received as *A. marginata*.

A few pollinated pistils were processed for observing pollen germination and pollen tube growth by fluorescence microscopy according to the technique of Shivanna and Sastri (1981).

The following treatments, were adopted, with a minimum of 25 pistils per treatment.

Mentor pollen was prepared by treating the compatible pollen with anhydrous methanol for about 1 min and then evaporating the solvent. This was mixed with incompatible pollen (1:1) before pollinations. Mentor pollen leachate was prepared according to the method of Sastri and Shivanna (1980) and applied to the stigma before incompatible pollinations.

Three different auxins (indole-3-yl acetic acid (IAA), 1-naphthyl acetic acid (NAA), and 2,4-dichlorophenoxy acetic acid (2,4-D), each at 10, 25, and 50 mg l^{-1}), gibberellic acid (GA) at 10, 22, 43 and 87·5 mg l^{-1} and two cytokinins (6-furfurylaminopurine (Kn), and 6-benzylaminopurine (BAP) each at concentrations 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M were used.

A piece of absorbant cotton wool was wetted with an aqueous solution of the hormone, and wrapped around the base of the hypanthium of the incompatibly pollinated flower (Plate 2A). In one treatment, the cotton wool was rewetted with the same hormone solution daily for 7 d. Two controls were maintained, incompatible pollinations without hormone application and hormone application without pollinations.

Buds were also pollinated 2 d and 1 d before anthesis. Delayed pollinations were performed 1 d after anthesis.

RESULTS

Pollen germination and pollen tube growth

Comparison of pollen behaviour in compatible and incompatible pollinations revealed that in the former, the pollen tubes were characteristically lined with callose and had small, evenly distributed callose plugs (Plate 1A). In the incompatibly pollinated pistils the tubes were

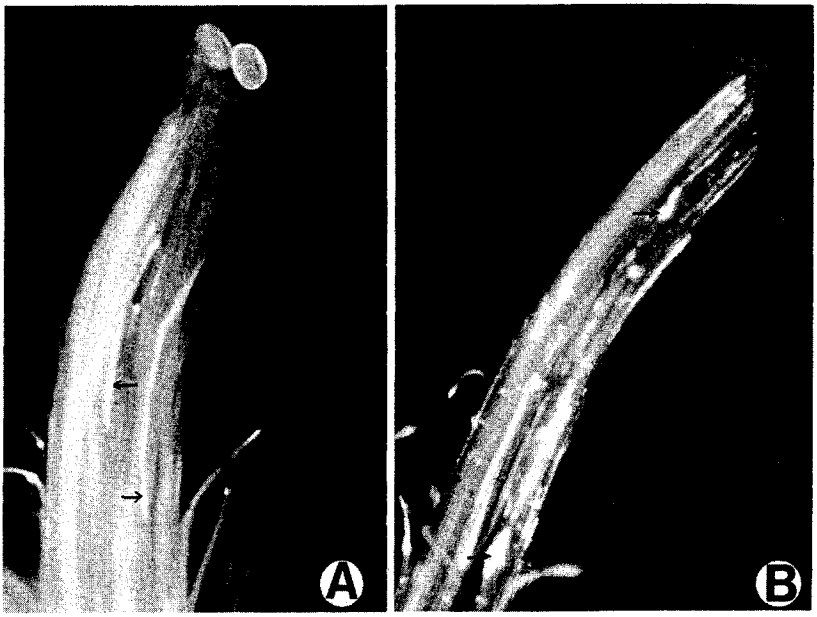

PLATE 1. Aniline blue fluorescence in compatibly and incompatibly pollinated pistils in *Arachis* (×180). (A) Pollen tubes in *A. monticola* pistil pollinated with *A. monticola* pollen. Note the small callose plugs (arrows) evenly distributed in the pollen tubes. (B) Pollen tubes in *A. monticola* pistil pollinated with *A.* sp. P.I. No. 276233 (RHIZOMATOSAE). Note irregularly distributed large callose plugs (arrows) in pollen tubes; compare with those in 1A.

abnormal, being swollen in places due to large callose plugs irregularly distributed along the pollen tubes (Plate 1B). However, in some pistils pollen tubes were observed in the ovary after incompatible pollinations. When flowers were pollinated 1 d after anthesis, or buds pollinated 2 d or 1 d before anthesis, there was no germination of pollen, even in controls pollinated with compatible pollen, indicating that the stigma is not receptive before or after the day on which anthesis occurs.

Gynophore formation after incompatible pollinations

A few gynophores (pegs) (up to 21% in one combination, Table 2) were formed after incompatible pollinations. However, all these pegs degenerated before they grew long enough to enter the soil, and no pods were obtained.

Effects of mentor pollen and mentor pollen leachate

Mentor pollen was used in three crosses and increased the number of pegs formed in two of them. Mentor pollen leachate was applied in only one cross and was found to increase pegging considerably. However, the pegs dried before penetrating the soil as occurred in the untreated controls and in the mentor pollen treatment (Table 2).

Effect of hormones

(a) *Peg initiation and elongation:* No pegs were produced following hormone treatments of emasculated unpollinated flowers. IAA and 2,4-D were ineffective when applied to *A. monticola* or *A. hypogaea* used as female parents in incompatible crosses, but pegs were formed after treatment with kinetin, GA and NAA. NAA at 25 mg l^{-1} produced twice the number of pegs as compared with the controls when *A. hypogaea* or *A. monticola* was crossed

TABLE 2 Per cent pegging in intersectional crosses in *ARACHIS*

Crosses / Method	*monticola* × P.I. No. 276233	*monticola* × *glabrata*	*monticola* × P.I. No. 262848	*monticola* × *A.* sp. (Coimb)	*monticola* × P.I. No. 262844	*hypogaea* × P.I. No. 276233	*hypogaea* × *glabrata*	*hypogaea* × P.I. No. 262844
Control[a]	19	0	6	0	21	16	9	
Mentor pollen[a]	15	3	NA	9	NA	NA	NA	NA
Mentor pollen leachate[a]	NA	22	NA	NA	NA	NA	NA	NA
Mentor pollen leachate + kinetin 10^{-6} M	NA	53	NA	NA	NA	NA	NA	NA
Kinetin 10^{-5} M	16	1	NA	NA	NA	36[b]	NA	NA
Kinetin 10^{-6} M	21	15[b]	28	14	NA	46[b]	NA	NA
Kinetin 10^{-7} M	16	NA	NA	NA	NA	62[b]	NA	NA
Gibberellic acid 87·5 mg l^{-1}	89	***	55	60	***	33	72	58
Kinetin 10^{-7} M + gibberellic acid 87·5 mg l^{-1}	41	***	***	***	***	11	38	8
Naphthalene acetic acid 25 mg l^{-1}	33	***	NA	***	***	42	0	NA

[a] The pegs dry before penetrating the soil.
[b] Some embryos cultured and plants obtained.
*** Current emphasis is being placed on NAA, GA, Kn and hormone mixture.
NA, Not attempted.

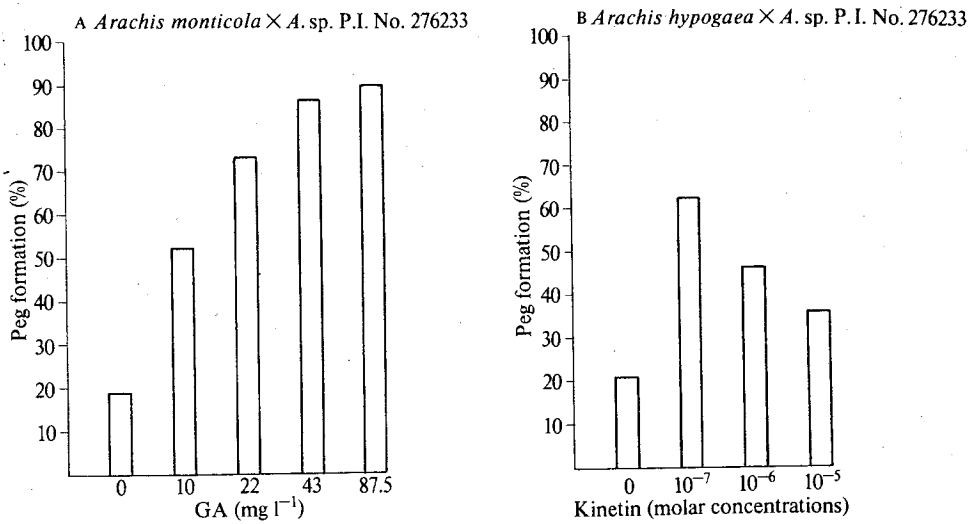

FIG. 1. (A) Effect of gibberellic acid (4 concentrations) on percent peg formation in incompatible pollinations, *A. monticola* × *A.* sp. P.I. No. 276233. (B) Effect of kinetin (3 concentrations) on peg percentages in *A. hypogaea* cv. Robut 33-1 × *A.* sp. P.I. No. 276233, an incompatible cross.

with *A.* sp. P.I. No. 276233. The kinetin concentrations applied were ineffective with *A. monticola* as the female parent, but were effective in *A. hypogaea* × *A.* sp. P.I. No. 276233. The number of pegs formed in this cross was dependent on kinetin concentration and increased to a maximum of 62% at a concentration of 10^{-7} M (Fig. 1B).

Gibberellic acid application at four concentrations stimulated peg formation; this depended on concentration (Fig. 1A), giving optimum results of 90% pegs in *A. monticola* × *A.* sp. P.I. No. 276233 at the highest concentration used (87·5 mg l^{-1}). GA at 87·5 mg l^{-1} increased peg formation in all six crosses tried.

Most pegs formed following hormone treatments elongated and penetrated the soil.

(b) *Effect of hormone mixtures:* Flowers were treated with a mixture of two different hormones at concentrations which were found to be effective in the previous experiment. Of the combinations used (Kn 10^{-7} M + NAA 25 mg l^{-1}; Kn 10^{-7} M + GA 87·5 mg l^{-1}; GA 87·5 mg l^{-1} + NAA 25 mg l^{-1}) only the combination of GA and Kn was effective in inducing peg formation; however it was less effective than GA alone (Table 2).

(c) *Pod initiation and development:* Most pegs which developed after hormone treatments elongated sufficiently to penetrate the soil. To assist penetration of pegs, branches were held close to the soil surface by wire loops, and were periodically observed for pod initiation and development.

The ratio of pods initiated to pegs formed depended on the hormone used (Table 3). Kinetin at all three concentrations stimulated pod formation in up to 53% of pegs formed. NAA at 25 mg l^{-1} also produced pods in about 42% of the pegs. Most of these pods developed slowly, and very rarely reached maturity (Plate 2B). Pods with slowly developing healthy ovules were observed after kinetin treatments and most of these pods had embryos at heart stages. The embryos and ovules very rarely developed beyond this stage in any of the treatments. Most pods degenerated if left in the soil for more than 60 d, and in subsequent treatments they were therefore removed for embryo culture before this occurred. In compatible crosses between cultivars of *Arachis hypogaea* the pods reached maturity in about 40 d after pollination.

TABLE 3. *Effect of some auxins and kinetins on pegging and pod set in an incompatible cross* A. hypogaea *cv. Robut 33–1* × A. *sp. P.I. No. 276233*

Treatment control	No. of pollinations	No. of pegs		Number of pods			Pods/pegs (%)		
		No.	%	Mature	Immature	Total	Mature	Immature	Total
Kn 10^{-5} M	81	21	26	2	7	9	10	33	43
Kn 10^{-6} M	89	19	21	3	7	10	16	37	53
Kn 10^{-7} M	87	37	43	2	17	19	5	46	51
Kn 10^{-7} M IAA 25 mg l^{-1}	158	36	23	2	2	4	6	6	11
IAA 10 mg l^{-1}	91	8	9	0	2	2	0	25	25
IAA 25 mg l^{-1}	155	22	15	0	4	4	0	18	18
IAA 50 mg l^{-1}	93	4	4	0	2	2	0	50	50
NAA 10 mg l^{-1}	52	10	19	0	7	8	0	70	80
NAA 25 mg l^{-1}	108	31	29	1	10	13	10	32	42
NAA 50 mg l^{-1}	27	1	4	0	0	0	0	0	0

Hybrid plants

In preliminary experiments, pegs were left in the soil for long periods, and mature pods were very rarely formed. Those that did mature were harvested and their seeds sown. A seed from a pod formed following the cross *A. monticola* × *A.* sp. P.I. No. 276233 (with Kn 10^{-6} M treatment) germinated and produced a plant (Plate 2D). Its hybrid nature was confirmed by morphological and electrophoretic studies. This plant survived for 22 months, 12 months longer than its female parent (Sastri and Moss, 1981). Similarly, a hybrid between *A. hypogaea* and *A.* sp. P.I. No. 276233 survived for 11 months, at least 6 months longer than its female parent.

Embryo culture

Pods were removed from the plants about 60 d after pollination, and their embryos cultured when possible. Most treatments produced pods which were thick-shelled and contained immature ovules. Attempts to culture such ovules have been unsuccessful (Sastri, Nalini, and Moss, 1980), although the technique used was that reported by Martin (1970) to be suitable for ovule culture.

Embryos at heart and early cotyledonary stages have been cultured from 20 pods, 14 of which have given rise to plants (Plate 2C). Embryos at an earlier stage have been dissected and cultured; callus has been observed in very rare cases but this always becomes necrotic. If callus growth from such very young embryos can be maintained, hybrid plants could be generated in much larger numbers, as has been done from callus derived from other tissues (Sastri, Nalini, and Moss, 1981).

DISCUSSION

Reproductive isolation of a number of wild species in the genus *Arachis* has often been mentioned (Hull and Carver, 1938; Gregory, 1946; Smartt and Gregory, 1967; Gregory and Gregory, 1979) but no concerted attempts to develop techniques for successful hybridization have been made. This is probably due to lack of sufficient information on the nature of the incompatibility. All that is known is that there is a hypertrophy of integuments in such crosses (Johansen and Smith, 1956; Murty, Rao, Kirti, and Bharathi, 1980). There are also pre-fertilization barriers in wide crosses in *Arachis*, which do not allow a normal interaction

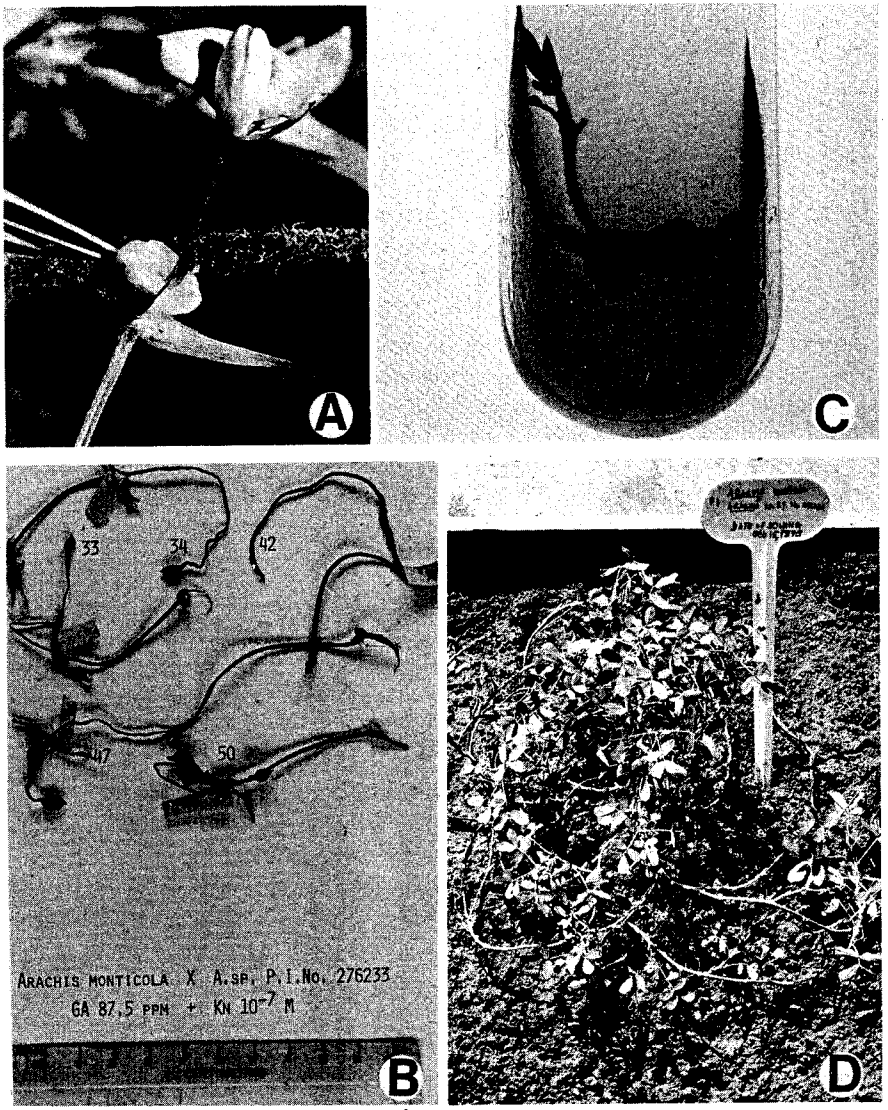

PLATE 2. Efficacy of hormones in overcoming interspecific incompatibility in *Arachis*. (A) A flower with its base wrapped with cotton soaked in a hormone solution (× 1·0) (B) Slow pod development in hormone-treated incompatibly pollinated flowers. *A. monticola* × *A.* sp. P.I. No. 276233 (×0·6). (C) A hybrid seedling by culture of immature embryos or slowly developing pods from hormone-treated incompatible pollinations *A. hypogaea* cv. Robut 33–1 × *A. glabrata*, treated with kinetin 10^{-7} M (×2·0). (D) *A. monticola* × *A.* sp. P.I. No. 276233, F_1 hybrid 15 months old (×0·18).

between pollen and pistil, as revealed by fluorescence microscopy (Anon, 1981). This results in a delay in fertilization and reduces the frequency of fertilization. This conclusion is also supported by the observations of Murty *et al.* (1980), who found that fertilization occurred even 48 h after pollination, in contrast to 6 h in compatible pollinations.

The present studies have created the possibility of producing hybrids from these much desired crosses by the use of plant growth hormones.

Hormone application was adopted when the preliminary attempts with other techniques did not yield satisfactory results, as hormones have been used profitably in some incompatible

crosses in a number of other genera (Nettancourt, 1977). Further, the post-fertilization morphogenesis of the ovaries into fruits has been shown to be hormonally regulated in a number of plants. There have been reports on the use of some plant hormones on the vegetative and reproductive growth of groundnut plants (Amir, 1969; Ketring and Schubert, 1980, 1981) but their uses in incompatible crosses have not been attempted or suggested. Ziv and Zamski (1975) have discussed some factors for pod formation in *A. hypogaea in vitro* and *in vivo*, and have attributed a role to hormones. They found that kinetin at 0·1 mg l^{-1}, with NAA at the same or lower concentration, promoted elongation of cultured selfed gynophore tips, but at higher concentrations pod formation was observed.

In the present study it was not the objective to determine the hormonal control mechanisms in geocarpy (peg and pod formation) but to be able to produce hybrids between incompatible species in order to transfer pest and disease resistance from wild species to *A. hypogaea.*

The conclusions that can be drawn from these experiments are that hormones can be used to induce pegs and pods in incompatible crosses between sections ARACHIS and RHIZOMATOSAE[1] and that peg initiation and elongation, and pod development are under different hormonal influences. One significant effect of hormonal application is to prolong the life of the peg allowing the hybrid embryo to develop further *in vivo*, thus increasing the chances for successful manipulation under *in vitro* conditions. Gibberellin has been found to increase peg numbers as well as their lengths after incompatible pollinations. Kinetin, although it does not produce as many pegs per 100 pollinations as GA, does stimulate more pegs to produce pods (Table 3). This suggests that the major effect of GA is on peg production, whereas kinetin influences pod production. The concentrations and types of auxins used have not given results which denote a specific role for auxins, although Jacobs (1951) reported that auxin application to the gynophore inhibited gynophore elongation. However, there are some indications that auxins also have a role in pod and seed development. NAA for example (at 25 mg l^{-1}) stimulated pod development on 32% of pegs (Table 3), while with IAA (at 50 mg l^{-1}), although only four pegs were formed, two of these produced pods.

Attempts are being made to elucidate the hormonal factors favouring normal pod and embryo development *in vivo* so that a larger number of pods with well developed seeds can be obtained from incompatible crosses. This would facilitate the production of a large number of hybrid plants to be incorporated into a breeding programme.

[1] In ARACHIS × TRISEMINALE (the latter a monotypic section) also.

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