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INTERGENERIC HYBRIDIZATION IN PIGEONPEA. I. EFFECT OF HORMONE TREATMENTS

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ABSTRACT

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Attempts to cross *Cajanus cajan* with *Atylosia albicans*, *A. cajanifolia*, *A. grandifolia*, *A. mollis*, *A. platycarpa*, *A. sericea* and *A. volubilis* resulted in varying degrees of success. Hormone treatment (gibberellic acid and kinetin) increased pod-set and the number of seeds per pod. Hormone treatment among the unsuccessful crosses delayed bud drop by 3—4 days, which prolonged ovule development. Our results indicate that treatment with hormones helps post-fertilization development and leads to improvement in the rate of crossing success.

INTRODUCTION

The cultivated pigeonpea, *Cajanus cajan* has many related wild species in the genus *Atylosia*. Some of these have desirable characters such as disease and pest resistance (Remanandan, 1980; Reddy et al., 1980), high protein content (Reddy et al., 1979) and photoperiod insensitivity (Ariyanayagam and Spence, 1978) which we would like to transfer to pigeonpea. Earlier attempts at *Cajanus—Atylosia* hybridization have been helpful in the assessment of relationships among species (Reddy, 1973; Pundir, 1981) and in the study of the genetics of pod and seed characters. In these earlier studies the rate of pod-set was low in most of the successful combinations and zero in several other combinations (Pundir, 1981). However, the reasons for the low rate of pod-set among the successful combinations (Reddy et al., 1980; Pundir, 1981) and the failures of other combinations are not known.

The use of gibberellic acid (GA3) in interspecific or intergeneric hybridization was suggested by Larter and Enns (1960) and was used successfully to improve seed set and embryo yield in *Hordeum* interspecific crosses

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(Subrahmanyam and Kasha, 1971; Kasha et al., 1978) and in barley—rye crosses (Kruse, 1967; Fedak, 1977). Other growth regulators, such as cytokinins and auxins, have also been used in other crop plants (Al-Yasiri and Coyne, 1964; Chen et al., 1978; Sastri and Moss, 1982) to increase the rate of success in interspecific crosses.

The use of *Atylosia* species in pigeonpea breeding programmes has been difficult because of problems encountered in obtaining sufficient hybrid seed in successful *Cajanus—Atylosia* crosses, and the lack of compatibility in several other *Cajanus—Atylosia* combinations. The present investigation with hormones was initiated to improve the production of mature seed in successful combinations and to determine the likelihood of producing new *Cajanus—Atylosia* hybrid combinations.

MATERIALS AND METHODS

The species used in crosses are listed in Table 1. Pigeonpea cultivars exhibit wide variation in their ability to cross with *Atylosia* spp. Based on preliminary data, we chose the cultivar 'Pant A-2' because of its better crossability with different *Atylosia* spp.

Seeds of all the *Atylosia* spp. except *A. platycarpa* were sown at the same time, in the field at ICRISAT, during 1981—82. Since *A. platycarpa* flowers in about 60 days, its seeds were sown later, to synchronize its flowering with that of the other species. Flower buds of appropriate size on the female parents were emasculated by hand between 08.00 and 12.00 h and their stigmas immediately dusted with pollen from the male parent.

Gibberellic acid (GA3) and kinetin were used independently at concentrations ranging from 10 μg ml^{-1} to 80 μg ml^{-1} and as a 1:1 mixture at the same concentrations. No. 22 hypodermic needle was used to fill the bud cavity, surrounding the pistil, with the hormone solution. The treatments were given twice, at 24 h and at 48 h after pollination. Care was taken to avoid physical injury to the buds during the hormone application. One hundred pollinations were made for each treatment.

TABLE 1

Species of *Atylosia*[a] used in crosses with *Cajanus cajan* (L.) Millsp.

Species	Accession No.	Habit
A. albicans (W. & A.) Benth.	NKR- 177	Perennial climber
A. cajanifolia Haines	PR-4876	Erect shrub
A. grandifolia F.V. Muell. ex Benth.	PR-4221	Bushy shrub
A. mollis Benth.	JM-4331	Climber
A. platycarpa Benth.	PR-4557	Herbaceous creeper
A. sericea Benth. ex Bak.	JM-1961	Erect shrub
A. volubilis (Blanco) Gamble	JM-4208	Perennial climber

[a]All provided by the Genetic Resources Unit, ICRISAT.

Records were kept of the commencement of bud drop, pod-set, pod length and the number of seeds per pod, for each cross and each treatment.

RESULTS

Crossability of Cajanus cajan *with* Atylosia *spp.*

We obtained different levels of success with the crosses attempted using cultivar Pant A-2 as the female parent. In the absence of hormone treatment 1—7% of the crosses with *A. albicans*, *A. grandifolia*, *A. cajanifolia* and *A. sericea* were successful (Table 2). The rate of success in intercultivar crosses of Pant A-2 ranged from about 18 to 80% averaging 40% depending on the parental genotypes and the skill of the crosser. Crosses of Pant A-2, using *A. mollis*, *A. platycarpa* and *A. volubilis* as pollen parents failed, although two reciprocal crosses viz. *A. mollis* × Pant A-2 and *A. volubilis* × Pant A-2 resulted in normal pod development. However, seeds from these two crosses were extremely shrivelled and did not germinate.

Effect of hormone treatments

The successful cross combinations exhibited a uniform response to hormone treatments. GA3 was found to be superior to kinetin irrespective of the cross combination. For example, when *A. grandifolia* was the pollen parent, pod-set increased from 2% in the control to 14% when treated with 50 μg ml^{-1} of GA3. But higher concentrations of GA3 decreased pod-set. Kinetin treatments showed no consistent response at the concentrations tested. Treatments with the GA3 + kinetin mixture also did not improve pod-set at any concentration and were detrimental at higher concentrations. Similar trends were apparent in crosses of Pant A-2 with *A. albicans*, *A. cajanifolia* and *A. sericea* as male parents (Table 2).

In the four successful crosses, GA3, alone or in combination with kinetin, increased pod length and seeds per pod (Fig. 1). In these crosses the average pod length at physiological maturity was increased from 4.5—5.5 cm in controls, to 7 cm, which is comparable to the size of selfed pods on the female parent without hormone treatment, while the Pant A-2 × *A. albicans* cross was the only one with pods comparable to the selfed pods on Pant A-2. Furthermore, the number of seeds per pod increased from the range of 1.6—2.2 in controls to 3.5—4.0 when GA3 or GA3 + kinetin treatments were given. However, hormone treatments did not influence seed size.

The hybrid seeds from all the successful combinations were viable (41—86% depending upon the cross combination) and all of the F1 plants were partially sterile, ovule sterility ranging from 29—80% depending upon the hybrid.

Among the unsuccessful crosses bud drop commenced within two days

TABLE 2

Percent pod-set in intergeneric crosses of *Cajanus cajan* and *Atylosia* species followed by hormone treatments

Hormone conc. ($\mu g\ ml^{-1}$)	*C. cajan* × *A. albicans*			*C. cajan* × *A. cajanifolia*			*C. cajan* × *A. grandifolia*			*C. cajan* × *A. sericea*		
	GA_3	Kin	GA_3+Kin	GA_3	Kin	GA_3+Kin	GA_3	Kin	GA_3+Kin	GA_3	Kin	GA_3+Kin
Control	7	7	7	2	2	2	2	2	2	1	1	1
10	8	8	6	3	2	1	1	3	1	2	0	1
20	8	5	8	2	3	2	2	2	2	3	2	1
30	10	7	7	4	2	1	4	2	1	3	2	2
40	13	6	8	11	3	1	5	1	0	7	3	1
50	17	8	3	7	3	0	14	2	0	5	2	1
60	4	5	1	3	2	0	3	3	0	10	1	0
70	1	4	0	0	2	0	0	2	0	0	2	0
80	0	5	0	0	1	0	0	2	0	0	1	0

after pollination (Fig. 2a). GA3 treatment prolonged ovary development and delayed bud drop by varying periods depending upon the cross combination and the concentration of the hormone treatment (Fig. 2a). For example, when *A. platycarpa* and *A. volubilis* were pollen parents, bud drop commenced two days after pollination in the control. GA3 prolonged this period up to 5 days when used at 60 μg ml^{-1} in *A. volubilis* and when used at 50 μg ml^{-1} in *A. platycarpa*. When *A. mollis* was the male parent bud drop was delayed by 3—4 days. Increase in ovule size was evident in those cross combinations where bud drop had been delayed following hormone applications.

In all the reciprocal crosses there was variation in response to hormones (Fig. 2b). *A. sericea* and *A. grandifolia* failed to respond to any of the three hormone treatments. In *A. cajanifolia* GA3 delayed bud drop while the kinetin and GA3 + kinetin treatments were ineffective, a trend which was similar to that observed in unsuccesssful *Cajanus* crosses. Kinetin at a low concentration (10 μg ml^{-1}) prolonged ovary development by three days in *A. platycarpa* and four days in *A. albicans*.

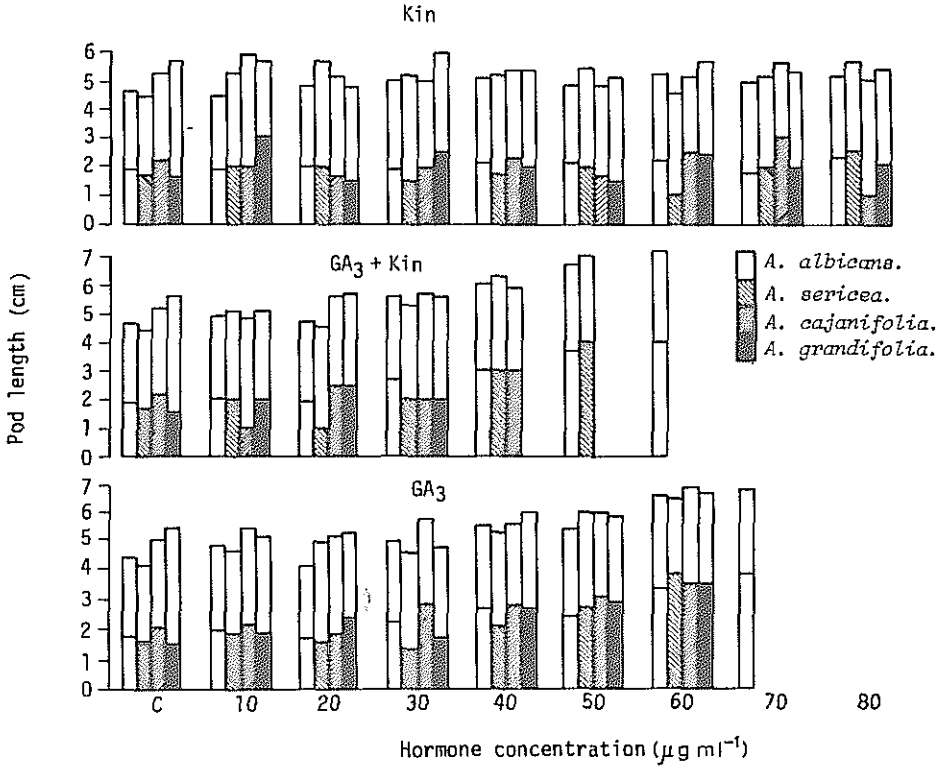

Fig. 1. Effects of gibberellic acid and/or kinetin on pod length and seed set in four *Cajanus—Atylosia* crosses. Insert in pod length = seeds per pod (1 seed = 1 cm).

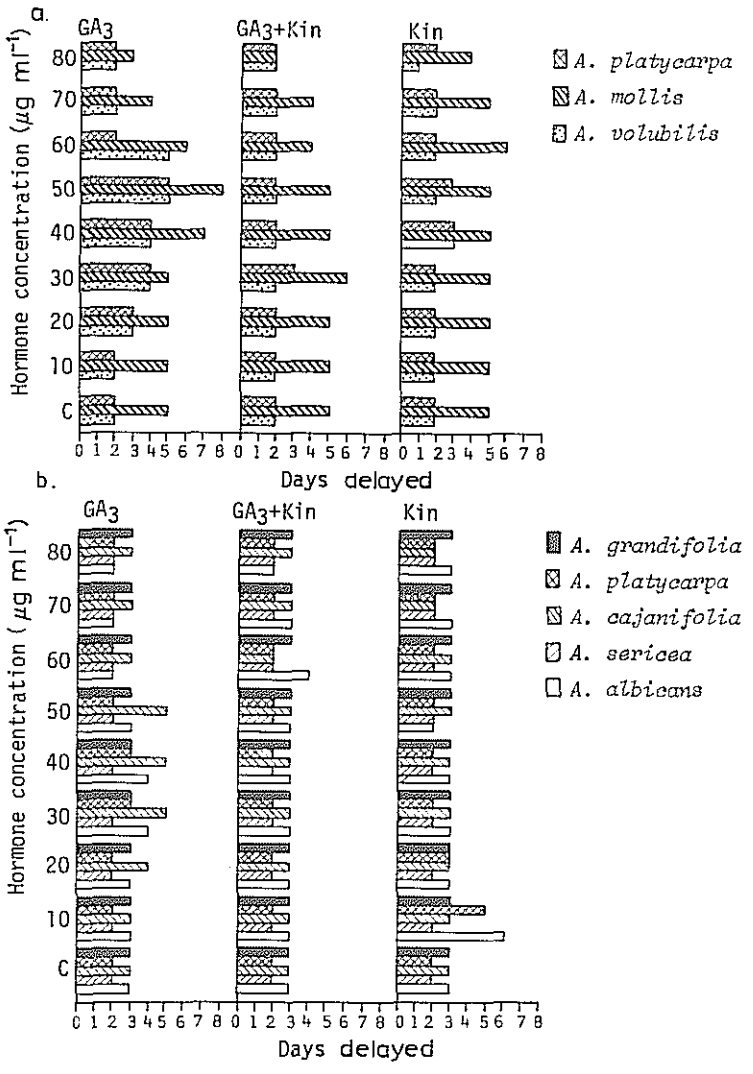

Fig. 2. Effects of gibberellic acid and/or kinetin on delaying bud abscission, with (a) *Cajanus cajan* as female parent; (b.) *Cajanus cajan* as male parent.

DISCUSSION

The results show that in the intergeneric crosses of *Cajanus* and *Atylosia* the rate of success can be increased with hormone treatments. These increase the percentage of pod-set, pod length and number of seeds per pod in the cross combinations. Moreover, the onset of flower drop among unsuccessful crosses was delayed following hormone treatments. The success rate in the untreated controls was low.

The increased pod set associated with most of the GA3 or GA3 + kinetin treatments is likely to be due to enhanced post-fertilization development of both ovary and ovule(s), since treatments commenced one day after pollination, when fertilization would have been completed. The increase in pod length and number of seeds per pod following hormone treatments further substantiate such an interpretation. Higher concentrations of GA3 and GA3 + kinetin reduced the rate of success, but promoted pod length and the number of seeds per pod, wich again indicates the role of the hormones in post-fertilization development.

Kruse (1967) and Kasha et al. (1978) attributed increased success in obtaining progeny from intergeneric or interspecific crosses to the check on post-fertilization breakdown exerted by exogenous GA3 supplied to the florets. The increase in pod length in our *Cajanus* × *Atylosia* crosses may reflect increased cell number and/or cell elongation in the ovary wall after hormone treatments. Kasha et al. (1978) observed increased mitotic activity following GA3 treatments in interspecific crosses of *Hordeum*. Sastri and Moss (1982) found increases in peg number as well as peg length following GA3 treatments in *Arachis* interspecific crosses. Al-Yasiri and Coyne (1964) also recorded a positive influence of hormones on pod length and diameter in a *Phaseolus* spp. cross. These results are consistent with our findings in pigeonpea intergeneric crosses.

The failure to increase further the number of successful cross combinations emphasizes the role of hormones only in the post-fertilization processes. Since hormones did not increase the size of mature seeds, the increase in ovule size in unsuccessful crosses is most likely due to the delay in bud drop, permitting the ovule to develop for a longer period. In the unsuccessful combinations GA3 treatments helped to delay the commencement of flower bud abscission. In crosses between *Phaseolus vulgaris* and *P. acutifolius*, a combination of GA3 and NAA was used to overcome pod abscission (Al-Yasiri and Coyne, 1964). Emsweller and Stuart (1948) reported the influence of growth regulators on retarding the senescence of the embryo sac, and on the length and diameter of capsules in *Lilium longiflorum*. Although kinetin treatments (10—80 $\mu g\ ml^{-1}$) did not improve pod-set or the number of seeds per pod, treatment with the lowest concentration (10 $\mu g\ ml^{-1}$) was effective in delaying bud drop in the *A. albicans* × Pant A-2 and *A. platycarpa* × Pant A-2 crosses.

The data show that, among the crosses studied here, response to hormone treatments is determined by the female parent, irrespective of the pollen parent used. The response of *A. cajanifolia* as a female parent was similar to that of Pant A-2. These two species have more morphological similarities than do the other species used in this study and are considered one genus by some taxonomists (van der Maesen, personal communication, 1982). In the unsuccessful combinations, in spite of increased ovule size following hormone treatments, attempts to culture, aseptically, ovules recovered from such treatments have failed. Furthermore, premature (< 11-days-old)

selfed embryos placed on defined media have not developed into plants (unpublished results). The present study shows that hormones can increase the percentage of success in crosses where the degree of success would otherwise be low. Based on these results it would now be worth trying other hormones, and their combinations, in an attempt to increase the number of successful crosses, using further refinements in the embryo culture technique.

REFERENCES

Al-Yasiri, S. and Coyne, D.P., 1964. Effect of growth regulators in delaying pod abscission and embryo abortion in the interspecific cross *Phaseolus vulgaris* × *P. acutifolius*. Crop Sci., 4: 433—435.

Ariyanayagam, R.P. and Spence, J.A., 1978. A possible gene source for early, day-length neutral pigeonpeas, *Cajanus cajan* (L.) Millsp. Euphytica, 27: 505—509.

Chen, N.C., Parrott, J.F., Jacobs, T., Baker, L.R. and Carlson, P.S., 1978. Interspecific hybridization of food legumes by unconventional methods of plant breeding. In: Report of First International Mungbean Symposium, 1977, Univ. Philippines, Los Bănos, pp. 247—252.

Emsweller, S.L. and Stuart, N.W., 1948. Use of growth regulating substances to overcome incompatibility in *Lilium*. Proc. Am. Soc. Hort. Sci., 51: 581—589.

Fedak, G., 1977. Haploids from barley × rye crosses. Can. J. Genet. Cytol., 19: 15—19.

Kasha, K.J., Subrahmanyam, N.C. and Ali, A., 1978. Effect of gibberellic acid treatment and nutrient supply through detached tillers, upon haploid frequency in barley. Theor. Appl. Genet., 51: 169—175.

Kruse, A., 1967. Intergeneric hybrids between *Hordeum vulgare* L. spp. distichum (V. pallas, $2n = 14$) and *Secale cereale* L. (V. petkus, $2n = 14$). Kgl. Vetlandbohojsk Arsskr., 82—92.

Larter, E.N. and Enns, H., 1960. The influence of gibberellic acid on the development of hybrid barley ovules in vitro. Can. J. Genet. Cytol., 2: 435—441.

Pundir, R.P.S., 1981. Relationships among *Cajanus*, *Atylosia* and *Rhynchosia* species. Ph.D. thesis, Banaras Hindu University, Varanasi, India.

Reddy, L.J., 1973. Inter-relationships of *Cajanus* and *Atylosia* species as revealed by hybridization and pachytene analysis. Ph.D. thesis, Indian Institute of Technology, Kharagpur, West Bengal, India.

Reddy, L.J., Green, J.M., Singh, U., Bissen, S.S. and Jambunathan, R., 1979. Seed protein studies on *Cajanus cajan*, *Atylosia* and some hybrid derivatives. In: Proceedings on Seed Protein Improvement in Cereals and Grain Legumes, IAEA, Vienna, 2: 105—117.

Reddy, L.J., Green, J.M. and Sharma, D., 1980. Genetics of *Cajanus cajan* (L.) Millsp. × *Atylosia* spp. Proc. Inter. Workshop on Pigeonpeas, ICRISAT, India, 2: 39—50.

Remanandan, P., 1980. The wild gene pool of *Cajanus* at ICRISAT, present and future. Proc. Inter. Workshop on Pigeonpeas, ICRISAT, India, 2: 29—38.

Sastri, D.C. and Moss, J.P., 1982. Effect of growth regulators on incompatible crosses in the genus *Arachis* L. J. Expt. Bot., 33: 1293—1301.

Subrahmanyam, N.C. and Kasha, K.J., 1971. Increased barley haploid production following gibberellic acid treatment. Barley Genet. Newslett., 1: 47—50.