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CROSSABILITY RELATIONSHIPS AMONG *CAJANUS, ATYLOSIA* AND *RHYNCHOSIA* SPECIES AND DETECTION OF CROSSING BARRIERS

R. P. S. PUNDIR[1] and R. B. SINGH[2]

Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi 221 005, India

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INDEX WORDS

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SUMMARY

Crossability of two cultivars of *Cajanus cajan,* eight species of *Atylosia* and one of *Rhynchosia* was investigated. Of the 73 combinations attempted, success was achieved in 12 cases. *C. cajan* crossed successfully with *A. albicans, A. cajanifolia, A. lineata, A. scarabaeoides,* and *A. trinervia.* Within the genus *Atylosia, A. lineata* crossed with *A. albicans* and *A. scarabaeoides,* and *A. scarabaeoides* with *A. sericea.* Three species – *A. platycarpa, A. volubilis* and *R. rothii* did not cross with any other one. In most of the unsuccessful combinations, although the pollen germinated on the receiving stigmas, the pollen tube growth was inhibited inside the stigma or in the stylar tissue.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) MILLSP.), a foremost grain legume of the Indian subcontinent, is the only cultivated species of the substribe *Cajaninae,* which is a group of about 13 closely related genera mainly distributed in the tropics (REMANANDAN, 1981). *Cajanus cajan* shows close relationship with *Atylosia, Dunbaria* and *Rhynchosia* species and these are considered wild relatives of pigeonpea. Many of the desired features in the cultivated pigeonpea such as higher pod index, disease and insect resistance and early flowering are well distributed in the wild relatives. Crossability of some *Atylosia* species with pigeonpea has already been demonstrated (DEODIKAR & THAKAR, 1956; KUMAR & THOMBRE, 1958; KUMAR et al., 1958; REDDY, 1973; DE, 1974; ARIYANAYAGAM & SPENCE, 1978; REDDY et al., 1981). However, there are no reports for several of the other *Atylosia* and *Rhynchosia* species.

In the present study, two cultivars (cvs.) of pigeonpea, eight species of *Atylosia* and one of *Rhynchosia* were cross-pollinated in all possible combinations and their crossability analysed. The crossing barriers of the failing crosses were also investigated.

[1] Present address: Genetic Resources Unit, ICRISAT, Patancheru, A.P. 502 324, India.
[2] Present address: FAO, RAPA, Bangkok-2, Thailand.

MATERIALS AND METHODS

The source of the experimental seeds was the Genetic Resources Unit, ICRISAT, Patancheru, (A.P.). The materials included *Cajanus cajan* (L.) MILLSP. cvs. Pant A2 and UPAS 120, *Atylosia albicans* (W. & A.) BENTH. (JM 2356), A. *cajanifolia* HAINES (JM 2739), *A. lineata* W. & A. (ICP 7469), *A. platycarpa* BENTH., *A. scarabaeoides* (L.) BENTH. (ICP 7464), *A. sericea* BENTH. ex. BAKER (ICP 7470), *A. trinervia* (D.C) GAMBLE (JM 2668), *A. volubilis* (BLANCO) GAMBLE (JM 1984), and *Rhynchosia rothii* BENTH. (JM 2296). The experiment was conducted at the Agricultural Research Farm of Banaras Hindu University, Varanasi during the 1977–79 seasons.

The seeds were scarified to ensure timely and even germination and sown in small earthen-ware pots filled with a soil and farmyard manure (3:1, v/v) mixture. Seedlings with three to four leaves (15–20 days) along with the soil adhering to the roots, were transplanted to the field with pulverized soil at an appropriate depth. The plant to plant and the row to row spacings were 1 m and 2 m respectively. Care was taken to raise a normal crop under near normal growing conditions, including the necessary support for the creeping and the climbing forms.

All the species except *A. trinervia* were cross-pollinated with each other in a complete diallel fashion. As *A. trinervia* failed to flower under Varanasi conditions, its pollen was collected from plants at a site in Ootacamund, Tamil Nadu (77E, 11S & 2200msl) from where this accession was originally procured, however, the pollen was sufficient for only one line (UPAS 120).

The pollinations were made between October 1977 and February, 1978 in the following manner: the buds which would bloom the next day were emasculated. Pods, flowers and the buds around each emasculated bud were removed. With the help of a pair of forceps the buds were opened through the keels and the anthers were removed without injuring any other part of the flower. The tip of the forceps was dipped in spirit after every emasculation to avoid any unwanted pollinations. Each individual plant or row of plants was labelled to monitor the cross and a small coloured thread was tied on the pedicel of the emasculated bud for identification purposes.

The pollinations were preferably done just after emasculation, or within a few hours of emasculation. For pollination, flowers at anthesis (with their petals held downward or removed to expose the dehisced anthers) were brushed on the stigma of the emasculated bud. Each flower provided enough pollen grains for 4–5 stigmas. While the emasculation can be done any time of the day, the pollen grains are available normally from 0800 to 1600 hrs with some variation depending upon weather and season.

The seeds collected from the possibly crossed pods and those of the parents were germinated in small pots in June 1978 and later the plants were transplanted into the field. The F1 plants were detected by comparing them with their respective parents. The percentage success of each cross was calculated as below:

$$\text{a) Number of crossed pods} = \frac{\text{Number of F1 plants}}{\text{Number of total plants}} \times \text{Number of possibly crossed pods harvested}$$

$$\text{b) Crossability (\%)} = \frac{\text{Number of crossed pods}}{\text{Number of pollinated flowers}} \times 100$$

In failing combinations, pollinations were repeated to observe pollen grain germina-

tion and pollen tube growth. Stigmas with styles were fixed in F.P.A (formalin 5: propionic acid 5:70% alcohol 90, v/v), 4–6 hours after pollination. The tissues were cleared in hot lactophenol (16 hours), mounted on glass slides in cotton blue (1% solution in lactophenol) and observed under a microscope for germination of the pollen grains. This and other staining procedures did not reveal pollen tubes through the stigmatic tissue and style. Fluorescence microscopy was therefore adopted following the procedure of GHOSH & SHIVANNA (1977).

RESULTS

Only 73 cross combinations could be attempted (Table 1). Besides *A. trinervia,* the three species, *A. platycarpa, A. lineata,* and *A. sericea* could not be cross-pollinated in all the possible combinations owing to their season-specific and short-duration flowering. The number of pollinations made for a given cross-combination ranged from 10 (*A. lineata* × cv. UPAS 120) to 1193 (*A. cajanifolia* × *A. scarabaeoides*). In the 43 cross combinations, the number of pollinations attempted was greater than 300. Of the 73 combinations attempted, only in 12 cases F1 plants were obtained. The

Table 1. Number of pollinations made and percent success (between parentheses) between *Cajanus, Atylosia* and *Rhynchosia* species.

♀ \ ♂	cv. Pant A 2	cv. UPAS 120	*A. albi.*	*A. cajif.*	*A. lin.*	*A. platy.*	*A. scar.*	*A. ser.*	*A. volub.*	*R. rothii*
Cv. Pant A 2	–	–	663 (0.9)	674 (0)	75 (0)	55 (0)	409 (0.7)	669 (0)	225 (0)	661 (0)
Cv. UPAS 120	–	–	430 (0.9)	669 (0)	90 (1.1)	128 (0)	376 (0)	472 (0)	381 (0)	713 (0)
A. albi.	626 (0)	364 (0)	–	220 (0)	105 (0)	–	183 (0)	409 (0)	57 (0)	374 (0)
A. cajif.	876 (2.2)	714 (1.1)	653 (0)	–	79 (0)	149 (0)	1193 (0)	954 (0)	457 (0)	1004 (0)
A. lin.	168 (1.2)	10 (10.0)	25 (12.0)	53 (0)	–	–	67 (4.5)	25 (0)	219 (0)	–
A. platy.	12 (0)	–	–	80 (0)	–	–	–	–	–	–
A. scar.	162 (0)	157 (0)	187 (0)	39 (0)	–	–	–	182 (0.6)	164 (0)	273 (0)
A. ser.	478 (0)	348 (0)	377 (0)	571 (0)	–	–	561 (0)	–	–	508 (0)
A. volub.	674 (0)	745 (0)	752 (0)	369 (0)	362 (0)	–	599 (0)	759 (0)	–	703 (0)
R. rothii	475 (0)	758 (0)	551 (0)	911 (0)	140 (0)	90 (0)	856 (0)	986 (0)	419 (0)	–

Note: cv. UPAS 120 × *A. trinervia* – 36 flowers pollinated, crossability percent 8.3.

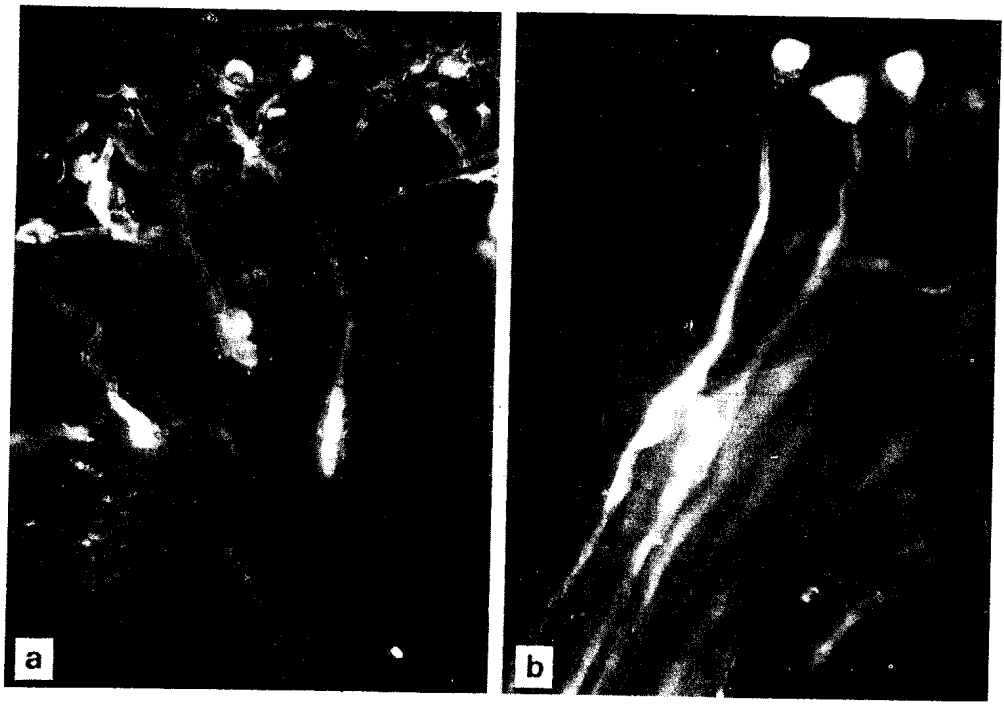

Fig. 1. Pollen tube growth through the stylar column in pollinations involving distant relatives. a) *A. cajanifolia* × *R. rothii;* inhibition of pollen tube growth manifested in club-shaped tips. b) *A. albicans* × *A. volubilis*; near normal growth.

percent success of crossability ranged from 0.6 (*A. scarabaeoides* × *A. sericea*) to 12.0 (*A. lineata* × *A. albicans*).

In the cross cv. UPAS 120 × *A. trinervia* (the only cross involving *A. trinervia*), 36 flowers were pollinated and three crossed pods were obtained. The cv. UPAS 120 could be successfully pollinated with *A. albicans* and *A. lineata*. The other cv. Pant A 2 set pods when crossed with *A. albicans* and *A. scarabaeoides*. When these cultivars (Pant A 2 and UPAS 120) were used as pollen parents, only *A. cajanifolia* and *A. lineata* set pods. Both the cultivars failed to cross with *A. platycarpa*, *A. volubilis* and *R. rothii* in either ways. Among the *Atylosia* species, only three combinations, namely, *A. lineata* × *A. albicans* (12.0%), *A. lineata* × *A. scarabaeoides* (4.5%) and *A. scarabaeoides* × *A. sericea* (0.6%) resulted in fertilization.

Three species – *A. platycarpa, A. volubilis* and *R. rothii* did not cross with any other species.

Pollen tube growth in unsuccessful combinations. It is evident from Table 1 that in only 12 cross-combinations, F1 hybrids were produced. In order to determine the stage and causes of the barrier(s) in the failing combinations, the stylar tissues were observed under a microscope for pollen germination and pollen tube growth. In all crosses,

pollen grains germinated on the receiving stigmas, but in 27 of the 28 failing combinations the pollen tubes were inhibited in the stigmatic tissue (Fig. 1a). In many cases the tips of the pollen tubes were bulged and were rarely seen inside the style. Only in one combination, *A. albicans* (♀) × *A. volubilis* (♂), normal pollen tube growth was observed at least up to the mid stylar region (Fig. 1b).

DISCUSSION

Crossability is a pre-requisite for gene transfer. However, an understanding of the extent of and barriers to crossability among the species has been helpful not only in choosing methods for producing hybrids and their derivative materials, but also in tracing phylogenetic relations. The significance of differentiation of cytoplasm and the use of specific female parents for producing a successful F1 can hardly be over-emphasised in choosing the parents for hybridization and also to understand the mode of differentiation at the cytoplasmic and genetic levels and their interactions. *Cajaninae* species produce many flowers, but as much as 26–81 percent of the flowers are shed (PUNDIR, 1981). The fruiting coefficient also varies under different climatic conditions and agronomic practices. The situation is further aggravated when artificial pollination, either intra-specific or inter-specific is attempted. In the 12 successful crosses of the 73 combinations attempted, the crossability varied from 0.6 to 12.0%. Only *C. cajan* and *A. lineata* produced pods in both ways; the other successes were unilateral. Successful crosses between *C. cajan* and the male parents *A. scarabaeoides, A. lineata, A. albicans,* and *A. trinervia,* as in the present case, were also obtained by REDDY (1973), and REDDY et al. (1979,1981). Unlike in the present study, REDDY (1973) was unable to cross *A. lineata* (♀) with *C. cajan* (♂). Successful crosses *A. scarabaeoides* (♀) × *C. cajan* (♂) and *C. cajan* (♀) × *A. sericea* (♂) were reported by ARIYANAYAGAM & SPENCE (1978) and REDDY (1973), respectively, but attempts in the present investigation were not successful. We obtained a successful cross between *A. cajanifolia* and *C. cajan* only when the former was the female parent whereas REDDY et al. (1981) reported success in the reverse direction. The good results of *A. lineata* (♀) × *A. scarabaeoides* (♂), *A. scarabaeoides* (♀) × *A. sericea* (♂) and *A. lineata* (♀) × *A. albicans* (♂) are reported here for the first time. In the present study, *A. platycarpa, A. volubliis* and *R. rothii* did not cross with any other species. The cross *A. platycarpa* (♀) × *C. cajan* (♂) was reported by ARIYANAYAGAM & SPENCE (1978), but the results were subsequently identified as selfs (pers. communication).

Crossing barrier. Crossig failures may exist at the gametic, zygotic or post-zygotic levels including hybrid sterility and weakness. In the present study, in 28 unsuccessful cross-combinations, the pollen germination of the stigma occurred in every case. The characteristic feature of the pollen tube growth inhibition at stigmatic surface suggests that interspecific/intergeneric incompatibility exists in the material studied. In the cross of *A. albicans* (♀) and *A. volubilis* (♂) (which did not produce F1 plants), pollen tubes were normal. Since the time given after the pollination was low (6 hrs), these grew only upto the upper region of the style. By allowing more time after making the pollinations, the styles and ovaries may be examined for further pollen tube growth and fertilization, which was not done in the present study.

Most of the *Atylosia* species and *R. rothii* which failed to hybridize with cultivated *C. cajan* contain useful genes. In view of the inhibition of pollen tube growth as the barrier to crossability, breeders may have to resort to some simple manipulations such as artificial nutrition, amputation, and use of mentor pollen (DE NETTANCOURT, 1977; SASTRI, 1984). Unconventional techniques of somatic hybridization or even DNA transfer for effecting gene transfers across the existing barriers may be resorted to.

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