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Intra species variation in *Atylosia scarabaeoides* (L.) Benth., a wild relative of pigeonpea (*Cajanus cajan* (L.) Millsp.)

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Summary

Atylosia scarabaeoides (L.) Benth., a wild relative of pigeonpea, possesses several useful genes which can be utilized for pigeonpea improvement. In the present study, 33 accessions of *A. scarabaeoides* were evaluated at ICRISAT Center during the 1987 rainy season for variation in some useful traits to identify parents for inter-generic hybridization. A large variation was observed for leaf components, seed size, pod length, seeds/pod, days to flowering, seed protein, sulphur amino acids, resistance to cyst nematode, phytophthora blight, sterility mosaic, fusarium wilt, pod borer, pod fly, and pod wasp. Only four accessions were found to have more than 28% protein content. Methionine and cystine contents were marginally higher than in pigeonpea but the variation was not large enough to utilize them in the breeding program. In *A. scarabaeoides,* accessions resistant to fusarium wilt, phytophthora blight, sterility mosaic, and cyst nematode were detected. Compared to pigeonpea, the *A. scarabaeoides* accessions were less susceptible to lepidopteran borer and were immune to pod fly damage. Accessions ICPW 89 and ICPW 111 in short- (100–120 days), and ICPW 94 and ICPW 118 in medium-duration (140–180 days) were identified as potential parents for use in inter-generic hybridization.

Introduction

The genetic potential of wild relatives in the improvement of economically important crops is now well established since they harbour some useful genes not available in the germplasm of the cultivated species. Breeders have attempted hybridizations with wild relatives in various food crops mainly for transferring resistance to economically important diseases and/or to create additional genetic variation. The transfer of specific genes from wild species into commercial varieties has been very successful in crops such as cotton, maize, sugarcane, tobacco, and wheat (Stalkar, 1980). Crosses between a cultivated species and its wild relatives are capable of producing new plant characters (Stebbins, 1977) or extreme forms (Barbaki et al., 1976). Harlan (1976) and Stalkar (1980) published research reviews wherein the wide hybridizations have produced lines adapted to biotic and abiotic stress environments, high productivity, or with improved quality.

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a monotypic genus (Reddy, 1973) and it is logical to seek additional variation in the genus *Atylosia* which is closely related to *Cajanus*. The close affin-

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ity between these two genera has been demonstrated by their successful hybridization by many researchers (Dundas, 1984; Kumar, 1985).

Pigeonpea characteristically lacks vigor during seedling and early vegetative (juvenile) phases (Brakke & Gardner, 1987) and the extra-short-duration pigeonpea varieties, which mature in about 90–100 days and are cultivated in pure stands, are unable to produce adequate biomass to support higher levels of grain productivity. One of the avenues to improve the biomass production in pigeonpea is to increase their growth rate by incorporating genes from their wild relatives as suggested by Frey (1985). The Genetic Resources Unit of ICRISAT has 271 accessions of 47 wild species related to *Cajanus* (Remanandan et al., 1988). Among these, *Atylosia scarabaeoides* (L.) Benth. is the most widely distributed and is predominant in the Indian subcontinent and Australia. Since *A. scarabaeoides* can easily be crossed with pigeonpea, its useful genes can be utilized for improvement of the latter. At present, ICRISAT maintains 77 accessions of *A. scarabaeoides* collected from a wide eco-geographical range. Except the morphotaxonomical description of the species (Maesen, 1986), no information is available on the variation within the species for economic traits. In the present study 33 accessions of *A. scarabaeoides* have been assessed for 18 plant and seed characteristics. This information will be useful in identifying potential parents for breeding program involving intergeneric hybridization.

Materials and methods

Thirty-three *Atylosia scarabaeoides* genotypes, acquired from the Genetic Resources Unit of ICRISAT, were evaluated at ICRISAT Center for leaf characters, seed yield components, seed protein, sulphur amino acid content and resistance to major diseases and pests. For evaluation of leaf components, seed and pod traits and protein and amino acid determination, the materials were grown in Vertisols during the 1987 rainy season in two replications in a randomized complete block design. Each plot comprised of a 4 m long single row. Spacing between and within the rows was kept at 60 and 30 cm respectively. Three competitive plants in each plot were identified randomly for recording observations on leaf and seed characters. As our earlier observations in pigeonpea (Saxena & Sharma, 1981) indicated, leaf components in mature leaf samples obtained from different positions of the plant did not differ significantly, a sample of five fully expanded leaves was taken at random from each of the three plants at the time of flowering. The leaf samples were placed in moist plastic bags before processing for leaf fresh weight (mg), petiole length (mm), and petiole fresh weight (mg). Leaf area (mm^2) was measured using a Delta-T automatic leaf area meter. Thereafter, the samples were transferred to a glassine bag and dried at 80° C for 40 h before recording leaf and petiole dry weights. Specific leaf weight (mg/mm^2) was calculated by dividing the sample leaf dry weight by its area. Data on pod length (mm) and seeds/pod were recorded on five fully developed pods harvested from each plant. Seed mass (g/100-seeds) was recorded on the individual plant basis. Seed protein content was evaluated in plot bulk samples by determining the nitrogen content in decorticated dry split cotyledons (dhal) and multiplying it by factor 6.25. The content of sulphur amino acids, methionine and cystine together, was determined by using a Beckman 119 CL Amino Acid Analyzer. Defatted dhal samples were refluxed in 6N HCl for 24 h. After evaporating the HCl from the hydrolysate, the residue was taken in citrate buffer (pH 2.2).

Only 12 accessions were evaluated for pest damage assessment. For each genotype, an unreplicated single row was grown under insecticide-free conditions. From each plot all the pods from a randomly selected plant were harvested at maturity. The pods were sorted out for the damage caused by lepidopteran borer (mainly *Heliothis armigera*), pod fly (*Melanagromyza obtusa*), and pod wasp (*Tanaostigmodes cajaninae*).

Evaluation for resistance to phytophthora blight (*Phytophthora drechsleri f. sp. cajani*), wilt (*Fusarium oxysporum f. sp. udum*), sterility mosaic, and cyst nematode (*Heterodera cajani*) was carried out in pot culture in a glasshouse. Twenty seeds of each accessions were sown for screening of each disease.

For phytophthora blight, 15-cm diamter plastic pots filled with sterilized red soil were used. One week after sowing the seedlings were drench inoculated with mycelial suspension of P2 isolate of *Phytophthora drechsleri* var. *cajani* from a 15-day old culture multiplied on pigeonpea-meal-broth (Nene et al., 1981). The mortality due to blight was recorded 10 days after inoculation when the susceptible check (ICP 7119) showed 100% mortality. For sterility mosaic screening, 15-cm diameter plastic pots filled with sterilized black soil and sand mixture were used. At primary leaf stage, the seedlings were staple-inoculated with sterility mosaic infected leaves carrying the eriophyid mite vectors (Nene & Reddy, 1976). Disease observations were recorded one month after inoculation when the susceptible check (ICP 8863) showed 100% incidence. For fusarium wilt screening, 60-cm diameter earthen pots filled with wilt-sick soil collected from a Vertisol wilt-sick plot were used. Wilt observations were recorded three months after sowing when the susceptible cultivars (ICP 2376 and LRG 30) showed near 100% disease. Screening for cyst nematode was done in 10-cm diameter plastic pots filled with nematode infested soil with an infestation level of 21 eggs and juveniles per cm^3 of soil. Thirty to fortyfive days after germination, the root systems were examined for the presence of young cysts by carefully inverting the pot soil in a container and gently washing the root system in water. The young cysts shine as white pearl-like bodies on the roots. The number of young cysts on each root system was counted and rated on 1–9 scale with 1 being immune (no cyst) and 9 highly susceptible (>30 cysts). Maximum ratings of different accessories are mentioned in Table 2.

Results and discussion

Leaf characters

Studies on the significance of leaf characters to determine yield of crop plants are of interest to breeders. In soybean, Auckland & Lambert (1974) suggested the use of petiole length and petiole weight in selecting for yield. Among the *A. scarabaeoides* genotypes used in this study, significant differences were observed for all the leaf components (Table 1). For leaf area, a large variation was observed; ICPW 110 had the largest (1166 mm^2) leaves, while the smallest (385 mm^2) leaves were found in ICPW 96. Leaf area was positively associated with leaf weight ($r = 0.84^{**}$), petiole length ($r = 0.63^{**}$), and petiole weight ($r = 0.38^{*}$) and negatively related with specific leaf weight ($r = -0.55^{**}$). Mean petiole weight in different accessions ranged from 3.0 (ICPW 111) to 6.2 mg (ICPW 119) and was found to be negatively associated with seed size ($r = -0.36^{*}$) and positively related with leaf weight ($r = 0.50^{**}$) and petiole length ($r = 0.51^{**}$). In pigeonpea, however, Saxena & Sharma (1981) reported a positive correlation between petiole weight and seed size. The contrasting nature of this relationship in pigeonpea and *A. scarabaeoides* and its magnitude suggests that petiole weight cannot be used as a reliable selection criterion for seed size in segregating inter-generic populations. Specific leaf weight has been considered as a selection criterion in alfalfa (Pearce et al., 1969) and pigeonpea (Saxena & Sharma, 1981). Variation for specific leaf weight in the *A. scarabaeoides* accessions was limited and it had no significant relationship ($r = -0.21$ to 0.18) with any agronomic trait.

Flowering and yield components

A wide range was observed for days to flowering (Table 1.) Of the 33 accessions evaluated, ICPW 89 from Himachal Pradesh, ICPW 85 and ICPW 132 from Orissa and ICPW 82 and ICPW 111 from Maharashtra flowered in less than 60 days. Days to flowering was not related to any leaf or yield components. For seeds/pod also, a large variation among the genotypes was observed. A Burmese collection (ICPW 95) had the smallest (3.1 seeds/pod) pods while the longest (6.2 seeds/pod) pods were found in ICPW 122, a collection from Kerala. Compared with pigeonpea, the seed size in *A. scarabaeoides* was very small, ranging from 1.1 (ICPW 94) to 3.0 g/100-seeds (ICPW 111). The mean pod length (2.1 cm) and mean seed size (1.9 g/100-seeds) of *A. scarabaeoides* were smaller than those

of pigeonpea cultivars and germplasm (Remanandan et al., 1988). Unlike pigeonpeas (Saxena & Sharma, 1981), seed and pod size were independent (r = 0.15) of each other in *A. scarabaeoides* accessions.

Seed protein and amino acids

Genetic variation for seed protein in pigeonpea germplasm is limited and *A. scarabaeoides* has been successfully used as donor parent for transferring high protein genes to pigeonpea at ICRISAT Center (Saxena et al., 1987a). The range for protein content (Table 1) in the *A. scarabaeoides* accessions indicated the presence of a continuous variation from 23.5 to 29.3 percent and out of 33 genotypes tested only five had 28% protein or more. The highest protein content (29.3%) was recorded in ICPW 89, a collection from Himachal Pradesh followed by ICPW 121 (28.9%), ICPW 84 (28.3%), ICPW 82 (28.1%), and ICPW 88 (28.0%). On the contrary, ICPW 86 had as low as 23.5% protein which is similar to those of pigeon-

Table 1. Mean values for some leaf and seed characteristics in *Atylosia scarabaeoides* (L.) Benth. accessions

Accession	Origin	Leaf area (mm^2)	Leaf dry wt. (mg)	Specific leaf wt. (mg/mm^2)	Petiole length (mm)	Petiole dry wt. (mg)	Seeds per pod	Pod length (mm)	100 seed mass (g)	Seed protein (%)	Methionine + cystine (g/100 g protein)	Days to flowering
ICPW 115	Assam, India	745	66	0.09	19.6	4.0	3.6	19.7	2.0	26.1	2.55	120
ICPW 116	Sikkim, India	1008	120	0.12	23.9	5.0	3.8	19.7	2.2	24.4	2.74	125
ICPW 84	Bhutan	860	76	0.09	19.1	4.3	4.0	20.2	2.2	28.3	2.49	123
ICPW 89	Himachal Pradesh, India	894	82	0.09	20.8	5.2	4.2	21.4	2.0	29.3	2.65	55
ICPW 90	Himachal Pradesh, India	519	62	0.12	15.4	3.4	4.8	19.9	1.8	26.0	2.49	130
ICPW 92	Himachal Pradesh, India	838	73	0.09	21.1	5.1	3.9	21.2	2.0	26.6	2.62	66
ICPW 91	Punjab, India	960	75	0.08	19.4	5.2	4.3	18.5	1.7	26.2	2.91	73
ICPW 96	Uttar Pradesh, India	385	47	0.12	15.2	4.0	4.5	20.4	1.6	25.2	2.57	73
ICPW 98	Uttar Pradesh, India	766	79	0.10	21.2	6.0	3.9	18.7	1.9	25.9	2.64	91
ICPW 85	Orissa, India	772	80	0.10	21.5	5.1	4.2	19.9	2.2	25.3	2.89	56
ICPW 118	Orissa, India	783	76	0.10	23.6	5.2	4.7	22.6	2.1	25.4	2.95	87
ICPW 132	Orissa, India	1004	100	0.10	25.2	5.0	4.4	22.3	2.0	27.1	2.83	56
ICPW 82	Maharashtra, India	998	79	0.08	18.9	4.1	4.9	24.7	2.2	28.1	2.94	57
ICPW 83	Maharashtra, India	703	74	0.10	20.2	4.1	4.2	20.7	2.1	26.0	2.49	88
ICPW 111	Maharashtra, India	738	65	0.09	14.4	3.0	4.7	22.4	3.0	26.7	2.37	54
ICPW 112	Maharashtra, India	830	65	0.08	16.7	4.0	4.4	20.5	2.5	25.0	2.34	61
ICPW 86	Karnataka, India	755	73	0.10	25.8	4.1	4.1	20.2	2.1	23.5	2.91	74
ICPW 87	Tamil Nadu, India	910	69	0.08	26.0	3.0	4.1	21.0	2.5	25.2	3.06	97
ICPW 88	Andhra Pradesh, India	809	73	0.09	22.1	4.1	4.9	21.5	1.9	28.0	2.95	89
ICPW 117	Tamil Nadu, India	617	56	0.09	16.7	4.0	4.9	23.3	1.8	25.5	2.83	88
ICPW 121	Karnataka, India	843	69	0.08	21.8	5.0	5.2	20.9	1.9	28.9	2.97	98
ICPW 122	Kerala, India	1123	96	0.09	23.7	6.0	6.1	25.5	1.6	24.9	2.63	111
ICPW 109	Karnataka, India	695	67	0.10	21.9	4.9	4.9	22.3	1.6	26.3	2.85	104
ICPW 110	Andhra Pradesh, India	1166	91	0.08	24.8	4.1	4.0	22.4	1.7	25.6	2.83	105
ICPW 126	Andhra Pradesh, India	1013	90	0.09	24.2	7.0	3.6	19.5	1.7	26.9	2.70	89
ICPW 130	Andhra Pradesh, India	1000	92	0.09	19.2	3.4	4.4	22.0	2.0	25.1	2.65	75
ICPW 95	Burma	1126	96	0.09	24.9	5.3	3.1	18.3	1.7	25.6	2.45	71
ICPW 119	The Philippines	930	92	0.10	23.3	6.2	4.7	23.0	2.0	25.4	2.48	79
ICPW 133	Australia	832	73	0.09	20.5	3.6	4.6	21.5	2.2	26.0	2.94	66
ICPW 94	Sri Lanka	483	49	0.10	20.0	4.2	4.6	18.8	1.1	25.6	2.73	88
ICPW 124	Uttar Pradesh, India	844	84	0.10	24.7	6.1	4.2	19.5	1.6	24.6	2.58	128
ICPW 125	Tamil Nadu, India	658	63	0.10	16.0	4.0	5.1	21.1	1.4	25.5	2.83	108
ICPW 128	–	997	87	0.09	24.8	5.2	4.3	20.3	2.1	26.5	2.88	67
SE		±25.6	±3.4	±0.01	±0.57	±0.17	±0.12	±0.58	±0.04	±0.47	±0.06	±2.3
Mean		836.0	76.8	0.09	21.09	4.61	4.42	21.02	1.92	26.07	2.72	86.4
CV (%)		4.3	6.2	7.50	3.81	5.18	4.01	3.90	3.02	2.54	3.16	3.7

pea cultivars. Protein data of the present investigation showed that all the *A. scarabaeoides* accessions tested were not rich in seed protein, as was believed earlier (Reddy et al., 1979) by pigeonpea breeders, and that the genes for high protein were not concentrated in the materials from a particular geographical region. Similar to those of inter-generic high-yielding high protein derivatives (Saxena et al., 1987b), the protein content in *A. scarabaeoides* was independent (r = 0.12) of seed size.

Sulphur-containing amino acids are important for enhancing the biological value of legume seed proteins. In *A. scarabaeoides,* the content of sulphur amino acids (methionine and cystine to-

Table 2. Screening of *A. scarabaeoides* accessions against phytophthora blight, sterility mosaic, fusarium wilt, and cyst nematode (*Heterodera cajani*)

Accession	Phytophthora blight (%)	Sterility mosaic +	Fusarium wilt (%)	Cyst nematode rating (1–9 scale)
ICPW 115	72.2	R	0.0	5
ICPW 116	48.0	S	0.0	7
ICPW 84	52.2	S	0.0	5
ICPW 89	20.8	S	14.3	5
ICPW 90	59.8	S	0.0	7
ICPW 92	66.1	S	0.0	5
ICPW 91	28.5	S	0.0	7
ICPW 96	100.0	–	0.0	5
ICPW 98	32.1	S	33.9	–
ICPW 85	62.5	S	27.3	9
ICPW 118	16.5	S	7.1	7
ICPW 132	95.0	–	50.0	7
ICPW 82	60.0	S	20.0	7
ICPW 83	25.0	S	40.0	7
ICPW 111	35.8	S	28.6	3
ICPW 112	74.2	S	0.0	5
ICPW 86	70.0	S	0.0	7
ICPW 87	50.0	S	0.0	5
ICPW 88	75.0	S	50.0	7
ICPW 117	50.0	S	66.7	7
ICPW 121	61.5	R	30.0	7
ICPW 122	68.2	S	28.6	7
ICPW 109	68.5	S	0.0	7
ICPW 110	67.2	S	0.0	7
ICPW 126	91.6	S	0.0	7
ICPW 130	23.0	S	11.1	7
ICPW 95	72.7	S	0.0	7
ICPW 119	54.1	S	0.0	5
ICPW 133	83.3	S	66.7	7
ICPW 94	47.2	S	0.0	3
ICPW 124	32.0	R	0.0	7
ICPW 125	43.3	S	25.0	7
ICPW 128	45.3	S	100.0	5
Pigeonpea (checks)				
ICP 8863	–	S	–	–
LRG 30	–	–	92.6	–
ICP 2376	–	–	64.7	–
ICPL 87	–	–	–	9

+ R = Resistant, S = Susceptible, Rating 1 = No cyst and 9 = > 30 cysts/root.

gether) ranged between 2.45 and 3.06 g/100-g-protein showing a small variation. These amino-acid values are slightly higher than those reported earlier for normal- and high-protein pigeonpea cultivars (Singh et al., 1989), but not large enough to warrant breeding efforts. In grain legumes, with the exception of pigeonpea, negative relationship has been reported between protein percentage and methionine content per unit of protein (Singh & Eggem, 1984). As in pigeonpea, in *A. scarabaeoides* also, protein and sulphur amino acid contents were not correlated (r = 0.09), suggesting that *A. scarabaeoides* can be used for increasing protein content in pigeonpea without adversely affecting its quality in terms of sulphur amino acids.

Disease and pest resistance

Resistance to fusarium wilt was fairly common in *A. scarabaeoides* and of the 33 accessions evaluated, 20 were resistant (Table 2). In contrast, only three accessions ICPW 115, ICPW 121, and ICPW 124 were resistant to sterility mosaic. ICPW 89 and ICPW 118 were found to be resistant to phytophthora blight. ICPW 94, a collection from Sri Lanka, and ICPW 111, an Indian collection, showed resistance to cyst nematode (*Heterodera cajani*) (Table 2).

Table 3. Percentage of pod damage due to pests in 12 accessions of *A. scarabaeoides* grown under insecticide-free conditions

Accession	Pod borer (%)	Pod fly (%)	Pod wasp (%)	Total (%)
ICPW 83	4.1	0.0	53.4	57.5
ICPW 90	0.0	0.0	0.0	0.0
ICPW 94	0.5	0.0	28.8	29.3
ICPW 95	0.0	0.0	50.0	50.0
ICPW 91	2.4	0.0	71.4	73.8
ICPW 115	4.2	0.0	12.5	16.7
ICPW 116	11.1	0.0	48.9	60.0
ICPW 117	16.7	0.0	33.3	50.0
ICPW 118	2.2	0.0	24.4	26.7
ICPW 109	3.1	0.0	10.8	14.8
ICPW 126	2.6	0.0	58.9	61.6
ICPW 132	4.1	0.0	30.1	32.9
Pigeonpea (Check) T.21	33.2	15.0	1.1	49.6

The pest damage data (Table 3) indicated that as compared to the check pigeonpea cultivar, most of the *A. scarabaeoides* accessions were relatively less susceptible to the lepidopteran borer attack and surprisingly all the accessions were immune to pod fly damage. With the exception of ICPW 90, ICPW 109, and ICPW 115, all the accessions showed a high level of susceptibility to pod wasp attack.

Sources of earliness and resistance to common diseases are available in pigeonpea germplasm and, therefore, *A. scarabaeoides* genes may be used to enrich the existing genetic variability for these traits. For incorporating characters such as increased vegetative growth index, resistance to cyst nematode, and high protein content, which are lacking or limited in pigeonpea, breeders can utilize the *A. scarabaeoides* gene pool. However, in the light of the present results, it appears necessary to select an appropriate *A. scarabaeoides* line for *Cajanus – A. scarabaeoides* introgression to maximize the economic gains. The accessions identified as potential parents for inter-generic hybridization are: ICPW 89 for earliness, high protein, resistance to cyst nematode and fusarium wilt; ICPW 94 for resistance to cyst nematode, pod borer, pod fly, and fusarium wilt; and ICPW 111 for earliness, resistance to cyst nematode, and ICPW 118 for phytophthora blight resistance. This, besides contributing increased vegetative growth index t short-duration pigeonpeas, can also impart other traits such as high protein content and resistances to diseases and pests.

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