

Relationship between seed size and protein content in newly developed high protein lines of pigeonpea

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Abstract. To develop high protein lines, several crosses were made between *Cajanus* as a source of acceptable seed size and *Atylosia* as a source of high protein. In the present study, 1974 single F7 plants from these intergeneric crosses were examined. Correlation coefficients among these crosses between seed size and seed protein content ranged from -0.30 , ($P < 0.01$) to $+0.28$ ($P < 0.01$). Two crosses had significant negative correlations and one showed a significant positive correlation while in the remaining two no significant association was detected. Based on all the selections there was a highly significant negative correlation (-0.13 , $P < 0.01$) between seed size and protein content. However, the extent of variation that could be attributed to this association was small. Observations indicated that in pigeonpea, unlike other legume and cereal crops, simultaneous improvement can be made for seed size and protein. Some high protein lines (HPL 2, HPL 7, HPL 40, and HPL 51) have been identified. Protein content of these lines ranged between 27.0 and 29.0% and 100-grain weight between 10.0 and 12.1 g.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.), a protein rich pulse crop of tropical and sub-tropical regions, is among the most important grain legumes in the diet of people in India, Africa, and the Caribbean. India accounts for about 85% of the world's supply of pigeonpea, where it is mostly consumed after dehusking in the form of dhal (decorticated split cotyledons). Factors affecting the protein quality of pigeonpea have recently been highlighted and their improvement through effective breeding has been suggested [1]. Pigeonpea protein content ranges between 18 to 26% [2]. Some *Atylosia* spp that are related to pigeonpea, have been found to have 29 to 30% protein [3]. However, the seeds of these *Atylosia* spp are very small (2–3 g/100 seeds) and are of no economic value. Reddy et al. [3] crossed three of these *Atylosia* spp to adapted pigeonpea cultivars for improving the protein level of the latter. There was some concern when making these crosses that the high protein level of the *Atylosia* would remain associated with small seed size in the derived lines because of the negative correlation between seed size and protein content often found both in cereals [4, 5] and in legumes [6, 7]. This is of concern because a

small seeded pigeonpea is not acceptable to most consumers. Usually, a size of about 10 g/100 seeds is required, although in parts of Africa and the Caribbean larger seed of about 18 g/100 seeds is preferred. Therefore the present study of the relationship between seed size and protein content in derivatives of *Cajanus* × *Atylosia* crosses was made to determine the possibility of developing high protein pigeonpea lines with large seeds.

Materials and methods

The high protein breeding material (F7) developed by Reddy et al. [3] from five intergeneric crosses (Baigani × *A. scarabaeoides*, T.21 × *A. sericea*, Pant A-2 × *A. albicans*, T.21 × *A. scarabaeoides* and Paut A-2 × *A. scarabaeoides*) was grown at ICRISAT Center in progeny rows during 1982. Baigani, Pant A-2 and T.21 are the varieties of pigeonpea commonly grown in India. In this material, a few plants (about 3%) were found to have undesirable characters of *Atylosia* spp, such as vining growth, dwarf plants, and small hairy pods. Therefore, 1974 single plants that appeared similar to *Cajanus* in plant and grain characteristics were selected for evaluation. After their 100-seed weight was recorded, the seeds were dehulled in a small laboratory pearling mill and the protein percentage of the dhal (decorticated split seed) was determined using a Technicon Auto Analyzer [8]. Protein values were obtained by multiplying the nitrogen value with 6.25. Results were expressed as protein percent in the sample, as is basis. No moisture correction was applied. Simple correlation coefficients between seed size and protein percentage were estimated. To examine this relationship within different seed size groups, the selections were subdivided into large (> 8.5 g/100 seeds), medium (6.5–8.4 g/100 seeds) and small (< 6.5 g/100 seeds) seeded groups.

Results and discussions

The correlation coefficients among the crosses ranged from -0.30 , ($P < 0.01$) to $+0.28$ ($P < 0.01$). Of the five crosses examined, two had significant negative correlations, one showed a significant positive correlation, while in the remaining two crosses, no significant association was detected between seed size and protein percentage (Table 1).

A highly significant positive association ($r = 0.28$, $P < 0.01$) between seed size and protein content was observed in the large seed size group of the cross T.21 × *A. scarabaeoides*. The largest number of selections was from the cross Baigani × *A. scarabaeoides* and the correlation in these selections was highly significantly negative ($r = -0.12$, $P < 0.01$). However, none of the correlation coefficients calculated for the different seed size groups, within this cross, was significant (Table 1). Within the large

Table 1. Correlation between seed size and protein percentage in high protein derivatives from five intergeneric crosses grown at ICRISAT during 1982-83

Cross/seed size group	n	100-seed wt. (g)		Percent protein		r
		Mean	Range	Mean	Range	
Baigani ×						
<i>A. scarabaeoides</i>						
Large	382	9.7	8.5-14.2	26.0	20.0-32.7	0.04
Medium	584	7.4	6.5-8.4	26.2	18.8-33.3	-0.08
Small	265	5.7	3.8-6.4	26.8	21.4-35.6	0.04
Total	1231	7.8	3.8-14.2	26.3	18.8-35.6	-0.12**
Pant A-2 ×						
<i>A. albicans</i>						
Large	41	9.3	8.5-12.0	25.7	21.8-30.3	0.02
Medium	94	7.4	6.5-8.4	26.3	22.4-31.2	0.01
Small	30	5.8	5.0-6.4	26.1	23.5-28.6	0.12
Total	165	7.6	5.0-12.0	26.1	21.8-31.2	-0.07
Pant A-2 ×						
<i>A. scarabaeoides</i>						
Large	17	9.2	8.5-10.7	26.6	23.2-28.3	-0.17
Medium	169	7.2	6.5-8.4	26.4	22.2-30.4	0.06
Small	82	5.8	4.5-6.4	26.5	22.3-29.2	0.08
Total	268	6.9	4.5-10.7	26.4	22.2-30.4	-0.07
T.21 ×						
<i>A. sericea</i>						
Large	67	9.2	8.5-10.4	26.6	22.0-30.1	0.04
Medium	22	8.0	6.5-8.4	27.3	24.2-30.6	-0.72**
Small	2	5.8	5.3-6.2	28.4	27.1-29.7	na
Total	91	8.9	5.3-10.4	26.8	22.0-30.6	-0.30**
T.21 ×						
<i>A. scarabaeoides</i>						
Large	154	9.7	8.5-13.7	25.2	20.7-30.1	0.28**
Medium	61	7.7	6.5-8.4	24.4	19.9-30.3	-0.02
Small	4	6.0	5.7-6.2	24.9	24.1-26.6	na
Total	219	9.1	5.7-13.7	25.0	19.9-30.3	0.28**
Over all the						
Crosses						
Large	661	9.6	8.5-14.2	25.8	20.0-32.7	-0.08*
Medium	930	7.4	6.5-8.4	26.2	18.8-33.3	-0.08*
Small	383	5.7	3.8-6.4	26.6	21.4-35.6	-0.02
Total	1974	7.8	3.9-14.2	26.2	18.8-35.6	-0.13**

*, ** significant at 5% and 1% respectively.

na = not appropriate due to small sample size.

Table 2. Protein content and seed size of newly developed high protein lines and their parents

Species/ genotype	Protein* (%)	100 seed wt. (g)	Protein/seed (mg)
<i>Cultivated spp.</i>			
Baigani	23.7	11.2	26.5
Pant A-2	22.7	7.5	17.0
T.21	24.4	7.5	18.3
<i>Wild spp.</i>			
<i>A. scarabaeoides</i>	28.4	2.3	6.5
<i>A. sericea</i>	29.4	1.9	5.6
<i>A. albicans</i>	30.5	2.8	8.5
<i>High protein lines</i>			
HPL 2	29.0	12.1	35.1
HPL 7	28.0	10.0	28.0
HPL 40	27.0	10.4	28.1
HPL 51	27.9	10.6	29.6

*Decorticated seed (N × 6.25).

seed size group in the cross T.21 × *A. sericea* the protein content and seed size were found to be independent of each other, while in the medium seeded group this association was highly significantly negative ($r = -0.72$, $P < 0.01$). Although over all crosses there exists a significant negative correlation ($r = -0.13$, $P < 0.01$) between seed size and protein content, the selection of genotypes with increased protein content and acceptable seed size is possible because of the low magnitude of this association.

In pigeonpea, Dahiya and Brar [9] found no correlation between seed size and protein in 220 germplasm accessions, while Reddy et al. [3] reported negative association between these two variables in T.21 × *A. sericea* derivatives. Similarly, Singh et al. [10] concluded from their studies on 14 genotypes of pigeonpea that no quality character such as protein percentage, amino acid ratios, and cooking period was associated with seed size.

In pigeonpea, the association between seed size and protein content appears to be partly genetic in origin. K.B. Saxena (unpublished) examined the relationship between seed size and protein at phenotypic, genotypic, and environmental levels in 10 late maturing yield trials involving a total of 192 cultivars. He observed that in general phenotypic as well as genotypic correlations were negative. The environmental correlations in his study were of a lower order indicating little common environmental effect on seed size and protein content.

According to Bahl et al. [11] a negative relationship between seed size and protein implies that seed increases in size as a result of the deposition of an increased amount of starch altering the starch:protein ratio. However, it has been found in *Vicia faba* [12], *Phaseolus vulgaris* [13] and soybean [14] that the negative correlation can be changed through breed-

ing, and it has been possible to identify exceptional genotypes in segregating generations that appeared more efficient than expected in their protein synthesis.

The correlation values obtained in the present studies indicate that in pigeonpea improved seed size and protein can be selected simultaneously. From the intergeneric crosses between *Cajanus* and *Atylosia* some promising high protein lines identified are HPL 2, HPL 7, HPL 40, and HPL 51 with respectively, protein percentage and seed size (g/100 seed) values of 29.0 and 12.1, 28.0 and 10.0, 27.0 and 10.4, and 27.9 and 10.6 (Table 2). This table also shows that protein per seed is higher in high protein lines than their parents.

In pigeonpea, seed size within certain ranges is also associated with yield. Sharma and Saxena [15] showed that these two variables are independent of each other in the seed size range of 9–12 g/100 seeds. However, this relationship was positive in material smaller and negative in material larger than the seed size range indicated above. This observation indicates that in the medium seed size material simultaneous improvement can be made for seed size as well as yield.

The new high protein large seeded lines are being used in our breeding program. Considering the associations of seed size with yield and protein content the development of high yielding and high protein cultivars with acceptable seed size appears to be feasible. Among important food legumes, pigeonpea contained the lowest amount of limiting sulphur amino acids, methionine and cystine implicating the importance of these amino acids in protein quality improvement program [1]. Our preliminary results (unpublished) have shown that these high protein lines are comparable with adopted pigeonpea cultivars with respect to their methionine and cystine contents. However, additional efforts to evaluate protein quality of the high protein lines in terms of their biological value, true digestibility, and net protein utilization would be most useful.

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