P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.14

Indian Journal of Plant Genetic Resources

SHORT COMMUNICATION

Transgressive Segregation for Seed Protein in an Inter-specific *Cajanus* Cross

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Abstract

Three inter-specific *Cajanus* crosses were assessed for their intra-population variation for protein content. In the unselected F_7 bulk population of a cross involving a wild species (*Cajanus scarabaeoides*) and an early maturing cultivar, "Baigani," the transgressive segregation for both high as well as low protein contents was observed. The high protein (35.6%) transgressive segregant produced 24% more protein than the wild species donor parent (28.7%). Similarly, the low-protein (18.8%) transgressive segregant had 17% less protein than the low-protein parent (22.7%). It is concluded that the parents of this cross carried different sets of dominant and recessive protein-controlling alleles; their complementary and/or additive gene effects produced these transgressive segregants.

Keywords: *Cajanus scarabaeoides, Cajanus cajan,* High protein, Genetic control, Segregation.

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Received: 05/06/2022 **Revised:** 15/12/2022

Accepted: 19/12/2022

How to cite this article: Saxena, K.B., Srivastava, N., Kumar, R.V. (2023). Transgressive Segregation for Seed Protein in an Inter-specific *Cajanus* Cross. *Indian J. Plant Genetic Resources*. 36(2), 313-316.

DOI: 10.5958/0976-1926.2023.00036.2.14

Introduction

Among the rainfed legume crops pigeon pea occupies a high place due to its adaptation to subsistence agriculture, diverse utility applications, high protein (20–22%), and its role in combating malnutrition in different tropical and sub-tropical countries (Saxena *et al.* 2021). It is, therefore, believed that breeding high-protein pigeon pea cultivars may help further enhance protein availability. In the past, the lack of suitable high-protein pigeon pea donors restricted the breeders from taking any protein enhancement programme. The identification of certain *Cajanus* wild species as potent high-protein donors (Reddy *et al.*,1997) has regenerated interest in breeding high-yielding high, protein pigeon pea cultivars. In an inter-specific cross involving a pigeon pea [*Cajanus cajan* (L.) Mill sp.] cultivar and its wild relative [*C. scarabaeoides*] events of transgressive segregation were observed. In such genetic events a hybrid population produces individual(s) with extreme values outside the range of the parents. This paper reports the selection of transgressive segregants for seed protein within an F_7 bulk population for both high as well as low protein contents.

Materials and Methods

The Parents

A high-protein wild relative of pigeonpea (*Cajanus scarabaeoides*) was selected for this research. This species is a creeper (Figure 1) and has several undesirable agronomic

Figure 1: Plants of *C. scarabaeoides* (source: ICRISAT photo lab)

traits, such as photosensitivity, excessive biomass, and pod shattering. Flowering in this species is profuse but seed yield is low. The pods of *C. scarabaeoides* have prominent dense hairs with 3 to 5 ovules in each. Its seeds are small (2–3 g/100 seeds), dark brown and unfit for consumption but rich (28.7%) in protein content. The seed coat is hard and takes over three weeks to germinate. To generate inter-specific breeding populations this species was crossed with three early maturing pigeon pea cultivars (Baigani, T 21, and Pant A2), known for their good seed traits, adaptation, and yield.

Field Plot Technique

A basal doze of fertilizer @100 kg/ha DAP was applied and for good drainage, ridges 75 cm apart, were constructed in field along the slope. The seeds were manually placed at 2 to 3 cm depth with an inter-hills distance of 30 cm. The experimental area was irrigated for uniform germination and further irrigations were given as and when required. From flowering to maturity, the crop was monitored by plant protection team and sprayed with chemical insecticides to control pod borers.

Development of Breeding Populations

Due to hard seed coat, the seeds of *C. scarabaeoides* were scarified with a sharp blade to enable them to germinate rapidly. All the four genotypes were sown in a crossing block. In all the crosses, the wild species was used as male parent. The floral buds of pigeon pea cultivars were emasculated and pollinated simultaneously using fresh pollen collected from the wild species. In each flower bunch, only two buds were used in crosses. In each cross, most of the pollinated buds dropped and the 8 to 11% hybridization success was achieved. All the hybrid seeds were sown in pots, and kept inside a glasshouse. The matured pods were handpicked from each plant, and the plants were left to produce a second flush of flowers to fetch more F_2 seeds. All the F_2 plants were harvested at maturity, and their seeds bulked for generation advance. All the subsequent generations were raised in vertisols field. Since pigeon pea flowers are cross-pollinated by bees, mainly *Megachile* and *Apis* species, the breeding materials were grown each year under nylon field nets fixed on aluminum frames. The breeding materials were advanced through bulk method of breeding up to F_5 generation. A handful of pods were harvested from each plant and threshed separately. Subsequently, 20 seeds from each plant were bulked for generation advance. In F_6 generation, all the bulks were grown and F_7 single plants were harvested for protein determination and selection.

Protein Estimations

The fresh seeds harvested from single plants were cleaned and oven-dried at 55°C for 24 hours. An electronic seed counter was used to draw the samples of 100 seeds. The testa of each seed was removed using a tangential abrasive de-hulling device (TADD) and grinding of the decorticated grains was carried out using Udy cyclone mill. The nitrogen estimations of the samples were done with the help of Technicon Auto Analyzer and the protein values were determined by multiplying the nitrogen readings by a factor 6.25 (Singh and Jambunathan1982). For each plant two determinations were done, and their mean values were used for the study.

Results and Discussion

In the F_7 bulk of cross Baigani x *C. scarabaeoides,* a total of 1231 plants were assessed for their protein content. In cross T. 21 x *C. scarabaeoides* and Pant A 2 x *C. scarabaeoides* respectively, 219 and 268 plants were involved (Table 1). The results showed a wide range for protein content in all three crosses. In crosses T. 21 x *C. scarabaeoides* and Pant A 2 x *C. scarabaeoides,* the parental type segregants were recovered and the promising segregants were marginally (5–6%) better than the high protein donor *C. scarabaeoides.*

The cross Baigani x *C. scarabaeoides*, appeared best with protein content ranging between 18.8 to 35.6%. This range extended the parental values by significant margins in both directions. These observations indicated the emergence of transgressive segregants for protein content in this population. The highest protein value recorded

Table 1: Transgressive segregation for seed protein (%) in three inter-specific crosses

Cross	Cultivar	Wild species	Mid-parent	Protein range	Transgressive segregants		
					ID	%Protein	%Gain
Baigani x *C. scarabaeoides*	22.7	28.7	25.70	18.8–35.6	B-1	35.6	24.04
T. 21 x *C. scarabaeoides*	20.4	28.7	24.55	19.9–30.3	T-1	30.3	5.57
Pant A 2 x *C. scarabaeoides*	21.7	28.7	25.20	22.2–30.4	P-1	30.4	5.92

in a segregant was 35.6%. This unique recombinant had 24.04% greater protein than the wild species donor (28.7%). Similarly, at the other end, the segregant with the lowest (18.8%) protein had 17.18% less protein than the low-protein parent Baigani (22.7%). When the individual(s) in a segregating hybrid population exhibit extreme values outside the parental range, the situation is described as "transgressive segregation". Such segregants are produced when the alleles of a given trait in the two parents are different. They combine to produce a unique recombinant using their complementary and/or additive effects. In the other two crosses, where the same high protein donor was used, such transgressive segregants were absent; but some recombinants with slightly greater protein content than the donor were recovered (Table 1). This indicated that in comparison to CV Baigani, the genetic constitution of cvs. T.21 and Pant A2 was different as far as the genes controlling protein content is concerned.

Saxena *et al.* (communicated, unpublished) tested the progeny of this genotype and a control at three locations. They reported significantly large gains in protein over the control. This selection recorded, respectively 32.1, 32.2, and 32.3% protein at Gulbarga (17.4°N), Jalna (19.8°N), and Gwalior (26.2°N). The control values ranged between 22 and 23%. These data indicated significant superiority and high stability this high protein selection.

Dahiya *et al*. (1977), while studying the genetics of high-protein trait in pigeon pea reported the presence of 3 to 4 genes. Reddy and Singh (1981) further revealed that high protein genes are dominant in nature. Durga (1989) concluded from her studies that these genes are additive and/or complementary in nature. Rick and Smith (1953), Grant (1975), and Vega and Frey (1980) reported that the transgressive segregation or generation of extreme variability in a population is a consequence of complementary gene actions and product-wise, such events could appear on positive, negative, or both the sides of the performance-curve. The emergence of transgressive segregants is generally correlated with the genetic divergence of the parental lines and the presence of certain recessive complementary alleles whose expression is masked by the major/dominant alleles. These conclusions were further confirmed through marker-based QTL studies (De Vicente and Tanksley 1993). In the present case both low and high-protein transgressive segregants were recovered. Thus, it can be postulated that complementation of high-protein alleles from the wild species and CV. Baigani together produced the transgressive segregants. To understand the true genetic nature of protein content in pigeon pea some targeted genetic and molecular studies are needed.

Saxena and Sawargaonkar (2015) reported that this protein trait is highly stable across locations and years. Also, Singh *et al.* (1990) revealed that the key nutritional efficiency parameters such as true protein digestibility, biological value, and net protein utilization of the high protein selections, were similar to the control cultivar. Hence, their cumulative utilizable proteins and sulfur-containing amino acids are considerably greater than the commonly grown pigeon pea cultivars. Therefore, based on the overall assessment, the high-protein transgressive selections were nutritionally superior to the present cultivars. Such high-protein genetic stocks are valuable to pigeon peas' genetic wealth. Hence irrespective of their agronomic traits, they should be preserved for future protein-related genetic studies or breeding programmes. Since these selections also carry a portion of the cultivated type of genome, they will not suffer from any hybridization incompatibility or linkage drag which are common issues in any inter-specific or inter-generic breeding programme.

Conclusion

Here, we report the selection of a high protein (35.6%) transgressive segregant from an inter-specific *Cajanus* cross. This is the highest protein value ever recorded in pigeon pea. Besides this, the protein quantity present in this line is nutritional efficient and stable across the environments. These traits make it a valuable germplasm resource for use in future pigeon pea breeding programmes.

Acknowledgments

The authors acknowledge Dr JM Green and Dr DG Faris, Principal Pigeonpea Breeders, ICRISAT, for their guidance and support in conducting this research.

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