

## INVITED REVIEW

Special Section: Breeding for Plant Based Proteins and Industrial Application of Pulse Crops

Genetic enhancement of seed protein in pigeonpea [*Cajanus cajan* (L.) Millspaugh Maesen]K. B. Saxena<sup>1</sup> | R. K. Saxena<sup>2</sup>  | N. Srivastava<sup>1</sup> | R. V. Kumar<sup>1</sup><sup>1</sup>International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, India<sup>2</sup>ICAR-Indian Agricultural Research Institute (IARI), Gogamukh, India

## Correspondence

K. B. Saxena, International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, 502 324, Telangana, India.

Email: [kbsaxena1949@gmail.com](mailto:kbsaxena1949@gmail.com)

R. K. Saxena, ICAR-Indian Agricultural Research Institute (IARI), Assam 787035, India.

Email: [Rachit.K.Saxena@gmail.com](mailto:Rachit.K.Saxena@gmail.com)

Assigned to Associate Editor Rebecca J. McGee.

## Abstract

Protein-energy malnutrition is a widespread social issue, particularly in Asia and Africa, where the availability of protein is about one-third of the natural requirements. Legumes are known for their high-protein grains, but the current protein harvests from this group of crops are not enough to meet the nutritional demand of the growing population. In this context, genetic enhancement of protein in pulses offers hope for additional protein supplies. International Crops Research Institute for the Semi-arid Tropics (ICRISAT) made efforts in this direction by breeding high-protein pigeonpea [*Cajanus cajan* (L.) Millspaugh Maesen] cultivars. The new genotypes were bred using three wild species as protein donors. These inbreds not only were high yielding (1700–2100 kg ha<sup>-1</sup>) but also had high protein (27%–30%) content, reflecting significant genetic advance for this trait. It was also estimated that cultivation of such cultivars on one hectare would yield an additional 80,000–100,000 g of protein for consumption. Also, their biological assessment, using Wistar male rats, revealed that the estimates of protein digestibility, biological value, and net protein utilization were similar to that of the popular cultivar. But, on account of greater availability of protein, the newly bred genotypes can be rated nutritionally superior to the present-day cultivars. Therefore, it is concluded that in pigeonpea, the genetic enhancement of seed protein without sacrificing yield is a viable plant breeding option. This process can be enhanced if the recently evolved genomics knowledge and technologies are used to assist in achieving nutritional food security.

## Plain Language Summary

Protein-energy malnutrition is a major challenge in Asia and Africa, where the available protein is far below the required levels. Legumes, known for their protein-rich grains, can help address this issue, but current harvests are insufficient to meet growing nutritional needs. To tackle this, researchers have developed new high-protein pigeonpea varieties by using wild relatives as donors. These improved varieties not only produced high yields (1700–2100 kg ha<sup>-1</sup>) but also contained 27%–30%

Abbreviations: MAS, marker-assisted selection; QTL, quantitative trait locus; SPC, seed protein content.

© 2025 The Author(s). Crop Science © 2025 Crop Science Society of America.

protein—significantly more than existing varieties. Growing these cultivars on one hectare could provide an additional 80–100 kg of protein, contributing to better nutrition. Tests on animals showed that the protein quality was comparable to popular cultivars, but the higher protein content made these new varieties nutritionally superior. This review shows that increasing protein levels in pigeonpea through breeding is possible without reducing yield.

## 1 | INTRODUCTION

The statistics published by FAO (2023) revealed that in the year 2022, over 700 million people in the world faced hunger, and this number is 122 million more than those who faced hunger in 2019. The Food and Agriculture Organization also projected that in 2030 almost 600 million people will be chronically undernourished, and about half of such children may fail to survive due to severe poverty-driven malnutrition. This scenario assumes even greater significance in the backdrop of the fact that the present per person protein availability in tropical and subtropical region is 24 g day<sup>-1</sup> and it is half of the normal requirement of an adult (FAO, 2023). It is generally believed that such a situation has emerged due to uncontrolled population growth, stagnation in production of protein-laden food, and increasing cost of animal protein resources.

In the past, the researchers demonstrated that enhancement of pulse supplement in cereal-based diets can markedly improve its nutritional quality (Daniel et al., 1970; Hulse, 1977; Kurien et al., 1971). Unfortunately, for low-income group of masses, such food standards are also too luxurious to afford on a sustainable basis. A survey of Indian villages carried out by Bidinger and Nag (1981) revealed that most of the regular rural diets are inadequate and provide only 10% of the protein, 5% of energy, and 21.7% of the required lysine. Shalendra et al. (2013) reported that over the decade from 2000 to 2010, there has been a decline in the consumption of cereals, pulses, and sugar in both rural and urban India, particularly the consumption of pulses, which falls significantly below the recommended daily intake of 42 g of protein per person in rural areas, dropping to 23 g in 2009 and 27 g in urban areas. This indicates a serious challenge regarding food and nutritional security in India, emphasizing the urgent need to promote the consumption of both cereals and protein-rich pulses.

Pulses are a known source of protein, and among these, pigeonpea [*Cajanus cajan* (L.) Millspaugh Maesen] is rated high because of its drought tolerance, soil-enriching properties, high protein, and multiple usages (Saxena et al., 2021). Globally, pigeonpea is cultivated on 6.36 million ha in 22 countries with production of 5.48 million tonne and average productivity of 0.86 tonne ha<sup>-1</sup>. India ranks first in

pigeonpea production with 4.34 million tonne grain harvested from 4.98 million ha land with mean yield of 0.87 tonne ha<sup>-1</sup> (Government of India, Ministry of Agriculture and Farmers Welfare, 2021). The other major pigeonpea-producing countries are Malawi (451,000 tonne), Myanmar (307,000 tonne), Tanzania (196,000 tonne), and Kenya (104,000 tonne). Besides these, Uganda, Mozambique, the Caribbean islands, and some South American countries also produce substantial amounts of pigeonpea.

The popular pigeonpea cultivars have 20%–22% seed protein (Jha et al., 2022), but it is not high enough to supplement the ever-expanding nutritional requirements. Therefore, augmenting protein yields from the new crop varieties emerges as a potential strategy in enhancing the role of pigeonpea in combating malnutrition. Since the land area will always remain limited for the low-yielding pulses, the genetic enhancement of seed protein offers hope for additional harvests of home-grown protein. This review aims to comprehensively assess the accomplishments in breeding high-protein pigeonpea cultivars while also evaluating their nutritional quality through biological assessments. Additionally, it highlights recent genomic advancements and explores their potential integration into future breeding strategies for enhanced protein content.

## 2 | PIGEONPEA IN HUMAN NUTRITION

Pulses are the main protein supplier, and the important crops in this group include *Vigna mungo* (25%–28% protein), *Cajanus cajan* (20%–22% protein), *Pisum sativum* (14%–31% protein), *Lens culinaris* (21%–31% protein), *Phaseolus vulgaris* (20%–30% protein), *Vigna unguiculata* (15%–25% protein), and *Cicer arietinum* (25%–29% protein) (Jha et al., 2022). Among these, *Cajanus cajan* (pigeonpea) is the most important pulse of subsistence agricultural systems.

Both dry and immature seeds of pigeonpea (Figure 1) are consumed by human beings, while pod shells, milling by-products, and foliage make a healthy fodder/feed for domestic animals. It is to be noted that the progenitor species of this pulse, *Cajanus cajanifolius*, is also consumed as a fresh vegetable by the tribes inhabiting the area of its origin, located on the east coast of central India (van der Maesen, 1986).

The first pioneering research on nutritional qualities of pulses was carried out by Pal (1939), and the results showed that pigeonpea was the best source of biological protein for humans and suggested that it should be eaten with rice to make a balanced diet. In India, de-hulled split cotyledons of pigeonpea are cooked into a thick soup (locally called as “dal”) and eaten with bread and rice. In Africa and South America, whole dry pigeonpea grains are cooked into a porridge-like dish. Besides these, its fully grown but immature seeds are harvested about a month after flowering, and these are consumed as fresh, frozen, or canned vegetables (Saxena et al., 2021). Both the dry and fresh grains of pigeonpea are nutritive, but they differ quantitatively with respect to certain nutrients.

By weight the mature pigeonpea seeds contain about 85% cotyledons, 14% seed coat, and 1% embryo (Table 1). The edible portion in pigeonpea seed is its two large cotyledons, which are rich in both carbohydrates (65%–70%) and proteins (20%–22%). Its embryo is small and predominantly (about 50%) made up of proteins (Faris & Singh, 1990). On the other hand, seed coat contains 30%–35% fiber and negligible protein. Pigeonpea protein comprises four primary components, namely, albumin, globulin, glutelin, and prolamin. Notably, pigeonpea contains a significant proportion of lysine (Singh & Jambunathan, 1982). The values of globulin, the sulfur-deficient amino acid, were high in the proteins from seed, cotyledons, and embryo. In these entities the proportions of prolamin were <5%. Methionine and cysteine, the sulfur-containing amino acids, are present in the cotyledons and embryo but only in about 1% of the proportion.

### Core Ideas

- Genetic enhancement of protein in pulses offers hope for additional protein harvests.
- Pigeonpea is rated high because of its resilience and high protein availability.
- To encounter the issues of hunger and malnutrition, increases in productivity and protein harvests are required.

In comparison to mature seeds, the fresh peas contain greater proportions of crude fiber and fat, with high protein digestibility. Also, they contain significant amounts of trace and mineral elements, including phosphorus, potassium, zinc, copper, and iron. Pigeonpea seeds are also rich in vitamins A<sub>1</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, B<sub>9</sub>, C, and E (June 2024). The vegetable pigeonpeas also have a nutritional edge over garden peas (*Pisum sativum*). A comparative analysis of the two species shows that the vegetable pigeonpea excels over the garden pea in having more than five times  $\beta$ -carotene, three times thiamine (vitamin B<sub>1</sub>), riboflavin (vitamin B<sub>2</sub>), and niacin, two times more of ascorbic acid, Ca, and Cu (Faris et al., 1987).

In addition to essential nutrients, mature pigeonpea seeds also contain certain anti-nutritional elements, as documented in various studies (Harris et al., 2014; Singh, 1988; Talari & Shakappa, 2018). These compounds encompass oligosaccharides (such as raffinose, stachyose, and verbascose), enzyme



**FIGURE 1** A commercial crop and marketable splits of pigeonpea (above) and vegetable-type pods and seeds (below). Source: RV Kumar.

**TABLE 1** Nutritional parameters recorded in different parts of mature pigeonpea seeds.

| Constituents <sup>a</sup>            | Seed | Cotyledons | Embryo | Testa |
|--------------------------------------|------|------------|--------|-------|
| Protein                              |      |            |        |       |
| Total (%)                            | 20.5 | 22.2       | 49.6   | 4.9   |
| Albumin (%)                          | 10.2 | 11.4       | 17.0   | 2.6   |
| Globulin (%)                         | 59.9 | 64.5       | 52.7   | 26.3  |
| Glutelin (%)                         | 17.4 | 18.2       | 21.3   | 32.8  |
| Prolamin (%)                         | 3.0  | 3.5        | 2.7    | 4.2   |
| Key amino acids<br>(g/100 g protein) |      |            |        |       |
| Lysine                               | 6.8  | 7.1        | 7.0    | 3.9   |
| Threonine                            | 3.8  | 4.3        | 4.7    | 2.5   |
| Methionine                           | 1.0  | 1.2        | 1.4    | 0.7   |
| Cysteine                             | 1.2  | 1.3        | 1.7    | –     |

<sup>a</sup>Source: Faris and Singh (1990); Singh and Jambunathan (1982).

inhibitors (including trypsin, chymotrypsin, and amylase inhibitors), and polyphenols (like phenols and tannins). However, most of these anti-nutritional components are typically eliminated when pigeonpea seeds are consumed in the form of decorticated cooked splits.

### 3 | PIGEONPEA IN ANIMAL NUTRITION

The Indian Council of Agricultural Research-Indian Grassland and Fodder Research Institute reported that India suffers from a deficit of green fodder, dry fodder, and concentrate mixture to the tune of 35.6%, 10.95%, and 44.0%, respectively. Hence, to narrow down this gap, some non-conventional fodder/feed resources can be exploited for livestock feeding. Pigeonpea has potential to provide quality legume forage in livestock diets as the main protein resource (Mekonen et al., 2022; Phatak et al., 1993). During the process of preparing pigeonpea splits from dry grains for marketing and consumption, the mills produce about 15% of cotyledonous powder, and it gets mixed with husk and embryo trash. The powder is rich in protein and fiber and can directly be used as pulse protein-based value-added products. Studies conducted on protein resources also revealed that seeds and leaves of pigeonpea can be incorporated up to 20% and 30% in the diets of lactating cows and goats, respectively (Mekonen et al., 2022; Phatak et al., 1993).

In the post rainy season, when the main fodder crops disappear from fields, pigeonpea can provide a good grazing option for cattle, sheep, and goats. For stall feeding the pigeonpea plants with soft branches, dry leaves, flowers, and young pods are harvested and finely chopped and mixed with grass fodder in a proportion of about 20%. This fodder mixture is being used to partially fulfill the protein requirements of dairy

industry located in hilly, dry areas of southern China. Corriher et al. (2010) observed that pigeonpea seeds can be incorporated at the rate of 20% in a maize silage-based diet without any detrimental effect on weight milk production in Holstein cows. The dairy farmers also preserve the pigeonpea fodder in silos and salted fodder bricks for use in dry season (Saxena et al., 2021).

The protein content of fresh forage ranges between 10% and 25%. As compared to older leaves, the younger green leaves are more palatable and contain greater amounts of protein. The pigeonpea hay contains 9.3% crude protein, 9.3% ash, 78.6% neutral detergent fiber, 60.2% acid detergent fiber, and 2.2 M cal kg<sup>-1</sup> dry matter metabolizable energy. Squibb et al. (1950) opined that the dry pigeonpea leaves can replace alfalfa as a source of carotene and other essential nutrients in chicken rations. According to Wallis et al. (1986), pigeonpea seeds, pods, and milling trash can also be used as alternate to soybean and maize in pig and poultry industries. Raw pigeonpea seeds can be included up to 20% in diets of growing pig. Raw and processed pigeonpea seeds can also be included up to 10% and 20%, respectively, in poultry diets (Ali et al., 2020). However, the deficiency of certain amino acids may limit the effectiveness of this resource unless suitable additives are incorporated during feed processing.

### 4 | GENETIC REGULATION OF SEED PROTEIN CONTENT (SPC) IN PIGEONPEA

Understanding the genetic regulation of a given trait is advantageous for its improvement, particularly when devising its mating and selection strategies. Qureshi et al. (2013) while reviewing the subject concluded that in different legumes the protein content is typically governed by dominant, partially dominant, additive, and/or non-additive genetic mechanisms. In pigeonpea the information on genetic control of protein is scarce, that too with inconsistent results. This could be a consequence of low priority or limitation of funding resource for research.

#### 4.1 | Pigeonpea crosses

Dahiya and Brar (1977) and Durga (1989) found significant maternal inheritance of seed protein in F<sub>1</sub> generation. Dahiya et al. (1977) reported the presence of 3–4 protein regulating genes in pigeonpea, while Durga (1989) reported that the protein content in pigeonpea was under the control of additive and complementary gene action with dominance of low protein. According to Casey and Domoney (1984) there were a number of storage protein genes in pigeonpea with co-dominant inheritance. Further investigation into the inheritance of protein content revealed the importance of

dominance and epistatic effects, suggesting polygenic control (Obala et al., 2018); they also proposed the use of reciprocal recurrent selection to simultaneously enhance seed yield and protein in pigeonpea.

## 4.2 | Inter-specific crosses

Reddy et al. (1979) observed that in an interspecific cross the high protein was controlled by two recessive alleles. In contrast, Reddy and Singh (1981) working with another inter-specific cross, reported that the high-protein trait was controlled by dominant genes with quantitative variation being expressed in  $F_2$  generation. Recently, Saxena, Srivastava, Reddy, et al. (2023) revealed that two independent dominant genes with complementary effects controlled the high protein trait. They concluded that the protein content in pigeonpea is rather simply inherited and high yielding high-protein cultivars can be bred through pedigree breeding. Also, to maintain high breeding values of the selections, the protein estimation methods should be reliable. This would make the screening of segregating populations effective.

## 4.3 | Screening of primary gene pool for high protein donors

To breed high yielding high-protein pigeonpea cultivars, the availability of stable high protein donors was the primary requisite. For this, a search for useful donor parent with distinctly high protein content was made, first, the primary gene pool was screened. In the past half-century, the genetic variation for protein content in pigeonpea was studied by Tripathi et al. (1975), Hulse (1977), Narsimha and Desikachar (1978); Manimekalai et al. (1979), Remanandan et al. (1988), Srivastava and Vasishtha (2012), Obala et al. (2018), and Choi et al. (2020). The inference drawn from these reports was that the variation for protein content in the primary *Cajanus* gene pool is limited (17%–22%) and, thus, this resource cannot be used as protein donor in the targeted high protein breeding programs.

## 4.4 | Exploring secondary gene pool of wild species for high protein donors

It is important to highlight the efforts aimed at developing high-yielding pigeonpea cultivars with enhanced protein content. This endeavor involved transferring high-protein genes from wild *Cajanus* species into cultivated pigeonpea. After an unsuccessful search for high-protein donors within the primary gene pool, pigeonpea breeders shifted their focus toward utilizing the secondary gene pool. They screened several wild

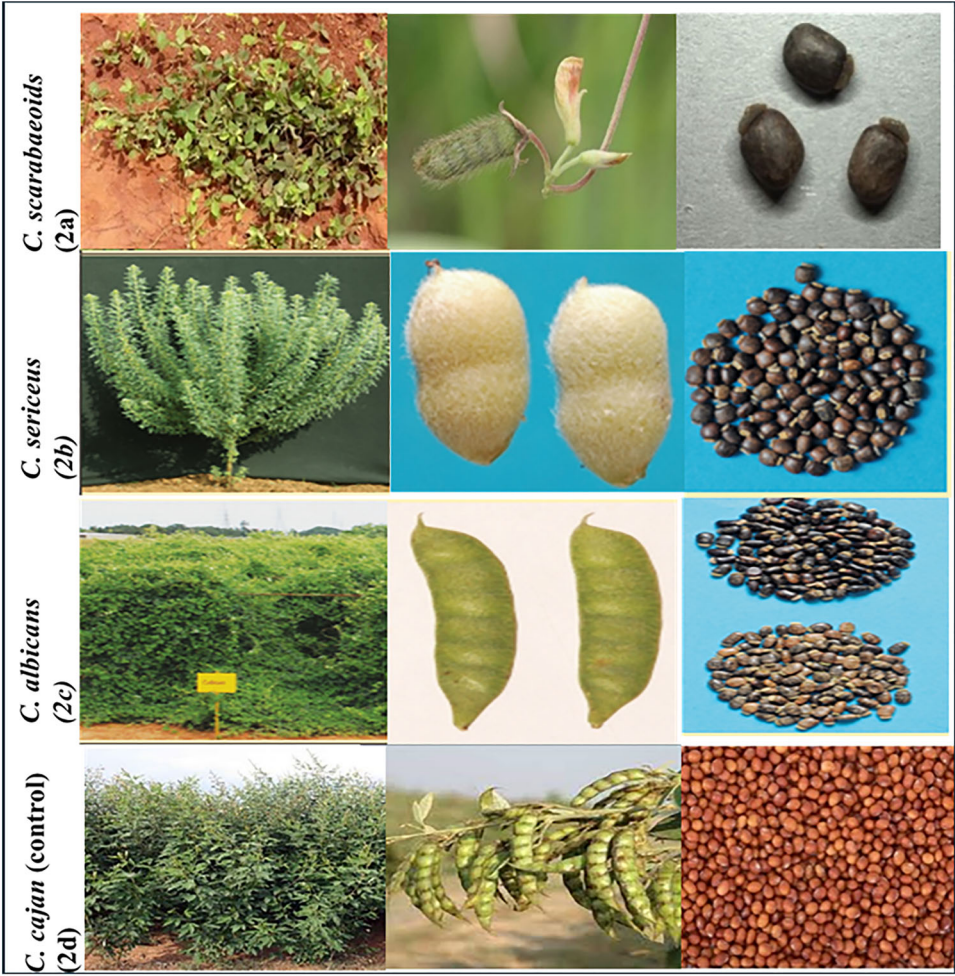
relatives of pigeonpea for SPC and identified three high-protein wild species that could be successfully crossed with cultivated varieties. In the interspecific hybridizations, these wild species were used as female parents, and their emasculated floral buds were hand-pollinated with fresh pollen from cultivated pigeonpea flowers. In  $F_1$  generation, each seedling was examined for leaf marker; and those resembled their respective maternal parent were considered self-pollinated. The success rate of hybridization, based on the count of true hybrid plants, ranged from <5% to 35% (Reddy & Singh, 1981). The selected high-protein donor species were *Cajanus scarabaeoides* (L.), *C. sericeus* (Benth.), and *C. albicans* (W. & A.). The key traits of these wild species, as described by van der Maesen (1986), are briefly summarized here for quick reference.

*Cajanus scarabaeoides* is widely distributed in parts of Asia, Australia, and Africa. It is generally found growing in open grassland, dry scrub vegetation and deciduous monsoon forests (Figure 2a). The pods are 1–2 cm long and each pod, on average, produces 3–5 dark grey small (2.5 g 100 seeds<sup>-1</sup>) grains. Protein content of its decorticated seeds is high (28.4%) (Table 2). The native range of *C. sericeus* is India and Myanmar. It is adapted to seasonally dry tropical biome. The plants are about 1 m tall with densely erect striate branches with dense, short, and whitish silky hairs (Figure 2b). Its racemes are sessile, axillary, and 1–3 yellow flowers are borne in leaf axils. The oblong pods are small (11–13 mm) and, on average, contain two rectangular-round grey/black seeds which contain 29.4% protein. The third wild species, *C. albicans*, is distributed in the tropical dry deciduous forests of peninsular India and Sri Lanka (Figure 2c). It is a perennial climber with woody base and long branches with whitish pubescent leaves. The pods are 3–4 cm long and, on average, they contain 5–7 small grey seeds and they contain about 30% protein. The SPC in above mentioned studies was estimated following Singh and Jambunathan (1981).

## 4.5 | Hybridization and selection

Due to insect-aided cross-pollination, all the breeding activities including seed multiplication were carried out inside insect-proof cages fixed over aluminium frames. First, based on protein data, the best accession of each wild species was selected for hybridization as female parent. The details of different breeding activities and procedures are described by Saxena, Srivastava, Kumar, et al. (2023).

The  $F_1$  hybrid plants were raised inside a glasshouse and multiple pod harvesting was done for raising large  $F_2$  populations. From  $F_2$  to  $F_5$  generations pedigree breeding was adopted and selection for protein (>25%), seed size (>6 g 100 seeds<sup>-1</sup>), pod size (>4 seeds pod<sup>-1</sup>), seed color (white or brown), and normal plant type was exercised. In the follow



**FIGURE 2** Plants, pods, and seeds of three high protein donor wild species (2a,b, and c) compared with a popular pigeonpea cultivar (2d). Source: International Crops Research Institute for the Semi-arid Tropics (ICRISAT).

**TABLE 2** Selection of three high-protein wild species from secondary Cajanus gene pool.

| Cajanus wild species | Plant type | 100-seed weight (g) | Seed color | Protein (%) |                      |
|----------------------|------------|---------------------|------------|-------------|----------------------|
|                      |            |                     |            | Seeds       | % Deviation from (C) |
| C. scarabaeoides     | Trailing   | 2.5                 | Dark       | 28.4        | 28.5                 |
| C. sericeous         | Erect      | 1.9                 | Dark       | 29.4        | 33.0                 |
| C. albicans          | Creeper    | 2.8                 | Dark       | 30.5        | 38.0                 |
| BDN 1 (C)            | Spreading  | 9.8                 | Brown      | 22.1        | —                    |

up F<sub>6</sub>–F<sub>9</sub> generations, selections for high (26%–32%) protein and favorable morphological traits were carried out.

4.6 | Transgressive segregation for protein content

In cross Baigani × *C. scarabaeoides*, the protein content ranged between 18.8% and 35.6%; and it extended the parental values by significant margins at either end of the curve.

These observations pointed toward the presence of transgressive segregants for protein content. The highest protein value recorded in one of the segregant was 35.6%. This unique recombinant had 24.04% greater protein over the wild species donor (28.7%) (Saxena, Srivastava, Kumar, et al., 2023). Theoretically, such segregants are produced when the genes for a given trait in the two parents are vastly different and occasionally combine to produce such unique recombinants to produce unexpected results using complementary and additive gene effects.

**TABLE 3** Comparison of high protein selections with control for protein, yield, maturity, and seed size.

| Item                    | High protein line HPL 8 | High protein line HPL40 | Local control |
|-------------------------|-------------------------|-------------------------|---------------|
| Morphological traits    |                         |                         |               |
| Yield (kg/ha)           | 1660                    | 2100                    | 2060          |
| Seed size (g/100 seeds) | 10.5                    | 9.6                     | 9.6           |
| Maturity (days)         | 163                     | 169                     | 168           |
| Nutrient constituents   |                         |                         |               |
| Starch (%)              | 54.3                    | 55.6                    | 59.3          |
| Protein (%)             | 28.7                    | 31.1                    | 23.1          |
| Albumin (%)             | 9.1                     | 8.0                     | 8.6           |
| Globulin (%)            | 63.5                    | 66.2                    | 60.3          |
| Glutelin (%)            | 20.2                    | 19.7                    | 22.8          |
| Prolamin (%)            | 2.9                     | 3.2                     | 2.1           |
| Cysteine                | 0.8                     | 0.8.                    | 0.7           |

Source: Singh et al. (1990).

#### 4.7 | Chemical composition of high protein selections

The protein contents of the high protein selections were about 20% greater than the local control (Table 3). Besides protein, the seeds were also analyzed for their macro and micro-nutrients (Faris & Singh, 1990). The starch content in the selections was lower by about 8%. The greater proportion of globulin, the major component of storage protein, in the high-protein genotypes, was associated with their low glutelin fraction. The amino acid composition (100 g protein<sup>-1</sup>) of the high-protein genotypes was comparable with those of the control. However, the sulfur-containing amino acids methionine and cystine were noticeably higher in the high-protein genotypes (Singh et al., 1990).

#### 4.8 | Distribution of protein granules within high- and low-protein pigeonpea seeds

To study the distribution patterns of protein and starch granules within the low (22.48%) and high (29.64%) protein seeds, 10- to 12- $\mu$ m-thick sections were prepared. Significant differences were observed in the organizational set up of cellular protein and starch molecules in low and high-protein pigeonpea genotypes (Reddy et al., 1979).

Each cell of the low protein line had 7.47 starch grains and they occupied 645.34  $\mu$ m<sup>2</sup> area. In contrast, the corresponding values in the high protein selections were 3.88 and 296.82 ( $\mu$ m<sup>2</sup>), respectively. In the high protein selections these values were significantly low, suggesting greater loading of protein granules in the cells of high-protein genotypes. Besides these,

the intra-cell staining pattern in the high- and low-protein lines also differed considerably. The microscopic observations showed that the intensity of starch staining increased gradually toward inner layers of cells. This study also showed that in the high-protein lines the starch grains in the peripheral layers of cotyledonary cells were few or absent; and their concentration gradually increased toward the inner layers of the cells (Reddy et al., 1979).

The high concentration of protein molecules toward periphery of the seed may worry some. This is because the majority of the produce is milled and polished to produce quality splits and it may machine-off the valuable protein-rich outer layers. This will necessitate appropriate adjustments in the milling unit to minimize such nutrient losses.

#### 4.9 | Stability of high protein trait in diverse environments

Sham (1976) and Jain et al. (1986) reported significant adverse effect of environment on the stability of seed protein in pigeonpea. Hence, the stability of high protein trait was also studied at diverse locations spread over six Indian provinces at the latitudes ranging from 17.3 to 29.1 °N. The results showed that despite some variation (28.9%–30.4%) among the locations, at each site the high-protein lines maintained their superiority over the control (Table 4). Among the test lines, HPL 24 appeared to be the best; and its protein showed a little variation (31.3%–32.3%) with a mean value of 31.6%. Such a genotype can be used as donor for future breeding programs.

#### 4.10 | Yield assessment of high protein selections

The first set of high protein F<sub>10</sub> inbreds were evaluated for their productivity and other key parameters. The results were encouraging and among non-determinate lines, the test lines HPL 40–5 and HPL 40–17 produced, respectively, 2100 and 2070 kg ha<sup>-1</sup>, and these yields were at par with the control cultivar BDN 1 (2020 kg ha<sup>-1</sup>). The protein content of these selections; however, was significantly greater than the control (Table 5). Similarly, the determinate selection HPL 8–10 was similar in productivity to the control but significantly superior in the protein content.

The advantage of high-protein lines was also reflected in the total protein harvest from a unit land. For example, selection HPL 40–5 produced an estimated protein yield of 452 kg ha<sup>-1</sup>; it was 21.2% greater than the control BDN 1. These results suggested that breeding of high-protein cultivars is nutritional viable and such activities could be undertaken in pigeonpea without any significant yield penalty.

**TABLE 4** Protein percent of four high-protein lines (HPL) in six diverse locations.

| Province       | Location   | Lat. (°N) | HPL 24 | HPL 25 | HPL 26 | HPL 28 | Check | Standard error |
|----------------|------------|-----------|--------|--------|--------|--------|-------|----------------|
| Telangana      | Patancheru | 17.3      | 31.3   | 28.6   | 29.7   | 27.8   | 23.3  | ±0.3           |
| Karnataka      | Gulbarga   | 17.4      | 32.1   | 29.9   | 29.6   | 27.6   | 23.0  | ±0.5           |
| Maharashtra    | Jalna      | 19.8      | 32.2   | 28.9   | 29.7   | 30.4   | 23.1  | ±0.7           |
| Gujarat        | SK Nagar   | 24.5      | 30.9   | 28.4   | 29.0   | 27.3   | 21.4  | ±0.4           |
| Madhya Pradesh | Gwalior    | 26.2      | 32.3   | 30.4   | 28.2   | 27.3   | 22.0  | ±0.7           |
| Haryana        | Hisar      | 29.1      | 31.1   | 29.6   | 31.7   | 29.2   | 24.5  | ±0.5           |
| Mean           |            |           | 31.7   | 29.30  | 29.66  | 28.27  | –     | –              |

Source: Pigeonpea breeding unit, International Crops Research Institute for the Semi-arid Tropics (ICRISAT).

**TABLE 5** Seed yield and protein harvest from high-protein lines.

| Genotype                          | Maturity (days) | 100-seed wt. (g) | Yield (t ha <sup>-1</sup> ) | Protein (%) | Protein yield (kg ha <sup>-1</sup> ) |
|-----------------------------------|-----------------|------------------|-----------------------------|-------------|--------------------------------------|
| <b>Non-determinate selections</b> |                 |                  |                             |             |                                      |
| HPL 40–5                          | 169             | 9.6              | 2.10                        | 26.9**      | 452                                  |
| HPL 40- 17                        | 169             | 8.5*             | 2.07                        | 26.5**      | 440                                  |
| BDN 1 (C)                         | 168             | 9.6              | 2.02                        | 23.2        | 373                                  |
| <b>Determinate selections</b>     |                 |                  |                             |             |                                      |
| HPL 8–10                          | 163             | 10.5**           | 1.66                        | 26.5**      | 353                                  |
| HPL 8–16                          | 162             | 10.5**           | 1.57                        | 27.4**      | 344                                  |
| ICPL 211 (C)                      | 162             | 14.3             | 1.46                        | 21.6        | 251                                  |

Significant deviation from control (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

Source: Singh et al. (1990).

**TABLE 6** The estimated parameters (g/100 g) were recorded for evaluating biological efficiency of high protein selections.

| Material | Line    | Protein % | True protein digestibility | Biological value | Net protein utilization | Utilizable protein |
|----------|---------|-----------|----------------------------|------------------|-------------------------|--------------------|
| Whole    | HPL-40  | 27.3*     | 58.5                       | 70.5**           | 40.9                    | 11.2*              |
|          | LPL C11 | 21.9      | 59.5                       | 64.3             | 38.3                    | 8.4                |
| Splits   | HPL-40  | 31.1*     | 69.8                       | 73.6             | 51.4                    | 16.0*              |
|          | LPL C11 | 24.8      | 72.3                       | 73.6             | 53.2                    | 13.2               |

\* $p < 0.05$ . \*\* $p < 0.01$ .

Source: Partially adapted from Singh et al. (1990).

#### 4.11 | Biological assessment of high-protein genotypes

Breeding of high-protein pigeonpea lines with good yield potential is considered an important breakthrough in the breeding history of the crop. The second necessary step is to verify their potential role in the enhancement of nutritional parameters and related health benefits. In this context, various key biological parameters such as true protein digestibility, biological value, net protein utilization, and utilizable protein were determined by conducting a laboratory feeding trial using metabolic cages and Wistar male rats (Singh et al., 1990) (Table 6). Results of this experiment showed that (i)

the estimates of utilizable protein were significantly superior to the local normal protein cultivar, (ii) the protein values recorded for whole seeds samples were significantly inferior to the decorticated splits, due to the presence of polyphenol and fiber present in the seed coat, (iii) the polyphenols tend to decrease protein digestibility by inhibiting the production of digestive enzymes and increasing fecal nitrogen (Bressani et al., 1988; Singh, 1988), (iv) the levels of various nutritional attributes of high and normal (control) protein genotypes are quite comparable, and (v) the high-protein genotypes are nutritionally superior to normal-protein cultivars as they contain quantitatively more utilizable protein and sulfur containing amino acids.

## 5 | DISCUSSION AND OUTLOOK

Food and nutritional security is defined as the availability of adequate food in terms of quantity, quality, safety, and acceptability at all times to ensure a healthy and active life. However, many countries continue to struggle to meet this benchmark. According to the United Nations, factors such as inexorable population growth, limited availability of vegetable protein, and the rising costs of animal protein contribute significantly to nutritional insecurity, particularly among poor populations. Additionally, the rapid expansion of industrialization and urbanization has led to shrinking croplands, limiting the scope for horizontal agricultural expansion. To address the pressing challenges of hunger and malnutrition, increasing protein harvests per unit area is imperative.

In this context, advances in pigeonpea genomics offer a promising avenue for accelerating genetic gains, particularly in enhancing SPC (Bohra et al., 2020; Varshney et al., 2012). Genomic tools such as marker-assisted selection (MAS) and high-throughput sequencing technologies have enabled the identification of sequence-based markers and candidate genes associated with SPC, providing opportunities for targeted trait improvement (Jamedar et al., 2024; Obala, et al., 2019). Whole-genome resequencing studies have further pinpointed sequence variations influencing SPC, laying the groundwork for genomics-assisted breeding programs. These research efforts have also elucidated the genetic architecture of SPC and its relationship with agronomic traits. Through quantitative trait locus (QTL) analysis and genotyping-by-sequencing, major QTLs and epistatic QTLs influencing SPC and related traits have been identified (Obala et al., 2020). Identified genomic resources can be systematically applied in MAS to further improve pigeonpea protein content in upcoming breeding programs. Additionally, in the future, non-destructive phenomics approaches could enhance the efficiency of identifying high-protein segregants.

Genetic enhancement of pulses, particularly pigeonpea, is a crucial strategy for combating poverty, malnutrition, and starvation. Identifying stable high-protein sources, understanding the genetic control of SPC, and successfully transferring high protein traits into adapted genotypes indicate that high-protein pigeonpea cultivars can be developed without compromising grain yield. Pigeonpea, despite being a low-yielding pulse crop, contains approximately 22% grain protein, making it a vital source of dietary protein, especially in rainfed farming systems. Given the unpredictable nature of agriculture in such regions—often plagued by intermittent drought and other climatic challenges—pigeonpea remains one of the most adaptable pulse crops due to its drought tolerance and soil-ameliorating properties (Saxena et al., 2021).

In the present research, efforts were made to enhance SPC in pigeonpea through breeding. High protein traits

were successfully introgressed from wild species of the secondary gene pool, followed by the elimination of undesirable wild traits through large-scale selection in segregating populations. The success in developing high-protein genotypes without a significant yield penalty marks a major achievement in plant breeding. These genotypes should be conserved for future breeding programs as donor parents and for advanced genetic and molecular studies. Ensuring the genotypic purity of these high-protein lines over generations is crucial, as natural outcrossing poses a threat to their genetic stability. To mitigate this, multiplication should be carried out under insect-proof cages or in isolated environments.

Since pigeonpea grains and milling by-products are commonly used as feed for both ruminants and non-ruminants (Saxena et al., 2021), high protein derivatives could serve as an excellent source of digestible protein. Further studies may be required to assess the biological value of these emerging genotypes. The newly developed transgressive segregants with exceptionally high protein content represent valuable germplasm for future breeding programs aimed at improving resistance to biotic and abiotic stresses. Based on these pigeonpea breeding advancements, it is estimated that cultivating high-protein pigeonpea on a one-hectare plot could yield an additional 80–100 kg of digestible protein. As an affordable and homegrown protein source, large-scale cultivation and consumption of these high-protein lines could significantly contribute to alleviating nutritional insecurity in rural populations.

## AUTHOR CONTRIBUTIONS

**K. B. Saxena:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing—original draft; writing—review and editing. **R. K. Saxena:** Conceptualization; formal analysis; methodology; project administration; resources; supervision; writing—original draft; writing—review and editing. **N. Srivastava:** Data curation; methodology; resources; writing—original draft. **R. V. Kumar:** Investigation; methodology; resources; writing—original draft.

## ACKNOWLEDGMENTS

We would like to acknowledge all the authors of cited research in this MS and support from ICAR, DBT, ICRISAT, and several partners from different institutions. RKS also acknowledges the support from Department of Science and Technology (DST), Government of Gujarat, and Gujarat Biotechnology University (GBU).

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## ORCID

R. K. Saxena  <https://orcid.org/0000-0002-9405-3570>

## REFERENCES

- Ali, S. T., Molla, B., Abto, A., Asres, Z., Liuel, Y., & Mesifen, L. (2020). Effect of feeding pigeon pea (*Cajanus cajan*) seed meal on nutrients intake and growth performances of broiler chicken breeds. *Scientific Papers: Animal Science and Biotechnologies*, 53, 1–5.
- Bidinger, P. D., & Nag, B. (1981). The role of pigeonpeas in village diets. In *Proceedings of the international workshop on pigeonpeas* (pp. 357–364). ICRISAT.
- Bohra, A., Saxena, K. B., Varshney, R. K., & Saxena, R. K. (2020). Genomics assisted breeding for pigeonpea improvement. *Theoretical and Applied Genetics*, 133, 1721–1737. <https://doi.org/10.1007/s00122-020-03563-7>
- Bressani, R., Hernandez, E., & Braham, J. E. (1988). Relationship between content and intake of bean polyphenolics and protein digestibility in humans. *Plant Foods for Human Nutrition*, 38, 5–21. <https://doi.org/10.1007/BF01092306>
- Casey, R., & Domoney, C. (1984). The genetics of legume storage proteins. *Philosophical Transactions of the Royal Society of London. Biological Sciences*, 304, 349–358.
- Choi, Y. M., Lee, S., Yoon, H., Lee, M. C., Oh, S., Ko, H. C., Shin, M. J., Hur, O., Yi, J. Y., & Kebede, T. D. (2020). Agricultural characters, phenolic and nutritional contents, and antioxidant activities of pigeonpea (*Cajanus cajan*) germplasms cultivated in the Republic of Korea. *Korean Journal of Plant Resources*, 33, 50–61.
- Corriher, V. A., Hill, G. M., Bernard, J. K., Jenkins, T. C., West, J. W., & Mullinix, B. G. Jr. (2010). Pigeonpeas as supplement for lactating dairy cows fed corn silage-based diets. *Journal of Dairy Science*, 93, 5309–5317. <https://doi.org/10.3168/jds.2010-3182>
- Dahiya, D. S., & Brar, J. S. (1977). Diallel analysis of genetic variation in pigeonpea (*Cajanus cajan*). *Experimental Agriculture*, 13, 191–200. <https://doi.org/10.1017/S0014479700007808>
- Dahiya, D. S., Brar, J. S., & Bhullar, B. S. (1977). Inheritance of protein content and its correlation with grain yield in pigeonpea (*Cajanus cajan* L. Millsp.). *Qualitas Plantarum—Plant Foods for Human Nutrition*, 27, 327–334. <https://doi.org/10.1007/BF01092325>
- Daniel, V. A., Narayanaswamy, D., Desai, B. L. M., Kurien, S., Swaminathan, M., & Parpia, H. A. B. (1970). Supplementary value of varying levels of red gram (*Cajanus cajan*) to poor diets based on rice and ragi. *Indian Journal of Nutrition and Dietetics*, 7, 358–362.
- Durga, B. K. (1989). *Genetic studies on protein content and nitrogen accumulation in pigeonpea* [Ph.D. Thesis, Osmania University].
- FAO. (2023). *FAOSTAT statistical database*. Food and Agriculture Organization of the United Nations. <http://www.fao.org/faostat/>
- Faris, D. G., Saxena, K. B., Mazumdar, S., & Singh, U. (1987). Vegetable pigeonpea—A promising crop for India (Information Bulletin No. 3). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).
- Faris, D. G., & Singh, U. (1990). Pigeonpea: Nutrition and products. In Y. L. Nene, S. D. Hall, & V. K. Sheila (Eds.), *The pigeonpea* (pp. 401–434). CAB International.
- Government of India, Ministry of Agriculture and Farmers Welfare. (2021). <https://www.agricoop.nic.in>
- Harris, K. K., Sahu, M., & Verma, D. (2014). Phytochemical analysis of the leaf, stem, and seed extracts of *Cajanus cajan* L. (dicotyledoneae, Fabaceae). *World Journal of Pharmacy and Pharmaceutical Sciences*, 3, 694–733.
- Hulse, J. H. (1977). Problems of nutritional quality of pigeonpea and chickpea and prospects of research. In J. H. Hulse, K. O. Rachie, & L. W. Billingsley (Eds.), *Nutritional standards and methods of evaluation for food legume breeders* (pp. 88–100). IDRC.
- Jain, K. C., Saxena, K., Singh, U., Reddy, L. J., & Faris, D. G. (1986). Genotype-environment interactions for protein content in pigeonpea. *International Pigeonpea Newsletter*, 5, 18–19.
- Jamedar, H. V. R., Gandham, P., Bajaj, P., Thati, S., Rao, V. S., & Varshney, R. K. (2024). Identification of superior haplotypes for seed protein content in pigeonpea (*Cajanus cajan* L.). *Journal of Plant Biochemistry and Biotechnology*, 33, 178–188.
- Jha, U. C., Nayyar, H., Parida, S. K., Deshmukh, R., von Wettberg, E. J. B., & Siddique, K. H. M. (2022). Ensuring global food security by improving protein content in major grain legumes using breeding and ‘omics’ tools. *International Journal of Molecular Science*, 23, 7710. <https://doi.org/10.3390/ijms23147710>
- Kurien, P. P., Narayanaswamy, D., Daniel, V. A., Swaminathan, M., & Parpia, H. A. B. (1971). Supplementary value of pigeonpea (*Cajanus cajan*) and chickpeas to poor diets based on kaffir corn and wheat. *Nutritional Reports International*, 4, 227–232.
- Manimekalai, G., Neelakantan, S., & Annapan, R. S. (1979). Chemical composition and cooking quality of some improved varieties of red gram dhal. *Madras Agricultural Journal*, 66, 812–816.
- Mekonen, T., Tolera, A., Nurfeta, A., Bradford, B., Yigrem, S., & Vipham, J. (2022). Effects of pigeon pea leaves and concentrate mixture on feed intake, milk yield, and composition of crossbred dairy cows fed native pasture hay. *Animal*, 16, 100632–100638. <https://doi.org/10.1016/j.animal.2022.100632>
- Narsimha, H., & Desikachar, H. S. R. (1978). Objective methods for studying cookability of tur pulse (*Cajanus cajan* L. Millsp.) and factors affecting varietal differences in cooking. *Journal of Food Science and Technology*, 15, 47–50.
- Obala, J., Saxena, R. K., Singh, V. K., Kale, S. M., Garg, V., Kumar, C. V. S., Saxena, K. B., Tongoon, P., Sibiya, J., & Varshney, R. K. (2020). Seed protein content and its relationships with agronomic traits in pigeonpea is controlled by both main and epistatic effects QTLs. *Scientific Reports*, 10, 214. <https://doi.org/10.1038/s41598-019-56903-z>
- Obala, J., Saxena, R. K., Singh, V. K., Kumar, C. V. S., Saxena, K. B., Tongoon, P., Sibiya, J., & Varshney, R. K. (2019). Development of sequence-based markers for seed protein content in pigeonpea. *Molecular Genetics and Genomics*, 294, 57–68. <https://doi.org/10.1007/s00438-018-1484-8>
- Obala, J., Saxena, R. K., Singh, V. K., Vechalapu, S., Das, R., Rathore, A., Kumar, C. V. S., Saxena, K., Tongoon, P., Sibiya, J., & Varshney, R. K. (2018). Genetic variation and relationships of total seed protein content with some agronomic traits in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Australian Journal of Crop Science*, 12, 1859–1865. <https://doi.org/10.21475/ajcs.18.12.12.p1138>
- Pal, R. K. (1939). A review of the literature on the nutritive value of pulses. *Indian Journal of Agricultural Sciences*, 9, 133–137.
- Phatak, S. C., Nadimpalli, R. G., Tiwari, S. C., & Bhardwaj, H. L. (1993). Pigeonpeas: Potential new crop for the southeastern United States. In J. Janick & J. E. Simon (Eds.), *New crops* (pp. 597–599). Wiley.
- Qureshi, I. A. M., Wani, S. A., Lone, A. A., Dar, Z. A., & Nehvi, F. A. (2013). Breeding for quality traits in grain legumes. In C. P. Malik, A. M. Wadhwani, R. K. Mahajan, R. P. Saharan, & P. Kumar (Eds.), *Conventional and nonconventional interventions in crop improvement* (pp. 1–20). MD Publishers.

- Reddy, L. J., Green, J. M., Singh, U., Bissen, S. S., & Jambunathan, R. (1979). Seed protein studies on *Cajanus cajan*, *Atylosia* spp. and some hybrid derivatives. In *Seed protein improvement* (pp. 105–117). International Atomic Energy Agency.
- Reddy, L. J., & Singh, U. (1981). Improving protein quality in pigeonpea through the use of wild relatives. *International Pigeonpea Newsletter*, 1, 22–23.
- Remanandan, P., Sastry, D. V. S. S. R., & Mengesha, M. H. (1988). *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)* (p. 90). ICRISAT Pigeonpea Germplasm Catalogue.
- Saxena, K. B., Choudhary, A. K., Dalvi, V. A., Saxena, R. K., Ghosh, J., Singh, S., Verma, P., & Kumar, S. (2021). Pigeonpea is significantly more than just a delicious pulse. *Journal of AgriSearch*, 8, 177–187.
- Saxena, K. B., Srivastava, N., & Kumar, R. V. (2023). Transgressive segregation for seed protein in an inter-specific *Cajanus* cross. *Indian Journal of Plant Genetic Resources*, 36, 313–316. <https://doi.org/10.61949/0976-1926.2023.v36i02.14>
- Saxena, K. B., Srivastava, N., Reddy, L. J., & Saxena, R. K. (2023). Inheritance of seed protein in two interspecific pigeonpea (*Cajanus cajan*) crosses. *Plant Breeding*, 142, 500–505. <https://doi.org/10.1111/pbr.13100>
- Shalendra, G., Gmmagolmath, K. C., Sharma, P., & Patil, S. M. (2013). Role of pulses in the food and nutritional security in India. *Journal of Food Legumes*, 26, 124–129.
- Sham, N. L. (1976). Effect of nitrogen, phosphorus, and sulphur on protein content of arhar (*Cajanus cajan* L.). *Seed Farming*, 2, 37–39.
- Singh, U. (1988). Anti-nutritional factors of chickpea and pigeonpea and their removal by processing. *Plant Foods for Human Nutrition*, 38, 251–261. <https://doi.org/10.1007/BF01092864>
- Singh, U., & Jambunathan, R. (1981). Methods for the estimation of protein in pigeonpea (*Cajanus cajan* L. Millsp.) and the relationship between whole grain and dhal protein contents. *Journal of the Science of Food and Agriculture*, 32, 705–710. <https://doi.org/10.1002/jsfa.2740320711>
- Singh, U., & Jambunathan, R. (1982). Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). *Plant Foods for Human Nutrition*, 31, 347–354. <https://doi.org/10.1007/BF01094046>
- Singh, U., Jambunathan, R., Saxena, K. B., & Subrahmanyam, N. (1990). Nutritional quality evaluation of newly developed high protein genotypes of pigeonpea (*Cajanus cajan*). *Journal of the Science of Food and Agriculture*, 50, 201–209. <https://doi.org/10.1002/jsfa.2740500208>
- Squibb, R. L., Falla, A., Fuentes, J. A., & Love, H. T. (1950). Value of desmodium, pigeonpea fodder, and Guatemalan and United States alfalfa meals in rations for baby chicks. *Poultry Science*, 29, 482–485. <https://doi.org/10.3382/ps.0290482>
- Srivastava, R. P., & Vasishta, H. (2012). Genotypic variation in pigeonpea for protein, dietary fibre, fatty acids and lectins. *Indian Journal of Agricultural Biochemistry*, 25, 111–115.
- Talari, A., & Shakappa, D. (2018). Role of pigeon pea (*Cajanus cajan* L.) in human nutrition and health: A review. *Asian Journal of Dairy and Food Research*, 37, 212–220.
- Tripathi, R. D., Srivastava, G. P., Misra, M. C., & Sinha, S. C. (1975). Comparative studies in the quality characteristics of early and late cultivars of red gram (*Cajanus cajan* L.). *Indian Journal of Agricultural Chemistry*, 8, 57–61.
- van der Maesen, L. J. G. (1986). *Cajanus DC. and Atylosia W. & A. (Leguminosae)*. Wageningen Agricultural University.
- Varshney, R. K., Chen, W., Li, Y., Bharti, A. K., Saxena, R. K., Schlueter, J. A., Donoghue, M. T. A., Azam, S., Fan, G., Whaley, A. M., Farmer, A. D., Sheridan, J., Iwata, A., Tuteja, R., Penmetsa, R. V., Wu, W., Upadhyaya, H. D., Yang, S. P., Shah, T., ... Jackson, S. A. (2012). Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nature Biotechnology*, 30, 83–89. <https://doi.org/10.1038/nbt.2022>
- Wallis, E. S., Faris, D. G., Elliot, R., & Byth, D. E. (1986). Varietal improvement of pigeon pea for small holder livestock production systems. In *Proceedings of the Workshop on Crop-Livestock Systems Research* (pp. 536–553). <http://oar.icrisat.org/id/eprint/4416>

**How to cite this article:** Saxena, K. B., Saxena, R. K., Srivastava, N., & Kumar, R. V. (2025). Genetic enhancement of seed protein in pigeonpea [*Cajanus cajan* (L.) Millspaugh Maesen]. *Crop Science*, 65, e70130. <https://doi.org/10.1002/csc2.70130>