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# Electrophoresis of the Seed Protein of Cajanus Cajan L.

**Kamlesh Kumari[1], Dr. Akhilesh Dogra[2]**

[1]Research Scholar, Department of Botany, B.R.A.B.U. Muzaffarpur (India)
Email: *kumarikamleshmishra[at]gmail.com*

[2]Head of Department, Department of Botany, L.N.T. College Muzaffarpur, B.R.A.B.U. Muzaffarpur (India)

**Abstract:** ***I have studied about the electrophoresis of seed proteins in Cajanus cajan L., focusing on genetic diversity and protein composition. The analysis reveals that wild genotypes demonstrate higher protein content than cultivated varieties, particularly highlighting the prominent protein bands in the wild genotype Cajanus scarabaeoid. The evaluation of tris-soluble protein banding patterns emphasizes the effectiveness of SDS-PAGE for protein characterization compared to water-soluble proteins. Additionally, the paper discusses isozymes as variants of enzymes that perform identical functions but differ in amino acid sequences, offering insights into metabolic tuning in various developmental stages. The research enhances understanding of the genetic potential and adaptability of pigeonpea through electrophoretic analysis.***

**Keywords:** electrophoresis seed, proteins, Cajanus Cajan, genetic diversity, SDS-PAGE, isozymes, pigeon pea protein composition, wild genotypes

## 1. Introduction

Electrophoresis of seed proteins of Cajanus cajan L. is an essential technique for analyzing genetic diversity and protein composition in this significant legume crop. Examining protein band migration and molecular weights has clarified the genetic relationships and protein profiles of various pigeonpea genotypes. Analysis indicates that wild genotypes have higher protein content than cultivated varieties, with the wild genotype Cajanus scarabaeoid showing the most prominent protein bands. I have also worked for the diagnostic value of tris-soluble protein banding patterns, which were more pronounced than those of water-soluble proteins, highlighting the superiority of SDS-PAGE for protein characterization. These insights advance our understanding of pigeon pea's genetic capacity and environmental adaptability.

## 2. Isoenzymes

In biochemistry, isozymes (also called isoenzymes or simply isoforms) are enzymes that vary in amino acid sequence but perform the same chemical reaction. Isozymes often exhibit distinct kinetic properties (e.g., differing Km values) or unique regulatory mechanisms. They enable metabolic fine-tuning to meet the specific demands of a tissue or developmental phase. Isozymes are usually encoded by homologous genes that have diverged. Enzymes catalyzing the same reaction are called isozymes if encoded by different genes, or allozymes if by different alleles of one gene; the terms are often used interchangeably. Isozymes were first described by Hunter et al. (1957), who defined them as different variants of the same enzyme with identical functions and present in the same individual.

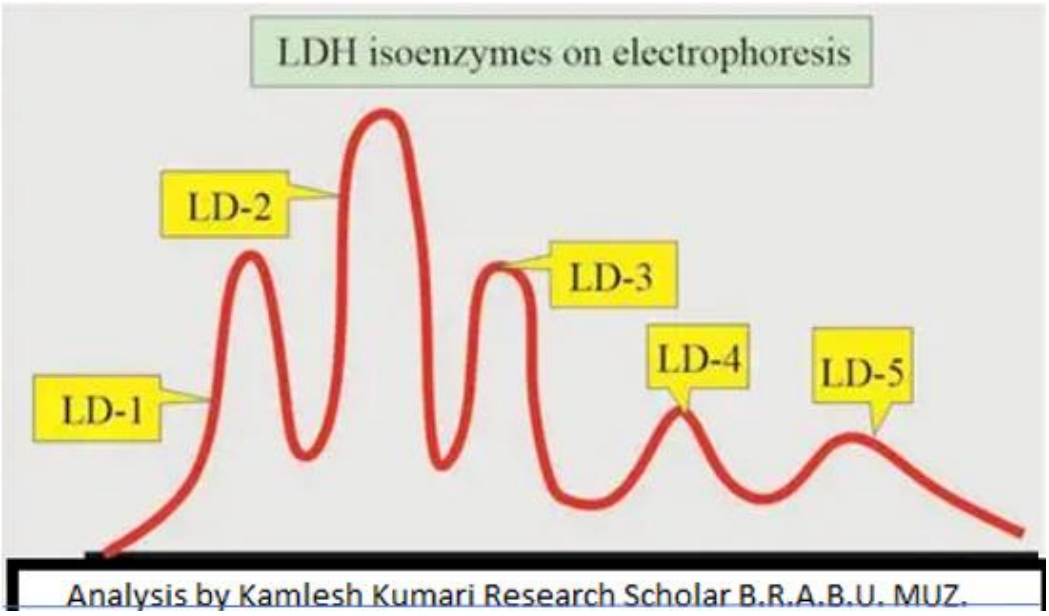


**Figure 1:** LDH Isoenzymes on Electrophoresis

This definition encompasses

1) Enzyme variants that are the product of different genes and thus represent different loci (the physical position of a gene on a chromosome; described as isozymes) .
2) Enzymes that are the product of different alleles (alternative forms) of the same gene (described as allozymes).

Isozymes typically arise from gene duplication but may also develop through polyploidization or nucleic acid hybridization. Over evolutionary time, if a new variant retains the original's function, it may be lost due to mutation accumulation, forming a pseudogene. If mutations alter function or gene expression, both variants may be selected for and specialize in new roles. For example, isozymes may be expressed at different developmental stages or in different tissues. Allozymes may result from point mutations (changes in a single DNA base) or insertion-deletion (indel) events (insertion or deletion of DNA bases) that affect the gene's coding sequence. As with any other new mutation, there are three things that may happen to a new allozyme:

- It is most likely that the new allele will be non-functional, in which case it will probably result in low fitness and be removed from the population by natural selection.
- Alternatively, if the amino acid residue changed is in a relatively unimportant part of the enzyme (for example, far from the active site, where the chemical reaction occurs), the mutation may be selectively neutral and subject to genetic drift (random changes in allele frequency).
- In rare cases, the mutation may result in an enzyme that is more efficient or catalysis a slightly different chemical reaction, in which case the mutation may increase fitness and be favored by natural selection.

An example of an isozyme is glucokinase, a variant of hexokinase that is not inhibited by glucose 6-phosphate. Its distinct regulatory features and lower affinity for glucose (compared to other hexokinases) allow it to serve different functions in cells of specific organs, such as controlling insulin release by beta cells of the pancreas or initiating glycogen synthesis in liver cells. Both processes must occur only when glucose is abundant.

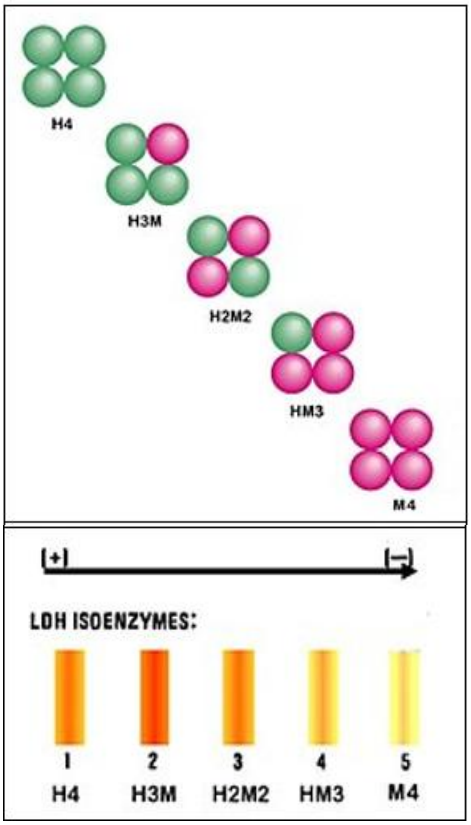


**Figure 2: (above) and 3 (below) 5 isozymes of LDH by electrophoresis**

The enzyme lactate dehydrogenase is a tetramer made of two different subunits, the H-form and the M-form. These combine in different combinations depending on the tissue.

**Table 1:** Distinction between five isozymes using Electrophoretic Mobility

| Type | Composition | Location | Electrophoretic Mobility | Whether destroyed by Heat (at 60 °C) | Percentage of normal serum in humans |
|---|---|---|---|---|---|
| $LDH_1$ | HHHH | Heart and Erythrocyte | Fastest | No | 25% |
| $LDH_2$ | HHHM | Heart and Erythrocyte | Faster | No | 35% |
| $LDH_3$ | HHMM | Brain and Kidney | Fast | Partially | 27% |
| $LDH_4$ | HMMM | Skeletal Muscle and Liver | Slow | Yes | 8% |
| $LDH_5$ | MMMM | Skeletal Muscle and Liver | Slowest | Yes | 5% |

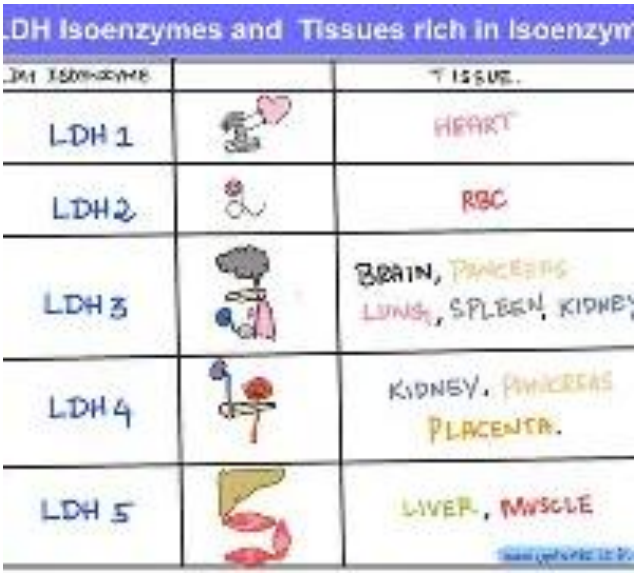


**Figure 3 and 4 LDH Isoenzyme and Tissue**

### 2.1 Isoenzymes of creatine phosphokinase

Creatine kinase (CK) or creatine phosphokinase (CPK) catalyzes the interconversion of phospho-creatine to creatine. CPK exists in 3 isoenzymes. Each isoenzyme is a dimer of 2 subunits

**Table 5:** Dimer of isoenzymes
M (muscle), B (brain), or both

| Isoenzyme | Subunit | Tissue of Origin |
|---|---|---|
| CPK1 | BB | Brain |
| CPK2 | MB | Heart |
| CPK3 | MM | Skeletal muscle |

Isoenzymes of alkaline phosphatase: Six isoenzymes have been identified. The enzyme is a monomer, and the isoenzymes result from differences in carbohydrate content (sialic acid residues).

The most important ALP isoenzymes are α1-ALP, α2-heat-labile ALP, α2-heat-stable ALP, pre-β ALP, and γ-ALP. An increase in α2-heat-labile ALP suggests hepatitis, whereas pre-β ALP indicates bone diseases.

## 3. Pigeonpea Genotypes

Pigeonpea (Cajanus cajan L.) is a crucial legume with diverse genotypes contributing significant genetic variation. These genotypes underpin breeding efforts to boost yield, nitrogen fixation, and waterlogging resistance. The following are some of the notable pigeonpea genotypes identified through genetic studies:

MWPLR 14: Identified as an early maturing variety with high yield potential.
ICEAP 01170: Another early maturing variety with high yield potential.
ICEAP 871091: Identified as an early maturing variety with high yield potential.
ICEAP 01285: Identified as an early maturing variety with high yield potential.
Kachangu: Identified as a high-yielding genotype.
MWPLR 16: Identified as a high-yielding genotype.
TZA 5582: Identified as a high-yielding genotype.
No. 40: Identified as a high-yielding genotype.

These genotypes are selected for their capacity to enhance yield and other important pigeonpea traits, making them invaluable for breeding. Their genetic diversity is vital for creating adaptable varieties that support food security and livelihoods.

## 4. Seeds of Cajanus Cajan L.

- Common Names: Arhar, Congo pea, no eye pea, pigeon pea
- Assamese: arahar, mirai-maha
- Bengali: arahar Gujarati: tuver
- Hindi: arhar, tuvar
- Kannada: togari bele, togari kalu
- Konkani: tori
- Malayalam: adhaki, tuvara
- Manipuri: Mairongbi
- Marathi: tur
- Nepali: rahar
- Oriya: har-har, kakshi, tubara
- Sanskrit: adhaki, kakshi, tuvari
- Tamil: adhaki, iruppuli, kaycci, tuvarai
- Tangkhul: Khaithei
- Telugu: adhaki, kandi, togari, tuvaramu
- Urdu: arhar, tuar
- Botanical name: Cajanus cajan Family: Fabaceae (Pea family)
- The Arhar plant is an erect annual or short-lived perennial reaching a height of 3-10 feet.

**Table 4:** Clustering pattern of 50 pigeonpea [Cajanus cajan]

| Cluster No. | No. of Genotypes | Number of the Genotypes |
|---|---|---|
| I | 9 | IC- 525402, IC- 525409, IC- 525410, IC- 73791, IC- 525571, IC- 385885, WRG- 27, LRG-41, WRG-55 |
| II | 5 | IC- 525520, IC-525529, IC-525521, IC- 73868, WRGE- 90 |
| III | 8 | IC-383168, IC- 361457, IC-525407, IC-73871, IC- 525572, IC- 525465, IC- 525509, IC- 525584 |
| IV | 9 | IC- 525570, IC- 525522, ICPL- 96053, IC- 525412, LRG-52, IC-525529, ICP- 7035, ICPL- 99004, IC- 525581 |
| V | 15 | ICPL 87119, BSMR- 853, ICP- 72751, IC- 525408, IC- 525411, IC- 73322, IC- 525418, IC- 525423, IC-525526, IC- 525567, IC- 525573, WRG-65, WRG- 79, IC-525477 |
| VI | 3 | IC- 525574, IC- 525466, IC- 525528 |
| VII | 1 | IC- 73752 |

## 5. Zymograms of different isozymes

Zymograms visually represent variations in enzyme activity among isozymes of the same enzyme. Isozymes, which differ in amino acid sequence but catalyze identical reactions, often result from gene duplication or divergence. Zymograms facilitate detection and interpretation of these variations by revealing enzyme activity patterns on electrophoresis gels, which may reflect differences in alleles or allozymes.

Studying isozymes is essential for understanding genetic variation and can provide key insights into population genetics and enzyme function. For more information, refer to the relevant literature.

## 6. Protein Types in Cultivars

The FAO (2023) statistics revealed that in 2022, over 700 million people worldwide faced hunger, 122 million more than 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 capita protein availability in tropical and subtropical regions is 24 g day1, which is half 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 the production of protein-rich foods, and the increasing cost of animal protein resources. In the past, researchers demonstrated that the addition of pulse supplements to cereal-based diets can markedly improve their nutritional quality (Daniel et al., 1970; Hulse, 1977; Kurien et al., 1971).

Unfortunately, for the low-income masses, such food standards are also too luxurious to afford sustainably. A survey of Indian villages conducted by Bidinger and Nag (1981) revealed that most regular rural diets are inadequate, providing only 10% of the protein, 5% of the 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, par ticularly 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.)] is rated high for its drought tolerance, soil-enriching properties, high protein content, and multiple uses (Saxena et al., 2021). Globally, pigeonpea is cultivated on 6.36 million ha in 22 countries with production of 5.48 million tones and average productivity of 0.86 tons ha−1.

India ranks first in pigeonpea production with 4.34 million tons of grain harvested from 4.98 million ha of land with a mean yield of 0.87 tons ha−1 (Government of India, Ministry of Agriculture and Farmers Welfare, 2021). The other major pigeonpea-producing countries are Malawi (451,000 tones), Myanmar (307,000 tones), Tanzania (196,000 tones), and Kenya (104,000 tones). Beside 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 land area will always remain limited for low-yielding pulses, genetic enhancement of seed protein offers hope of additional home-grown protein harvests. This review aims to comprehensively assess accomplishments in breeding high-protein pigeon pea cultivars and to evaluate their nutritional quality through biological assessments. Additionally, it highlights recent genomic advances and explores their potential integration into future breeding strategies to enhance protein content. The first pioneering research on the nutritional qualities of pulses was conducted by Pal (1939), who found 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, dehulled split pigeon pea cotyledons are cooked into a thick soup (locally called “dal”) and served 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 consumed as fresh, frozen, or canned vegetables (Saxena et al., 2021). Both the dry and fresh grains of pigeonpea are nutritious, but they differ quantitatively in 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, the 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 amount of lysine (Singh et al., 1982). The values of globulin, the Sulphur-deficient amino acid, were high in the proteins from the seed, cotyledons, and embryo. 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 A1, B1, B2, B3, B6, B9, C, and E (June 2024). The vegetable pigeon peas 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 β-carotene, three times thiamine (vitamin B1), riboflavin (vitamin B2), 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).

## 7. Conclusion

This study highlights the effectiveness of electrophoresis, particularly SDS-PAGE, in analyzing the seed protein profiles of Cajanus cajan L. It underscores the existence of greater protein content and distinct banding patterns in wild genotypes, notably Cajanus scarabaeoid, which reflect underlying genetic diversity and potential adaptability. The emphasis on tris-soluble proteins provides valuable diagnostic insights, contributing to a better understanding of the genetic and metabolic variations within pigeonpea. Overall, this research advances our knowledge of the protein composition and genetic relationships in pigeonpea, offering a foundation for future breeding and conservation efforts aimed at harnessing genetic diversity for crop improvement.

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