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Advances in Bioresearch

## ORIGINAL ARTICLE

# Seed Storage Protein Analysis by SDS-PAGE in Three Species of Genus *Cassia*

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**ABSTRACT**
*Seed storage proteins are stable biochemical markers widely used in taxonomic and phylogenetic investigations. The present study aimed to analyze interspecific variation and evaluate genetic relationships among three species of genus Cassia Cassia fistula L., Cassia glauca Lam., and Cassia tora L. using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). A total of 17 protein bands with distinct relative mobility (Rf) values were detected. C. fistula and C. glauca exhibited 14 bands each, while C. tora showed 15 bands. Species-specific as well as common bands were observed, indicating both similarity and divergence. Similarity index analysis revealed the highest affinity between C. tora and C. glauca (48.27%), followed by C. fistula and C. tora (41.37%), while the lowest similarity was recorded between C. fistula and C. glauca (37.93%). The results support modern taxonomic segregation within the traditional genus Cassia and demonstrate that SDS-PAGE is an effective and economical method for biosystematics studies.*
***Keywords:*** *Seed storage protein, SDS-PAGE, Cassia fistula, Cassia glauca, Cassia tora, Similarity index, Genetic diversity*

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## INTRODUCTION

The genus Cassia (Family: Caesalpiniaceae; currently placed within subfamily Caesalpinioideae of Fabaceae under APG IV classification) comprises nearly 600 species distributed across tropical and subtropical regions worldwide. Members of this genus are ecologically and economically important due to their medicinal, ornamental, and pharmacological significance [1]. However, taxonomic circumscription within *Cassia* has long been controversial, resulting in segregation into related genera such as Senna and Chamaecrista [2,3].

*C. fistula* is a well-known ornamental and medicinal tree with reported antimicrobial, antioxidant, hepatoprotective, and antidiabetic activities. *C. tora* is an annual herb widely used in traditional medicine and as a leafy vegetable, while *C. glauca* (often treated under Senna surattensis) is a shrub or small tree with medicinal applications. Despite morphological distinctions, overlapping characteristics often complicate species delimitation.

Modern plant systematics increasingly integrates molecular phylogenetics; however, biochemical techniques such as seed protein electrophoresis remain valuable due to their simplicity, reproducibility, and cost-effectiveness [4,5]. Seed storage proteins are genetically controlled and relatively unaffected by environmental factors, making them suitable markers for taxonomic and diversity studies [6,7].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) separates proteins based primarily on molecular weight by denaturing them and imparting uniform negative charge (Laemmli, 1970). Although molecular DNA-based markers such as RAPD, ISSR, and sequencing techniques have gained prominence, protein profiling remains a valuable complementary method in resource-limited settings [8,9,10].

Recent studies emphasize integrative taxonomy combining morphological, biochemical, and molecular evidence for accurate phylogenetic inference [11]. Therefore, the present investigation aims to:

Analyze seed storage protein banding patterns of three *Cassia* species.

Detect interspecific variation.
Evaluate genetic affinity using similarity index calculations.
Contribute biochemical evidence to ongoing taxonomic clarification within the genus.

## MATERIAL AND METHODS

### Collection of Plant Material

Mature pods of the three species were collected from different locations in Samastipur and Darbhanga districts. Seeds were separated, washed with sterilized distilled water, air-dried, and stored in labeled plastic pouches until analysis.

### Protein Extraction

Approximately 0.5 g of seed powder was homogenized in extraction buffer containing:

- 0.5 M Tris-HCl (pH 6.8)
- 5% β-mercaptoethanol
- 10% glycerol
- 2% SDS
- Trace amounts of bromophenol blue

The homogenate was centrifuged at high speed for 20 minutes. The supernatant was transferred to fresh tubes and heated in a water bath for 5 minutes to ensure complete protein denaturation.

### SDS-PAGE Procedure

Protein separation was performed using a vertical slab gel electrophoresis system.

- Separating gel: 10% polyacrylamide
- Stacking gel: 4% polyacrylamide
- Polymerization agents: Ammonium persulphate (APS) and TEMED
- Running condition: 30 mA for approximately 2.5 hours

Samples were loaded in duplicate lanes for each species. Electrophoresis continued until the tracking dye reached the bottom of the gel.

### Staining and Documentation

Gels were stained with 0.1% Coomassie Brilliant Blue for 12 hours and subsequently destained using a mixture of methanol, acetic acid, and water. Protein bands were visualized under white light and photographed [12].

### Similarity Index Calculation

The similarity index (S) was calculated following Sneath and Sokal (1963):

$$S = \frac{2N_{ab}}{N_a + N_b} \times 100$$

Where:

$N_{ab}$= number of common bands

$N_a$, $N_b$= total number of bands in each species

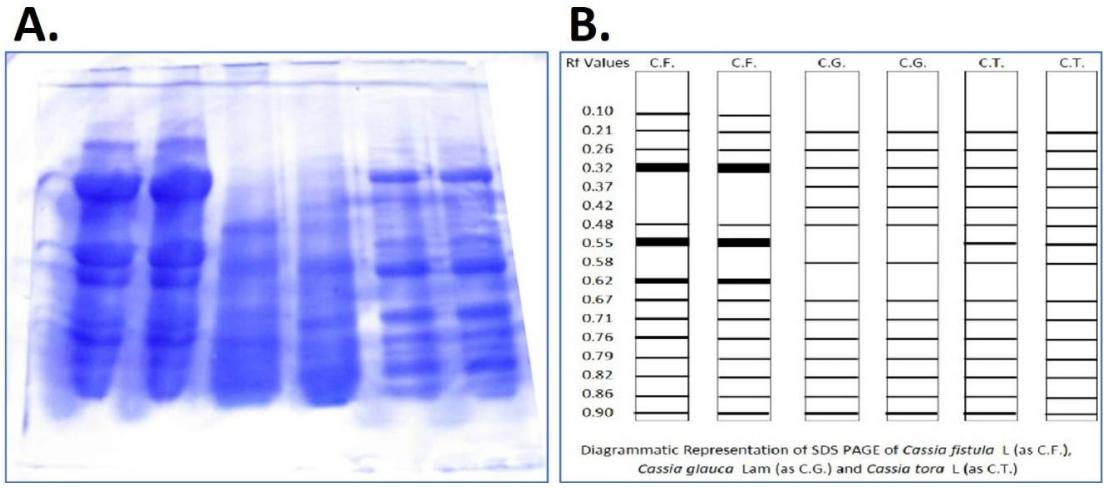
## RESULTS AND DISCUSSION

### SDS-PAGE Protein Banding Pattern

The SDS-PAGE profiling of seed storage proteins in *Cassia fistula* L., *Cassia glauca* Lam., and *Cassia tora* L. revealed clear and reproducible banding patterns. A total of 17 distinct protein bands were detected with relative mobility (Rf) values ranging from 0.10 to 0.90.

- *C. fistula* showed 14 bands.
- *C. glauca* showed 14 bands.
- *C. tora* showed 15 bands.

Most bands were common among the three species, indicating shared genetic components. However, several bands were species-specific, demonstrating interspecific polymorphism (Figure 1A and B).




**Figures 1:** Photograph showing seed storage protein banding after SDS-PAGE in *Cassia fistula* L (in first two lanes) *Cassia glauca* Lam (in the middle two lanes) & *Cassia tora* L (in the last two lanes).

The protein band at Rf value 0.1 and Rf 0.62 were only present in *Cassia fistula* L but absent in *Cassia tora* L and *Cassia glauca* Lam. Similarly in protein band at Rf 0.37, Rf 0.42 and Rf 0.58 were present in *Cassia glauca* Lam and *Cassia tora* L but absent in *Cassia fistula* L. Also, protein band at Rf value 0.55 was present in *Cassia fistula* L and *Cassia tora* L but absent in *Cassia glauca* Lam (Table 1).

**Species-Specific and Shared Bands**

The SDS–PAGE analysis revealed distinct species-specific bands, such as Rf 0.10 and 0.62 in *Cassia fistula*, indicating unique protein expression patterns. Shared bands at Rf 0.37, 0.42, and 0.58 between *Cassia tora* and *Cassia glauca* reflect closer genetic affinity. These variations demonstrate both conserved and divergent protein fractions, supporting interspecific differentiation within the genus (Table 1).

**Table 1:** Showing protein bands and their respective Rf values in *Cassia fistula* L, *Cassia glauca* Lam and *Cassia tora* L.

| Rf Value | *C. fistula* | *C. glauca* | *C. tora* | Interpretation |
|---|---|---|---|---|
| 0.10 | Present | Absent | Absent | Unique to *C. fistula* |
| 0.37 | Absent | Present | Present | Shared by *C. glauca & C. tora* |
| 0.42 | Absent | Present | Present | Shared by *C. glauca & C. tora* |
| 0.55 | Present | Absent | Present | Shared by *C. fistula & C. tora* |
| 0.58 | Absent | Present | Present | Shared by *C. glauca & C. tora* |
| 0.62 | Present | Absent | Absent | Unique to *C. fistula* |

The presence of unique bands (e.g., Rf 0.10 and 0.62 in *C. fistula*) indicates species-specific gene expression. Such protein polymorphism reflects genetic differentiation at the biochemical level (Table 1).

**Similarity Index Analysis**

The calculated similarity index values ranged from 37.93% to 48.27% among the studied species. The highest similarity was observed between *Cassia tora* and *Cassia glauca*, indicating closer genetic relatedness. In contrast, the lower similarity involving *Cassia fistula* suggests greater evolutionary divergence within the genus.

**Table 2:** Showing three species of *Cassia* in different pairs and their respective similarity index.

| Name of species | Similarity index |
|---|---|
| *Cassia fistula* L-*Cassia glauca* Lam | 37.93 |
| *Cassia fistula* L-*Cassia tora* L | 41.37 |
| *Cassia tora* L-*Cassia glauca* Lam | 48.27 |

Similarity and dissimilarity in protein bounding will help in better understanding of evolutionary process in a taxon [13,14].

**Integrated Interpretation with Current Research**

Seed storage proteins are direct products of gene expression and are highly conserved, yet capable of revealing interspecific variation. Because they are relatively unaffected by environmental conditions, they serve as reliable biochemical markers for taxonomic studies [15].

The moderate similarity between *C. tora* and *C. glauca* aligns with modern phylogenetic treatments of Leguminosae. Molecular phylogenomic studies demonstrate that traditional *Cassia* is polyphyletic and that several species, including *C. tora* and *C. glauca*, are more closely related within the genus *Senna* [16,17]. The biochemical similarity observed in this study supports these genomic findings.

The lower similarity of *C. fistula* suggests greater evolutionary divergence. According to integrative taxonomy frameworks, congruence between biochemical and molecular data strengthens phylogenetic inference [18,19].

Although advanced DNA sequencing techniques now dominate plant systematics, SDS–PAGE remains valuable in preliminary diversity screening, especially in developing laboratories. Recent reviews emphasize that classical protein profiling still contributes to germplasm characterization and validation of molecular results [20,21].

**Evolutionary and Taxonomic Implications**

Protein band similarity corresponds to shared structural genes, whereas absence or presence of bands reflects mutation, gene duplication, or divergence. The higher similarity between *C. tora* and *C. glauca* suggests a closer common ancestry compared to *C. fistula* [22].

These findings support taxonomic segregation within the genus and demonstrate that biochemical evidence parallels molecular phylogenomic classification [11,16, 23].

## CONCLUSION

The present investigation demonstrates that seed storage protein profiling using SDS–PAGE is an effective and reliable biochemical approach for assessing interspecific variation within the genus *Cassia*. The detection of 17 distinct protein bands across *Cassia fistula* L., *Cassia glauca* Lam., and *Cassia tora* L. highlights both conserved and polymorphic protein fractions, reflecting underlying genetic similarities and differences among the studied taxa. The highest similarity index observed between *C. tora* and *C. glauca* (48.27%) indicates a closer genetic affinity between these two species compared to *C. fistula*. This biochemical evidence supports modern taxonomic interpretations that segregate members of the traditional genus *Cassia* into distinct but related genera. The comparatively lower similarity of *C. fistula* suggests a more distant evolutionary relationship within the group. Importantly, the study reaffirms that seed storage proteins, as stable products of gene expression, serve as dependable markers for biosystematic and phylogenetic studies. Although advanced molecular techniques such as DNA sequencing provide higher resolution, SDS–PAGE remains a cost-effective, reproducible, and accessible tool—particularly valuable in preliminary diversity assessments and in laboratories with limited resources. Overall, the findings contribute meaningful biochemical evidence to the understanding of evolutionary relationships within *Cassia*. The integration of classical protein profiling with modern molecular approaches will further enhance taxonomic clarity and germplasm characterization in future research.

## DECLARATION OF INTEREST

The authors have no competing interests to declare relevant to this article's content.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## AUTHOR'S CONTRIBUTIONS

Chandra Shekhar Singh- conceptualization, methodology, analysis and writing the original draft preparation. Khalid Anwer- Providing research guidance, constructing the study, revising the manuscript.

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