Received: 28 November 2024 | Accepted: 3 March 2025

DOI: 10.1002/tpg2.70027

The Plant Genome

REVIEW

Seed protein electrophoresis in plant genetics: Commemorating the pioneering contributions of Prof. Chittaranjan Kole and team to the foundation of plant proteomics

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Assigned to Associate Editor Manish Pandey.

Abstract

Despite the rapid progress of proteomics in human and other model organisms, plant proteomics has advanced at a comparatively slower pace. This review aims to highlight the pioneering work on seed protein markers detected by employing gel electrophoresis primarily by a team of Indian scientists that paved the way for elucidation of intervarietal and interspecific variation, evolution, and phylogenetic relationship of species and their association with resistance to pest and diseases. Far from being replaced, gel electrophoresis remains as an excellent supporting and different approach, offering a pathway to a more profound visualization and understanding of the cell proteome. This review focuses on how, from a historical standpoint, gel electrophoresis has significantly contributed to plant proteomics and other biological research. Acknowledging the pioneering work on seed storage proteins, this review serves as both a congratulatory gesture and a tribute to the eminent scientist Prof. Chittaranjan Kole and his team who pioneered the strategy of seed protein electrophoresis in crop biology research. Their findings, both directly and indirectly, have proven invaluable, particularly for those who ventured into proteomics without easy reach to sophisticated and expensive instruments/equipment to pursue DNA-based genomics research. This gel electrophoresis-based plant proteomics review includes the evolution of gel-based proteomics, their contribution to crop biology research, and future directions. It stands not only as a retrospective analysis but also as a testament to the enduring significance of gel electrophoresis in shaping the landscape of crop proteomics.

Abbreviations: 2D-DIGE, two-dimensional differential gel electrophoresis; 2-DE, two-dimensional gel electrophoresis; CLS, Cercospora leaf spot; CNE, clear native electrophoresis; DIA, data-independent acquisition; DIGE, differential gel electrophoresis; GLH, green leafhopper; MYMV, Mungbean yellow mosaic virus; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Sarita Pandey and Shalini Tiwari contributed equally to this work.

Plain Language Summary

This article explores the importance of gel electrophoresis, a laboratory technique used to study proteins in plants, and its impact on plant biology research. While newer technologies have accelerated studies on human and model organism proteins, research on crop plants has often relied on more accessible techniques like gel electrophoresis. Led by pioneering work from Prof. Chittaranjan Kole and his team, gel-based proteomics has been essential for studying differences between plant varieties and understanding how plants evolve to resist pests and diseases. This technique has helped researchers without advanced equipment make important discoveries, providing insights into crop resilience and adaptation. The article reviews the evolution of this method, its contributions, and future possibilities, highlighting how gel electrophoresis remains a valuable tool for advancing plant science and sustainable agriculture.

1 | INTRODUCTION

The role of protein markers in crop improvement has gained increasing significance in recent years, offering crucial insights into the genetic, physiological, and biochemical attributes of plants. As global agricultural challenges intensify due to climate change, soil degradation, and population growth, researchers seek innovative strategies to enhance crop yield, nutritional content, and resilience to environmental stressors. Protein markers have emerged as essential tools in this pursuit, enabling scientists to decipher molecular mechanisms governing plant development and stress responses. By leveraging protein markers, researchers can identify desirable traits, optimize breeding strategies, and accelerate the development of improved crop varieties (Eldakak et al., 2013).

Among the diverse applications of protein markers, seed proteins play a crucial role in both plant growth and human nutrition. Seed proteins not only provide essential nutrients for the developing plant embryo but also serve as valuable dietary protein sources. The composition of seed proteins varies among species, with key classes including albumins, globulins, prolamins, and glutelins (Rasheed et al., 2020). Advances in proteomic technologies have significantly enhanced our understanding of these proteins, particularly through gel-based proteomics. This field has transformed seed protein research, enabling the identification of key proteins linked to stress tolerance, disease resistance, and nutrient efficiency.

Prof. Chittaranjan Kole, a distinguished plant geneticist, has made significant contributions to the field of crop improvement, particularly through his work in genomics and molecular breeding. His research has emphasized the integration of cutting-edge molecular techniques to enhance agricultural productivity and address food security challenges. Building upon such foundational work, this review aims to provide a comprehensive examination of gel electrophoresis in crop proteomics research, specifically in the context of seed proteins.

By exploring the evolution of proteomic techniques, with a particular emphasis on two-dimensional gel electrophoresis (2-DE), this review seeks to highlight how advancements in proteomics have expanded our understanding of seed proteins and their applications in agriculture. Given the growing need for sustainable and high-yielding crops, a deeper comprehension of seed protein composition and function is essential for breeding resilient varieties. Ultimately, this review underscores the transformative potential of protein markers in modern crop improvement, offering new avenues for enhancing agricultural sustainability and food security.

1.1 | Role of seed proteins in plant growth and development

Seed proteins are vital for plant growth and development, serving as crucial sources of amino acids and energy for the developing embryo. They are broadly categorized into several types, each with distinct functions and characteristics (Khalid, Hameed, & Tahir, 2023). For example, storage proteins that accumulate in seed tubers, or other plant storage organs, serve as a reserve of amino acids for germination and early seedling growth. Types of storage proteins include albumins, globulins, prolamins, and glutelins (Fujiwara et al., 2002). Notable examples include soybean glycinin and conglycinin (globulins), wheat glutenin and gliadin (prolamins), and rice glutelins. Similarly, structural proteins provide support and rigidity to plant cells and tissues. They are important for maintaining the shape and integrity of plant structures. Cellulose synthases (involved in cellulose formation), tubulins and actins (cytoskeletal proteins), and extensins (involved in cell wall reinforcement) are some known structural proteins.

Proteins that function as enzymes also play crucial roles in plants in various metabolic pathways, including photosynthesis, respiration, and biosynthesis of secondary metabolites (Quinn et al., 2024), for example, Rubisco (involved in photosynthesis), amylase (involved in starch breakdown), and catalase (involved in detoxifying reactive oxygen species) (Shewry et al., 1995). Defense proteins such as pathogenesis-related (PR) proteins, protease inhibitors, and antimicrobial peptides protect plants against pathogens, herbivores, and environmental stresses. They are part of the plant's immune system. Transport proteins facilitate the movement of substances, such as ions and small molecules, within the plant (Oliveira et al., 2022). They are involved in nutrient uptake, translocation, and distribution. Examples include aquaporins (which facilitate water transport), ion channels, and nutrient transporters. Signal transduction proteins relay signals within the plant, regulating various physiological processes in response to external stimuli. Key examples are receptor kinases, G-proteins, and transcription factors involved in plant hormone signaling. Photosynthetic proteins are involved in the process of photosynthesis, capturing light energy and converting it into chemical energy (Oliveira et al., 2022). Examples include chlorophyll-binding proteins, photosystem proteins, and enzymes of the Calvin cycle. Regulatory proteins control gene expression and coordinate various cellular processes. They play a role in plant development and responses to environmental stimuli. For example, transcription factors, protein kinases, and other proteins involved in gene regulation. Some plant proteins can trigger allergic reactions in sensitive individuals (Oliveira et al., 2022). Understanding allergenic proteins is crucial for food safety. Proteins in nuts (e.g., peanuts and tree nuts), certain fruits, and grains (e.g., wheat gluten) can be allergenic. Seed maturation proteins are expressed during seed development and are involved in processes related to seed maturation and desiccation tolerance. For instance, late embryogenesis abundant proteins also known as LEA proteins, seed storage proteins, and oleosins (Rasheed et al., 2020).

1.2 | Evolution of proteomics—Biomarker discovery to gel-based proteomics

The timeline of developments in gel electrophoresis for protein study began in the 1960s with the introduction of horizontal slab gels, which simplified protein separation based on size. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the predominant gel electrophoretic technique for protein analysis, relies on the separation of proteins by size and assists in the determination of their relative molecular mass (Lan et al., 2024) (Supporting information). This method hinges on the action of SDS, an anionic detergent that binds tightly to proteins, inducing their denaturation and linearization into polypeptide chains. Typically, one SDS molecule associates with every two amino acids, with a constant ratio of approximately 1.4 g of SDS/g of protein. The resulting protein-SDS complexes carry a net negative charge, propelling them toward the anode during electrophoresis (Otzen et al., 2022).

Core Ideas

- Gel electrophoresis continues to play an essential role in plant proteomics and broader biological research, offering valuable insights despite the slower progress compared to human and model organism proteomics.
- The pioneering work on seed protein markers, led by Prof. Chittaranjan Kole and his team, paved the way for understanding intervarietal and interspecific variations, species evolution, and resistance to major pests and diseases.
- This review explores the evolution, contributions, and future directions of gel-based proteomics, emphasizing its enduring significance in the field. It highlights how gel electrophoresis has remained a critical tool, especially for researchers without access to advanced and costly genomic equipment, shaping the landscape of crop proteomics and plant biology.

The advent of gel-based proteomics marked a revolutionary shift in the analysis of seed proteins. Techniques named 2-DE have been instrumental in separating and identifying complex protein mixtures based on their isoelectric point and molecular weight (Oliveira et al., 2022). This groundbreaking technique combined isoelectric focusing with SDS-PAGE, allowing for the high-resolution separation of complex protein mixtures. This method, developed in the early 1980s, allowed scientists to visualize and compare protein profiles from various seeds, uncovering critical details about protein composition and function (Dunn & Burghes, 1986; Oliveira et al., 2022).

Pioneers in gel-based proteomics have made significant contributions by utilizing 2-DE to characterize seed storage proteins and identify variations related to different growth conditions and developmental stages. This has led to enhanced understanding of protein functions and interactions, which is crucial for developing crops with improved nutritional content, stress tolerance, and overall quality (Oliveira et al., 2022). The 1990s introduced advancements like differential gel electrophoresis (DIGE), which enabled the simultaneous comparison of multiple samples. The 2000s further advanced with the integration of advanced imaging technologies and

mass spectrometry (MS) for detailed protein identification (Hirano & Shirakawa, 2022).

In the last 30 years, the molecular biology research landscape has been significantly influenced by omics approaches, with a notable emphasis on proteomics (Wang et al., 2024). This trend aligns with the completion of numerous genome sequences, the widespread adoption of next generation sequencing for transcript analysis, advancements in MS and associated equipment, and the development of bioinformatics tools and pipelines (Tebani et al., 2016; Wang et al., 2024). From a methodological perspective, proteomics has evolved swiftly from the classical 2DE-MS (first generation) to isobaric or isotopic labeling strategies (Abdallah et al., 2012; Wang et al., 2024) (second generation), then to shotgun or gel-free/label-free techniques (third generation), and finally, to targeted, mass-western, or selective reaction monitoring/multiple reaction monitoring approaches (fourth generation). A noteworthy advancement in proteomics methodology is observed in shotgun proteomics, which is currently undergoing a paradigm shift with the adoption of data-independent acquisition (DIA). This approach depends on the presence of proteotypic, specific protein species, and the development of spectral libraries (Wang et al., 2024).

More recent developments include the use of DIA techniques, enhancing the quantitative analysis and precision of protein profiling. Each of these milestones has significantly enhanced our ability to study and understand proteins in greater detail. In Figure 1, we highlighted the key milestones in the development of gel electrophoresis systems for protein analysis, illustrating the evolution from early techniques to contemporary advancements.

2 | PLANT PROTEOMIC STUDIES BASED ON GEL ELECTROPHORESIS

The essential role of molecules in supporting life has been documented since the early stages of biological research. The term "protein," as coined by Berzelius in 1838, originates from the Greek word "proteios," meaning "the first rank" (Cristea et al., 2004). In the early 1990s, Prof. Chittaranjan Kole conceptualized the importance of proteins analysis in plant biology research and formulated a research project on "Genetic Characterization of Electrophoretic Banding Patterns of Seed Proteins in Mungbean: Molecular Implications and Seed Protein Improvement" that he operated from June 01, 1994 to May 31, 1997 with funding from the Indian Council of Agricultural Research assisted by his then-colleague, Mr. B.S. Naik. However, describing the overall protein content of a cell in terms of localization, interactions, post-translational modifications, and turnover at a specific time, the "proteome" was described by Marc Wilkins in 1996 as the "PROTein complement of a genOME" (Wilkins et al., 1996). Proteomics involves the comprehensive characterization of the proteome, encompassing the expression, structure, functions, interactions, and modifications of proteins at any stage (Mani et al., 2022). The proteome undergoes fluctuations over time, from cell to cell, and in response to external stimuli. Eukaryotic cell proteomics is particularly complex due to post-translational modifications occurring at various sites through numerous pathways (Krishna & Wold, 2013). Proteomics is a crucial approach for understanding gene function, though it is considerably more complex than genomics (Lander et al., 2006; Mani et al., 2022). Variations in gene expression levels can be identified by analyzing the transcriptome or proteome, allowing comparison between two cellular states. For large-scale examination of the entire transcriptome, microarray chips have been developed. Proteins serve as executors of biological functions, and their levels are influenced not only by corresponding mRNA but also by host translational regulation and control mechanisms. Consequently, proteomics is often regarded as the most relevant dataset for characterizing a biological system (Cox & Mann, 2007; Mani et al., 2022).

2.1 | Methods for comprehensive analysis of protein

This section explores the diverse applications of proteomic techniques in enhancing crop improvement (Figure 2). By analyzing protein/seed protein expression profiles, modifications, and interactions, researchers can gain insights into stress responses, disease resistance, and nutrient utilization. Traditional protein purification techniques rely on chromatography methods such as ion exchange chromatography, size exclusion chromatography, and affinity chromatography (Hage et al., 2012; Jungbauer & Hahn, 2009; Voedisch & Thie, 2010). To analyze specific proteins, methods like enzyme-linked immunosorbent assay and western blotting are commonly used (Mani et al., 2022). Although these methods can target a few specific proteins, they are limited in determining overall protein expression levels (Kurien & Scofield, 2006; Lequin, 2005). Techniques such as SDS-PAGE, 2-DE, and two-dimensional differential gel electrophoresis (2D-DIGE) are employed to separate complex protein samples (Dunn & Burghes, 1986; Issaq & Veenstra, 2008; Marouga et al., 2005). In the early and late 1990s, advanced versions of bi-dimensional gel electrophoresis emerged, expanding its analytical potential. One such advancement, blue-native polyacrylamide gel electrophoresis, enabled detailed analysis of protein complexes and their components (Ebhardt et al., 2015; Knezevic et al., 2001; Rosenberg & Utz, 2015). Simultaneously, fluorescence DIGE surfaced, streamlining experimental setups, elevating reproducibility and sensitivity, and reducing variability between replicates

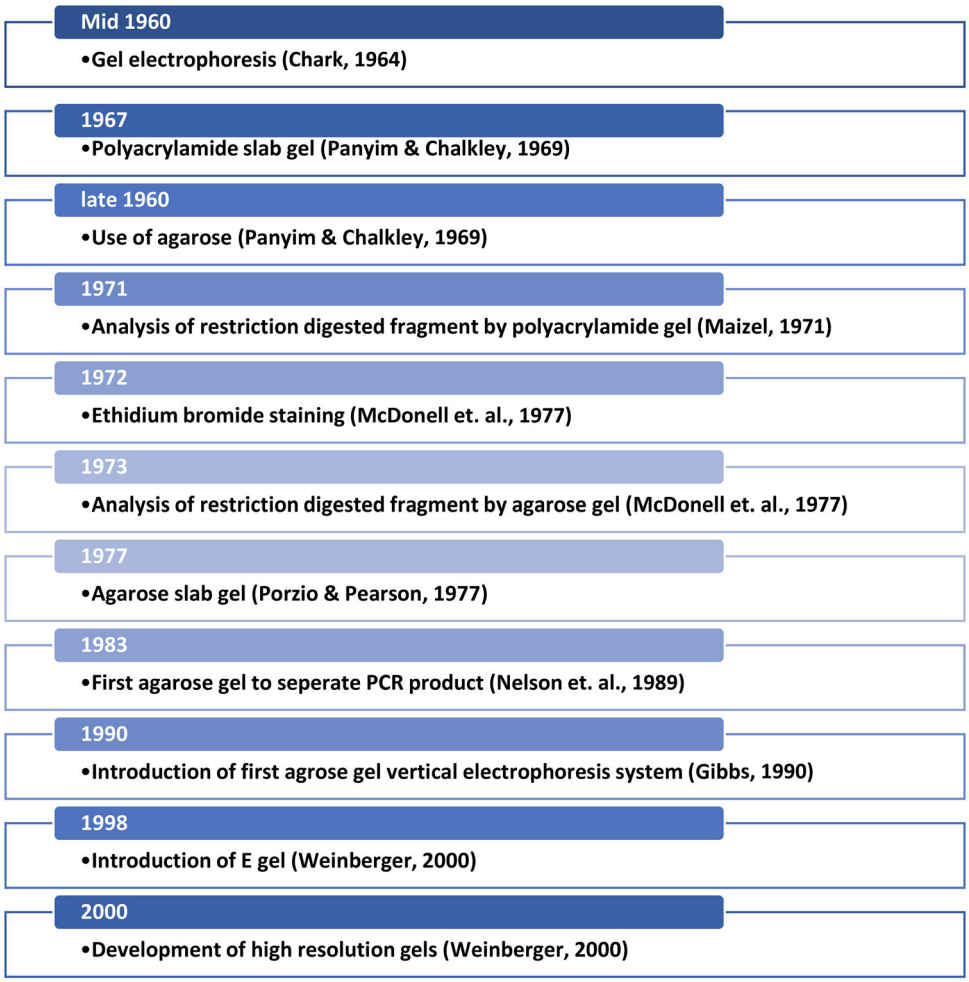

FIGURE 1 The flowchart visualizes the key milestones in the development of gel electrophoresis systems for protein study, showing the progression from early techniques to modern advancements.

(Sanchez-Carbayo et al., 2006). These methodologies can be effectively combined for comprehensive analyses (Delehanty & Ligler, 2002). The 2D-DIGE technique was initially embraced in human and model organism research, and this technique was later adopted by the plant community (Popescu et al., 2007; Tibes et al., 2006; Ummanni et al., 2014; Zhu et al., 2001). Additionally, two less commonly employed variants of 2-DE have been reported, mainly in membrane protein complex studies. Clear native electrophoresis (CNE) and high-resolution CNE are methods that exclude dye to prevent interference with fluorescence detection or enzymatic assays. Though occasionally applied in plant studies (Jovanovic et al., 2007; Sadia et al., 2009), these techniques are not widely used. Protein microarrays, or protein chips, have been developed for rapid and high-throughput expression analysis. Advanced proteomics techniques, including MS, allow for the analysis of complex protein mixtures with enhanced sensitivity (Yates, 2011). Additionally, Edman degradation is utilized for sequencing amino acids of specific proteins (Smith, 2001). Quantitative proteomics methods, such as isotope-coded affinity tag labeling, stable isotope labeling with amino acids in cell culture, and isobaric tags for relative and absolute quantitation, have recently been introduced (Ong et al., 2006; Shiio & Aebersold, 2006; Wiese et al., 2007). X-ray crystallography and nuclear magnetic resonance spectroscopy are two essential high-throughput techniques that reveal a protein's three-dimensional structure, aiding in the understanding of its biological function (Smyth & Martin, 2000; Wiese et al., 2007). Proteome analysis offers a comprehensive view of cellular structural and functional information, as well as insights into cellular responses to various stressors and drugs, through single or multiple proteomics techniques.

2.2 | Contribution of pioneering work on seed proteins by Prof. Chittaranjan Kole and his co-workers

The exploration of seed proteins has been instrumental in advancing agricultural science and crop improvement,

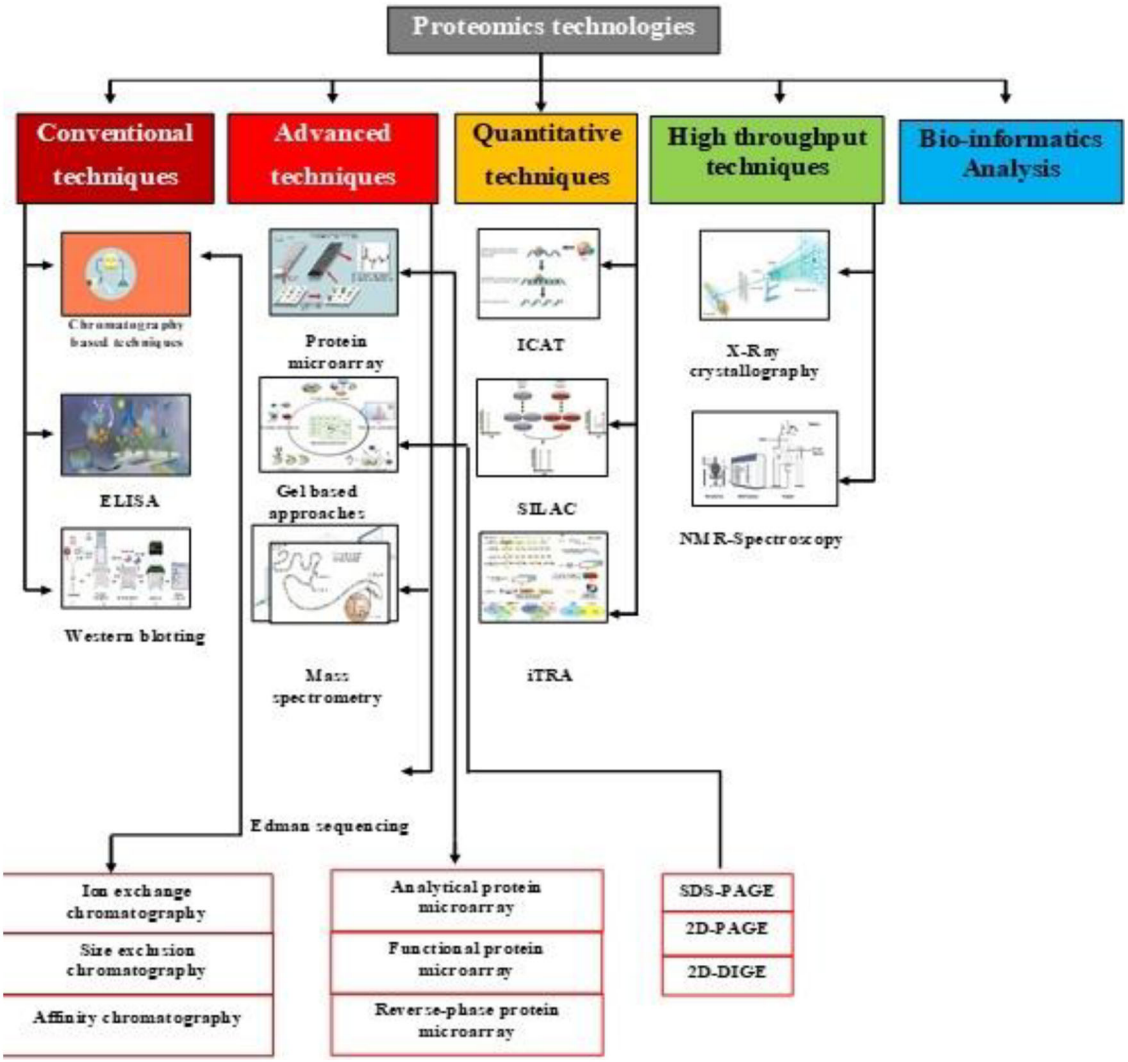

FIGURE 2 Various applications of proteomic methodologies in crop improvement. ELISA, enzyme-linked immunosorbent assay; ICAT, isotope-coded affinity tag; iTRAQ, isobaric tags for relative and absolute quantitation; NMR, nuclear magnetic resonance; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SILAC, stable isotope labeling with amino acids in cell culture.

largely due to the pioneering efforts of researchers who laid the foundation for understanding and utilizing these crucial biomolecules (Chavhan et al., 2024). The significance of researching seed storage proteins in agriculture and crop enhancement is well-established. Recognizing the transformative potential of proteomics, Prof. Chittaranjan Kole and his team have been instrumental in unraveling enigmatic aspects at the protein level in crops essential for ensuring food security and promoting sustainable agriculture (Figure 3). In this pursuit, they played a key role and made significant contributions to studies related to seed storage proteins in various crops, such as mungbean (*Vigna radiata*) (Chand & Kole, 2002; Naik, 1998; Naik et al., 2000; Naik & Kole, 2002), urdbean (*Vigna mungo*) (Kole et al., 2005, 2006), pigeonpea (*Cajanus cajan*) (Panigrahi et al., 2001a, 2001b, 2002), and rice (*Oryza sativa*) (Padmavathi et al., 1999, 2001, 2002).

Until 2012, gel electrophoresis, specifically the 2-DE version, has remained the predominant and nearly exclusive platform in plant proteomics research. Today, it remains widely employed on its own or alongside other platforms, ranking among the most commonly used methods for plant proteome analysis. Since its introduction in the early 2000s, this technique has significantly advanced our molecular understanding of plant biology, offering promising applications in agriculture and environmental sciences. Prof. Kole's Laboratory of Molecular Biology and Biotechnology (LMBB) in the Odisha University of Agriculture and Technology has been at the forefront of seed protein-based proteomics, with a primary focus on mungbean, urdbean, pigeonpea, and rice. Therefore, further, we discussed the role of seed proteins in various aspects of plant biology, as researched in Prof. Kole's lab.

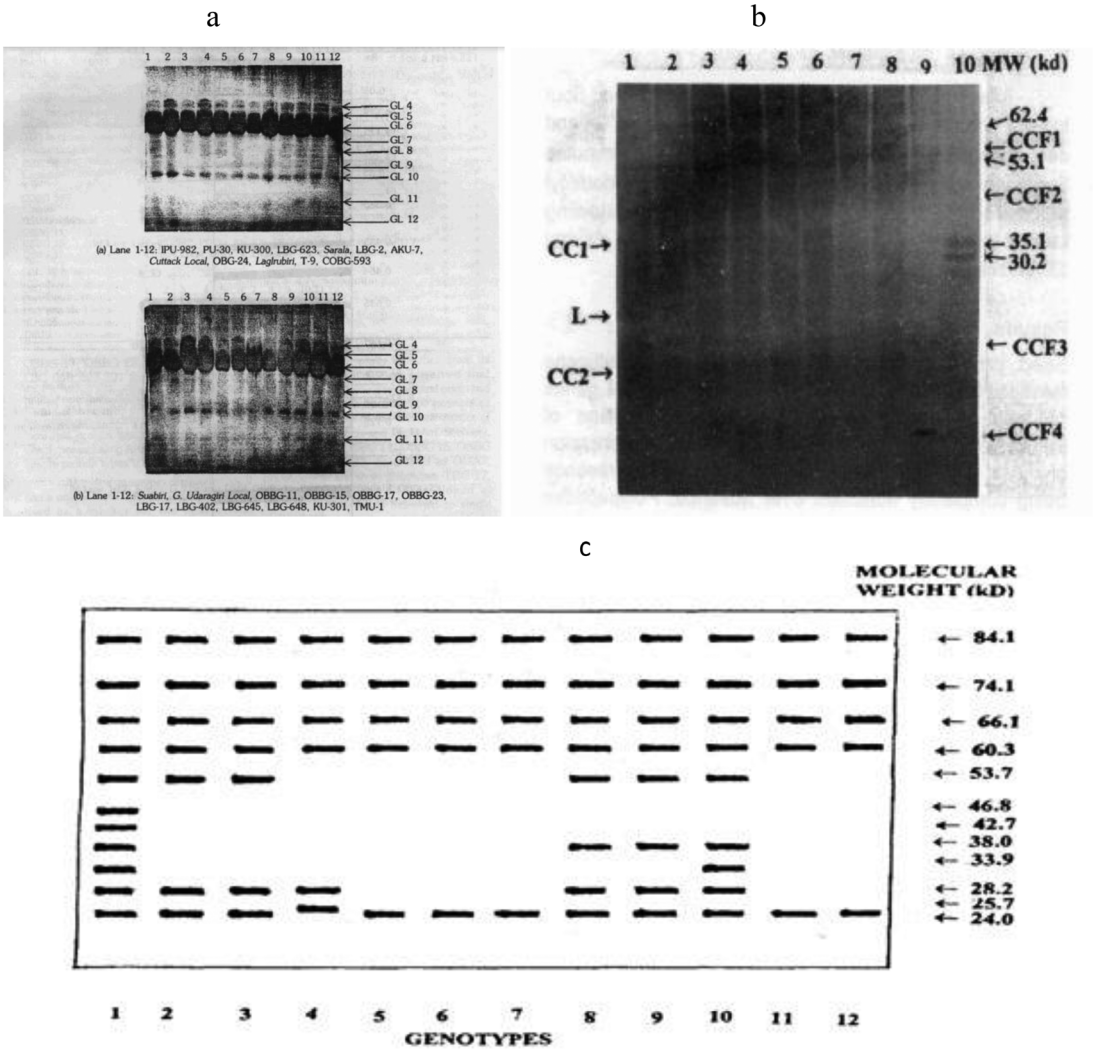

FIGURE 3 The figure illustrates the early gel-based research in different crops by Prof. Kole and team. (a) Seed globulin of 24 urdbean derived from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (b) Electropherogram of polypeptide banding pattern of 8 genotypes of *Cajanus cajan* and *C. cajanifolius*. (c) Seed protein profile of 12 genotypes studied for resistance to green leafhopper (Padmavathi et al., 1999).

3 | PROTEIN PROFILING/MARKERS FOR ASSESSING GENETIC DIVERSITY

The genetic diversity enables breeders to enhance crop resilience to environmental stresses, increase yield potential, and enhance nutritional profiles (Hafeez et al., 2023). Moreover, diverse crop varieties offer adaptability to various agro-ecological conditions and consumer preferences, thereby bolstering food security and sustainability. Particularly in the case of mungbean, urdbean, cowpea, and *Vigna sublobata*, earlier studies unveiled extensive diversity both between and within species, leading to profound insights. Sahai and Rana (1977) observed almost congruous protein profiles of two varieties of mungbean and completely similar banding patterns of two varieties of urdbean. In mungbean, Thakare et al. (1988) found uniform banding pattern of vicilin protein of four cultivars. They, however, observed two out of 86 accessions of urdbean to differ from the rest. The banding patterns of three accessions of *V. sublobata* were altogether different. Gomathinayagam and Ramaswamy (1994) found all the nine bands of the two varieties of cowpea to be uniform. Prasadi et al. (1996), while studying genetic variation in six varieties of cowpea in relation to insect resistance, observed the major variation in protein bands of molecular weight above 24 kD. Later on, Prof. Kole's research, particularly on mungbean and rice proteomics, has revealed the presence of wide diversity between and within species leading to significant discoveries. In an electrophoretic analysis study of seed albumins and globulins from six *Vigna* species and four synthetic allopolyploids, 20 albumin and 16 globulin polypeptides were identified. This study supported the hypothesis that *Vigna glabrescens* had originated through natural allo-polyploidization involving *V. radiata* and *Vigna umbellata* (Kole & Panigrahi, 2001). Variability

studies based on gel electrophoresis by Prof. Kole and his team in 37 local landraces of mungbean collected from different parts of Odisha and selections from them revealed a significant association of protein content with early flowering, pod length, pod number, seed number, and yield per plant (Naik et al., 2000). The utilization of gel electrophoresis for basic genetic diversity analysis in cereals and legumes has been instrumental in laying the groundwork for modern research in proteomics. By visualizing DNA or protein fragments based on their size and charge, gel electrophoresis enables researchers to assess the genetic diversity present within different crop populations (Hameed et al., 2012). This foundational knowledge provides a basis for subsequent proteomic investigations, allowing researchers to delve deeper into the functional aspects of genetic diversity at the protein level. Proteomic techniques have revolutionized the study of crop diversity by enabling the comprehensive analysis of protein expression patterns across diverse crop populations (Davidson et al., 2012; Hooper et al., 2020; Lim et al., 2010). Later, several works on seed storage protein were reported in rice, mungbean, pigeonpea, and *Cicer* across the globe (Dhawale et al., 2015; Jugran et al., 2010; Khan et al., 2013; Khalid et al., 2023; Nayak et al., 2022; Panigrahi et al., 2007). Panigrahi et al. (2007) elucidated the phylogenetic relationship among 11 species of the genus *Cajanus* using seed albumin and globulin markers, and their findings were well corroborated on the basing of single nucleotide polymorphism-based phylogenomic studies (Kassa et al., 2012). Similarly, proteomic studies on other crops such as maize, soybean, and tomato have revealed unique protein profiles linked to traits including disease resistance, nutrient efficiency, and abiotic stress tolerance (Davidson et al., 2012; Hooper et al., 2020; Jaradat & Goldstein, 2018; Lim et al., 2010. These examples underscore the importance of proteomic approaches in elucidating the functional implications of genetic diversity within crops, thereby informing breeding strategies aimed at developing resilient and high-performing varieties tailored to meet the challenges of modern agriculture.

3.1 | Identification of resistance sources

The identification of resistance sources in crops, such as in the case of rice against the green leafhopper (GLH), holds significant promise for boosting agricultural yields and enhancing crop resilience. By pinpointing specific protein markers associated with resistance, researchers can develop targeted breeding strategies aimed at selecting resistant traits. For instance, in a study by Padmavathi et al. (1999), specific protein bands unique to susceptible rice varieties were identified as potential markers for screening GLH resistance. In another study, Prof. Kole and team demonstrated the inheritance of protein markers detecting polymorphism among rice genotypes with contrasting host responses to GLHs (Padmavathi et al., 2001). They demonstrated the monogenic control of two albumin polypeptide bands (46.8, 42.7 kD) through linkage analysis employing an F_2 population derived from the cross TN1/IET15120 (GLH susceptible). These findings not only enable the development of marker-assisted breeding programs to confer resistance to GLH but also provide insights into the underlying genetics of resistance mechanisms. Electrophoretic analysis by Panigrahi et al. (2001a) of seed albumin from six varieties of *C. cajan*, along with two landraces and its putative progenitor *Cajanus cajanifolius*, revealed 16 distinct polypeptide bands. Among these, four bands specific to *C. cajanifolius* can serve as reliable markers for verifying hybridity in wide hybridization aimed at the introgression of stress resistance genes. Kole et al. (2000), based on seed protein type, deciphered that among different local landraces of mungbean, Jhainmung and Kendrapara 2 were resistant to Mungbean yellow mosaic virus (MYMV) and Cercospora leaf spot (CLS), while Bahalmung, Ratila 1, and Karlakhaman were resistant to only MYMV. In 2002, Chand and Kole detected two polypeptide bands in seed protein through SDS-PAGE that differentiate resistant and susceptible mungbean genotypes. The band with Rm 0.790 was exclusive to resistant genotypes, while the Rm 0.781 band was found only in susceptible ones. These markers aid in screening for CLS resistance in mungbeans. Similarly, Pattnaik and Kole (2002) detected an albumin polypeptide of Rm 0.202 present only in the susceptible varieties of mungbean that will be useful for negative selection of resistance against MYMV, the most destructive disease in mungbean and other pulse crops. Overall, the identification of resistance sources based on approaches such as seed storage gel electrophoresis offers several advantages in crop improvement efforts. This technique allows for the visualization and characterization of protein profiles associated with resistance traits directly from seeds, offering a convenient and efficient means of screening large populations for resistance. Moreover, seed storage gel electrophoresis facilitates the identification of novel resistance genes and the characterization of genetic diversity within crop germplasm, thereby expanding the genetic resources available for breeding programs. Previously, in French bean (Phaseolus vulgaris L.), presence of arcelin polypeptides was found to be associated with resistance to bruchids (Osborn et al., 1986).

Recent studies have demonstrated the effectiveness of identifying resistance sources through seed storage gel electrophoresis in various crop species. For example, in a study by Dhokane et al. (2016), integrated metabolon-transcriptomic was utilized to identify potential resistance sources for Fusarium head blight in wheat, leading to the discovery of novel resistance alleles. Similarly, in a study by Du Plessis (2013), biochemical characterization was employed to identify

resistance sources for Fusarium head blight resistance in wheat, facilitating the development of Fusarium head blight resistant cultivars. These examples highlight the importance of leveraging seed storage gel electrophoresis in identifying resistance sources and accelerating crop improvement efforts to enhance agricultural productivity and sustainability.

3.2 | Assessing genetic purity

The assessment of genetic purity, denoting the degree of uniformity and absence of genetic contamination within crop cultivars, is pivotal for ensuring the integrity and performance of agricultural germplasm (Hafeez et al., 2023). Panigrahi et al. (2001b) conducted the first electrophoretic analysis of seed protein fractions to verify hybridity in wide crosses. They analyzed seed globulins to identify markers for hybridity verification in two interspecific crosses involving two pigeon pea varieties as female parents and *C. cajanifolius* as the male parent. Later on, Mishra et al. (2012) characterized the interspecific hybrid (*C. cajan* × *C. scarbaeoides*) using both seed albumin and seed globulin markers. In a study by Mohanty et al. (2001), a distinctive electrophoretic pattern based on albumin and globulin profiles was observed across 24 mungbean cultivars. This research underscored the utility of these unique polypeptide patterns as markers for the precise identification and preservation of genetic purity within mungbean cultivars. Similarly, Naik and Kole (2001) provided the first comprehensive report on the electrophoretic banding patterns of mungbean, encompassing 37 Indian cultivars, further highlighting the significance of protein markers in maintaining genetic purity. Genetic purity in crops refers to the absence of genetic contamination or admixture within a cultivar, ensuring the retention of desired traits and characteristics (Hafeez et al., 2023). It is essential for preserving the genetic integrity of cultivars and preventing unintended changes in agronomic performance, nutritional composition, and other important traits. Maintaining genetic purity is crucial for seed production, breeding programs, and the commercialization of crop varieties, as any deviation from the desired genetic makeup can lead to reduced yield potential, decreased quality, and compromised market value (Hafeez et al., 2023).

Recent research has continued to leverage protein markers for the identification and maintenance of genetic purity in crop cultivars. For instance, in a study by Tripathy et al. (2015), protein profiling was utilized to assess the genetic purity of upland rice cultivars, revealing distinct protein markers associated with specific genetic backgrounds in upland rice. Similarly, in a study by Manivannan (2017), seed storage protein markers were employed to evaluate the genetic purity of pearl millet hybrids, enabling the identification of protein markers for hybrid verification and quality assurance. Moreover, Naik et al. (2019) had characterized the intraspecific hybrids in *Clitoria ternatea* (a medicinal legume) using seed protein markers. These recent advancements underscore the ongoing importance of protein markers in ensuring genetic purity and enhancing the reliability and effectiveness of crop breeding and seed production programs.

3.3 | Varietal identification and study on genetic inheritance

Seed protein markers have been utilized in inheritance studies to examine seed protein expression or genetic linkage in various crop plants, including *Solanum tuberosum* (Rickeman & Desborough, 1978), common bean (Koenig & Gepts, 1989; Osborn et al., 1986; Romero Andreas et al., 1986), soybean (Chen & Shoemaker, 1998), sunflower (Anisimova, 2003; Anisimova & Yang, 2004; Serre et al., 2001), rice (Padmavathi et al., 2001), urdbean (Bashir et al., 2005), *Fagopyrum esculentum* (Pan & Chen, 2010; Zeller et al., 2004), and sweet chestnut (Martín et al., 2012). In 2002, Kole et al. (2002) assessed genetic variation in 20 *V. mungo* genotypes for protein content, protein yield, and seed yield. Protein yield showed a significant positive association with seed yield, protein content, seed weight, and pods/plant suggesting a quantitative inheritance pattern for protein content. A globulin profile was depicted for the first time based on the electrophoretic banding pattern for urdbean leading to the identification of the two genotypes, AKU 7 and LBG 402, with unique protein profiles (Kole et al., 2006). In a similar study by Padmavathi et al. (2002), variation in water soluble seed albumin banding patterns between and within a set of 12 aromatic and non-aromatic rice genotypes was observed. They reported the presence of nine polypeptide bands of various molecular weights ranging from 21.4 to 93.3 kD among the aromatic and non-aromatic varieties elucidating variability in genotypes with respect to albumin fractions. Quantitative variation in total seed protein content was studied in urdbean (Kole et al., 2005). The first report on the use of SDS-PAGE to delineate the inheritance of protein expression and linkage of seed protein was reported by Prof. Kole and team in the year 2002 in mungbean (Naik & Kole, 2002). They observed a specific polypeptide subunit of 63.1 kD present in non-aromatic varieties only, which could be used as marker for hybridity confirmation in the crossing program (Padmavathi et al., 2002). In addition, Rath et al. (2015) also reported the inheritance and genetic linkage of seed protein (albumin and globulin) markers with host resistance to pod borer locus (PPB1) in pigeonpea. These pioneering works on seed protein provided a foundation for modern-day phylogenetic and diversity studies based on seed protein. Further, they also showed that protein studies can effectively be utilized to understand complex genetic diversity within and between species.

3.4 | Contributions of foundational research toward advancements in crop improvement

The groundbreaking work led by Prof. Chittaranjan Kole and his team underscores the versatility of seed protein analysis, with far-reaching applications in plant genetics, taxonomy, and breeding. Seed proteins play a pivotal role in studies related to genetic diversity, variety identification, phylogenetic relationships, evolution, and trait mapping and have become a cornerstone in advancing our understanding of plant biology (Hirano & Shirakawa, 2022). SDS-PAGE has emerged as a potent tool for discerning genetic diversity through the identification of seed storage proteins, given their resilience to environmental fluctuations. Its application extends to the analysis of variability in seed storage proteins across diverse crops and the differentiation of various varieties and germplasms (Hirano & Shirakawa, 2022; Lan et al., 2024). The first report on the detection of genotype-specific protein profile in mungbean through electrophoresis of seed albumins and globulins was published by Prof. Kole's lab (Mohanty et al., 2001). Later on, globally, the electrophoretic study of seed storage proteins was extensively conducted, contributing to genotype characterization in crops like wheat, mustard, *Solanum, Capsicum*, and *Vigna* (Geetha & Balamurugan, 2011; Ghafoor et al., 2002; Govindaraj et al., 2015; Malik et al., 2013; Mennella et al., 1999; Nagy et al., 2009; Rao et al., 1992; Siddiqui & Naz, 2009).

This electrophoretic approach proves invaluable due to the stability of protein profiles, enabling the study of crop evolution and origins. Beyond that, protein profiling of seed storage proteins facilitates variety identification, determination of phylogenetic relationships between species, germplasm characterization, and biosystematic analysis. Genetic relationship and phylogenetic relationship in mungbean were published in 1998 (Naik, 1998). In the year 2000, Prof. Kole questioned the rationale of assigning separate species to *V. mungo* and *V. radiata* through albumin marker analysis in *Vigna* sps. (Kole et al., 2000). The application of the electrophoretic approach was also done for germplasm evaluation in country bean by Prof. Kole and his team (Pujari, 2000; Shanmugam et al., 2000). Notable studies by other scientists also include the classification of wheat species based on seed storage protein profiling (Malik et al., 2013), identification of relationships among durum wheat genotypes (Pujar et al., 1999), and genetic diversity evaluation of wheat varieties (Shuaib et al., 2007). Noteworthy findings include Malik et al.'s (2013) classification of wheat species based on seed storage protein profiling, Pujar et al.'s (1999) identification of relationships among durum wheat genotypes, and genetic diversity evaluation of wheat varieties (Shuaib et al., 2007). In wheat breeding programs, pedigree-based association mapping, as demonstrated by Ishikawa et al. (2014), has proven advantageous. Genetic diversity analysis of *Brassica napus* genotypes emphasizes the efficacy of electrophoretic patterns of seed storage proteins for species differentiation (Choudhary et al., 2015). Khan and Ali recommend wheat endosperm protein for assessing genetic variability and cultivar identification in wheat breeding programs. Additionally, Prof. Kole's team reported that the mungbean (*V. radiata*) genotype BSN1, a pure line, has potential as a donor for traits such as pod length and seed weight. While it yields well in the spring season, it struggles in summer due to its susceptibility to MYMV (Naik & Kole, 2002). Later that year, Pattnaik and Kole (2002), identified a seed protein marker through electrophoretic analysis of seed albumins that distinguishes between MYMV-resistant and susceptible mungbean genotypes, thereby facilitating the incorporation of resistance genes through molecular breeding. In another study, Padmavathi et al. (1999) reported association of 46.8 and 42.7 kD polypeptides with susceptibility to GLH in rice, thereby differentiating susceptible and resistant varieties and subsequently their introgression into mega varieties through marker-assisted selection. Another team of Prof. Kole assessed interspecific hybrids of *C. cajan* and *C. cajanifolius* concerning various seed protein parameters, noting that a higher albumin-to-globulin ratio is a desirable trait for improving protein quality in grain legumes. They successfully developed high-yielding pigeonpea genotypes with enhanced seed protein content and quality through introgressive wide hybridization with *C. cajanifolius* (Panigrahi et al., 2002).

Till today, seed protein analysis is widely being used to assess genetic variation within and among plant populations in many crops including cereals, legumes, and oilseeds (Khalid et al., 2023; Pandey & Mann, 2015; Pandey et al., 2018). Such studies are crucial for germplasm characterization, genetic resource conservation, and the identification of diverse genetic pools that can be tapped for crop improvement (Chavhan et al., 2024). Seed protein profiling is being employed for the identification and authentication of different plant varieties, cultivars, and hybrids. Each variety often has a distinct seed protein profile, allowing for accurate identification (Chavhan et al., 2024). This is particularly important in agriculture for maintaining seed purity, preventing mislabeling, and ensuring the integrity of seed stocks. It supports the seed certification process for agricultural varieties. The genetic purity of sunflower hybrids was determined on the basis of isozymes and seed storage proteins and found to be an easy and accurate way of varietal identification (Nikolic et al., 2008). Various studies, such as Maccaferri et al. (2011) use of simple sequence repeat markers and Zhang et al. (2011) use of DArT markers for durum wheat genotypes, underscore the importance of differentiating origins, parentage, and distribution. Ishikawa et al. (2014) findings further emphasize the advantage of pedigree-based association mapping in breeding programs. Last, Manivannan's (2017) analysis of pearl millet cultivars highlights the efficiency of SDS-

PAGE in distinguishing varieties based on electrophoretic patterns, showcasing its utility in plant breeding programs and germplasm evaluation for various crops.

Seed protein studies contribute to the elucidation of phylogenetic relationships among plant species. By comparing seed protein profiles, researchers can infer evolutionary relationships and classify plants into taxonomic groups (Hafeez et al., 2024). This aids in refining plant taxonomy, understanding evolutionary patterns, and establishing phylogenetic frameworks for different plant families. SDS-PAGE, as highlighted by Chavhan et al. (2024), Hafeez et al. (2024), and Quenum and Yan (2017), consistently performs well in cluster analysis when cultivars belong to the same geographic location. This is corroborated by Akbar et al. (2012), and the technique has proven effective in assessing genetic diversity in various research studies (Kakaei & Kahrizi, 2011). Grewal et al. (2007) reported similar findings using random amplified polymorphic DNA (RAPD) markers for wheat genotypes. Seed protein analysis provides insights into the evolutionary history of plant species. Changes in seed protein composition over time can reveal evolutionary adaptations and relationships between different taxa. Studying the evolution of seed proteins helps trace the divergence of plant lineages, adaptation to ecological niches, and the selection pressures influencing seed protein profiles (Mueller et al., 2023). Despite the availability of numerous DNA markers for wheat, seed storage proteins remain highly valuable, as affirmed by Bean and Lookhart (2000). In the year 1999, Padmavathi and Kole identified protein markers for the screening of rice genotypes against GLN (Padmavathi et al., 1999). The seed protein markers streamline the breeding process by allowing for the selection of plants with specific protein profiles, expediting the development of new crop varieties with improved traits such as nutritional content or disease resistance.

4 | CONCLUSION

Gel electrophoresis, encompassing both 1D and 2D variations, remains a fundamental technique in plant proteomics, offering cost-effective and reliable insights into protein composition, genetic diversity, and trait selection. Despite its limitations in detecting low-abundance proteins and extreme isoelectric point proteins, its integration with gel-free methods has expanded the scope of proteome analysis, revealing post-translational modifications and protein interactions. The future of crop proteomics lies in miniaturization, nanotechnological advancements, and high-throughput MS, which promise deeper insights into seed protein functions. Integration with genomics and metabolomics will provide a holistic understanding of seed development and environmental responses, supporting precision breeding strategies and sustainable agriculture.

A pioneering force in this field, Prof. Chittaranjan Kole has significantly advanced seed protein research through electrophoretic profiling, enabling breakthroughs in genetic diversity assessment, resistance trait identification, and evolutionary studies. His foundational work in plant proteomics has not only provided a deeper understanding of seed storage proteins but has also paved the way for molecular breeding programs that address food security and climate resilience. His contributions continue to inspire innovations in plant molecular biology, reinforcing the relevance of gel electrophoresis as an indispensable tool for researchers worldwide. Looking ahead, the integration of functional genomics, precision agriculture, and bioinformatics-driven proteomic analyses will redefine crop improvement strategies. Collaborative efforts in data sharing and interdisciplinary research will be crucial in leveraging proteomics for enhanced agricultural productivity, nutritional security, and environmental sustainability.

AUTHOR CONTRIBUTIONS

Sarita Pandey: Conceptualization; data curation; project administration; writing—original draft. **Shalini Tiwari**: Writing—original draft; writing—review and editing. **Poulami Bhattacharjee**: Data curation. **Abhishek Anand**: Visualization. **Lekha T. Pazhamala**: Writing—review and editing. **Yasin Jeshima K.**: Writing—review and editing. **Roshan Kumar Yadav**: Conceptualization; Project administration; Supervision; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

There are no original data associated with this article. Referenced data are available in the literature.

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How to cite this article: Pandey, S., Tiwari, S., Bhattacharjee, P., Anand, A., Pazhamala, L. T., Yasin, J. K., & Yadav, R. K. (2025). Seed protein electrophoresis in plant genetics: Commemorating the pioneering contributions of Prof. Chittaranjan Kole and team to the foundation of plant proteomics. *The Plant Genome*, *18*, e70027. https://doi.org/10.1002/tpg2.70027