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# Assessment of genetic diversity in Sorghum genotypes using SDS-PAGE analysis of seed storage proteins

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## Abstract

*The successful genetic improvement of sorghum relies on the identification of genetic diversity within accessions. In this study, we investigated the total seed storage proteins of six sorghum genotypes, namely IS 3477, IS 33095, IS 7005 (non-pigmented), IS 28989, IS 7155, and IS 1202 (pigmented), sourced from India. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was employed to analyse the total seed protein bands, which were resolved using standard techniques on a 12% polyacrylamide gel. A total of 20 different protein bands were identified, with molecular weights ranging from 10 kDa to 110 kDa. Out of these, three genotypes exhibited monomorphism (40%), while the remaining three genotypes displayed polymorphism (60%). Notably, the polymorphic genotypes were primarily observed among the non-pigmented lines, while pigmented lines demonstrated lower levels of polymorphism. Our findings indicated that the SDS-PAGE method can be effectively employed to examine more diverse non-pigmented genotypes, thereby serving as a valuable technique for preserving, assessing, and utilizing sorghum germplasm. The utilization of this technique holds promise for the discovery of new cultivars through future breeding programs.*

**Keywords:** Sorghum, genetic diversity, SDS-PAGE, seed storage proteins, polymorphism, germplasm preservation, seed purity

## INTRODUCTION

Sorghum (*Sorghum bicolor* L.) serves as a staple food, supplying a significant proportion of calories and protein to populations in semi-arid tropical regions of Africa and Asia. The utilization of sorghum by-products, such as sorghum bran and spent grain, has gained attention due to their protein-rich content, low cost, and potential applications in animal feed and biopolymer production for food packaging (Van Heerden and Glennie, 1987; Cuq, *et al.,* 1998). Sorghum grain itself exhibits a protein content ranging from 6% to 16%, with an average of 13%. These proteins can be broadly classified into two categories: prolamin and non-prolamin proteins (Lasztity, 1996; Shewry, and Tatham, 1990). Kafirins, the major storage proteins in sorghum, fall under the category of prolamins. They are characterized by high levels of proline and glutamine and are soluble in non-polar solvents, including aqueous alcohols. Kafirins account for a substantial proportion, ranging from 77% to 82%, of the protein content in the endosperm. On the other hand, non-prolamin proteins, including albumins, globulins, and glutelins, constitute approximately 30% of the total proteins. The major storage proteins within the kafirin category are further classified into α, β, and γ based on their molecular weight and solubility (Belton, *et al.,* 2006; Thaddi and Nallamilli, 2018; Thaddi and Nallamilli, 2015). Sorghum proteins have demonstrated diverse applications, including the production of biodegradable films. These films can be manufactured using sorghum proteins extracted from sorghum bran, presenting an environmentally friendly alternative for packaging materials (Buffo, *et al.,* 1997a; Da Silva, and Taylor, 2005). Additionally, sorghum proteins have been identified as safe for individuals with celiac disease, an autoimmune reaction triggered by the consumption of prolamin proteins found in wheat (gluten), rye (secalin), and barley (hordein). Celiac disease affects a significant proportion of the population, with an estimated prevalence of 1 in 133 Americans (Ciacci, *et al.,* 2007; Fasano, *et al.,* 2003). In addition to their nutritional significance, sorghum grains are rich in polyphenols, tannins, flavonoids, and anthocyanins. These bioactive compounds have been investigated for their

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antioxidant properties, contributing to the overall health benefits associated with sorghum consumption (Naidu, *et al.,* 2009; Thaddi and Nallamilli, 2014a; Thaddi and Nallamilli, 2014b; Murty and Kumar, 1995).

Sorghum proteins possess a range of nutritional and functional properties that are essential for meeting dietary protein requirements in both human and animal diets. Furthermore, they play a significant role in malting and beer brewing processes. However, one major drawback of sorghum is its high content of phenolic compounds. Phenolic compounds, including phenolic acids, flavonoids, and tannins, are secondary metabolites that are predominantly present in sorghum. These compounds can interact with proteins, influencing their physical and biochemical properties. The interactions can lead to negative effects such as astringency and reduced nutritional value, or positive effects such as repellent characteristics (Taylor, 2016; Hahn, *et al.,* 1984; Haslam, *et al.,* 1992; Roger, *et al.,* 1980; Taylor and Schussler, 1984). Electrophoresis has proven to be an effective method for testing seed purity and identifying varieties in various cereal crops. However, reports on the use of electrophoresis for sorghum analysis are limited. The main challenge arises from the interference of polyphenols in sorghum varieties, which can hinder the extraction and separation of seed storage proteins using electrophoresis techniques (Cao, *et al.,* 2004). Consequently, the distribution and content of pigmented and non-pigmented varieties were investigated in this study, aiming to contribute to the understanding of sorghum protein composition and its potential applications. Sorghum cultivation spans across different regions worldwide, and it boasts an extensive germplasm collection comprising approximately 1,000,000 accessions. Seed purity and variety identification are critical for sorghum seed production, breeding programs, and cultivar identification. However, the reliability of electrophoresis as a tool for analysis is hampered by the presence of polyphenols in sorghum varieties. This study seeks to address these challenges by examining the distribution and content of pigmented and non-pigmented varieties.

## MATERIALS AND METHODS

**Plant Material:** Six different varieties of *Sorghum bicolor* (L.) Moench, including both non-pigmented and pigmented varieties, were used in this study. The varieties used were IS 3477, IS 33095, IS 7005 (non-pigmented), IS 2898, IS 7155, and IS 1202 (pigmented). The seed materials were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located in Patancheru, Hyderabad, Andhra Pradesh, India. Details of the varieties are provided in Table 1 and Figure 1. All the varieties were maintained through self-pollination, and cross-pollination was prevented by covering the panicles with brown paper bags. The plants were grown in the experimental farm of the Department of Botany, Andhra University, Visakhapatnam, for further analysis.

Table 1: List of Sorghum accessions used during the present study and their origin

| Accession | Obtaining Institute | Origin |
|---|---|---|
| IS 3477 | ICRISAT | India |
| IS 33095 | ICRISAT | India |
| IS 7005 | ICRISAT | India |
| IS 2898 | ICRISAT | India |
| IS 7155 | ICRISAT | India |
| IS 1202 | ICRISAT | India |

**Experimental Design:** The experimental design employed in this study involved the analysis of protein profiles in six different varieties of Sorghum bicolor. The varieties included three non-pigmented genotypes (IS 3477, IS 33095, IS 7005) and three pigmented genotypes (IS 2898, IS 7155, IS 1202). The plants were grown under controlled conditions in the experimental farm of the Department of Botany, Andhra University, Visakhapatnam. Randomized plot arrangements were used to minimize any potential bias in the analysis.

**Protein Extraction:** Protein extraction from the sorghum seeds was performed using a modified protocol. Mature panicles were collected, and one gram of seed from each variety was ground to a fine powder in liquid nitrogen using a mortar and pestle. The powder was then suspended in cold acetone containing 10% (w/v) trichloroacetic acid (TCA) and 1% (w/v) dithiothreitol (DTT).

After vortexing, the sample was kept at -20 °C overnight. Following centrifugation, the pellet was washed twice with cold acetone containing 1% DTT. After vacuum drying, the pellet was solubilized in a lysis buffer containing 8 M urea, 4% (w/v) CHAPS, 10 mM DTT, and 1% (v/v) ampholyte (pH 3–10). The resulting supernatant was used for protein content determination and subsequent analysis.

**Protein Quantification:** The protein concentration of the extracted samples was determined using a modified Bradford Assay (Kielkopf, *et al.,* 2020). Bovine serum albumin (BSA) standards were prepared in duplicate, and protein extracts were prepared in duplicate as well. The Bradford reagent was diluted with distilled water, and 900 µl of the diluted reagent was added to each standard and protein extract. After incubation, the absorbance was measured at 595 nm, and the concentrations of the unknown protein extracts were extrapolated from the standard curve.

**Acrylamide Stock Solution:** A 30% acrylamide and 1% bis (N, N' methylenebisacrylamide) stock solution was prepared. This stock solution, along with 10% ammonium persulfate (APS), was used for gel preparation in SDS-PAGE.

**Bradford Reagent Stocking Solution:** A stock solution for the Bradford reagent was prepared by mixing 95% ethanol, 85% phosphoric acid, and 0.01% Bradford reagent. The Bradford reagent was prepared by diluting 1 part BIO-RAD Protein Assay dye reagent concentrate with 4 parts distilled water. Additionally, a 5 mg/ml bovine serum albumin (BSA) stock solution was prepared in the urea buffer.

**Coomassie Brilliant Blue Staining Solution:** A staining solution containing 0.025% Coomassie Brilliant Blue R-250, 50% methanol, and 10% acetic acid was prepared. This solution was used to stain the SDS-PAGE gels for visualization of protein bands.

**Destaining Solutions:** Two destaining solutions were prepared. Destaining Solution I consisted of 40% methanol and 7% acetic acid, while Destaining Solution II contained 5% methanol and 7% acetic acid. These solutions were used to remove excess stain from the SDS-PAGE gels after staining.

**Electrophoresis Buffer:** The electrophoresis buffer used for SDS-PAGE contained 0.025 M Tris, 0.192 M glycine, 0.1% SDS, and had a pH of 8.3. This buffer facilitated protein separation during electrophoresis.

**Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):** Protein extracts were separated using 1D SDS-PAGE. The gel system consisted of stacking and resolving gels. The stacking gel concentrated the proteins into thin zones, while the resolving gel separated the proteins based on molecular weight. SDS-PAGE gels were cast on spacer glass plates, and resolving gels of 12% (v/v) and stacking gels of 5% (v/v) were prepared. Protein samples were mixed with 2X SDS sample loading buffer and denatured by heating. The samples were loaded onto the gels, and electrophoresis was performed using a Mini-PROTEAN$ 3 Electrophoresis Cell. The gels were then stained with Coomassie Brilliant Blue and destained using destaining solutions.

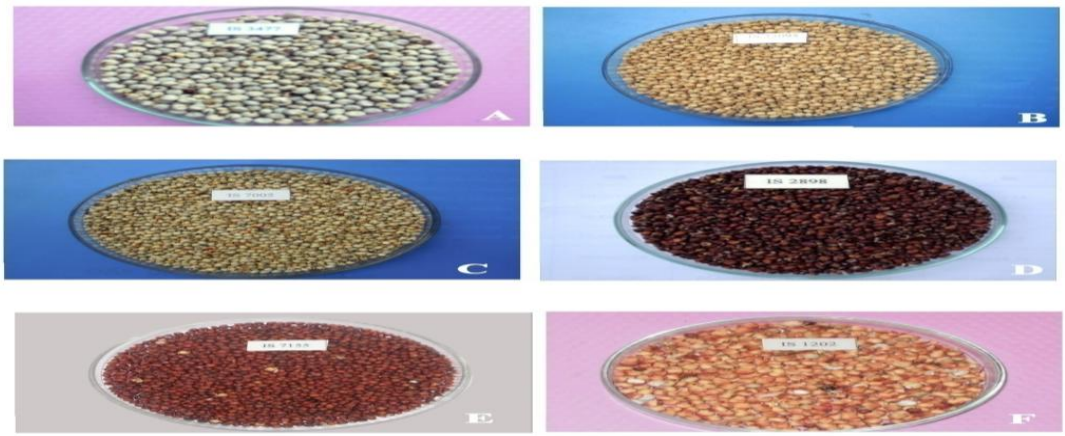

Figure 1: The genotypes are labelled as follows: A: IS 3477, B: IS 33095, C: IS 7155 (non-pigmented genotypes), D: IS 2898, E: IS 7005, F: IS 1202 (pigmented genotypes)

**Data Analysis:** Statistical Analysis: Statistical analysis was performed to evaluate the differences in protein profiles among the sorghum varieties. The banding patterns obtained from SDS-PAGE were analyzed using image analysis software. The presence or absence of protein bands was recorded, and the data were used to calculate the percentage of monomorphic and polymorphic protein subunits in each variety. Descriptive statistics, such as mean and standard deviation, were calculated to summarize the protein concentration data obtained from the Bradford Assay.

## RESULTS

**Protein Concentration in Sorghum Seed:** The protein concentrations of the tested sorghum varieties were determined using the Bradford Assay prior to SDS-PAGE analysis. As shown in Table 3, the protein concentration ranged from 9.5% to 12.5% on a dry weight basis. All varieties exhibited relatively low protein content, with none exceeding 12.5%. Notably, the non-pigmented varieties displayed higher protein percentages compared to the pigmented varieties.

**SDS-PAGE Analysis of Total Seed Storage Proteins:** Preliminary experiments utilizing 1-DGE aimed to differentiate the sorghum cultivars based on broad protein level differences and assess the quality and quantity of the protein extracts. However, the results from 12% SDS-PAGE analysis indicated minor differences in protein profiles between pigmented and non-pigmented cultivars, with no distinct variations observed. Figure 2 presents the CBB-stained SDS-PAGE banding pattern of the seed protein profiles of the six sorghum varieties (IS 3477, IS 33095, IS 7155, IS 2898, IS 7005, and IS 1202). Lane M represents the molecular weight marker, while lanes A-F correspond to the respective varieties' seed protein banding patterns. Each lane was loaded with 25 µg of total protein extracted from the corresponding tissue. The quality of the seed protein extracts was deemed satisfactory, as no visible signs of streaking or protein degradation were observed. In terms of protein expression, abundance, and banding patterns, the biological replicates of the experiment displayed high similarity, indicating reproducibility of protein preparation across distinct extractions. However, some minor variations were observed in the genotype-specific banding patterns within the molecular weight range of 20–100 kDa for the non-pigmented lines IS 3477 and IS 33095.

Comparison of protein loading across the biological replicates further confirmed the reproducibility of protein preparation. Protein extracts from all six varieties encompassed a wide range of molecular weights, ranging from 10 to 110 kDa, as shown in Figure 2. These results suggest that while there are minor variations in the protein profiles between pigmented and non-pigmented sorghum cultivars, overall, the protein content, expression, and banding patterns were consistent among the biological replicates. The SDS-PAGE analysis of sorghum seed proteins revealed the presence of 40 distinct protein bands, which were quantified and used as the basis for examining six sorghum genotypes. Among these protein components, 40% (18 protein subunits) were found to be monomorphic, consistently present across all genotypes, while 60% (22 protein subunits) were polymorphic, displaying variations among the genotypes. The gel was divided into four zones based on the polypeptide banding profiles, designated as zones 1, 2, 3, and 4 (Figure 2, Table 4).

Table 3: Estimation of Protein content in Sorghum by Bradford Assay method

| Varieties of Sorghum | Percentage of protein |
|---|---|
| IS 3477 | 12 % |
| IS 33095 | 12.5 % |
| IS 7005 | 11.5 % |
| IS 2898 | 10% |
| IS 7155 | 9.5% |
| IS 1202 | 11% |

The majority of the protein subunits were found in zones 2 and 4, while a smaller proportion was observed in zones 1 and 3. Notably, the different genotypes exhibited greater alterations in their seed proteins in the shorter polypeptide bands compared to the primary protein subunits. Zone 1, ranging from 100 to 110 kDa in molecular weight (MW), contained six protein subunits, of which three were monomorphic and three were polymorphic. Zone 2, ranging from 100 to 80 kDa in MW, contained twelve protein subunits, with four

being monomorphic and eight being polymorphic. Zone 3, ranging from 80 to 50 kDa in MW, comprised eight protein subunits, two of which were monomorphic and six of which were polymorphic. Finally, Zone 4, ranging from 50 to 10 kDa in MW, included fourteen protein subunits, with five being monomorphic and nine being polymorphic. Zones 2 and 4 displayed higher levels of polymorphism, making them potentially superior ranges for evaluating the genetic diversity of sorghum germplasm (Figure 3).

The Table 4 provides a detailed description of the sorghum seed storage subunits observed in the SDS-PAGE gel, categorized into different zones based on their molecular weight range. The total number of bands present in each zone, along with the corresponding molecular weight range, monomorphic bands, polymorphic bands, and the percentage of polymorphism, are presented. In Zone 1, which spans the molecular weight range of 100-110 kDa, a total of 6 bands were observed. Among these, 3 bands were monomorphic, meaning they were consistently present across all genotypes, while the remaining 3 bands were polymorphic, showing variations between genotypes. The percentage of polymorphism in Zone 1 was 50%. Zone 2 encompassed the molecular weight range of 100-80 kDa and contained 12 bands. Out of these, 4 bands were monomorphic, and the remaining 8 bands were polymorphic. The percentage of polymorphism in Zone 2 was not specified. Similarly, in Zone 3 (80-50 kDa), there were a total of 8 bands. Among them, 2 bands were monomorphic, and 6 bands were polymorphic. The percentage of polymorphism in Zone 3 was not specified. Zone 4 covered the molecular weight range of 50-10 kDa and exhibited 14 bands. Of these, 5 bands were monomorphic, while 9 bands were polymorphic. The percentage of polymorphism in Zone 4 was 65.5%. In total, the gel analysis revealed 40 distinct protein bands across all zones and molecular weight ranges. Among them, 18 bands were monomorphic, meaning they were consistently present, and 22 bands were polymorphic, indicating variations between genotypes. This comprehensive description of the banding pattern in the gel provides valuable insights into the genetic diversity and protein composition of the tested sorghum genotypes.

The current analysis revealed a significant level of genetic polymorphism within the tested sorghum germplasm. The division of the genotypes into a primary group and the convergence of non-pigmented genotypes contributed to this observed variation. It is possible that the restricted genetic background of the non-pigmented genotypes played a role in generating this level of variation. These findings highlight the presence of substantial genetic diversity in the examined sorghum germplasm, particularly within the shorter polypeptide bands. The identification of polymorphic protein subunits in specific molecular weight ranges suggests the potential use of SDS-PAGE analysis as a valuable tool for assessing and characterizing the genetic diversity of sorghum genotypes.

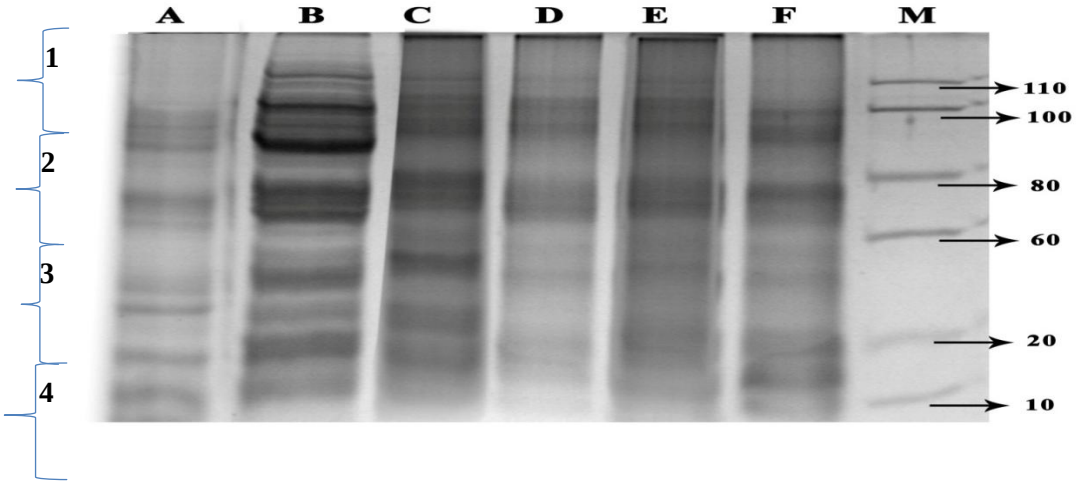


Figure 2: The figure depicts the results of SDS-PAGE analysis, showing the protein profiles of six different Sorghum genotypes. The protein ladder (M) is used as a molecular weight marker to determine the molecular weights of the proteins in the gel. The genotypes are labelled as follows: A: IS 3477, B: IS 33095, C: IS 7155, D: IS 2898, E: IS 7005, and F: IS 1202

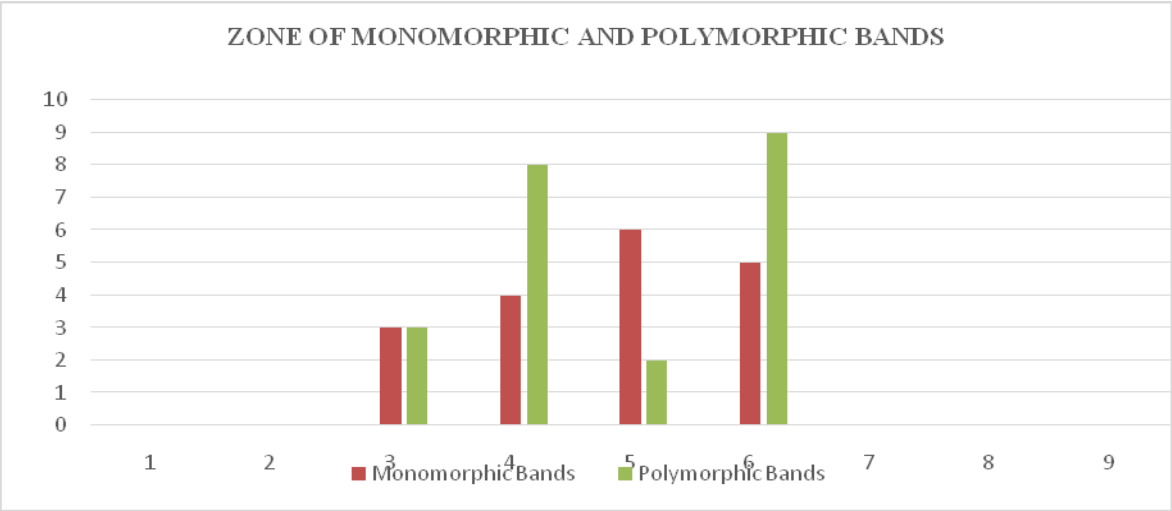


Figure 3: Different zones among all bands and polymorphic bands

Table 4: A description of each of the sorghum seed storage subunits of the banding pattern in the gel

| ZONE | Total Number of Bands in the gel pic | Molecular weight range in kDa | Monomorphic Bands | Polymorphic Bands | Percentage of Polymorphism |
|---|---|---|---|---|---|
| 1 | 6 | 100-110 | 3 | 3 | 50 |
| 2 | 12 | 100-80 | 4 | 8 | 65.5 |
| 3 | 8 | 80-50 | 6 | 2 | 35 |
| 4 | 14 | 50-10 | 5 | 9 | 75.5 |

## DISCUSSION

The current study aimed to investigate the variation in storage proteins of different sorghum cultivars using the SDS-PAGE method. Traditional protein analysis methods have often failed to reveal the extent of variations among germplasms, particularly cultivars. Understanding and preserving genetic diversity is essential for sustainable agriculture and the conservation of agricultural genetic resources. Germplasm serves as the primary resource for breeding programs to develop new varieties, emphasizing the importance of understanding genetic diversity and utilizing it for progeny assessment and utilization (Baranger, *et al.,* 2004; Nagy, *et al.,* 2009).

Proteins have been widely used as markers to estimate genetic diversity in various crop species, including sorghum. Decoding the data from DNA can provide valuable insights into the genetic diversity within sorghum. In this study, the total seed storage proteins exhibited significant levels of variability, with their performance regulated by genes, while the environment had minimal influence on their electrophoretic banding patterns in cereal crops. The seed storage protein banding pattern is often used for characterizing plant germplasm, identifying varieties, studying phylogeny, and conducting biosystematics analysis (Ramu, *et al.,* 2013; Ng'uni, *et al.,* 2012; Eswara Reddy, and Jacobs, 2002; Gepts, *et al.,* 1986; Assefa, *et al.,* 2011; D'Ovidio & Masci, 2004; Nkongolo, 2008). SDS-PAGE is a commonly used method have been investigated for seed storage proteins in cereals worldwide (Koranyi, 1989; Poperelya, *et al.,* 1989; Wang, *et al.,* 1994; Gorinstein, *et al.,* 1999; Shah, *et al.,* 2003; Abdel, 2004; Anjali and Sanjay, 2012). It is a simple and effective technique for assessing germplasm diversity. In this study, the SDS-PAGE method was employed to evaluate the genetic diversity of six sorghum genotypes, including both pigmented and non-pigmented germplasm. A total of 20 protein subunits were quantified. Among them, 38% (6 protein subunits) were found to be monomorphic, consistently present across all genotypes, while 62% (10 protein subunits) were polymorphic, displaying variations among the genotypes (Ghafoor, *et al.,* 2002). The protein banding patterns obtained from SDS-PAGE analysis revealed variation in the electrophoresis images, which is consistent with the approach used in this study. Some essential and non-

essential protein subunits exhibited significant variation, consistent with previous findings regarding their functional and structural properties. Genetic diversity was determined based on the appearance or disappearance of polypeptide bands. It was observed that none of the genotypes individually possessed all 20 protein subunits, resulting in distinct protein banding patterns among the six genotypes. This finding aligns with previous studies that highlight the stability and informativeness of seed protein electrophoresis as a tool for investigating the origins and evolution of cereals. Significant protein subunits exhibited less diversity compared to smaller polypeptides (Ghafoor, *et al.,* 2002; Rashed, *et al.,* 2010; Akbar, *et al.,* 2012; Gorinstein, *et al.,* 2004; Ladizinsky).

Notably, the non-pigmented genotypes (IS 3477 and IS 33095) exhibited a protein component with a molecular weight of 21 kDa among the major polypeptide chains. This suggests that only specific genotypes carried the gene encoding this particular protein component. Conversely, the absence of the 19 kDa major polypeptide chain in pigmented genotypes while present in the remaining germplasm indicates the presence of the gene for this protein in those genotypes. These findings support previous research demonstrating that the presence or absence of genes contributes to the genetic diversity of sorghum genotypes. Additionally, nine out of the thirteen minor protein components displayed genetic variation across the examined genotypes (Sharmila, *et al.,* 2013; Sanni *et al.,* 2008). It is important to note that the variability of total protein found in sorghum germplasm did not show a significant correlation with the accessions taken from the same area. Differences in the genetic diversity of the materials used, as well as the proportion and size of the gel used for protein separation, may account for the variation observed when comparing the results of the current study with previously published studies.

## CONCLUSION

The SDS-PAGE method proved to be a viable approach for preserving, assessing, and utilizing sorghum germplasm, which is the primary source for developing new varieties in future breeding programs. The analysis revealed significant genetic diversity within the tested sorghum germplasm, and the distinct protein banding patterns among different genotypes demonstrated the potential of SDS-PAGE for evaluating and characterizing genetic variation in sorghum.

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