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## RESEARCH ARTICLE

# VIGNA MUNGO (L.) HEPPER. PROTEIN SEPARATION AND ANALYSIS BY SDS-PAGE

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### ABSTRACT

Scientifically referred to as *Vigna mungo* (L.) Hepper, the mung bean is a significant leguminous crop prized for its high protein content. A recent study looked at the complex protein composition of mungo seeds using a method called SDS-PAGE. This study revealed the number and distribution of distinct protein components within the seeds, which also showed a variety of protein bands with differing molecular weights. This study's results can potentially transform leguminous crop quality and protein content through selective breeding methods. Furthermore, this vital research can help direct the creation of novel, nutritionally enhanced food products. Coomassie Brilliant Blue staining was used to visualize the protein (polypeptides) bands, which showed various bands with different molecular weights, suggesting a complex protein (polypeptides) profile in *Vigna mungo* seeds. The intensity and dispersion of protein bands were also examined to assess the relative quantity of various protein components. A subsequent densitometric analysis quantitatively revealed *Vigna mungo* seeds' protein content. This study adds to a comprehensive understanding of the protein composition of *Vigna mungo* seeds, which could lead to improved leguminous crop quality and protein content by selective breeding. Moreover, food technologists and nutritionists may use the insights gained in creating novel food products with enhanced nutritional profiles.

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## INTRODUCTION

Pulses are crucial in human diets, supplementing cereals and improving their nutritive values. They are rich in lysine, vitamins B, minerals, and fats and are the main source of protein in vegetarian Indian diets (Gurusamy et al., 2022). Pulses are cultivated in India for their high protein content. Mung beans are high in fiber, carbohydrates, energy, and vitamins, and they include minerals like magnesium, iron, phosphorus, potassium, zinc, and copper. Riboflavin and niacin are present in trace amounts (Varma et al., 2018). Sensitive consumers may experience rhinitis, asthma, or allergies while eating dry beans, despite their reputation for nutrient richness, health benefits, biodiversity, and climate resilience. Both black gram and mung beans are popular dry beans; however, because vicilin, a seed storage protein, is a major allergen in mung beans, they are linked to food allergies (Gupta et al., 2021). Mung beans are high in lysine but low in cysteine and methionine (Chunkao et al., 2020). They have 20.97-31.32% protein and 60% starch. Globulins comprise 60% of its proteins, albumins 25%, and other proteins 15%. The components of the globulins are 3.4% basic 7S, 7.6% 11S, and 89% 8S. 8S globulins are shown as four protein bands on sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) examination, whereas basic 7S globulins and 11S globulins comprise two polypeptides connected by disulfide bonds. Mung bean protein has many health advantages,

such as antihypertensive, anti-obesity, lipid-lowering, and antidiabetic qualities. Mung bean flour and several extraction techniques—such as water, alkaline, salt, and acid precipitation—are used in production (Zhou et al., 2024). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a widely used technique for separating proteins based on their molecular weight (Kielkopf et al., 2021). This method utilizes SDS, a detergent that denatures proteins and gives them a negative charge proportional to their size. The process involves preparing protein samples with SDS and a reducing agent, loading these samples onto a polyacrylamide gel, and applying an electric field to promote the separation of proteins (Wijethunga and Emenike, 2024). In this setup, smaller proteins (polypeptides) migrate faster than larger ones. After the proteins (polypeptides) have been separated, the gel is stained to visualize them, with their sizes estimated compared to molecular weight markers. SDS-PAGE has various applications, including protein identification, purity assessment, Western blotting, and protein quantification (Bass et al., 2017). However, it has some limitations, such as not providing information about protein function or native structure and having difficulty with proteins of similar sizes. Despite these drawbacks, SDS-PAGE remains a fundamental technique in molecular biology and proteomics, valued for its effectiveness in analyzing and characterizing proteins in various contexts (Rosenberg, 2013). Proteins can be qualitatively analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, or SDS-PAGE. To create polyacrylamide gel,

acrylamide monomer is polymerized with N-methyl bisacrylamide and additional cross-linking agents (Kouchmeshky, 2014). This reaction is an example of free radical catalysis (Wu *et al.*, 2024). The polymerization process is initiated by ammonium persulfate (APS) free radicals and stabilized by N', N'-Tetramethylethylenediamine (TEMED), which catalyzes the production of free radicals from persulfate ions (Liao *et al.*, 2024). In the protein synthesis process, excess soluble thiol ( $\beta$ -mercaptoethanol) and detergent SDS are employed to break up protein folding and subunits to resolve polypeptide chains using SDS-PAGE (Rajan, 2024). About half as many SDS molecules are attached to a polypeptide chain as there are residues of amino acids in the chain. Because the protein SDS complex has net negative charges, it moves toward the anode. The gel's pore size and polypeptide size determine how the separation occurs. Altering the concentration of Acrylamide and Bis-acrylamide will change the gel's pore size (Kielkopf *et al.*, 2021). The pore size and the concentration of Acrylamide are inversely correlated. Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) can be separated using PAGE because low gel concentration reduces the frictional effect. Elevated gel concentration results in a sifting action and divides oligomeric proteins based on their molecular weight (Guo *et al.*, 2021). The applied voltage, the molecule's net charge, and the frictional effect all affect how mobile polypeptide molecules are i.e. '(Applied voltage  $\times$  Net charge of molecules) / Friction of the molecules' is the formula for expressing the mobility of molecules.

## MATERIALS AND METHODS

**Preparation of stock solutions.** The process used to generate the monomer solution/acrylamide mix was 25 milliliters of Double distilled water (ddH<sub>2</sub>O), 14.6 grams of Acrylamide, and 0.4 grams of Bis. A brown bottle retained the volume at 4°C after it was filtered and brought up to 50ml with ddH<sub>2</sub>O. 1 liter of ddH<sub>2</sub>O and 36.3 grams of Tris were combined with 4X running gel (Resolving gel) buffer, pH 8.8, and 1.5M Tris. After adding 200 milliliters of ddH<sub>2</sub>O to bring the pH down to 8.8 using 6N HCl (Hydrochloric acid), the container was kept at 4°C. 25 milliliters of ddH<sub>2</sub>O and three grams of Tris were combined to create a 4X stacking gel buffer with a pH of 6.8 and 0.5MM Tris. The volume was made up to 50ml with ddH<sub>2</sub>O, and its pH was adjusted to 6.8 with 6N HCl and stored at 4°C. The 10% SDS was stored in room temperature, and 10% of APS (Ammonium persulfate) was freshly prepared. Preparation of water-saturated Butanol (by taking 50ml n-Butanol and 5ml ddH<sub>2</sub>O. It was well shaken, and the top phase was used for overlaying), 1% BPB (Dissolved in 10% alcohol first), and 1% Brilliant Blue was done. 12.5% TCA, and Tris-Glycine buffer (4XTank Buffer) was prepared having 0.025M Tris-CL, 0.192M Glycine, 10% SDS, and pH 8.3 (250ml dd H<sub>2</sub>O, 6g Tris, 28.8g Glycine and 20ml 10% SDS were mixed with ddH<sub>2</sub>O, the amount was increased to 500 ml. pH was adjusted to 8.3 and kept at 4°C for storage). 2X Treatment Buffer was prepared having 0.125M Tris-CL, 4% SDS, 20% Glycerol, 10% 2-mercaptoethanol, and pH 6.8. 2.5ml of Tris, 4ml of 10% SDS, 2ml of Glycerol, 1ml of 2-mercaptoethanol, and 0.5ml of ddH<sub>2</sub>O were mixed which is 10ml in volume. It was made 1X with 1% BPB just before use. The staining solution was prepared fresh. 180ml 12.5% TCA and 20ml 1% Brilliant Blue were mixed to make 200ml (Manns, 2011).

**Extraction of Total Seed protein.** 0.05g seed flour of Black mung i.e. *Vigna mung* (L.)Hepper was taken in an Eppendorf tube. 800 $\mu$ l of 0.5M NaCl (pH 2.4) was added to the sample. It was centrifuged at 12000rpm for 5min at 10°C. The supernatant was collected and stored at 4°C (Valizadeh, 2001).

**Extraction of seed Albumin and Globulin.** To an Eppendorf tube, 0.05g of seed flour was added. To the sample, 200 $\mu$ l of prechilled ddH<sub>2</sub>O was added. It was tipped every hour for four hours at a temperature of 4°C. Next, it was centrifuged at 4°C and 12,000 rpm for five minutes. Albumin was extracted (Table 1) from the supernatant and kept for denaturation at 4°C. After that, a pellet was obtained to extract globulin. ddH<sub>2</sub>O was used to wash the pellet. To

the Eppendorf tube, 800 $\mu$ l of 0.5M NaCl (pH 2.4) was added. It was shaken sporadically every 15 minutes and stored at room temperature for 45 minutes. It was centrifuged for five minutes at 4°C and 12,000 rpm. Globulin was extracted (Table 1) from the supernatant and kept for denaturation at 4°C (González-González *et al.*, 2020).

**Denaturation.** In an Eppendorf tube, 50 $\mu$ l of the protein sample and 50 $\mu$ l of the 1X cracking buffer (treatment buffer) were added. Twice, the tube was inverted to ensure a complete mixing. After that, it was denaturated for two minutes at 100°C in a water bath. The material was then kept until usage at 4°C.

**Preparation of SDS-PAGE.** The medium for the SDS-PAGE electrophoresis technique is a discontinuous gel made of polyacrylamide. It employs sodium dodecyl sulfate, or SDS, as a surfactant to conceal the intrinsic charge of the protein and enable accurate protein separation by mass. Positive charges are reduced by the basic pH range of the gel, and proteins move towards the anode at varying rates according to mass. Glass plates, spacer, and comb were cleaned thoroughly with teepol liquid, dried, and then again cleaned with rectified spirit. Spacers were put on the basal glass plate, and an upper glass plate was placed over it. These were sealed with adhesive tapes perfectly. The sandwich was clamped perfectly and sealed with 1% agar solution. 4.3 ml of ddH<sub>2</sub>O, 2.9 ml of 1.5M Tris HCl (pH 8.8), 4.2 ml of Acrylamide mix, 120  $\mu$ l of 10% SDS, 70  $\mu$ l of 10% APS, and 10  $\mu$ l of TEMED were used to create the resolving gel (12%). Using a safety pipette, the resolving gel was pipetted into the sandwich. After adding 50 milliliters of water-saturated butanol phase to the gel, polymerase was given 30 minutes. After decanting, the butanol was soaked on tissue paper and cleaned with ddH<sub>2</sub>O. 3 ml of ddH<sub>2</sub>O, 1.15 ml of Acrylamide mix, 0.75 ml of stacking gel buffer (pH 6.8), 50  $\mu$ l of 10% SDS, 50  $\mu$ l of 10% APS, and 6  $\mu$ l of TEMED were used to make 5% stacking gel. After covering the resolving gel with stacking gel and inserting the comb, the polymerase process was given 30 to 45 minutes. After adding tank buffer to both the upper and lower tanks, the gel was clamped using an electrophoresis device, and the comb was carefully removed. 10 $\mu$ l of denatured samples were loaded into each well. The sample was electrophoresed at 50V/100V till the movement of the dye up to 1cm above the lower end. Then the power supply was turned off. The gel unit was taken out of the electrophoresis equipment. Using a spatula, the plates were carefully separated. The gel was sliced near Well No. 1 in the lower left corner.

**Staining of gel.** The gel was placed in a tank containing 12.5% Trichloro acetic acid cycle (TCA) for forty minutes. For 2.5 hours, it was stained with a staining solution while being shaken periodically. 12.5% TCA was used to distemper it after being repeatedly changed, filtered, and reused. Under a transilluminator with white light, the gel was examined (Manns, 2011; Roy, 2014).

**Scoring of gel.** The distance was measured migrated by dye font and specific bands.

Relative mobility= $R_m$  = (Distance migrated by individual Polypeptide) / (Distance migrated by dye font)

Similarity Index (SI) = {(No. of similar bands)/(No. of similar bands + No. of dissimilar bands)} $\times$ 100

## RESULTS

The protein distribution patterns of lentil protein isolates (LPIs) were found to be identical, with four primary peaks (F1, F2, F3, and F4) eluting progressively at 650, 300, 160, and 36 kDa (Basset *et al.*, 2017). Peak F1 on SDS-PAGE comprises 60 kDa legumin-like subunits and 95 kDa lipooxygenase subunits. This peak's protein profile on SDS-PAGE was consistent with legumin-like globulin fractions; bands of about 60 kDa were decreased to around 40 and 20 kDa under reducing conditions. The peak F3 and F4 protein distribution was mostly influenced by vicilin 48 kilo Dalton (48 kDa), phytohemagglutinin (lectin) (32 kDa), and  $\gamma$ -vicilin (18 kDa) (Shrestha *et al.*, 2023). The range of total protein in mung beans was 7.67–27.30

mg BSAEs/g FW. Mung bean sprouts treated with 1% MeSA (methyl salicylate) exhibited the highest soluble protein concentration (27.30 mg BSAEs/g FW), with SA 20 millimeter (20 mM) therapy coming in second.

**Table 1. Protein samples taken in the wells**

Lane No.	Samples taken	Source
L	Protein standard	-
L <sub>1</sub>	Albumin with testa	Black gram
L <sub>2</sub>	Albumin without testa	Black gram
L <sub>3</sub>	Globulin with testa	Black gram
L <sub>4</sub>	Globulin without testa	Black gram
L <sub>5</sub>	Total protein with testa	Black gram
L <sub>6</sub>	Total protein without testa	Black gram

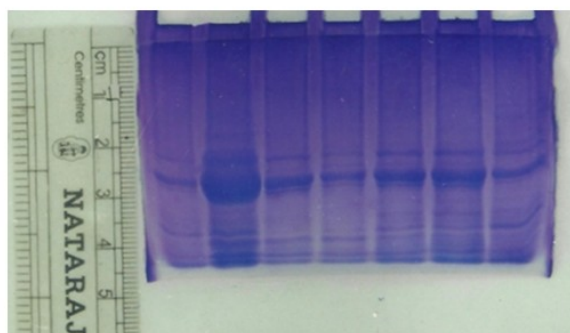
**Table 2. Relative mobility of polypeptide bands in different sample lanes (L<sub>1</sub> to L<sub>6</sub>)**

Band No.	Distance migrated (cm)	Relative mobility (R <sub>m</sub> )	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>	L <sub>6</sub>
1	1.4	0.28	✓	✓	×	×	✓	✓
2	1.5	0.30	×	×	×	×	✓	✓
3	1.6	0.32	✓	×	×	×	✓	✓
4	1.7	0.34	✓	✓	×	×	✓	✓
5	1.8	0.36	✓	×	×	×	✓	✓
6	1.9	0.38	✓	×	×	×	✓	✓
7	2.2	0.44	✓	✓	✓	✓	✓	✓
8	2.4	0.48	✓	✓	✓	✓	✓	✓
9	2.5	0.50	✓	✓	✓	✓	✓	✓
10	2.7	0.54	✓	✓	✓	✓	✓	✓
11	3.0	0.60	✓	✓	✓	✓	✓	✓
12	3.1	0.62	✓	✓	✓	✓	✓	✓
13	3.2	0.64	✓	✓	✓	✓	✓	✓
14	3.4	0.68	✓	✓	✓	✓	✓	✓
15	3.6	0.72	✓	✓	✓	✓	✓	✓
16	3.7	0.74	✓	✓	✓	✓	✓	✓
17	3.9	0.78	✓	✓	✓	✓	✓	✓
18	4.1	0.82	✓	✓	✓	✓	✓	✓
19	4.2	0.84	✓	✓	×	×	✓	✓
20	4.4	0.88	✓	✓	✓	✓	✓	✓
21	4.5	0.90	✓	✓	✓	✓	✓	✓

**Table 3. Similarity Index (SI) among six sample lane**

	L1	L2	L3	L4	L5	L6
L1		85	70	70	95.23	95.23
L2	85		82.35	82.35	80.95	80.95
L3	70	82.35		100	66.66	66.66
L4	70	82.35	100		66.66	66.66
L5	95.23	80.95	66.66	66.66		100
L6	95.23	80.95	66.66	66.66	100	

Scale L L1 L2 L3 L4 L5 L6



**Fig. 1 Separated polypeptide bands as seen under white light transilluminator**

Protein bands in mung bean sprouts with a MeSA (1%) concentration were more intense. The current study's overall SDS-PAGE protein profile results for both control and elicited sprouts showed that, in contrast to control, the intensity of protein bands increased in evoked

sprouts (Thappa *et al.*, 2023). The Similarity Index (SI) of Lane5-Lane6 (L5-L6) and Lane3-Lane4 (L3-L4) was 100%, suggesting there is no extractable protein in the testa. Most of the protein in the seed was found to be shared by albumin, such as L5 and L6, and Lane (L1) showed 95% similarity (Fig. 1, Table 2 and Table 3). The procedure adopted for the extraction of albumin somehow shows the presence of some proteins in testa as Lane 1 (L1) and L2 show 85% similarity. Lane2 (L2) shows similarity with L3 and L4 at 82.35 level, suggesting albumins without testa are different from globulin with testa and without testa because of the difference in polypeptides between albumin and globulin. Similarly, the SI of L2 with L5 and L6 suggests that the total protein polypeptides are greater than that of albumins. 70% similarity marked amongst L1 with L3 and L4 showed that albumin and globulins differ by their polypeptide constituents (Fig. 1). The 66% similarity marked between globulin and total protein confines the earlier conclusion that albumin composed the major constituents of total protein.

## DISCUSSION

The SDS-PAGE results of *Vigna mungo* (L.) Hepper demonstrates its diverse protein composition. Total protein in mung beans ranged from 7.67 to 27.30 mg BSAEs/g FW, with 1% MeSA (methyl salicylate) treatment yielding the highest concentration (27.30 mg BSAEs/g FW), followed by 20 mM salicylic acid. Protein bands in sprouts treated with MeSA were more intense, indicating increased protein levels compared to the control. The variability observed in band intensity may indicate potential differences in the levels of protein expression, which can be influenced by a variety of environmental conditions or developmental stages. For instance, certain stress conditions, such as temperature fluctuations or nutrient availability, have been linked to changes in protein synthesis. Notably, research has demonstrated that albumin levels tend to increase under these specific stress conditions, suggesting that this protein plays a role in an adaptive response mechanism. Understanding this mechanism could provide deeper insights into how organisms respond to environmental challenges. Furthermore, the analysis highlights the significant nutritional value of *Vigna mungo*, commonly known as black gram. This legume is particularly noteworthy because the proteins it contains are abundant in essential amino acids, which are amino acids that the body cannot synthesize and must obtain through diet. These essential amino acids play critical roles in numerous physiological processes, including protein synthesis, tissue repair, and hormone production. Consequently, *Vigna mungo* is recognized as an excellent source of nutrition, not just for human consumption, but also as a nutritious feed option for livestock, contributing to improved health and productivity in animal husbandry. Future research could concentrate on the functional characterization of these proteins to enhance our understanding of this plant's benefits. This would involve studying their roles in plant physiology, such as how they contribute to growth and development. Additionally, the exploration of these proteins could lead to innovative applications in food technology, potentially improving the quality and nutritional content of food products derived from this versatile legume. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) serves as an essential method for the comparative analysis of protein profiles across various samples. This technique not only facilitates the evaluation of protein purity but also aids in the estimation of molecular weights. Overall, SDS-PAGE provides critical insights into the protein composition of *Vigna mungo* (L.) Hepper, thereby supporting further functional research and potential applications in the fields of nutrition and agriculture.

## CONCLUSION

Size fractionation is achieved by separating proteins based on electrophoretic mobility using SDS-PAGE, a technique utilized in molecular biology, forensics, genetics, and biochemistry (Table 2). It's the most popular biochemical technique in the world. The study found that there is no extractable protein in the testa, with most protein in seeds shared by albumin. Total protein polypeptides were

more common than albumins, and 70% similarity between albumin and globulin indicated differences in polypeptide constituents (Fig.1). Albumin is composed of the major constituents of total protein. The seeds of black gram have more albuminous polypeptides than globulins polypeptides. In the future, we can analyze and compare varieties to determine the best choice while analyzing the chemical composition and functional properties that can aid the food industry in isolating desired components. Comparison of the viscosity of commercial concentrates at similar protein concentrations to that of minimally purified mung bean proteins is another avenue for further investigation.

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