





Evaluation of Genetic Diversity in Kidney Bean (*Phaseolus vulgaris* L.) Genotypes Based on their Protein Profile

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Abstract	Article History
<p>The phenotypes of organisms are expressions of their genotypes through proteins. Molecular marker like protein gives an indirect information about the structure of a plant. In this study, a total of ten (10) genotypes of kidney bean seeds consumed in Cameroon were studied to evaluate the genetic diversity and the systematic relationship that exist among them based on their buffer soluble protein profile using Native-PAGE and (SDS-PAGE) Sodium dedocyl sulphate polyacrylamide gel electrophoresis. The molecular weight of the proteins in the grains ranged from 17-30 kDa. The data matrix from the Native-PAGE revealed an average polymorphism of 32.90 % with the highest similarity index 100 % between Glp190 (V-naine) and PH237 while R-4 (V-naine) and R-6 showed the lowest similarity index of 14.30. The average polymorphism from the SDS-PAGE was 0.00 % with highest similarity index, 56.50 % between PH237 and R-43. The lowest was 4.80 % between R-4 (V-naine) and PH237. From the cluster analysis of buffer soluble proteins in Native PAGE, it showed that PH237 and Glp 190 (V-naine) are closely related than others and the furthest from R-6 and R-43. While from the SDS-PAGE, R-43 and PH237 are closer than any other. This investigation showed a wide range of variation among varieties and that analyses of these varieties based on morphology is very much limited to drawing conclusions. However, the numerical analysis of seed storage protein profiles offers no answer concretely to the identity of the genotypes studied though it offers extra banding for kidney bean genotypes variation grown in Cameroon.</p> <p>Keywords: Native-PAGE, SDS-PAGE, genotype, polymorphism, Kidney bean</p>	<p>Received: 16 May 2025 Accepted: 22 May 2025 Published: 29 Jun 2025</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

Primarily, Kidney beans is cultivated for its green pods and dry seeds. Preferences in consumption for seed color, shape, size, seed coat luster is a great deal which vary among consumers. The popularity of grain legumes in world is because of its impact in nutrition for man and animal. The interest in finding new sources of food to alleviate malnutrition and food security in developing countries is increasing significantly (Adam *et al.*, 2025). Legumes are one of the most important group of plants in the world and are second to cereals only in the world agriculture for the provision of food crops (Duranti, 2006). Kidney beans is a leguminous crop characterized with multiple diverse morphologies demonstrated with great variation in the growth habit, seed, flowering, pigmentation and flowering (Morojele and Mbewe, 2014). Legume seeds are being used as indispensable food crops to both man and animal (Morel *et al.*, 2012). Most legume seeds are an important food staple all over

the world especially in the developing world due to their high nutritional content. They are rich in lipid and proteins but the protein content is higher. A great role has been played in recent years by grain legumes in the search for vegetable protein sources due to the high protein content of their seeds ranging from 20% in pea to 40% in lupin (FAO, 1984; Mandal and Mandal, 2000). Kidney been grains are an unavoidable food staple in Cameroon especially in the student area as they prefer it to other food staples due to its high nutritional quality. Due to its high protein quality, it can be considered a better substituent in the diet for animal protein. They are as well used as rational nourishment components and food for vegetarians. Additionally, kidney beans have gain recognition with the ability in the management diabetes, promoting cardiovascular health, weight management, and exhibition of its anti-inflammatory and anticancer properties (Abirami and Jaspreet, 2023). Nevertheless, seed storage proteins of these legumes

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have low concentration of amino acids with sulfur and this problem has to be considered by plant breeders in their improvement programs (Summerfield and Roberts, 1985). The quest to reducing the risks relating to consumption of animal food products increases with the increase in the world's population. Thus, growth for production of plant protein could be the possible remedy.

The protein polymorphism, isozymes and productive features for the majority of grain legumes are well documented (De Falco *et al.*, 1991; Salmanowicz and Przybylska 1992; Labdi *et al.*, 1996; Singh *et al.*, 1994; Erskine and Muehlbauer, 1991; Koenig *et al.*, 1990). Even so, the comparative study of the variation of these protein genotypes is not well documented. Thus, it will be necessary to increase our knowledge on the genetic variation and similarity among these genotypes, using their seed storage proteins as molecular biomarker. Analyzing these grain species, will help document them and assist breeders in decision making of which once could result to a better breed if crossed bred.

Materials and Methods

Materials: β -mercaptoethanol (β -ME), Acrylamide, Bis-acrylamide, Coomassie Brilliant blue R250, Sodium dodecyl sulfate (SDS) and molecular weight markers (14-78 kDa) utilized for the preparation of reagents were of analytical grade and purchased from Sigma-Aldrich Chemical Co. St. Louis, MO, USA. All reagents were prepared fresh unless stated otherwise throughout the analysis, deionized water was used.

Sources of Samples, Identification and Characteristics: Ten kidney beans genotypes from different regions of Cameroon were bought from Bamenda food market, Northwest Region of Cameroon. They were identified by Dr. Dieudonné Betti Abessolo (botanist) at the Institute of Research and Agricultural Development (IRAD), Foubourt, West Region of Cameroon. Their common names were based on the morphological structures, pigmentation, color, shape and size.

Sample Collection and Processing

Using a commercial blender (Smart Grind, Black and Decker, Towson, MA, USA), the grains were dehulled and finely ground. They were defatted in three times hexane extractions (1 ml hexane/0.1 g flour) as described by Sammour *et al.* (1995). Each time, with slow stirring for two hours at 4°C. The flour was air-dried after the hexane layer was discarded. The flour was sieved via a net of 75 μ m mesh size. They were packaged in polyethylene bags of low density and stored in refrigerators prior to analysis.

Protein Extraction: From the ten different genotypes of beans, buffer soluble proteins were prepared and according to the method of Sammour *et al.* (1995) with few modifications. Exactly 60 mg of defatted flour was mixed with 1 ml of 50 mM Tris-HCl buffer at pH 8.0 in an Eppendorf tube at room temperature overnight. It was then centrifuged in micro-centrifuge machine (Eppendorf) at 15°C for 15 mins. The extracts were stored at -10°C until use. Protein concentration was estimated according to Lowry *et al.* (1951) with bovine serum albumin used as standard.

Electrophoresis: Proteins were separated using the TV50 Camlab Vertical Electrophoresis Unit. Electrophoresis of the buffer soluble grain proteins were performed at 5 % stacking gel and separating gel 12.5 % following the method of Laemmli (1970) with few modifications.

The polymerization mixture of Native PAGE consisted of 16.7 ml of 30 % acrylamide, 10 ml of 4X resolving gel buffer at pH 8.8, exactly 13.2 ml deionized water, 200 μ l of 10 % ammonium persulfate and 13.3 μ l of TEMED. The extracted water-soluble protein was mixed with equal volume of sample buffer made of 4X stacking gel buffer at pH 6.8, deionized water, glycerol (10 %) and 0.1 % bromophenol blue. Under reducing conditions for the Native PAGE, the sample buffer contained 5 % B-ME which was absent for the non-reducing conditions. The polymerization mixture of the SDS-PAGE consisted of 16.7 ml of 30 % acrylamide, 10 ml of 4X resolving gel buffer (pH 8.8), 0.4 ml of 10 % SDS, 12.8 ml of deionized water, 200 μ l of 10 % ammonium persulfate and 13.3 μ l of TEMED. The extracts (10 ml) of the buffer-soluble seed protein stored were solubilized in sample buffer consisting of 4 x stacking gel buffer (pH 6.8), 10 % SDS; 10 % glycerol and 0.1 % bromophenol blue. Using a water bath, the mixture was heated for 5 min, then placed on ice until they were applied to the gel. SDS PAGE was performed under reducing and non-reducing conditions. The molecular weight of each protein band were determined using molecular weight marker proteins ovotransferrin (78 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), actinidin (29 kDa), β -lactoglobulin (18 kDa) and lysozyme (14 kDa) as was similarly carried out by Munazza *et al.* (2009) and Sanjeev *et al.* (2012).

Electrophoresis was carried out using a current of 15 mA and 300 V of voltage (Consort E844 power pack) until the bromophenol blue tracker dye reached down the bottom of the gel. The gels were fixed and stained with 0.2 % Coomassie Brilliant blue R-250 in methanol: acetic acid: deionized water (5:4:1, v/v/v) overnight. The gels were destained afterwards using the solvent of the stain mixture; methanol: glacial acetic acid: deionized water (5:4:1, v/v/v) until the protein bands become clearly visible.

The gels were photographed and documented. In the data matrix, 1 (presence) and 0 (absence) were used as codes. A dendrogram was constructed with the software, PyElph version 1.4 based on the pattern of the total seed proteins of the bean cultivars, using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Average polymorphism was taken as total number of polymorphic bands (TNPB) divided by the total number of bands (TNB) (TNPB/ TNB) multiplied by 100. Jaccard's similarity index was calculated as ratio of similar bands to total bands between two genotypes multiplied by 100 (Jahangir *et al.*, 2014).

Results and Discussion

As environmental fluctuations barely influence the seed storage proteins, their profiling through electrophoresis technology is considered as a consistent tool for germplasm characterization (Kakaei *et al.*, 2012). The electrophoregram of buffer soluble protein profile of the ten kidney bean seed genotypes grown in Cameroon, obtained by one-dimensional Native and SDS-PAGE are presented in Figures 1 and 2 respectively. The protein patterns

of these genotypes were inspected virtually and compared to each other. In their slow-mobility range for Native-PAGE and fast-mobility range for SDS-PAGE, the proteins exhibited high similarities and variations. The slow mobility range is based on protein migration according to their charge and size and the fast mobility range is based only on their charges.

The Native and the SDS-PAGE of seed protein profile of the ten different kidney bean genotypes grown in Cameroon were carried out to evaluate at molecular level their genetic diversity and similarity. The profiling of the seed storage proteins showed distinct polymorphism in the banding patterns which led to the detection of 61 and 102 polypeptide bands in the Native and SDS-PAGE respectively. Out of the 61 bands detected (Table 1) in the Native Page, 20 were polymorphic and the rest monomorphic giving an average polymorphism of 32.9 % (Table 2). Also, a total of 102 polypeptide bands (Table 1) were detected in the SDS-PAGE but all were monomorphic giving an average

polymorphism of 0.00 % (Table 2). This could be because proteins separated through Native PAGE maintain their native conformation and charges. These results are not in line with Lukong *et al.* (2014) who electrophoretically, characterized and investigated the relationship between the water-soluble seed proteins of some legume species in Nigeria.

The more bands detected during the SDS-PAGE analysis suggested that the polymeric proteins contained disulphide bonds which were cleaved to smaller subunits of distinct molecular masses and the destabilization of the subunits by the SDS detergent, thus more polypeptide bands. The polymorphic index of 32.9 % for the Native PAGE and 0.00 % for the SDS-PAGE suggest that the ten genotypes have some identical protomers which are linked together by disulphide bridges to form similar polymeric proteins in the different hybrids. The unique polymeric proteins are responsible for the maintenance of their genetic and phenotypic diversity.

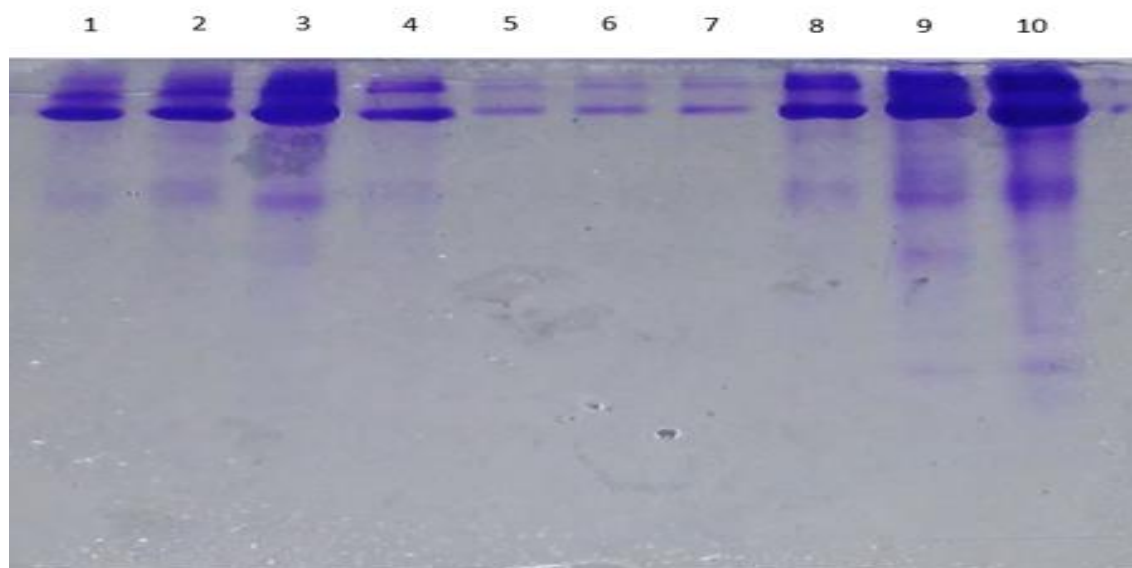


Figure 1: Electrophoregram of buffer soluble seed storage proteins using Native-PAGE (Lane 1 - 10). 1= (White beans long grain) Gl22; V-naine, 2= (Dwary mark mark grain) R-4; V-naine; 3= (White beans small grain) Mex142; V-grimpante, 4= (Medino) Glp190; V-naine, 5= (Meringe long grain) PH237, 6= (Meringe small grain) Meringe, 7= (Black small grain) PNN; V-naine, 8= (Black long grain) R-43, 9= (Yellow small grain) ECAPAN 017, 10= (Broad mark mark grain) R-6 and M molecular weight marker proteins.

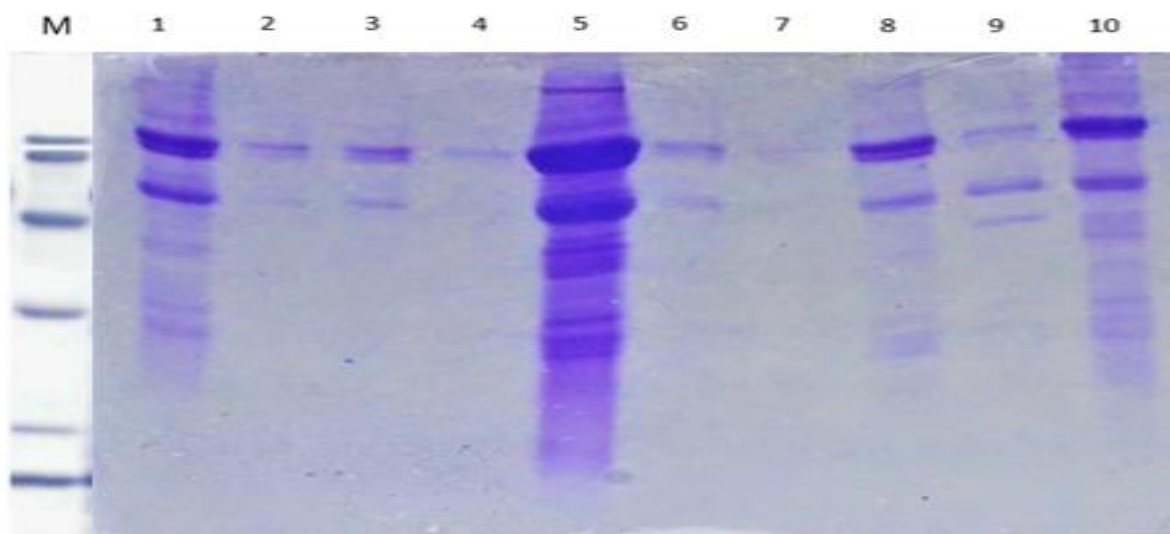


Figure 2: Electrophoregram of buffer soluble seed storage proteins using SDS-PAGE (Lane 1 - 10). 1= (White beans long grain) Gl22; V-naine, 2= (Dwary mark mark grain) R-4; V-naine; 3= (White beans small grain) Mex142; V-grimpante, 4= (Medino) Glp190; V-naine, 5= (Meringe long grain) PH237, 6= (Meringe small grain) Meringe, 7= (Black small grain) PNN; V-naine, 8= (Black long grain) R-43, 9= (Yellow small grain) ECAPAN 017, 10= (Broad mark mark grain) R-6.

Table 1: Data matrix of buffer soluble seed proteins of bean genotypes based on electrophoresis

Native PAGE										SDS-PAGE									
1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1
1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	0	1
1	1	1	1	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0
0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
1	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0	0	1	0	1
1	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0
1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	0
0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	1	1	1	0
0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0
1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	1
0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	1	0	0
0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	0	1	0	0
0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1
0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	1	1
0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1
1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	0	0
										1	0	0	0	0	0	1	1	0	0
										0	0	1	0	1	1	1	1	0	1
										1	0	0	0	0	0	0	0	0	0
										1	0	0	0	0	0	0	1	0	0
										0	0	0	0	1	0	0	0	0	1
										1	0	0	0	0	0	0	0	0	0
										0	0	0	0	1	0	0	1	0	1
										1	0	0	0	1	0	0	1	0	1
										1	0	0	0	0	0	0	0	0	0
										0	0	0	0	1	0	0	1	0	0
										1	0	0	0	0	0	0	0	0	0
										0	0	0	0	0	0	0	0	0	1
										0	0	0	0	1	0	1	0	0	0

1= (White beans long grain) Gl22; V-naine, 2= (Dwary mark mark grain) R-4; V-naine, 3= (White beans small grain) Mex142; V-grimpante, 4= (Medino) Glp190; V-naine, 5= (Meringe long grain) PH237, 6= (Meringe small grain) Meringe, 7= (Black small grain) PNN; V-naine, 8= (Black long grain) R-43, 9= (Yellow small grain) ECAPAN 017, 10= (Broad mark mark grain) R-6.

Table 2: Average Polymorphism of Kidney Bean Genotypes in Native- and SDS-PAGE experiments

Experimental Conditions	TNB	TNPB	AP (%)
Native-PAGE for buffer soluble proteins	61	20	32.9
SDS-PAGE for buffer soluble proteins	102	00	00.0

In Table 3, the Native-PAGE, Gl22 (V-naine) showed a high similarity index of 85 % with R-4 (V-naine) and Mex142 (V-grimpante). This could be an explanation why the varieties differ phenotypically just by size, color and pigmentation. However, the highest similarity index was 100 %, observed between Glp190 (V-naine) and PH237. The 100 % could be that all the buffer soluble proteins of both varieties are identical. Phenotypically, these two genotypes differ just by pigmentation on the Glp190 (V-naine). The least similarity index was 14.3 % between R-4 (V-naine) and R-6.

In the SDS-PAGE, the highest similarity index was 56.5 % between PH237 and R-43. These two differ phenotypically by color. The highest similarity index could be that their monomeric proteins are more similar than the polymeric proteins that showed just 22.2 % of similarity in the native. The lowest similarity index in the SDS-PAGE was 4.8 between R-4 (V-naine) and PH237 (Table 3)

Table 3: Similarity Index Buffer Soluble Proteins of Ten Kidney beans Genotypes Grown in Cameroon

Code	Native-PAGE										Code	SDS-PAGE									
	1	2	3	4	5	6	7	8	9	10		1'	2'	3'	4'	5'	6'	7'	8'	9'	10'
1	100										1'	100									
2	75	100									2'	14.3	100								
3	85.7	85.7	100								3'	8.9	44.4	100							
4	66.7	57.1	66.7	100							4'	17.6	33.3	10	100						
5	57.1	57.1	66.7	100	100						5'	21.4	4.8	25	11.1	100					
6	57.1	37.5	42.9	60	60	100					6'	16.7	37.5	40	14.3	9.5	100				
7	57.1	37.5	42.9	60	60	60	100				7'	14.3	20	25	28.6	26.3	30	100			
8	27.3	27.3	18.2	22.2	22.2	37.5	37.5	100			8'	50	14.3	22.7	15.8	56.5	25	30	100		
9	25	16.7	18.2	20	20	33.3	33.3	50	100		9'	21.1	22.2	27.3	50	21.1	44.4	30	26.3	100	
10	23.1	14.3	16.7	18.2	18.2	30	30	77.8	41.7	100	10'	24	5.6	15	13.3	47.6	17.6	10.5	37.5	25	100

1= (White beans long grain) GI22; V-naine, 2= (Dwary mark mark grain) R-4; V-naine, 3= (White beans small grain) Mex142; V-grimpante, 4= (Medino) Glp190; V-naine, 5= (Meringe long grain) PH237, 6= (Meringe small grain) Meringe, 7= (Black small grain) PNN; V-naine, 8= (Black long grain) R-43, 9= (Yellow small grain) ECAPAN 017, 10= (Broad mark mark grain) R-6.

A dendrogram was constructed using unweighted paired group mean and arithmetic average (UPGMA) with the data obtained from the analysis of Native and SDS-PAGE. The cluster analysis from the Native PAGE in figure 3, revealed that some genotypes were closely related to each other (PH237 and Glp 190 (V-naine), Mex142 (V-grimpante) and GI 22 (V-naine), PNN (V-naine) and Meringe, R-6 and R-43. From the SDS-PAGE, the cluster analysis revealed that some genotypes [R-43 and PH237, Mex 142 (V-grimpante) and R-4 (V-naine), ECAPAN 017 and Glp 190 (V-naine)] were closely related (Figure 4).

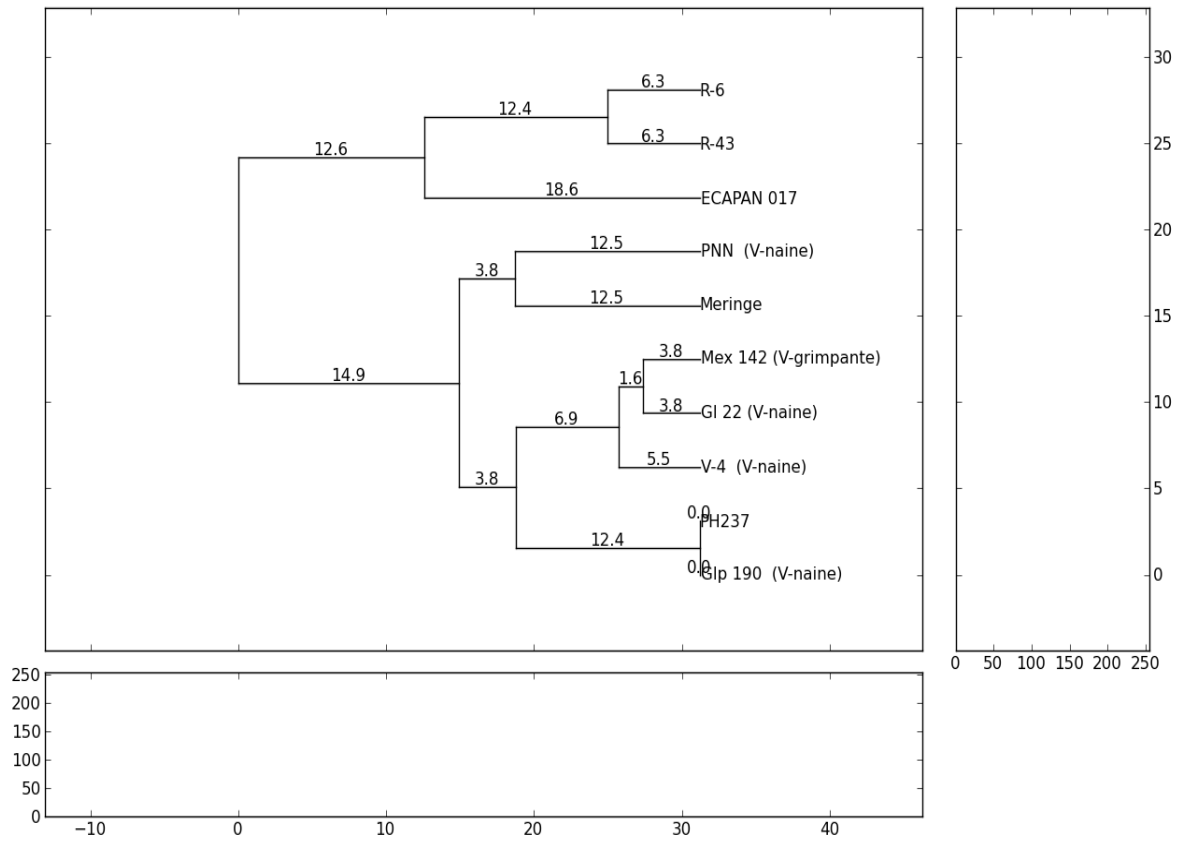


Figure 3: Dendrogram of UPGMA depicting phylogenetic relationships among ten kidney bean genotypes soluble in 50mM Tris-HCl buffer (pH 8.0) obtained by Native PAGE.

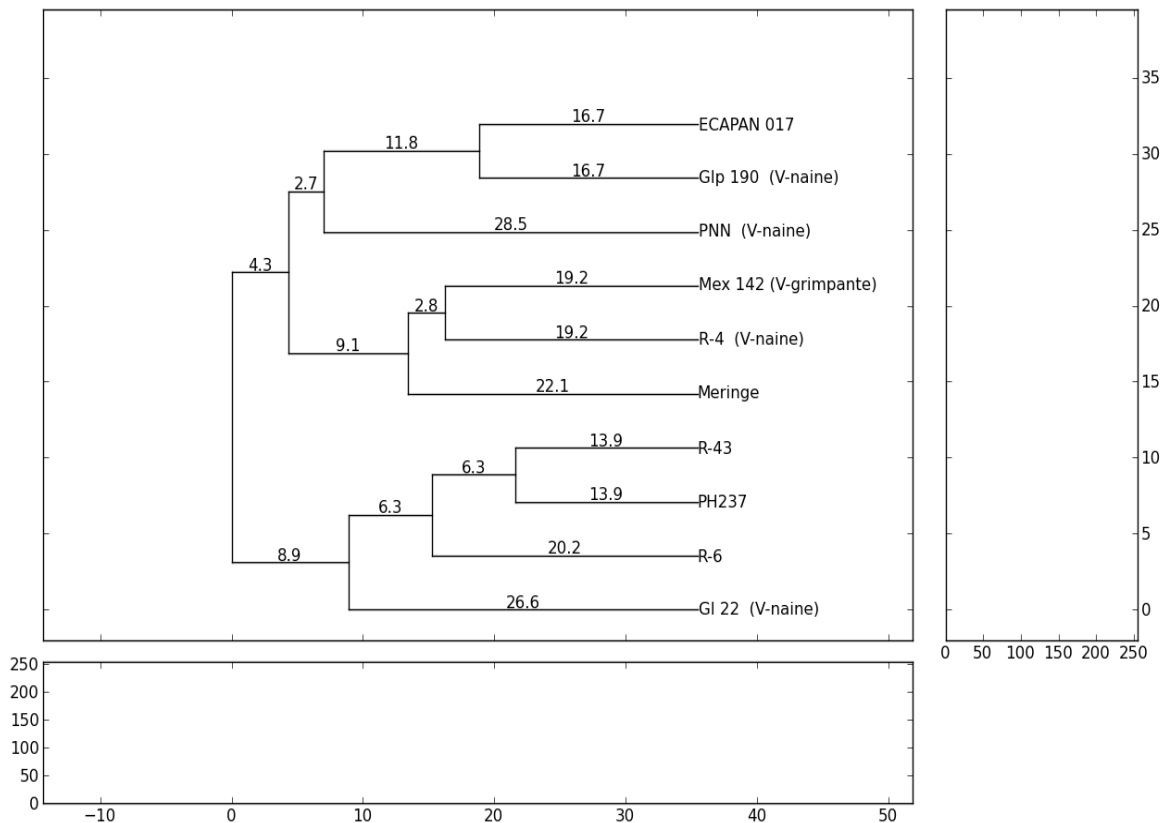


Figure 4: Dendrogram of UPGMA depicting phylogenetic relationships among ten kidney bean genotypes soluble in 50mM Tris-HCl buffer (pH 8.0) obtained by SDS-PAGE.

