

## Estimation of Genetic Variation in Rapeseed Germplasm Using Total Seed Proteins Profile

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### Abstract:

*Genetic diversity among 114 rapeseed (*Brassica campestris* L.) accessions was investigated for seed protein profiling using Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS-PAGE). In total, 16 protein bands were observed with molecular weight ranging from 20 kDa to more than 66 kDa. Among total protein bands, 12 (75%) were polymorphic which resolved taxonomic variation. The data was analyzed statistically using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). A dendrogram was formed and on the basis of banding pattern, the data was clustered into 4 regions. All the regions exhibited polymorphism for one or the other protein band.*

**Key words:** Genetic diversity, rapeseed, total seed proteins, SDS-PAGE, Pakistan

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## Introduction

Rapeseed is the major crop of oilseed that is cultivated worldwide and consumed as edible oil [1]. Huge amount of foreign exchange is being spent every year on the import of edible oil in Pakistan. To reduce the import bill, domestic production of edible oil should be increased. Development of improved rapeseed cultivars is necessary to meet the increasing demands of edible oil [2]. Genetic diversity and its analysis is a pre-requisite in crop breeding for the development of improved and high yielding varieties [3]. It is important to distinguish between rapeseed cultivars because they differ in quality and other agronomic traits. Morphological characterization involves intensive data collection and requires a long time as the crop grows slowly. For this reason, SDS-PAGE is widely used technique for identification and differentiation of crop cultivars [4, 5].

SDS-PAGE is a reliable, stable, cheaper, and best option for the study of seed protein because seed proteins are not sensitive to environmental effects [6]. Banding pattern has been used for the identification cultivars [6]. Seed storage protein is convenient tool for estimation of genetic diversity in wild and cultivated rice [7]. However literature on the SDS-PAGE on Brassica species for genetic diversity is limited [8].

Variation in proteins exhibited by SDS-PAGE can distinguish between different cultivars of a crop [9]. Identification of *Brassica juncea* entries based on SDS-PAGE is useful to sort out desirable entries for various characters, which can be used as parents in hybridization program [10]. Ten polymorphic markers were identified using SDS-PAGE from seed protein and no identifiable polymorphic band was found from leaf protein in eighty-five cultivars of *Brassica rapa*, *B. juncea*, *B. napus*, *B. carinata*, *B. oleracea* and hexaploid brassic collected from Bangladesh, China , Denmark and Japan [11]. Cluster analysis based on polymorphic bands generated by

SDS-PAGE separated yellow sarson, self incompatible cultivars and brown sarson [12]. Cultivars of *Brassica rapa*, *Brassica napus*, *Brassica carinata* and *Brassica juncea* can be distinguished using SDS-PAGE [13]. In present studies, variation among 114 rapeseed (*Brassica campestris* L.) accessions was determined using SDS-PAGE.

## Materials and Methods

One hundred and fourteen accessions of rapeseed (*Brassica campestris* L.) were obtained from Plant Genetic Resources Program, National Agricultural Research Centre, Islamabad, Pakistan for investigation of genetic diversity for total seed proteins using SDS-PAGE.

For extraction of proteins, mortar and pestle was used to crush and grind seeds. Ten mg seed flour was placed into 1.5 ml microtube. To extract proteins, protein, 400 µl extraction buffer was added to the flour as an extraction liquid and mixed thoroughly in eppendorf tube with a small glass rod. Extraction buffer was constituted of 0.5M Tris-HCl (pH 8.0), 0.2% SDS, 5M Urea, and 1% 2-mercaptoethanol. Bromophenol blue was added to extraction buffer as a dye to point out the movement of protein in the gel. To purify extraction, the homogenate samples were mixed thoroughly by vortexing and centrifuged at 12,000 rpm for 10 minutes at room temperature (RT). Extracted crude proteins were found as clear supernatant and transferred into new 1.5 ml eppendorf tubes and stored at -20° C until gel electrophoresis.

SDS-PAGE of total seed proteins was performed in polyacrylamide slab gels in discontinuous buffer system. Vertical gel slabs were prepared in a glass sandwich tightened by a set of plastic clips lined with a band of foamed silicon rubber. Separating gels consisted of 20% by weight acrylamide and 0.135% by weight N,N-methylene-bis acrylamide in 0.5M Tris-HCl buffer (pH 8.8) with 0.27% SDS. The gels were

polymerized chemically by adding 15 $\mu$ l by volume of TEMED (Tetramethylethylene-diamine) and 10% APS (ammonium persulphate). Stacking gels contained 30% acrylamide and 0.8% N.N-methylene-bis-acrylamide in 0.25M Tris-HCl buffer (pH 6.8) containing 0.2% SDS. Polymerization of the stacking gel was made chemically in the same way as for the separation gels. Electrode buffer contained Tris-glycine (9.0g Tris-HCl and 43.2g glycine per 3 liters buffer solution at pH8.9) with 3.0g SDS (0.1%). Eight microliters ( $\mu$ l) of protein supernatant and 2  $\mu$ l of bromophenol blue which served as tracking dye were applied into the stacking gel sample wells with the help of a micropipette.

Electrophoresis was conducted at 100 V for approximately 2.5 hour until bromophenol blue marker went to bottom of gel. Molecular weights of the dissociated polypeptides were recorded by co-electrophoresis of molecular weight protein standards of "MW-SDS-70 kit" from Sigma Chemicals, USA and for SDS-PAGE comprising phosphorylase-b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and a-lactalbumin (14.4 kDa) from Pharmacia Fine Chemicals, USA.

After electrophoresis, stained gels with 0.2% (w/v) coomassie brilliant blue (CBB) R250 dissolved in a solution containing 10% (v/v) acetic acid, 40% (v/v) methanol and water in the ratio of 10:40:60 (v/v) for an hour. Gels were then destained by washing with a solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and water in the ratio of 5:20:75 (v/v) until background color was absent and bands were visible. Gel Drying Processor was used for about two hours to dry the gels after destaining.

The presence of bands was considered as an indicative. The scores were 0 for the absence and 1 for the presence of a band. Cluster analysis based on presence or absence of polypeptide bands was performed to distribute the accessions into different clusters. Only polymorphic bands were included

for cluster analysis. The analysis was performed using software “Statistica” version 6.0.

## **Results and Discussion**

Polyacrylamide gel was used for resolving protein fragments, and to investigate the optimum concentration, a series of experiments were performed using various concentrations. Among all the gel concentration (7.0% to 20.0%), 15% gel concentration gave the best resolution, hence all the analyses were conducted using 15% acrylamide gel concentration with 8  $\mu$ l of sample. The SDS-PAGE was performed on bulk seed samples. Table 1 indicated that 16 protein bands were observed with molecular weight from 20 kDa to more than 66 kDa (Fig. 1). Variation was observed in the density or sharpness of bands, but this variation was not considered due because the protein quantity was not measured either from the gel or prior to sampling. El-Beltagi [1, 14] also observed variations in different rapeseed cultivars using SDS-PAGE. Out of 16 protein bands, 12 were polymorphic, whereas other 25% of the bands were monomorphic, hence unable to resolve variation. Only polymorphic bands were used for construction of dendrogram through cluster analysis using UPGMA with Euclidian distances. On the basis of banding pattern, the gels were divided into 4 regions, i.e., Region A, B, C & D, and all the regions exhibited variation for one or the other fragment. Region A consisted of 1 polymorphic band of molecular weight higher than 66.0 kDa, whereas region B had 9 protein bands of molecular weight ranging from 29.0 kDa to 45.0 kDa, and among these 7 were polymorphic. The region C had 3 protein bands of molecular weight ranging from 24.0 kDa to 29.0 kDa and out of these two were polymorphic and one was monomorphic. Region D comprised of 3 protein bands of molecular weight ranging from 20.0 kDa to 24.0 kDa, out of which 2 were polymorphic and 1 monomorphic.

The data in the form of presence (1) or absence (0) were analyzed for cluster analysis using UPGMA base on Euclidian distances keeping in consideration polymorphic bands only. Based on Euclidian distances, the accessions were grouped into four clusters (Fig. 2). Cluster I comprised of 17 accessions, cluster II had 28 accessions, cluster III consisted of 25 accessions and there were 44 accessions in cluster IV (Table 2).

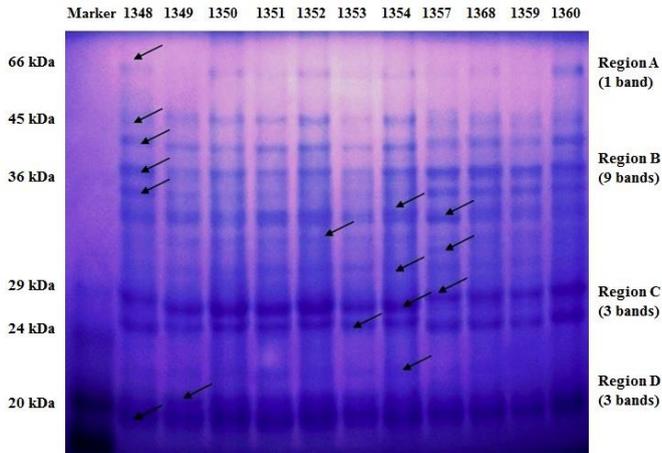
Protein banding pattern in rapeseed did not exhibit much variation and most of the proteins were conserved for the material under investigation. Some of the accessions were observed with few polymorphic bands therefore more germplasm is required to have maximum genetic diversity, especially for seed protein profiling. The gels were divided into four regions for convenience for data recording and many researchers have employed this technique in various crops such as mustard [15] those exhibited variation. Cluster analysis also exhibited some variation among the accessions as far as their banding pattern is concerned. Similarly as in our case, four regions of banding profiles were observed in 156 mustard accessions [15].

## **Conclusion**

The results of present study suggested that SDS-PAGE provided a useful tool in germplasm evaluation and discrimination based on genetic variation in seed storage proteins of *Brassica campestris* L. accessions.

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**Fig.1** Electrophoretic banding pattern generated by SDS-PAGE of seed storage proteins of *Brassica campestris* L. accessions. The arrows indicate 16 bands

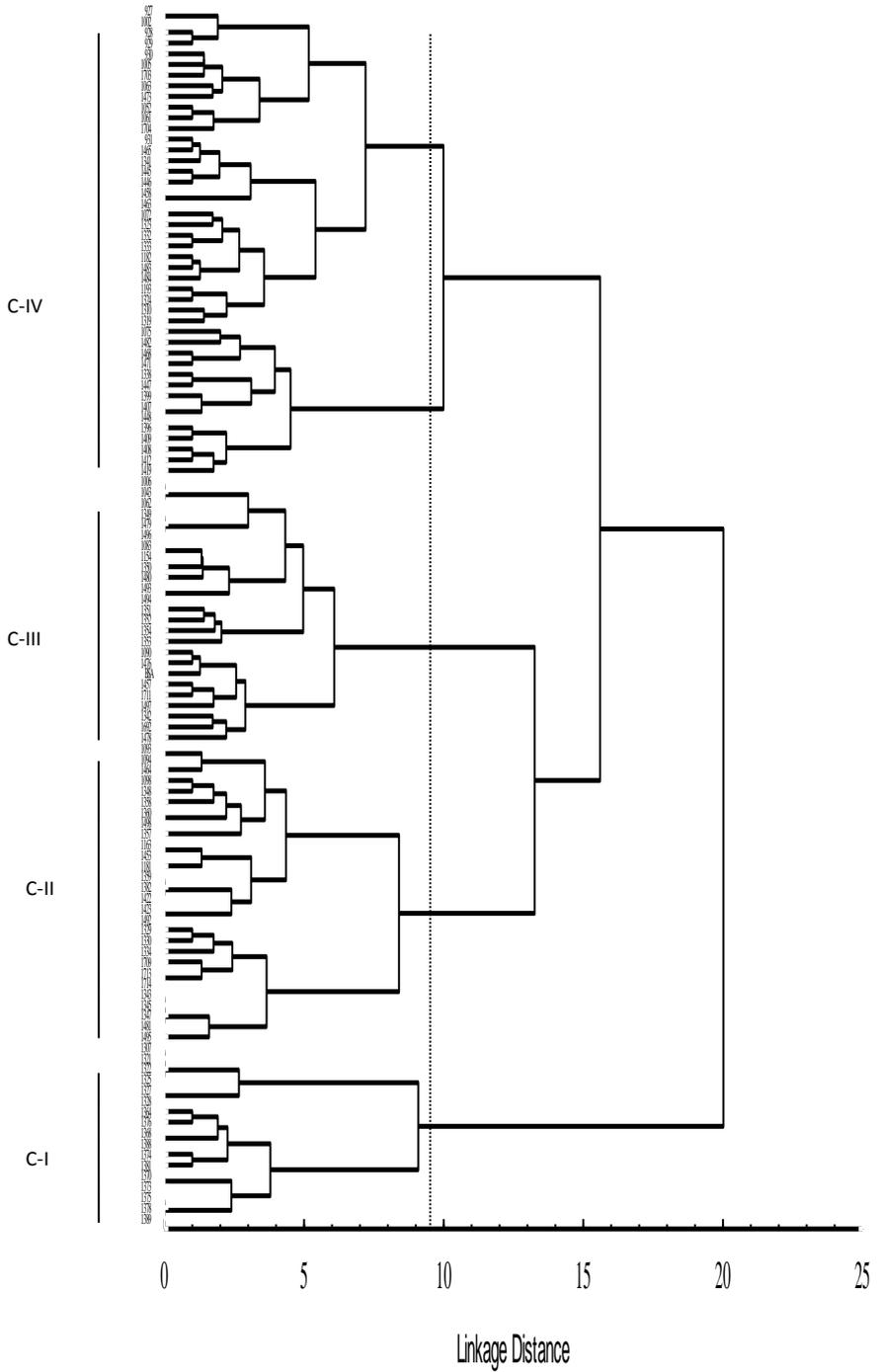
Protein region	Protein bands	Number of accessions	
		Presence	Absence
A	1	29	85
B	2	81	33
	3	114	0
	4	114	0
	5	87	27
	6	93	21
	7	63	51
	8	17	97
	9	12	102
C	10	18	96
	11	68	46
	12	84	30
D	13	114	0
	14	22	92
	15	87	27
	16	114	0

**Table-1.** Presence and absence of protein bands in SDS-PAGE analysis of *Brassica campestris* L. accessions

Cluster	Accessions	Number of accessions	Percentage
I	1307, 1321, 1322, 1325, 1327, 1328, 1364, 1368, 1370, 1373, 1374, 1375, 1376, 1378, 1381, 1388, 1389	17	14.91
II	1093, 1094, 1098, 1163, 1188, 1329, 1330, 1334, 1343, 1345, 1347, 1348, 1357, 1358, 1359, 1360, 1382, 1422, 1423, 1453, 1464, 1481, 1492, 1495, 1498, 1709, 1713, 1714	28	24.56
III	1006, 1043, 1062, 1083, 1090, 1154, 1342, 1349, 1350, 1351, 1352, 1353, 1354, 1457, 1476, 1478, 1479, 1480, 1493, 1494, 1496, 1497, 1692, 1711, BSA	25	21.93
IV	927, 928, 929, 930, 931, 1002, 1005, 1052, 1061, 1063, 1072, 1075, 1182, 1193, 1310, 1319, 1323, 1324, 1332, 1333, 1338, 1341, 1396, 1399, 1407, 1408, 1409, 1412, 1419, 1445, 1446, 1447, 1448, 1458, 1463, 1465, 1468, 1471, 1473, 1482, 1483, 1484, 1703, 1704	44	38.60

**Table-2. Accessions in each cluster by SDS-PAGE analysis of *Brassica campestris* L. accessions**

**Fig. 2. Dendrogram showing the relationships among *Brassica campestris* L. accessions based on SDS-PAGE of total seed Proteins**



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