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DETERMINING GENETIC DIVERGENCE AMONG *Brassica rapa* ECOTYPES THROUGH ELECTROPHORETIC MOBILITY OF TOTAL SEED PROTEINS

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ABSTRACT

Estimation of total seed proteins based variation among different *Brassica rapa* ecotypes is important for crop improvement and efficient utilization. In the present study, variations in total seed proteins among three ecotypes of *B. rapa* (brown sarson, yellow sarson and toria) were studied. Twenty different genotypes from all the ecotypes were analysed through SDS-PAGE and their phylogenetic relation was recorded. The polymorphism in protein size was investigated and they were divided into four main groups on the basis of molecular weight ranging from ~10 kDa to ~180 kDa. Group A comprised of large size proteins (~136 kDa to ~180 kDa), group D consisted of small size proteins (~10 kDa to ~19 kDa) whereas group B and C consisted of medium sized proteins (~26 kDa to ~115 kDa). The data of total soluble seed protein based variations were analysed through Unweighted Pair Group Method with Arithmetic Mean (UPGMA), which clustered all three ecotypes into four main groups. The cluster I and III contained one toria and brown sarson genotypes, respectively. These two groups showed maximum polymorphism as compared to other cluster groups. The clusters III and IV had all three ecotypes. The similarity coefficient values ranging from 47 to 100% were also recorded for all three types. The maximum similarity coefficient value was recorded as 100% for brown sarson and toria genotypes while the least similarity indices was recorded for brown sarson and yellow sarson ecotypes i.e. 47%. For the first time, we have reported considerable protein based variations in all three ecotypes of *B. rapa*. Our findings will be helpful as a preliminary study on the characterization of *B. rapa* ecotypes.

Key words: *B. rapa*, crop improvement, protein based diversity, phylogenetic relation.

INTRODUCTION

Genetic assessment of important Brassica species plays a key role in identification of genotypes. Various morpho-physiological and biochemical methods are used to screened improve cultivars among plant species/sub-species (Semagn *et al.*, 2006, Shatya *et al.*, 2015). Biochemical based method such as sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) is one of the efficient, quick, simple and accurate methods to study genetic divergence among plant species. SDS-PAGE analysis of seeds gives accurate polypeptide profile information which is not influenced by any environmental effect. This method has been used to study evolutionary and taxonomic relationships among crop species or sub-species (Das and Mukherjee, 1995).

SDS-PAGE method has been successfully employed by different researchers in many species of Brassica. Shinwari *et al.* (2013) studied genetic divergence among *Eruca sativa* L. genotypes through SDS-PAGE method. Maximum polymorphic protein bands having molecular weight ranges from 15-220 KDa were obtained. About 60-100% genetic similarity was found among all tested genotypes. Zada *et al.* (2013) characterized 94 different *Brassica carinata* L. (Ethiopian mustard) cultivars by using SDS-PAGE method. Thirty one different highly reproducible protein sub-units were noted. Among these proteins sub-units, 14 showed polymorphic patterns while rest of them are all monomorphic. While the genetic similarity remains from 50 to 100% in all tested genotypes. They concluded that genetic diversity varies among local and exotic genotypes. Khurshid and Rabbani (2012) characterized highly diverse Brassica genotypes *via* SDS-PAGE method. All the genotypes showed significant variation at protein level. Many small and large size proteins were evaluated among various Brassica species. All the genotypes were divided into three main regions. The first region contain highly polymorphic proteins, the second one showed minor polymorphism and the third region showed no or very low level of polymorphic pattern. The overall 91% genetic similarity was calculated among genotypes. Akbar *et al.* (2012) studied protein based variation among sesame (*Sesame indicum* L.) genotypes. High level of polymorphic bands (70%) was noted having protein band size ranging from 13.5 to 100 kDa. High similarity coefficient value 50 to 100 % was recorded.

Total seed storage proteins vary among plants species and sub-species. *B. rapa* is an important oil plant having many ecotypes. The three major ecotypes such as brown sarson, yellow sarson and Toria shows protein

based diversity. However there are insufficient reports available about total seed proteins based diversity among these ecotypes. Therefore the present study was designed to study total seed proteins based variation among three important ecotypes of *B. rapa* using SDS-PAGE analysis.

MATERIALS AND METHODS

Experimental Materials: All the experimental work was performed at Plant Genetic Resources Institute (PGRI), National Agriculture Research Centre (NARC), Islamabad, Pakistan. The fully mature seeds of three *B. rapa* ecotypes (brown sarson, yellow sarson and Toria) were acquired from the gene bank of PGRI, NARC, Islamabad, Pakistan. The genotypes list of all three ecotypes is given in Table 1.

Procedure of Protein Extraction: The mature 10-15 seeds of genotypes from all three *B. rapa* ecotypes were ground continuously for 5-10 minutes with the help of mortar and pestal. The ground materials were carefully transferred to 1.5 ml eppendorf tube along with 400 µl protein extraction buffer (0.5M Tris-HCl (pH 8.0), 0.2% SDS, 5 M urea, and 1% β -mercaptoethanol) and kept overnight at 20 ^{o}C. A bromophenol blue dye was also added along with protein extraction buffer with aim to check protein mobility. The samples were then mixed with vertexes at for 2-3 minutes followed by centrifugation at 12,000 rpm for 10 minutes. The clear upper layer containing pure proteins were separated from rest of extract and were stored in refrigerator at -20 ^{o}C.

Electrophoresis: The pure recovered proteins and a protein marker were then subjected to SDS-PAGE analysis to study protein based variation among genotypes according to Khurshid and Rabbani (2012). After running samples on gel it was stained with 0.5% Coomassie Brilliant Blue (CBB) G-250 dye dissolved in acetic acid, methanol and water at 3:22:25 ratio for 2-3 hours. After giving proper staining time, gel was then transferred into de-staining solution having same composition of staining solution but lack any dye. The banding pattern was recorded as 0 (absence of band/bands) and 1 (presence of band/bands) and molecular weight was compare with Fermentas page-ruler protein marker (SM#0671, Fermentas, USA).

Data Analysis: Data for similarity coefficients were generated using presence or absence of protein bands based on pair-wise comparison of genotypes. The genetic similarity estimates (F) were numerically calculated by using following equation of Nei and Li (1979)

Similarity (F) = 2Nab/ (Na+Nb)

Where Na = the number of scored fragments of individual ‘a’

Nb = the number of scored fragments detected in individual ‘b’

While;

Nab = the number of shared fragments between ‘a’ and ‘b’

The dendrogram were then constructed by using UPGMA (Unweighted Pair-Group Method with Arithmetic averages) (Sneath and Sokal, 1973). All he experimental work was analysed by using computational software NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

RESULTS AND DISCUSSION

***Brassica rapa* Ecotypes (Brown Sarson, Yellow Sarson and Toria) Shows Total Seed Proteins Based Variations Through SDS-PAGE Method:** In present study total seed proteins based variation among three important *Brassica rapa* ecotypes (brown sarson, yellow sarson and toria) were studied and maximum polymorphisms were recorded in all three types. The electrophorogram profile of protein bands is given in Fig. 1 (a,b) that showed maximum genetic diversity in all three tested ecotypes. Different genotypes gave different band patterns and its sizes varied from~ 10 kDa to ~180 kDa. A total of 15 bands were noted, in which 14 bands (93%) were highly polymorphic while 1 band (7%) was reported as monomorphic. The gel pattern showed four different regions (A-D) based on molecular weight and highly polymorphic polypeptides bands were recorded (Fig 1. a, b). The region A contains high molecular weight proteins ranging from ~136 kDa to ~180 kDa. In this region both monomorphic and polymorphic bands were noted. The region B contains 5 proteins sub units and its size varied from ~82 kDa to ~115 kDa. All polymorphic bands were recorded in this region. The region C contained medium size protein and their sizes vary from ~26 kDa to ~69 kDa and it consist 5 protein sub-units. In this region both types of bands pattern were recorded. In last part of gel which is mentioned is D regions contain small size proteins having molecular weight ranges from ~10 kDa to ~19 kDa of molecular weights. Total seed Proteins based variation is important to study phylogenetic relation between different crop species/sub-species. The critical analysis of total seed proteins gives maximum variation among different genotypes (Isemura *et al.,* 2001; Gepts and Bliss, 1988). Among various techniques used for estimation of crops genetic diversity, one is SDS-PAGE based method that was successfully used to study genetic relation among diverse range of genotypes. This method is useful than other methods as it is less expensive, need no DNA sample and show maximum tolerance to environmental conditions (Iqbal *et al.,* 2005; Javid *et al.,* 2004; Rahman and Hirata, 2004; Shtaya *et al.,* 2015; Jan *et al.,* 2016). The total seed proteins remain stable at different environmental condition that gives clear image of total protein of any plant species (Tanksly and Jones, 1980).

Zada *et al.* (2013) also conducted a study to evaluate *Brassica carinata* genotypes and recorded 31 different protein sub units. The findings of Shinwari *et al.* (2013) shows deviation from our results, that get total of 17 different polymorphic and a monomorphic bands in cultivar *Eruca sativa* by this method. Our results are in line with Turi *et al.* (2010) that recorded maximum genetic diversity among 234 genotypes of Brassica species and noted four different types of proteins. Maximum polymorphic and monomorphic bands were recorded in all tested genotypes. In another study by Rabbani *et al.* (2001) characterized local Pakistani Brassica genotypes *via* similar method. The small, medium and large proteins were separated and were ranked on the basis of their molecular weight. Different local and exotic plant species and even sub-species give different protein bands through SDS-PAGE method (Naz *et al.,* 2015; Kakaei and Kahrizi, 2011; Akbar *et al.,* 2012; Rabbani *et al.,* 2001).

Genetic Similarity Matrix and Cluster Analysis: The different protein bands pattern was recorded on the basis of similarity coefficient by using method of Nei and Li (1979). The similarity indices were considered among all four groups. The similarity coefficient values among all three ecotypes of *B. rapa* ranges from 0.47 (47%) to 1.00 (100%) (Table 3). The maximum similarity coefficient 100% was recorded among Br-538/Br-502, Br-519/Br-501 and Br-531/Br-557 genotypes, shows that there is similarity between brown sarson and yellow sarson ecotypes and among brown sarson types itself. The lowest similarity indices 47% was recorded among genotypes Br-517/Br-533 and Br-534/Br-517. These results indicates that, there is strong dissimilarity exist between brown sarson, yellow sarson and toria types of *B. rapa*. All three ecotypes showed maximum variation through this method that summarize that protein based variation varies among different sub-species of *B. rapa*.

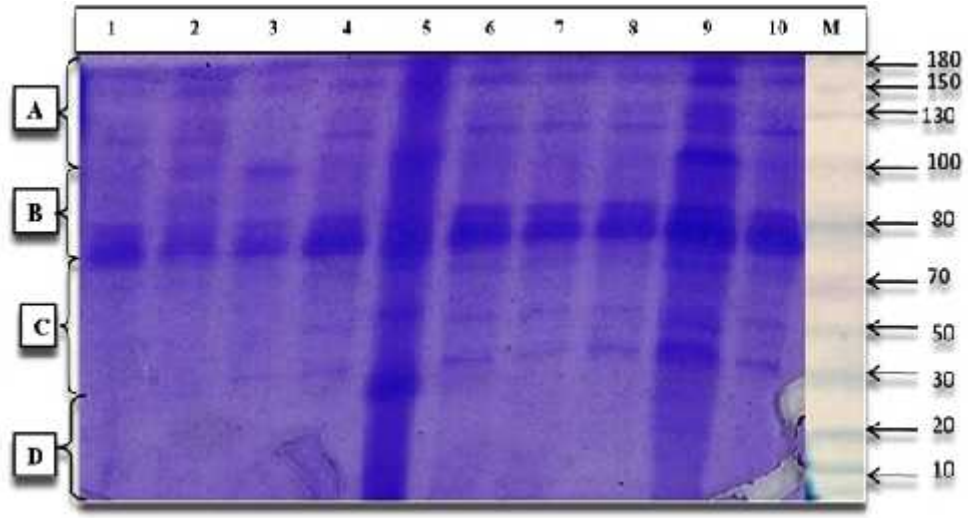

Fig. 1(a): Electrophoretic banding pattern of *B. rapa* genotypes generated through SDS-PAGE of total seed proteins. M, represents molecular size marker (~10 kDa to ~180 kDa), while numbers from 1-10 represent accessions Br-502, Br-529, Br-534, Br-501, Br-505, Br-519, Br-532, Br-531, Br-623 and Br-557, respectively.

In order to study phylogenetic based variation among three ecotypes of *B. rapa*, dendrogram was constructed through dissimilarity matrix by using UPGMA (Unweighted Pair Groups Method with Arithmetic averages). All 20 different genotypes from three different ecotypes were clustered into four major groups (Figure 2, Table 2). Cluster-I is highly polymorphic and consist only one toria genotype i.e. Br-517. In cluster-II mainly contain five brown sarson genotypes i.e. Br-505, Br-501, Br-519, Br-532 and Br-623. Similarly, cluster-III representing a diverse group that consist only one brown sarson type i.e. Br-503. The cluster-IV is largest among all four groups and consists of 13 different genotypes from all three ecotypes of *B. rapa* i.e. Br-502, Br-516, Br-529, Br-530, Br-531, Br-533, Br-534, Br-538, Br-556, Br-557, Br-558, Br-587 and Br-624. Two out of four groups showed maximum variation having only one genotype. The genotypes Br-503 (brown sarson) and Br-517 (toria) showed maximum protein based diversity in all tested genotypes (Table 2). Turi *et al.* (2010) noted 98% similarity among different Brassica cultivars. Our results are slightly different from Shinwari *et al.* (2013) who recorded 60% to 100% genetic similarity values for *Eruca sativa* species. This deviation from our study is due to different plants species used. Similarly during characterization of *Brassica carinata* by Zada *et al.* (2013) achieved 50 to 100 % similarity coefficient values. The phylogenetic tree showed four different cluster groups that showed different similarity indices values. Different clustering groups from our study

were recorded by Nasr *et al*. (2006) for *Brassica napus* L. and by Mukhlesur *et al*. (2004) for other Brassica genotypes.

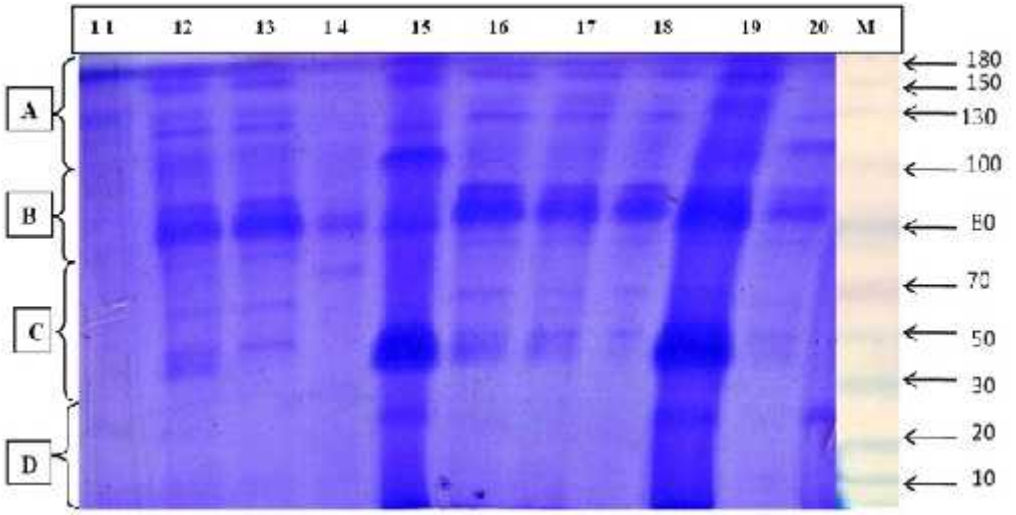

Fig. 1(b): Electrophoretic banding pattern of *B. rapa* genotypes generated through SDS-PAGE of total seed proteins. M, represents molecular size marker, while numbers from 11-20 represent accessions Br-517, Br-516, Br-533, Br-587, Br-503, Br-538, Br-530, Br-556, Br-558 and Br-624, respectively.

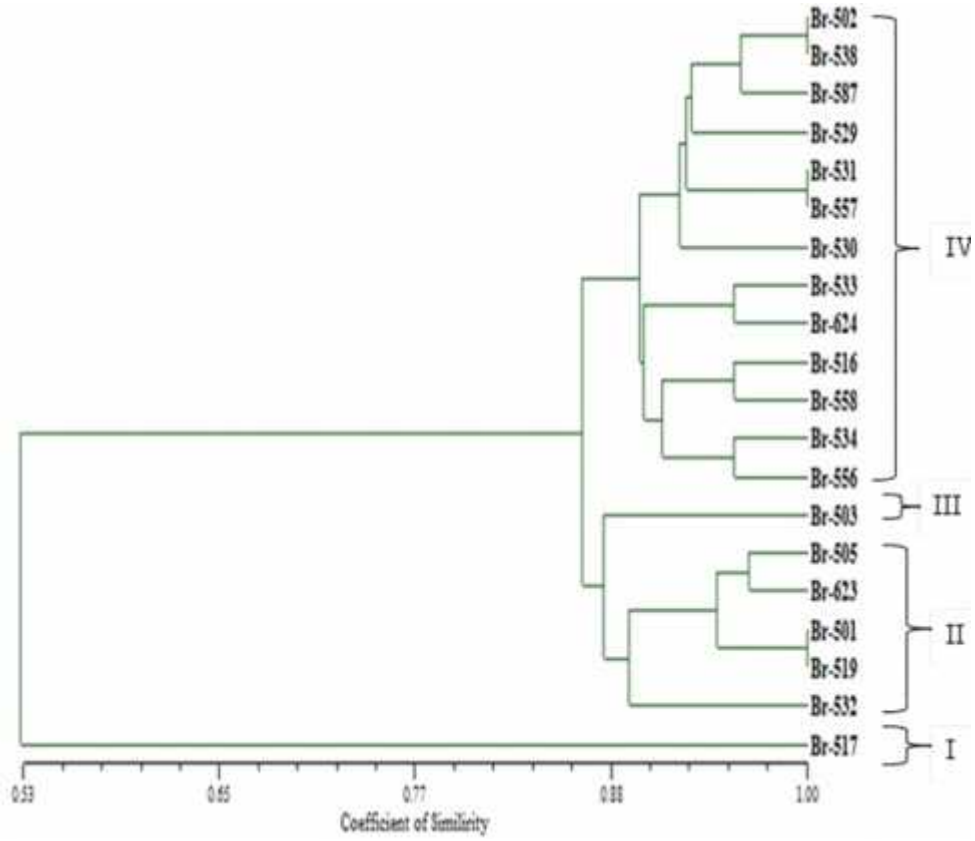

Fig. 2. Dendrogram indicating the phylogenetic relationships among three *B. rapa* ecotypes by using software program NTSys PC 2.1 to calculate DICE similarity coefficients.

Table 1. List of Accessions and ecotypes of *B. rapa*.

Sr. No.	Accession	Ecotype	Source
1	Br-502	Brown	NARC, Pakistan
2	Br-503	Brown	NARC, Pakistan
3	Br-505	Brown	NARC, Pakistan
4	Br-501	Brown	NARC, Pakistan
5	Br-519	Brown	NARC, Pakistan
6	Br-516	Toria	NARC, Pakistan
7	Br-517	Toria	NARC, Pakistan
8	Br-529	Yellow	NARC, Pakistan
9	Br-530	Brown	NARC, Pakistan
10	Br-531	Yellow	NARC, Pakistan
11	Br-532	Brown	NARC, Pakistan
12	Br-533	Yellow	NARC, Pakistan
13	Br-534	Brown	NARC, Pakistan
14	Br-538	Yellow	NARC, Pakistan
15	Br-556	Brown	NARC, Pakistan
16	Br-557	Brown	NARC, Pakistan
17	Br-558	Brown	NARC, Pakistan
18	Br-587	Yellow	NARC, Pakistan
19	Br-623	Brown	NARC, Pakistan
20	Br-624	Brown	NARC, Pakistan

Table 2. Grouping of 20 genotypes of *Brassica rapa* through cluster analysis based on SDS-PAGE method.

Clusters	No. of genotypes	Genotypes
I	1	Br-517
II	5	Br-505, Br-501, Br-519, Br-516, Br-623
III	1	Br-503
IV	13	Br-502, Br-516, Br-529, Br-530, Br-531, Br-533, Br-534, Br-538, Br-556, Br-557, Br-558, Br-587, Br-624

Table 3. Dice coefficient of similarity among three ecotypes of *B. rapa*.

Accession	Br-502	Br-503	Br-529	Br-505	Br-531	Br-501	Br-533	Br-587	Br-519	Br-538	Br-530	Br-516	Br-517	Br-532	Br-534	Br-556	Br-557	Br-558	Br-623	Br-624
Br-502	1.00																			
Br-503	0.80	1.00																		
Br-529	0.92	0.80	1.00																	
Br-505	0.92	0.89	0.92	1.00																
Br-531	0.92	0.88	0.92	0.92	1.00															
Br-501	0.88	0.85	0.88	0.96	0.88	1.00														
Br-533	0.92	0.80	0.92	0.92	0.92	0.88	1.00													
Br-587	0.96	0.85	0.96	0.96	0.96	0.92	0.96	1.00												
Br-519	0.88	0.85	0.88	0.96	0.88	1.00	0.88	0.92	1.00											
Br-538	1.00	0.80	0.92	0.92	0.92	0.88	0.92	0.96	0.88	1.00										
Br-530	0.92	0.80	0.92	0.92	0.92	0.88	0.92	0.96	0.88	0.92	1.00									
Br-516	0.87	0.75	0.87	0.88	0.87	0.92	0.87	0.92	0.92	0.87	0.87	1.00								
Br-517	0.59	0.56	0.59	0.53	0.59	0.56	0.47	0.56	0.56	0.59	0.59	0.50	1.00							
Br-532	0.83	0.88	0.83	0.92	0.83	0.88	0.83	0.88	0.88	0.83	0.92	0.78	0.59	1.00						
Br-534	0.92	0.80	0.92	0.92	0.92	0.88	0.92	0.96	0.88	0.92	0.92	0.87	0.47	0.83	1.00					
Br-556	0.87	0.75	0.87	0.88	0.87	0.83	0.87	0.92	0.83	0.87	0.87	0.91	0.38	0.78	0.96	1.00				
Br-557	0.92	0.88	0.92	0.92	1.00	0.88	0.92	0.96	0.88	0.92	0.92	0.87	0.59	0.83	0.92	0.87	1.00			
Br-558	0.92	0.80	0.92	0.92	0.92	0.88	0.92	0.96	0.88	0.92	0.92	0.96	0.47	0.83	0.92	0.96	0.92	1.00		
Br-623	0.89	0.93	0.89	0.97	0.89	0.93	0.89	0.93	0.93	0.89	0.89	0.85	0.50	0.89	0.89	0.85	0.89	0.89	1.00	
Br-624	0.87	0.75	0.87	0.88	0.87	0.83	0.96	0.92	0.83	0.87	0.87	0.91	0.38	0.78	0.87	0.91	0.87	0.96	0.85	1.00

Conclusion: We observed considerable biochemical variation viz-a'-viz total seed proteins among three ecotypes i.e. brown sarson, yellow sarson and toria. Highly polymorphic protein bands (small, medium and large) were recorded in all three ecotypes. The genetic variation of protein profiling is mainly attributed to unique agro-morphological nature of ecotypes as well as their geographical origin. However, we suggest more advanced tools i.e. 2-D gel electrophoresis and molecular marker etc. should be applied to investigate phylogenetic basis of divergence in the *B. rapa* at sub-species level.

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