



Assessment of divergence in electrophoretic mobility pattern of total seed protein in (*Brassica juncea* L.) germplasm using SDS-PAGE analysis

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Abstract

Genetic diversity of one hundred local Indian mustard (*Brassica juncea* L.) accessions was characterized for total seed storage protein via sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). The accessions used in the study were obtained from Gene bank of Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC), (Latitude 33.42° N; Longitude 73.08° E) Islamabad Pakistan. Total seed proteins were resolved on 12.25 % polyacrylamide gels generating a total of 21 bands on the basis of molecular weight within the range of 6 to ≈180 kDa. Among them seventeen bands (80.95%) were polymorphic and the remaining 4 (19.04%) were monomorphic showing high degree of variability viz- à -viz peptide mobility for studied germplasm. Similarity index among these accessions ranged from 0.62 to 1.0. Protein dissimilarity based dendrogram was generated through un-weighted pair group method with arithmetic average (UPGMA) which distributed all hundred accessions into 5 main clusters. Grouping pattern revealed moderate level of genetic divergence in seed protein profiling however it is suggested that 2-D gel-electrophoresis with other molecular techniques should be applied in future to explore genetic variation because only SDS-PAGE of seed protein is insufficient to completely estimate the genetic diversity present among these accessions.

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Introduction

Brassica is one of the earliest domesticated crop species according to written records, its domestication was practice back in 1500 BC (Prakash, 1980). It is comprised of 3,500 species and 350 genera, ordered into 10 tribes (Warwick *et al.*, 2000). Among oilseed Brassica species, *B. napus* L., *B. juncea* L. and *B. campestris* L. rank third highly essential source of edible oil in the world (Zhang & Zhou., 2006). Indian mustard (*B. juncea* L.) is pre-dominant species in the Indian sub-continent. In Pakistan it has been widely cultivated for thousands of years as an oil seed crop (Rabbani *et al.*, 1998). Among the Brassica family, *B. juncea* L. is magnificent crop because of its role in edible oil production (Farzinebrahimi *et al.*, 2012). In Pakistan after cotton and rape seed, Indian mustard is the third highly important source of oil (Abbas *et al.*, 2009).

Estimation of genetic diversity is the first step in every crop improvement program. Identification of superior genotypes requires diversity in the population (Murtaza, 2005). Genetic diversity of the *juncea* L. could assist plant breeders and geneticists to recognized the structure of germplasm to predict that, which combination would produce best progeny (Hu *et al.*, 2007), and also provided wide genetic basis for selection of breeding material (Qi, Yang & Zhang, 2008). There are different techniques available for estimation of crop genetic diversity, such as morphological, biochemical and molecular (DNA) markers. The electrophoresis of total seed storage proteins is a technique to investigate plant genetic diversity and classify plant germplasm (Isemura *et al.*, 2001). Electrophoretic seed protein analysis exposes high diversity in different genotypes in the oilseed Brassica mustard in Pakistan (Sadia *et al.*, 2009). Although SDS-PAGE technique has being utilized by several other plant breeders and scientists, they found it simple, more effective and comparatively inexpensive than agro-morphological techniques (Iqbal *et al.*, 2014; Khurshid *et al.*, 2013; Shinwari *et al.*, 2013; Zada *et al.*, 2013; Akbar *et al.*, 2012; Turi *et al.*, 2010;). Therefore, the present investigation was

performed to estimate and characterize the genetic variation and relationships on the basis of total seed storage proteins through SDS-PAGE analysis among *juncea* L. accessions.

Materials and methods

Experimental Materials

The present research study was carried out under laboratory condition at Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC), Islamabad (73° 06'E and 33° 33'N) during 2013. The experimental material comprised one hundred *B. juncea* L. was analyzed for biochemical characterization of total seed protein. The studied accessions were collected from gene bank of Plant Genetic Resources Institute (PGRI), NARC, Islamabad (Table 1).

All the studied genotypes were analyzed for total seed storage protein through SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis). Seed protein were extracted from 10 mg (0.1g) fine powder of each genotype into Eppendorf tube (1.5ml) with 400 µl of the protein extraction buffer (0.2% Sodium dodecyl sulphate (SDS), 1% 2-mercaptoethanol, 0.5M Tris-HCl (pH 8.0) and 5M urea) and mixed Bromophenol blue (BPB) dye as an indicator to detect the protein movement in the separation gel. The tubes were ccentrifuged at of 15000 rpm (revolution per minute) for 5 minutes at room temperature. The extracted proteins were resolved on 12.25% polyacrylamide slab type mini gel apparatus (AE-6530) at 90-100 voltage for three hours. Gels were stained with solution composed of 0.2 per cent (w/v) CBB (Coomassie Brilliant Blue) R250 (Dissolved in a solution containing 10 per cent (v/v) acetic acid, water with the ratio 5:20:75 (v/v) and 40 per cent (v/v) methanol) for one hour and the gels were de-stained with a solution (20% (v/v) methanol, water in the ratio of 5:20:75 (v/v) and 5% (v/v) acetic acid) for 1-2 hours. After de-staining, Electrophoretic bands on gel were visible and blue color of CBB (Coomassie Brilliant Blue) in the background disappeared. After clear visibility of the bands, the gels were dried and data were recorded for visible bands. The data were

recorded in binary data matrix (M.S Excel sheet 2007) for all the one hundred accessions. On the basis of the Electrophoretic band, Dice similarity coefficient matrix was calculated through statistical packages NTSys-pc, version 2.1 (Applied Biostatistics Inc, USA). Polymorphism in the banding pattern was calculated as suggested by Sneath and Sokal, (1973) and the dendrogram was developed through UPGMA (unweight pair-group method).

Results and discussion

During the present research work genetic variability was studied on the basis of total seed storage proteins for 100 *Brassica juncea* L. genotypes. The electrophoregrams revealed significant genetic variation at molecular level among *B. juncea* accessions. Although during the present study total twenty one polypeptide bands were found.

Table 1. Passport data of *Brassica juncea* L. accessions.

Accession	Genus	Species	Origin	Accession	Genus	Species	Origin
001061	<i>Brassica</i>	<i>Juncea</i>	Unknown	019500	<i>Brassica</i>	<i>juncea</i>	Unknown
001627	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019501	<i>Brassica</i>	<i>juncea</i>	Unknown
001628	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019503	<i>Brassica</i>	<i>juncea</i>	Unknown
001629	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019504	<i>Brassica</i>	<i>juncea</i>	Unknown
001631	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019507	<i>Brassica</i>	<i>juncea</i>	Unknown
001632	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019510	<i>Brassica</i>	<i>juncea</i>	Unknown
001633	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019513	<i>Brassica</i>	<i>juncea</i>	Unknown
001634	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019515	<i>Brassica</i>	<i>juncea</i>	Unknown
001635	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019516	<i>Brassica</i>	<i>juncea</i>	Unknown
001636	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019521	<i>Brassica</i>	<i>juncea</i>	Unknown
001637	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019528	<i>Brassica</i>	<i>juncea</i>	Unknown
001638	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019530	<i>Brassica</i>	<i>juncea</i>	Unknown
001639	<i>Brassica</i>	<i>Juncea</i>	Pakistan	022852	<i>Brassica</i>	<i>juncea</i>	Pakistan
001641	<i>Brassica</i>	<i>Juncea</i>	Pakistan	022854	<i>Brassica</i>	<i>juncea</i>	Pakistan
001642	<i>Brassica</i>	<i>Juncea</i>	Pakistan	022860	<i>Brassica</i>	<i>juncea</i>	Pakistan
001643	<i>Brassica</i>	<i>Juncea</i>	Pakistan	022862	<i>Brassica</i>	<i>juncea</i>	Pakistan
001644	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024910	<i>Brassica</i>	<i>juncea</i>	Pakistan
001646	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024911	<i>Brassica</i>	<i>juncea</i>	Pakistan
001647	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024914	<i>Brassica</i>	<i>juncea</i>	Pakistan
001649	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024919	<i>Brassica</i>	<i>juncea</i>	Pakistan
001650	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024920	<i>Brassica</i>	<i>juncea</i>	Pakistan
001651	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024922	<i>Brassica</i>	<i>juncea</i>	Pakistan
001652	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024924	<i>Brassica</i>	<i>juncea</i>	Pakistan
001653	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024925	<i>Brassica</i>	<i>juncea</i>	Pakistan
001654	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024927	<i>Brassica</i>	<i>juncea</i>	Pakistan
001655	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024929	<i>Brassica</i>	<i>juncea</i>	Pakistan
001656	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024931	<i>Brassica</i>	<i>juncea</i>	Pakistan
001657	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024932	<i>Brassica</i>	<i>juncea</i>	Pakistan
001658	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024933	<i>Brassica</i>	<i>juncea</i>	Pakistan
001659	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024934	<i>Brassica</i>	<i>juncea</i>	Pakistan
001660	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024938	<i>Brassica</i>	<i>juncea</i>	Pakistan
001661	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024939	<i>Brassica</i>	<i>juncea</i>	Pakistan
001664	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024947	<i>Brassica</i>	<i>juncea</i>	Pakistan
001665	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024950	<i>Brassica</i>	<i>juncea</i>	Pakistan
001666	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024953	<i>Brassica</i>	<i>juncea</i>	Pakistan
024954	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024986	<i>Brassica</i>	<i>juncea</i>	Pakistan
024955	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024990	<i>Brassica</i>	<i>juncea</i>	Pakistan
024958	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024991	<i>Brassica</i>	<i>juncea</i>	Pakistan
024959	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024992	<i>Brassica</i>	<i>juncea</i>	Pakistan
024961	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024994	<i>Brassica</i>	<i>juncea</i>	Pakistan
019506	<i>Brassica</i>	<i>Juncea</i>	Unknown	26810	<i>Brassica</i>	<i>juncea</i>	Unknown
024963	<i>Brassica</i>	<i>Juncea</i>	Pakistan	26819	<i>Brassica</i>	<i>juncea</i>	Unknown
024964	<i>Brassica</i>	<i>Juncea</i>	Pakistan	26823	<i>Brassica</i>	<i>juncea</i>	Unknown
024972	<i>Brassica</i>	<i>Juncea</i>	Pakistan	26828	<i>Brassica</i>	<i>juncea</i>	Unknown
024973	<i>Brassica</i>	<i>Juncea</i>	Pakistan	001674	<i>Brassica</i>	<i>juncea</i>	Unknown
024974	<i>Brassica</i>	<i>Juncea</i>	Pakistan	024952	<i>Brassica</i>	<i>juncea</i>	Pakistan
024976	<i>Brassica</i>	<i>Juncea</i>	Pakistan	001683	<i>Brassica</i>	<i>juncea</i>	Unknown
024977	<i>Brassica</i>	<i>Juncea</i>	Pakistan	001768	<i>Brassica</i>	<i>juncea</i>	Pakistan
024981	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019493	<i>Brassica</i>	<i>juncea</i>	Unknown
001667	<i>Brassica</i>	<i>Juncea</i>	Pakistan	019495	<i>Brassica</i>	<i>juncea</i>	Unknown

The protein bands size was determined through standard protein ladder (Bench Mark™ Pre-stained Protein Ladder, Lot No 1046147, Cat No 10748-010) having a range of 6 to ≈180 kDa molecular weight. Many other polypeptide protein sub units of lower molecular weight were also observed but they were not recorded because they were not reproducible. Although variability in the protein sub units sharpness and thickness was also found but this was not contributed as polymorphism. In the present study total 21 bands were observed, in which 17 (80.95%) were polymorphic and the remaining 4 (19.04%) were monomorphic (Figure 1).

Protein sub units were divided into 4 regions as A, B, C, D. The polymorphic protein bands were observed in these regions was in different numbers. In the region-A of the gel, total 4 protein sub-units were found. In which only 1 protein band were monomorphic, while all other 3 bands were polymorphic. These bands were found within the range of 180 kDa to 132 kDa. In region-B of the gel, total of 6 bands were observed in which 1 was monomorphic and five were polymorphic. These protein sub-units were calculated within the range of 115-72 kDa molecular weight. Region-C was the largest portion of the gel contained 8 protein sub units. These protein sub-units were found

polymorphic in nature and these polypeptide bands were observed within the range of 64 kDa to 19 kDa molecular weight. In the last region of the gel only 3 protein sub units were found, which were within the range of 17 to 6 kDa molecular weight. Among these protein bands 2 were monomorphic and only 1 was polymorphic (Figure 2).

Proteins banding patterning were designed and similarity matrix was measured among all studied accessions. And it was found that the range of similarity in these individuals of *Brassica juncea* L. was from 0.62 to 1.0, while the minimum 0.62 (62%) similarity among the accession 22860 and 24963 was recorded (Figure 3).

The studied dendrogram was developed through dissimilarity matrix using unweight pair groups method with arithmetic average (UPGMA) and all the individuals were divided into 5 main clusters (Table 2). The cluster-I, II and cluster-III were comprised only one accession 22860 (1%), 24920 (1%) and 19506 (1%) respectively. Totally 3 (3%) accessions 24974, 24990 and 24973 were comprised in the cluster-IV. The cluster-V was the largest group among the studied genotypes, which comprised 94 individuals (Table 2).

Table 2. Cluster pattern of 100 juncea L. accessions based on cluster analysis.

Clusters	No. of Acc.	Germplasm
Cluster-I	1	22860
Cluster-II	1	24920
Cluster-III	1	19506
Cluster-IV	3	24974, 24990, 24973
Cluster-V	94	1061, 1641, 1642, 1646, 1651, 1665 1666, 1636, 1649, 1637, 1634, 1632, 1629, 1638, 1659, 1635, 1633, 1656, 1667, 1657, 1655, 1647, 1631, 1658, 1683, 1653 1664, 1628, 1654, 1644 1639, 1661, 1652, 1650, 1643, 1627, 1660, 1674, 1768, 1748, 19521, 19503, 19530, 1940, 19493, 19507, 19515, 19501, 19516, 19500, 19504, 19510 19513, 19528, 22862, 22854 22852, 24981, 24972, 24939, 24994, 24911, 24931, 24963, 24933, 24924, 24976, 24919 , 24954, 24932, 24938, 24927, 24955, 24950, 24986, 24952, 24925, 24959, 24953, 24964, 24947, 24914, 24910, 24934, 24929, 24958, 24961, 24977, 24991, 24992, 26822, 26828, 26810, 26819

To estimate the genetic diversity and classification of different crop species, seed storage protein (SDS-PAGE) has a great applicability (Isemura *et al.*, 2001). Among different techniques, SDS-PAGE is one of the

greatest techniques for evaluation of genetic diversity through total seed protein, relatively less affected by environmental factors (Iqbal *et al.*, 2005 and Javid *et al.*, 2004).

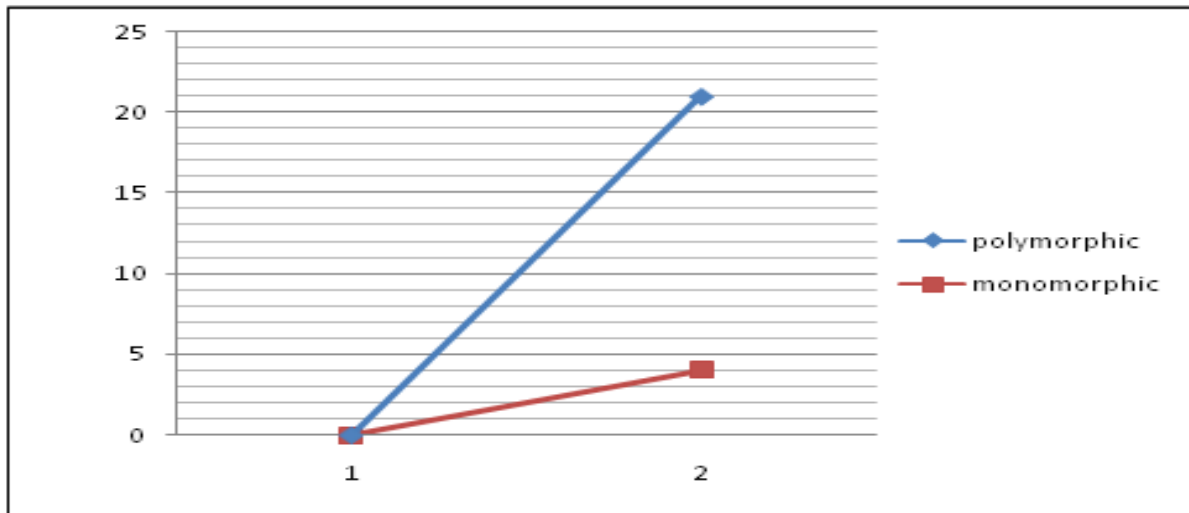


Fig. 1. Showing the Proportion of Polymorphic and Monomorphic Bands.

The protein sub-units observed in total seed protein are highly stable due to which the classification of different genotypes has done. It is comparatively simple and inexpensive technique. It has multiple significant advantages in plant breeding (Rahman and Hirata, 2004). Evaluation of genetic diversity through SDS-PAGE markers is helpful for crop species and their wild relatives (Thanh and Hirata, 2002).

In the present evaluation of *B. juncea* L. through SDS-PAGE technique, total of twenty one polypeptide bands were found, in which seventeen were polymorphic and four were monomorphic. Minimum 12 bands were observed in the accession 22860. The present study is in close agreement with that of Khan

et al., (2014) who found similar results in *B. napus* L. genotypes. Zada *et al.*, (2013) studied *B. carinata* L. genotypes through SDS-PAGE and calculated total of thirty one polypeptide bands. The results of Shinwari *et al.*, (2013) also support our present investigation, who found total of seventeen bands during estimation of *Eruca sativa* using SDS-PAGE markers. Similarly Turi *et al.*, (2010) evaluated different Brassica species and found twenty eight protein bands. Kakaie and Kahrizi, (2011) investigated *B. napus* L. germplasm and also found seventeen polypeptide bands. Our present finding was further strengthening by the finding of Akber *et al.*, (2012), who studied *Sesamum indicum* and found twenty proteins sub-units.

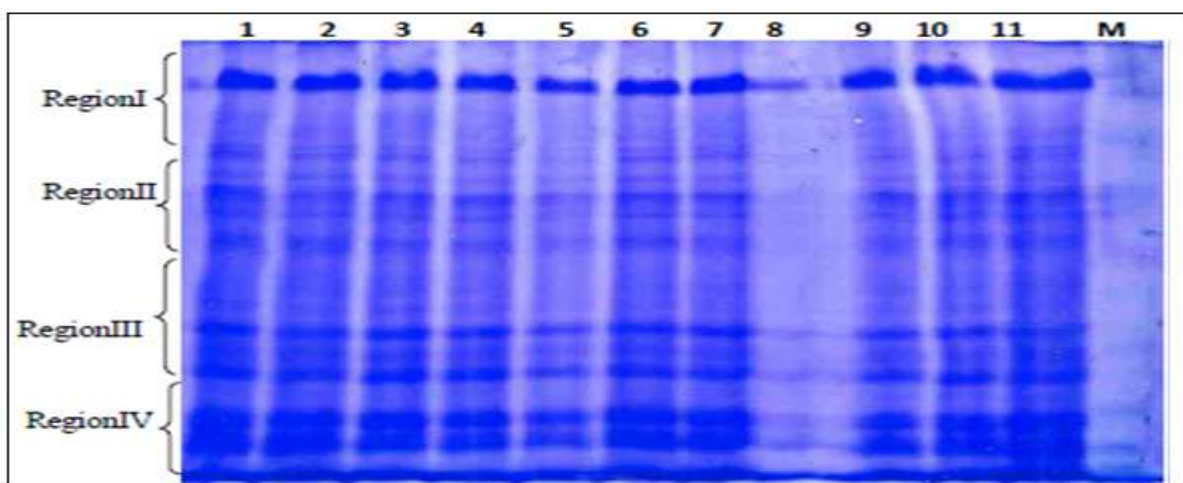


Fig. 2. Total seed protein gel samples 1-10 represent accessions i.e. 1638, 1642, 1655, 1651, 1628, 1641, 1656, 22854 and 19528.

Information about genetic diversity through SDS-PAGE of different Brassica species and within the species, small evidence available which agree with the present investigation of *B. juncea* L. accessions. There are still some problems to investigated genetic differences observed in Indian mustard accessions, which exhibit close relationship with each other through SDS-PAGE of seed protein. But protein

profile pattern of indigenous germplasm played potent role in the evaluation of genetic diversity (Rabbani *et al.*, 2001). The differences observed among the present study and previous results in number of protein bands may be due to diversity in studied accessions, different gel percentage used and selection of bands during data scoring.

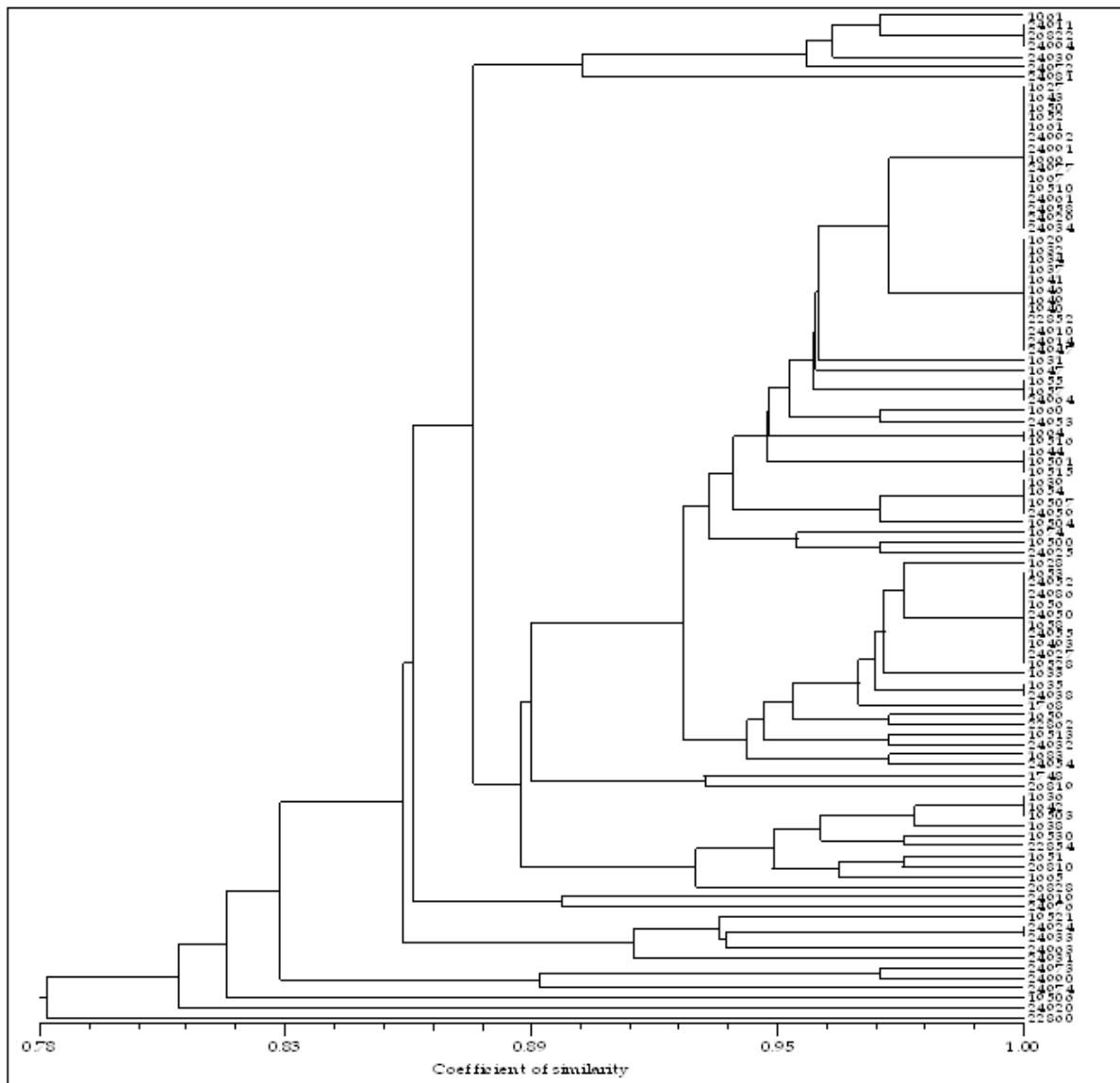


Fig. 3. Dendrogram showing genetic diversity through SDS-PAGE.

Genetic similarity matrix and cluster analysis

During the present study of *juncea* L. accessions were found to the similarity range of 62 to 100 percent. The present results were strengthened with that of Turi *et al.*, (2010) who found similarity within range of 45-98 percent during different Brassica germplasm

characterization. Our results were also close agreement with that of Shinwari *et al.*, (2013) found genetic similarity within the range of 60-100 percent during evaluation of *Eruca sativa*. Similarly Zada *et al.*, (2013) results also agreement with the present study, who found 50 to 100 percent genetic similarity

during investigation of *Brassica carinata* L. genotypes.

The dendrogram constructed by using dissimilarity matrix through unweighted pair group's method with arithmetic averages (UPGMA) divided all the accessions into five clusters. Zada *et al.*, (2013) calculated five clusters of their evaluated germplasm of *carinata* L. and constructed dendrogram using dissimilarity matrix through UPGMA. While similar findings were also noted by Nasr *et al.*, (2006) during *napus* L. germplasm evaluation and by Mukhlesur *et al.*, (2004) in *B. rapa* .

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