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ESTIMATION OF GENETIC DIVERSITY IN THE GERMPLASM OF *BRASSICA CAMPESTRIS* (L.) USING MORPHOLOGICAL AND BIOCHEMICAL MARKERS

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Abstract

A total of 60 genotypes of *Brassica campestris* (L) germplasm were acquired from National Research Centre (NARC) Islamabad, Pakistan for the evaluation of genetic diversity using the morphological characters and SDS-PAGE analysis. These genotypes were grown-up in Plant Garden Department of plant sciences, University of Malakand, Khyber Pukhtunkhwa, Pakistan. For the assessment of genetic diversity total of 18 morphological traits were scored and their phylogenetic relationship were plot through cluster diagram. For the estimation of genetic diversity, we used the most influential technique SDS PAGE. We observed six loci (bands) from the collected genotypes; locus-1 (band-1) contained 100% bands of protein and obvious as monomorphic locus while loci i.e. 2,3,4,5 and 6 showed 28%, 42%, 36%, 56% and 25%, respectively variation and were polymorphic. The inter species locus influence toward genetic assortment (LCTGA) was 83.33% in the attained genotypes. Profiling through SDS PAGE based on two-way cluster plotting resolved effectively the collected genotype into 4 cluster. We consider that this work provides a baseline for the understanding genetic diversity in the common crops used in Pakistan and warrants further investigation in this newly evolving field of study.

Key words: *Brassica campestris* (L), Genetic variability, new Chinese hybrid, SDS-PAGE, Locus variation

Introduction

Family Brassicaceae is a very important family among the crops predominantly for its high quality of edible oil. Rapeseeds, *Brassica campestris* L. and *Brassica napus* L., are also have pleasant feeling of yellow flowering blankets during blooms apart from its primary importance being nourishing and oil rich seed sources. Family *Brassicaceae* has about 350 genera and 3500 species (Christopher et al., 2005). The genus *Brassica* alone consists of about 100 species (Tanksley and Jones, 1981). About 40 species are commonly found in the Mediterranean region and 7 species are reported form Pakistan; in these 7 species two are wild while five species are commercially cultivated (Thanh and Hirata, 2002). All portions of the Brassica plant are edible like, root, stem, leaves, flower and seeds (Avrdca, 2000).

For a victorious crop development, genetic diversity is extremely significant. It protects our food provide by the expansion of the range of genes available to convene in the agricultural production (Pervaiz et al., 2010). Rapeseed (*Brassica campestris, Brassica napes* and *Brassica juncea*) is grown-up worldwide as a source of palatable and edible oil (Downey et al., 1990). The studied plant and species contain Sulphur compounds i.e. glucosinolates, which can create thyroid problem in livestock with using in large amounts (Ditomaso and Healy, 2007; Ali et al., 2017). In *Brassica* species great quantity of vitamin C and soluble fibre with numerous nutrients showing anticancer properties (Jain et al., 2011; Omar et al., 2009; Le et al., 2003). Beside used as vegetable for digestive ailments, oil is used a rubefacients, it is also used as counter irritants, provoke hair fall or hair loss, as it contains selenium. It is also used as aphrodisiac.

Mustard and rapeseeds cover 119.6 thousand hectares area in Khyber Pakhtunkhwa, Pakistan, giving 9.4 thousand tons of production of seed with an average production of 480 Kg/hec (Anon, 2006 and 2007). In Pakistan, the entire area for the crop is 23.68 million hectares in them 0.807 million hectares are oilseed crops which become approximately 3% of the entire cropped area. 21.17 million hectares are the cultured area in which approximately 4.92 million hectares is recent fallow. The cultivation of *B. campestris* is 4.88 million hectares (Sindh), 12.41 million hectares (Punjab), 1.96 million hectares (Baluchistan) and1.91 million hectares in Khyber Pakhtunkhwa (Anonymous, 2008). Brassica species is grown in 228 thousand hectares area in Pakistan (Anon, 2008). In all over the world, approximately 23 million hectares area is

used to grow *Brassica* oil seed crop annually with 36 million tons of oil production (F.A.O, 2004).

The appropriate investigation through morphological and biochemical markers offer baseline information for upcoming crop improvement programs. The study also has been organized to overcome on loss of genetic diversity in the locally grown gene bank of the food crops. In addition to this, the main intention of the recent trials is to assess the connotations among the genotype as well to find out the extent of innate nonconformity in the distant zone of Dir (Lower), KPK, Pakistan by the total seed protein analyses and using SDS-PAGE technique.

MATERIAL AND METHODS

Morphological characterization

The seeds for the trials were obtained from NARC Islamabad Pakistan. Experimental design and field trials were laid out at the Botanical Garden and Herbarium (BG&H) University of Malakand, Dir (L) Pakistan. In the design part, 2 blocks were designated with same length 86 feet. Each block comprised of 60 ridges having 1 feet distance between the two successive ridges. For the estimation of morphological characters, 60 genotypes with one control for the comparison were grown. Morphological characters recorded were both qualitative and quantitative. The parameters scored were i.e. days of germination, days of leaf emerging, days of flowering, days of fruiting, days of maturity, leaf length (cm), petiole length (cm), internodes length (cm), whole plant length with roots (cm), days of Harvesting, plant height (cm), 1000 seed wet weight (g), stem weight (g), siliqua weight per plant (g), total seeds weight per plant (g) and seeds and Siliqua weight per plant (g). Many qualitative character were recorded, i.e. Germplasm of the Plant (vigorous, very vigorous and weak), Leaf (Erect and Semi erect), leaf (Hairy or smooth), leaf types (Simple, Sinuate and Lobed) leaf colours (Green and Dark Green), Seed color (Red, Black and Brown).

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The seeds of *Brassica campestris* were examined through SDS-PAGE for protein analysis this method was described by Laemmli (1970), Payne et al., (1981). A concentration of 12.50% to 15% of separating gel and 4.50% of stacking gel were used for electrophoresis by a

discontinuous system of Laemmli (1970). A 0.05g powder of crushed seeds was kept in 1.5ml Eppendorf tube, 400 µl protein extraction buffer (PEB) was added then vertex to homogenize and centrifuge with 12000 rpm for 10 minutes. 70% of ethanol was used to wash the glass plates. With the completion of electrophoresis, the gel was stained in staining solution on Shaker at 46 rpm for 15 minutes, followed by the de-staining until the background of the gel disappeared.

RESULTS

A total of 18 morphological traits were scored, out of which 12 were quantitative and 6 were qualitative. The cluster analysis of 18 morphological characters was performed using the computer software PCORD. The result of cluster analysis is presented as phylogenetic tree (dendrogram) in Fig 2. The phylogenetic tree divided 60 genotypes into two linkages at linkage distance of 20%. The linkage-1 (L-1) is further divided into four clusters at genetic distance of 75.5%. Cluster-1 contained four accessions (BC mean *Brassica Campestris*) c i.e. BC31, BC14, BC13, BC11. Cluster-2 contained eight accessions i.e. BC47, BC41, BC21, BC25, BC22, BC10, BC32, BC3, Cluster-3 contain 9 i.e. BC59, BC58, BC57, BC44, BC60, BC49, BC48, BC23 and BC9. Cluster four contained eleven accessions i.e. BC30, BC29, BC27, BC24, BC23, BC18, BC17, BC15, BC5, and BC2.

The linkage-2 (L-2) is further divided into five clusters at the genetic distance of 75.5%. Cluster-5 contains four genotype which was BC52, BC51, BC53, BC54 and BC45. Cluster-6 contain four genotypes i.e. BC56, BC36, BC45 and BC19. Cluster seven contain ten genotypes i.e. BC56, BC50, BC37, BC36, BC35, BC34, BC15, BC8, BC7 andBC6. Cluster eight contains five genotypes i.e. BC42, BC43, BC20, BC25 and BC4. Cluster Nine contained four genotypes i.e. BC39, BC33, BC40 and BC1 (Figure 2 and 3).

Correlation analysis

The relationship of morphological parameters of *Brassica campestris* were computed and presented in Table 2. Stem length of the 1st month showed positive and extremely significant connection with stem length of the 2nd month. Stem length of 3rd month showed positive and very momentous association with stem length of 1st and 2nd months. Leaf length of the 1st month showed positive and highly significant association with stem length of 1st, 2nd and 3rd

months. Leaf length of 2nd month showed positive and highly significant association with stem length of 1st, 2nd and 3rd months and with Leaf length of 1st month. Leaf length of 3rd month showed positive and highly important association with stem length of 1st, 2nd and 3rd months and with Leaf length 1st and 2nd months.

Plant Biomass showed highly positive, significant association with the plant length in the roots. Stem mass presented positive and highly important association with Plant length with root and with Plant Biomass. Weight of siliqua per plant showed positive and highly significant association with Plant length with root and with Plant Biomass and also with Stem weight. Weight of seeds per Plant showed positive and highly significant association with Plant Biomass and with weight of siliqua per plant. Seeds and Siliqua weight exhibited positive and extremely significant association with Plant Biomass and with Weight of Siliqua per plant (Table 2).

Table 1. List of Abbreviations

S.N0	Full Name	Abbreviation
1	Plant Height	PH
2	Branch per Plant	Br/P
3	Pod per Plant	Pd/p
4	Seed per Plant	Se/P
5	1000 seed weight	1000 Sw
6	Gram yield per Plant	GY/P
7	Branch per Plant	B/P
8	Harvest index %	HI%

Table 2. Pearson correlation among quantitative traits of newly developed chines hybrid Brassica campestris

	PH	**Br/P**	**Pd/p**	**Se/P**	**1000 Sw**	**GY/P**	**B/P**	**HI%**
PH	1							
Br/P	0.047	1						
Pd/p	.721**	.300*	1					
Se/P	.420**	.271*	.519**	1				
1000 Sw	-.831**	-0.133	-.839**	-.625**	1			
GY/P	-0.245	0.052	-0.16	-0.209	.376**	1		
B/P	-0.12	0.034	-0.073	-0.174	.296*	.715**	1	
HI%	-0.222	0.055	-0.144	-0.117	0.226	.709**	0.014	1
**. Correlati								
*. Correlsignificant at the 0.05 level (2-tailed).								

SDS-PAGE analysis

All the genotypes were tested through proteomic assay, in order to evaluate the image of genetic assortment in seed storage protein. The SDS-PAGE was supported out in several combinations. It was determined that the 14% Acrylamide gel concentration and 20 µl samples gave the best resolution (Figure 1). Electropherogram (Figure 1) shows that out of the total 6 polypeptide bands, only band-1 is monomorphic because it is present in all genotypes while the remaining five bands including B-2, B-3, B-4, B-5 and band 6 are polymorphic. The data was recorded on the basis of presence and absence of protein bands i.e. 1 for present and 0 for absence. To find out the genetic diversity between *Brassica campestris*, a dendrogram were constructed (Figure 3). The dendrogram divided all the genotypes into two linkages i.e. Linkage-1 and linkage-2 at 25 % genetic similarity or 75% genetic disagreement distance. At 50% genetic similarity or 50% genetic disagreement level, Linkage-1 is further divided into cluster-1

and cluster-2, Cluster-3 and cluster-4. Similarly, Linkage 2 also at 50% genetic similarity or 50% genetic disagreement level divided into cluster-5, cluster-6, Cluster-7.Cluster-8 and Cluster-9 (Figure 3).

For confirmation of phylogenetic relationship among 60 cultivars of *Brassica campestris*, *a* scattered plot was constructed (see Figure 4). All the cultivars were clustered in four groups just like that of a phylogenetic tree. During the present study intra-specific locus variation among *Brassica* genotypes were also detected. Table 3 Represents intra-specific locus variation amongst 60 genotypes of *Brassica campestris*. Out of total loci (6 Loci), the locus-1 (band-1) is importantly monomorphic due to the occurrence of 100% protein bands and therefore marked as specie-specific locus. The variation found in L-2, L-3, L-4, L-5 and L-6 is 28%, 42%, 36%, 56% and 25%, respectively; while the genetic disagreement is 1.00, 0.72, 0.58, 0.64, 0.44 and 0.25, respectively. The comparative locus contribution towards the genetic disagreement is 83.33.

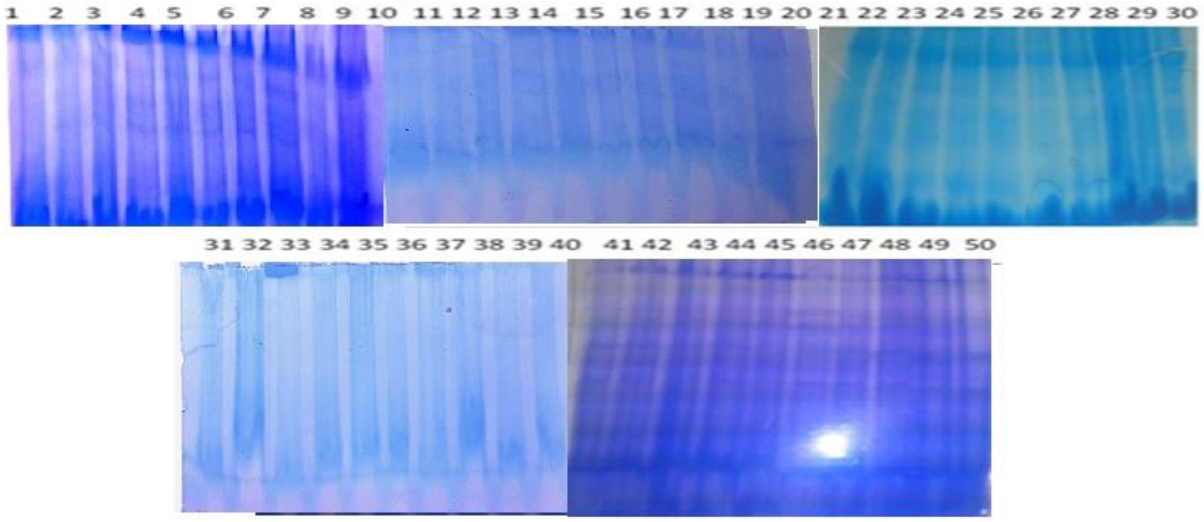

Figure 1. Electropherogram showing intra specific variation in 60 different genotypes of *Brassica campestris* collected from NARC Islamabad, Pakistan.

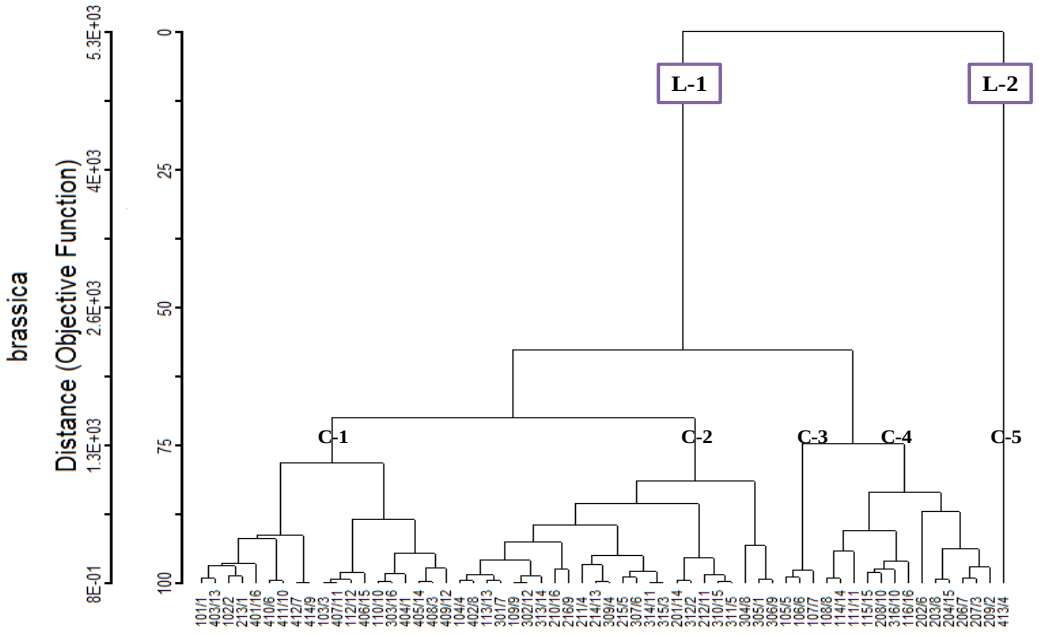

Figure 2. Intra-species Phylogenetic relationship noticed through morphological traits analysis in 60 different genotype of Brassica campestris collected from NARC Islamabad Pakistan

Table 3. Intra specific locus variation 60 genotypes of *Brassica campestris*.

Locus	**Present (%)**	**Absent (%)**	**Variation (%)**	**status**	**Genetic disagreement (band present)**
L-1(band-1)* (generic specific locus)	50(100%)	0(0.00%)	Nil	Mono	1.00
L-2(band-2)	36(72%)	18(32%)	28%	Poly	0.72
L-3(band-3)	29(58%)	21(42%)	42%	Poly	0.58
L-4(band-4)	32(64%)	14(36%)	36%	Poly	0.64
L-5(band-5)	22(44%)	28(56%)	56%	Poly	0.44
L-6(band-6)	25(50%)	25(25%)	25%	Poly	0.25
loci)100	83.33%				

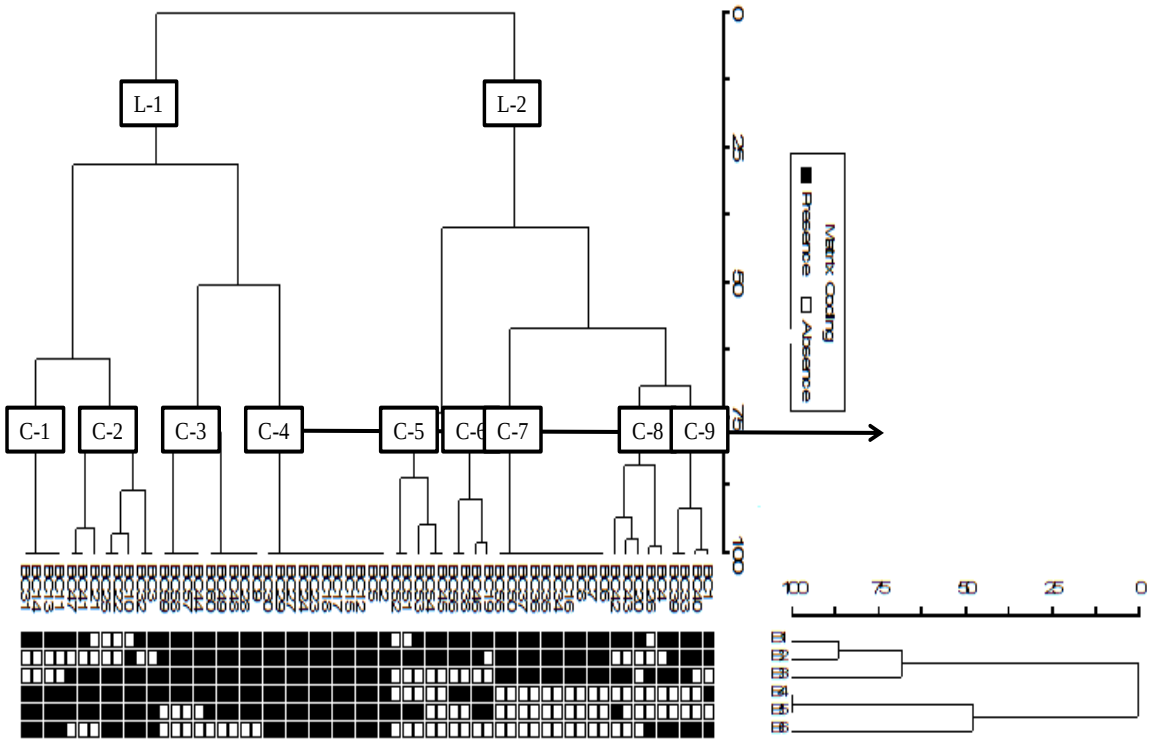

Figure 3. Phylogenetic relationship based on SDS-PAGE in 60 Genotype of Brassica campestris Collected from NARC Islamabad Pakistan.

Figure 4. Conformation of Phylogenetic relationship by scattered plot detected through SDS-PAGE in 60 cultivars of *Brassica campestris*.

Morphological traits

Plant height range from 3.00cm to 55.00cm with the mean value of 48.3, standard deviation of 6.94 and coefficient of variation for the trait was 14.38. Branches per plant ranged from 2.67cm to 4.33cm with the mean value of 3.51, standard deviation 0.36 and coefficient of

variation for the trait was 10.11. Pods per plant ranged shaier khan .from 24to 35with the mean value of 33.62, standard deviation 1.47 and coefficient of variation for the trait was 4.36. Seeds per plant ranged from 4.50 to 32.00 with the mean value of 24.90, standard deviation 4.13 and coefficient of variation for the trait was recorded to be 16.59.

Thousand seed weight ranged from 3.13 to 24.0gm with the mean value 4.34, standard deviation 2.64 and coefficient of variation for the trait was 60.81. Grain yield per plant ranged from 16.30 to 23.00 with the mean value of 19.20, standard deviation 1.43 and coefficient of variation for the trait was 7.46. Plant biomass range from 29.00 to 36.47 with mean value of 32.55 gm, standard deviation 1.66 and coefficient of variation for the trait was 5.10. Harvest index range from 50.25 to 68.00 with mean value of 58.99, standard deviation 3.17 and coefficient of variation for the trait was 5.37 (Table 4).

Table 4. Descriptive statistics for the comparative study of morphological traits.

Traits	Mean	Standard Deviation	Minimum	Maximum	CV%
PH	48.37	6.94	3.00	55.00	14.35
Br/P	3.51	0.36	2.67	4.33	10.11
Pd/p	33.62	1.47	24.00	35.33	4.36
Se/P	24.90	4.13	4.50	32.00	16.59
1000 Sw	4.34	2.64	3.13	24.00	60.81
GY/P	19.20	1.43	16.30	23.00	7.46
B/P	32.55	1.66	29.00	36.47	5.10
HI%	58.99	3.17	50.25	68.00	5.37

DISCUSSION

Intra-specific variations were recorded in the morphological analysis i.e. Qualitative and Quantitative parameters. Out of quantitative traits, stem length is ranging from 1.57 to 293.17 mean cm. Similarly, Leaf length, petiole length, inter-node length, harvesting days, plant length

with root, plant biomass, stem weight, Siliqua weight per pant, seed weight per plant, Siliqua + seed weight, 1000 seed weight is ranging from 0.73-17.50cm mean, 0.82-25.17cm mean, 0.37-42.27cm mean, 161-177 mean, 70.17-117.17cm mean, 18.50-45.0gm mean, 8.33-30.17gm mean, 5.67-14.33gm mean, 4.33-8.67gm mean, 9.11-19.33gm mean and 0.51-1.67gm mean respectively. Under hostile environmental conditions the propagation and seedling formation are the critical phases in the life cycle of a plant (Ashraf *et al.,* 2004). Salt stress affect the roots because of the direct interaction with salty soil and roots absorbed water from the soil to supply it to the whole plant, thus root and shoot lengths are the main and important traits to be observed. So, shoot and root lengths provide clues about the retort of plant to stress (Jamil and Rha, 2004).

Based on the scored morphological traits, dendrogram grouped all genotypes into three clusters. The genotypes in the same cluster showed similarity in their morphological traits. In 2009 Nisar et al., reported that morphological characterization is the first step to investigate genetic diversity, however, adversely affected by environmental fluctuations. On the other hand, biochemical markers such as SDS-PAGE are more accurate and evaluate correct genetic diversity index because it is free of environmental fluctuations (Akhtar, 2001; Rabbani et al., 2001). Since a many of the species are genetically confidentially related, it is often difficult to morphologically distinguish among species. Because SDS-PAGE of seed protein is a simple and consistent method, therefore, it is extensively used in studies on phenotypically close taxa and also used as genetic markers in the study of genetic variation (Sinha et al., 2012; Ali et al., 2017). The SDS-PAGE is also considered to be a practical and reliable method for species identification (Sinha et al., 2012). Electrophoresis of protein is a powerful tool for the assessment of genetic diversity and is typically measured as a dependable technology because seed storage proteins are highly independent of environmental variation (Javid et al., 2004; Iqbal et al., 2005; Nisar et al., 2007). Since in developed seeds, type and amount of proteins are more continuous with other plant tissues (Magni et al., 2007; Waqar et al., 2017). Different degree of genetic diversity was observed in *Brassica campestris* germplasms by using SDS-PAGE. Due to the absence of some Protein polypeptide bands in some lines, the remaining loci (L-2, L-3, L-4, L-5 and L-6) showed variation and therefore considered as polymorphic loci. 28% Variation in Locus-2 with 0.72 genetic disagreements, 42% variation in Locus-3 with a 0.58 genetic disagreement, 36% variation in Locus-4 with 0.64 genetic disagreement, 56% variation in Locus-5 with a 0.44

genetic disagreement and 25% variation in Locus-6 with 0.50 genetic disagreements is observed. Phylogenetic tree is arranged to four clusters for all for all accessions. The accessions in one cluster are mostly identical in their protein profile.

CONCLUSION

The genotypes collected from NARC Islamabad, Pakistan were evaluated for the estimation of genetic diversity and genetic homology. In this work we study variations, both morphological and molecular level. The result after the SDS-PAGE electrophoresis shows that this method provides a influential tool for the estimation of genetic diversity. All the genotypes and cultivars were tested through proteomic assay, in order to estimate the genetic diversity in seed stored protein. In the light of intra-specie locus variation, it is concluded that Locus-1 is monomorphic due to the presence of all protein polypeptide bands in all collected germplasm and marked as specie-specific locus for *Brassica campestris*. We also conclude that variation is present in the available *Brassica campestris* cultivars in Pakistan, at both morphological and molecular level. We also recommend that further trials to should be conducted at different microclimatic level to understand the behaviour of these cultivars to the environmental variability and climate change at the spatio-temporal scales.

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