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Investigation of genetic diversityin Pakistan I *Brassica Compestris* Landraces for the selection of high yielding genotypes

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Abstract

Brassica compestris vegetables are of great economic importance throughout the world and are utilized for Brassica oil seed. Its production has increased over the last 40 years and has become one of the most important world sources of vegetable oil after soya bean and cotton seed. Genetic diversity is important in selecting high yielding genotypes. For this purpose 50 genotypes of *Brassica campestris* seeds were collected from different region of Dir lower Khyber Pukhtun Khwa, Pakistan. These genotypes were grown in Botanical Gardan Department of Botany, University of Malak and Khyber Pukhtun Khwa, Pakistan, for the estimation of genetic diversity using the morphological traits and Biochemical marker. A total of 18 morphological parameters were scored for estimation of genetic diversity and phylogenetic relationship through cluster plotting. Similarly, six loci (bands) were detected in the collected genotypes, out of them locus-1 (band-1) contain 100% protein bands and marked as monomorphic locus. The remaining loci i.e. 2,3,4,5 and 6 were polymorphic and show 28%, 42%, 36%, 56% and 25% variation respectively. Inter specific locus contribution toward genetic diversity (LCTGD) was 83.33% in the collected genotypes. SDS-PAGE profiling based on Two-way cluster plotting successfully resolved the collected genotypes into 4 clusters. The present result shows that SDS PAGE is a powerful technique for the estimation of intra-specific Genetic diversity.

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Introduction

The genus *brassica* is highly diverse genus of family Brassicaceae (Avrdc, 2000). The family gathered 350 genera and 3500 species. Economically Brassicaceaeis one of the ten most important plant families with a wide range of agronomic traits (Christopher *et al*., 2005). All members of the family are widely used as vegetable, source of edible oil, forage and ornamentally. After soybean and palm, brassica oilseed species (*Brassica napus, B. campestris* and *B. juncea*) are the 3rd most significant source of edible oil (Turi *et al*., 2012).Mostly all parts of *Brassica* are edible including leaves, buds, stems, roots, seeds and flowers (Avrdc, 2000). *Brassica campestris* L. is mostly a winter or spring annual plant that produces large biomass, stem erect, stout, much-branched, leaves petioles, alternate with a few bristly hairs, especially along the veins, flowers bright yellow, pedicel late, four-petaled, deep taproot and a fibrous near-surface root system; and seeds small, round, pale or dark, and smooth (Sarwar *et al.,* 2013 b). Annually *Brassica* oilseed crops are cultivated on 23 million hectares that give over 36 million tones production to the world (Fao, 2004). In Pakistan the oilseed crop particularly *Brassica campestris* is annually cultivated on 21.16 million hectors area (Anonymous,2008).In crop plants genetic diversity is easily evaluated by using morphological characterization but keep in mind morphological traits are adversely affected by environmental changes (Nisar *et al* 2009). Using of biochemical technique such as SDS PAGE for the estimation of genetic diversity is more precise and accurate because biochemical techniques are free of environmental fluctuations (bretting *et al.,* 1995;Nisar *et al*., 2009).SDS-PAGE is a simple, reliable and widely used biochemical technology in telling the genetic structure of crop germplasm (Sultan *et al.,* 2006).Now days the SDS-PAGE technology is also used for species identification (sinha *et al* 2012).SDS-PAGE is an important genetic marker, especially in cereals in which their variability is related to technological properties of the species (Amar *et al* 2014).SDS-PAGE technique is also used to study taxonomical and evolutionary relations of several crop plants (Ghafoor *et al.,* 2002).In view of the importance of the oil crop, and the need to improve the scope and usefulness of its genetic resources, in this study we undertook to access and evaluate genetic diversity in *brassica campestris* using morphological and SDS-PAGE characterization.

Materials and methods

In 2014 and 2015 fifty different localities of District Dir Lower were visited for the collection of germplasm of locally cultivated *Brassica campestris*. The visited areas including Nasafatalash, Band agra, Khagram, Bajauro Talash, Kot, Mungai, Aspanr, Barjammakhai, Beyarai, Darmalbala, Derai, Gumbatbanda, Khalkandaro, Lajbok, Laram, pingal, sangolai, Tomang, Pato, Utala, Khalhayaserai, Luqmanbanda, Sayardara, Rabat, Gulmuqam, Alimast Chakdara, Agra gulabad, Ouch, Rehanpoor, Moonda, Amlookdara, Samarbagh, Zyamdara, Barmkai, Danda, Khadango, Nimazkot, Sairtormang, Nawagai, Chatpatpayeen, Korheraipayeen, Shagokhas, Khazana, Zangiankambat, Binshahi, Baghdushkhel, Sangulai, Kamarkotkai, Shalkandai and Khonatamergara. The collected germplasm from each locality were then cultivated in triplicate manner in the Botanical garden of Herbarium University of Malakand Khyber Pakhtunkhwa Pakistan.

Morphological characterization

For the estimation of intra specie genetic diversity morphological characterization were carried out by scoring 18 morphological traits. Out of total morphological traits, 12 are quantitative including (Stem, petiole, leaf, inter node, plant length, harvesting days, plant biomass, stem weight, siliqua weight, seed weight, siliqua+seed weight and 1000 seed weight) were documented in (Table 4) and 6 are qualitative traits which are (stem type, leaf shape, leaf hair, leaf type, leaf color and seed color) were recorded in (Table 3.2).

The data of total morphological traits were recorded and cluster analysis was carried out using the computer software PC-ORD.

SDS-PAGE characterization

For SDS-PAGE characterization seeds of each Locality were grinded into fine powder with the help of pastel and mortal. 0.05g of seed powdered was then added to 400µl protein extraction buffer (0.05 M Tris–HCl, 0.2% (w/v) SDS, 5 M Urea, 1% β-mercaptoethanol, 1 % (w/v) Bromophenol blue and maintain the pH 8). The mixture of seed powder and PEB was then vortexes thoroughly to homogenize and then centrifuge at 14000 rpm for 10 minutes.

The electrophoretic procedure was then carried out using 12% polyacrylamide gel, resolving gel (3.0M Tris-HCl) pH9, 0.4% SDS and 4.5% stacking gel (0.4M Tris-HCl pH 7.0, 0.4% SDS). Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125 % SDS) was added to the top pool of the apparatus. A 20µl of the supernatant was loaded with the help of micropipette into the wells of the gel. Apparatus was connected with uninterrupted electric supply (120 V) until the bromo-phenol blue (BPB) was reached to the bottom of gel plate. The gels were then stained for an hour with the solution containing 0.2% Commassie Brilliant Blue dissolved in 10% glacial acetic acid, 40% methanol and water in the ratio of 10:40:50. Gels were de-stained in a solution containing 5% acetic acid and 20% methanol. Gel evaluation for data scoring was done on a light box.

The experiment was repeated 3 times to check the reproducibility of the score-able protein bands. A band presence was coded (1), while the absence of bands scored as (0). The data was analyzed by software PC-ORD (McCune and Grace, 2005) due to complexity in the visual interpretation of SDS-PAGE of seed protein profiles.

Results

Morphological traits analysis

Total of 18 morphological traits were scored, out of which 12 were quantitative (Table 3.4) and 6 were qualitative (Table 2). Dendrogram were constructed (Fig 3.5) for the cluster analysis of 18 morphological characters in the form of phylogenetic tree.

Table 1. Intra specific locus variation 50 genotypes of *Brassica campestris* reported from Dir (l), Pakistan.

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
Locus contribution toward genetic diversity GD= (poly loci/total loci)100		83.33%			

Table 2. Qualitative characters of 51 genotypes of *Brassica campestris*.

S. No	STEM TYPE	LEAF SHAP	LEAF HAIRS	LEAF TYPE	LEAF COLOR	SEED COLOR
1	v. vigorous	Erect	Hairs	Simple	Green	Red
2	v. vigorous	Erect	Hairs	Simple	Green	Red
3	v. vigorous	Erect	Hairs	Simple	Green	Red
4	v. vigorous	Erect	Hairs	Simple	Green	Red
5	v. vigorous	Erect	Hairs	Simple	Green	Red
6	v. vigorous	Erect	Hairs	Simple	Green	Brown
7	v. vigorous	Erect	Hairs	Simple	Green	Brown

8	v. vigorous	Erect	Hairs	Sinut	Green	Black
9	v. vigorous	Erect	Hairs	Sinut	Green	Black
10	v. vigorous	Erect	Hairs	Sinut	Green	Brown
11	vigorous	Erect	Hairs	Sinut	Dark green	Red
12	vigorous	Erect	Hairs	Sinut	Dark green	Red
13	vigorous	Erect	Hairs	Sinut	Green	Black
14	vigorous	Erect	Nill	Sinut	Green	Black
15	vigorous	Erect	Nill	Sinut	Green	Black
16	vigorous	Erect	Nill	Sinut	Green	Red
17	vigorous	Erect	Nill	Sinut	Green	Red
18	vigorous	Erect	Hairs	Sinut	Green	Red
19	vigorous	Erect	Hairs	Sinut	Green	Red
20	vigorous	Erect	Hairs	Sinut	Green	Brown
21	v. vigorous	Erect	Hairs	Sinut	Green	Black
22	vigorous	Erect	Hairs	Simple	Green	Black
23	vigorous	Erect	Hairs	Lobed	Green	Red
24	vigorous	Erect	Hairs	Lobed	Green	Red
25	vigorous	Erect	Hairs	Lobed	Green	Red
26	v. vigorous	Erect	Hairs	Simple	Green	Brown
27	v. vigorous	Erect	Hairs	Simple	Green	Brown
28	v. vigorous	Erect	Hairs	Simple	Green	Red
29	v. vigorous	Erect	Hairs	Simple	Green	Red
30	v. vigorous	Erect	Hairs	Simple	Green	Brown
31	v. vigorous	Erect	Hairs	Simple	Green	Black
32	v. vigorous	Erect	Hairs	Simple	Green	Black
33	v. vigorous	Erect	Hairs	Simple	Green	Black
34	vigorous	Erect	Hairs	Simple	Green	Black
35	vigorous	Erect	Hairs	Simple	Green	Brown
36	v. vigorous	Erect	Hairs	Simple	Green	Black
37	weak	Semi-erect	Hairs	Simple	Green	Black
38	weak	Semi-erect	Hairs	Simple	Green	Black
39	weak	Erect	Nill	Simple	Green	Black
40	weak	Erect	Nill	Simple	Green	Brown
41	v. vigorous	Erect	Hairs	Simple	Green	Red
42	vigorous	Erect	Nill	Simple	Green	Red
43	v. vigorous	Erect	Nill	Simple	Green	Brown
44	vigorous	Erect	Nill	Simple	Green	Brown
45	vigorous	Erect	Nill	Simple	Green	Red
46	v. vigorous	Erect	Hairs	Simple	Green	Brown
47	vigorous	Erect	Hairs	Simple	Green	Brown
48	vigorous	Erect	Hairs	Simple	Green	Brown
49	vigorous	Erect	Hairs	Simple	Green	Brown
50	v. vigorous	Erect	Hairs	Simple	Green	Brown
Control	vigorous	Erect	Hairs	Simple	Green	Brown

The phylogenetic tree divided 51 genotypes into two linkages at linkage distance 25%.The linkage-1 (L-1) is further divided into two clusters at genetic distance of 87.5%. Cluster-1 contains thirteen accessions i.e.BC1, BC4, BC15, BC18, BC28, BC30, BC31, BC32, BC41, BC42, BC43, BC44, BC45 and BC49. Cluster-2 contain thirty-eight accessions i.e.BC2, BC3, BC5, BC6, BC7, BC8BC9, BC10, BC11, BC12, BC13, BC14, BC16, BC17, BC19, BC20, BC21, BC22, BC23, BC24, BC25, BC26, BC27, BC29, BC31, BC33,BC34, BC35, BC36, BC37, BC38, BC39, BC40, BC43, BC46, BC47, BC48 and BC51. The linkage-2 (L-2) is further divided into one cluster at genetic distance of 87.5% which contain one genotype which wasBC50. The percentage of each cluster in total population and locality of eachgeno type is present in (Table 3).

Table 3. Cluster analysis based on morphological traits.

Linkage	Genotypes	Cluster	Total population %	Genotype collected from
L-1	BC1, BC4, BC15, BC18,	C1	25.49%	Talashnasafa, Bajauro, laram, Tormang,
	BC28, BC30, BC31, BC32,			ouch, munda, Amlokdara, Samerbag,
	BC41, BC42, BC44, BC45,			ouch, munda, Amlokdara, Samerbag,
	BC49			kambat, Binshahi, Shalkandai.
L-2	BC2, BC3, BC5, BC6, BC7	C2	72.49%	Band Agra, Khagram, Kot, Mungai,
	BC13, BC14, BC16, BC17, BC19,			Darmalbala, Deritalash, Gumbat
	BC25, BC26, BC27, BC29,			Sangolai, Pato, Utala, khall Hayaserai,
	BC33, BC34, BC35, BC36, BC37,			Luqmanbanda, Sayardara, Rabat,
	BC38, BC39, BC40, BC43, BC46,			gullabad, Rehanpur, Zaimdara, Barmkai,
	BC47, BC48, BC51			Danda, Khadango, Nimazkot, Sair
	BC8, BC9, BC10, BC11, BC12,			Tormang, Nawagai, Chatpatpayeen,
				Khazana, Bag dushkhel, Sangulai,
				Kamarkotkai, control.
L-3	BC50	C3	1.96%	Khona Timergara,

Table 4. Quantitative characters and their mean of 51 genotypes of *Brassica campestris*.

Geno.no	S.L	L.L	Pet.L	Int.L	H.D	Plt.H	P/Bio M	St.W	Pod.w/p	Sed.W	Sed+Pod/W	1000g/w
1	38.18	6.01	15.06	5.620	177.0	95.17	40.67	17.83	14.330	8.670	9.110	0.610
2	30.23	6.55	12.93	6.403	177.0	72.67	21.33	9.17	7.000	5.170	12.170	0.650
3	29.70	6.90	13.20	5.997	177.0	75.50	19.67	8.33	6.170	5.170	11.330	0.740
4	34.47	6.95	15.14	5.137	177.0	92.00	22.83	9.67	7.330	5.830	12.830	0.580
5	31.59	6.52	14.37	5.647	177.0	83.83	20.33	8.33	6.170	5.830	12.000	0.670
6	29.39	5.01	13.47	5.477	167.7	75.00	18.50	7.33	5.670	5.500	11.170	0.670
7	30.56	5.34	14.30	4.950	163.0	90.83	26.67	12.00	9.000	5.670	14.670	1.020
8	29.22	5.37	14.20	5.027	163.0	76.50	22.67	10.00	7.000	5.670	12.670	1.180
9	28.95	5.56	14.50	5.287	172.3	77.67	21.00	9.00	6.000	6.170	12.170	0.590
10	30.09	5.64	15.24	5.400	177.0	70.17	19.00	8.00	5.830	5.170	11.000	0.620
11	32.85	6.06	16.64	5.423	172.3	81.83	27.00	10.83	9.500	6.830	16.330	0.760
12	31.77	5.63	16.47	5.603	177.0	73.83	22.33	9.67	6.830	6.000	12.670	0.500
13	29.23	6.19	16.14	5.713	177.0	74.50	19.50	8.50	6.170	4.830	11.000	0.650
14	31.62	6.12	17.25	5.833	177.0	79.00	24.67	10.50	7.500	6.670	14.170	0.850
15	43.92	9.33	22.75	8.200	177.0	85.00	23.00	9.83	7.670	5.500	13.170	0.580
16	32.92	6.47	18.46	6.157	164.3	85.00	34.67	15.33	12.500	6.830	19.330	0.860

17	32.61	6.39	18.67	5.893	166.3	79.83	23.33	10.50	8.330	4.500	14.000	0.760
18	32.76	6.26	19.01	5.977	174.3	99.50	25.17	11.00	8.830	5.330	14.830	0.940
19	31.72	6.94	19.22	5.900	177.0	77.67	25.83	11.17	8.170	6.500	12.000	1.670
20	32.38	6.03	19.47	5.807	177.0	75.33	21.00	9.33	6.670	5.000	11.670	1.210
21	31.47	6.45	19.64	5.467	177.0	80.83	22.33	9.50	7.670	5.170	12.500	0.660
22	31.60	6.65	20.08	5.957	169.0	72.50	22.17	9.33	7.330	5.500	12.830	0.720
23	34.21	5.92	21.04	6.033	166.3	81.00	22.00	10.17	7.500	4.500	12.330	0.560
24	36.28	5.80	22.03	5.910	161.0	86.17	25.33	11.83	8.330	4.330	13.170	0.770
25	33.48	6.10	21.53	5.727	162.3	82.00	24.00	9.67	7.670	6.670	13.500	0.670
26	32.02	6.13	21.38	6.097	177.0	78.17	20.83	8.83	6.500	5.500	12.000	0.570
27	32.63	6.62	22.08	6.110	177.0	77.33	21.33	9.33	7.000	5.000	12.000	0.630
28	35.28	6.60	23.29	5.313	177.0	85.83	22.33	9.67	7.170	5.670	12.830	0.580
29	33.78	5.70	22.83	5.300	177.0	83.33	25.67	11.33	8.330	5.830	14.330	0.570
30	33.43	5.53	22.99	5.967	177.0	90.00	24.50	11.00	8.000	5.670	13.500	0.510
31	45.79	6.98	27.92	5.853	177.0	117.17	45.00	30.17	8.830	6.000	14.830	1.150
32	32.37	6.24	23.54	5.973	177.0	102.67	28.17	12.50	10.000	5.670	15.670	1.440
33	32.03	5.80	23.61	5.873	177.0	77.50	24.67	10.50	7.670	6.670	14.330	0.730
34	34.13	6.00	24.71	5.740	177.0	81.00	22.00	10.17	7.330	4.500	11.830	0.950
35	33.29	6.72	25.00	5.597	177.0	80.67	25.83	11.50	8.500	6.000	14.500	0.650
36	32.20	6.75	24.98	5.730	177.0	75.00	22.17	10.00	6.500	5.670	12.170	0.720
37	34.92	6.30	26.07	5.367	177.0	77.83	26.83	11.67	9.170	6.000	15.170	0.730
38	32.56	6.53	25.70	5.107	177.0	77.33	24.67	11.50	7.500	5.670	13.170	0.570
39	34.33	5.97	26.43	5.527	177.0	85.00	25.67	11.00	8.670	6.000	14.670	0.810
40	33.70	6.35	26.68	5.753	167.7	78.00	26.00	11.33	8.830	5.500	14.330	0.780
41	34.11	6.16	27.09	5.070	170.0	91.83	37.50	17.00	12.330	8.000	20.330	0.680
42	35.88	6.29	28.06	5.157	174.7	86.67	22.67	10.17	7.500	5.000	13.170	0.690
43	34.30	5.94	27.75	4.677	177.0	77.17	22.33	9.83	6.830	5.670	12.330	0.680
44	38.37	6.24	29.54	5.213	177.0	87.17	25.00	10.50	8.670	5.830	14.000	0.750
45	38.39	5.93	29.77	5.217	177.0	88.83	22.83	10.00	7.830	5.000	13.000	1.060
46	31.39	5.30	27.56	5.607	177.0	75.00	22.83	9.83	7.330	5.670	13.000	0.790
47	33.22	6.23	28.81	5.230	177.0	81.83	23.83	10.00	7.670	6.170	13.830	0.710
48	33.82	6.43	29.42	5.113	177.0	82.33	23.50	10.33	7.170	6.000	13.170	0.770
49	35.67	6.68	30.45	5.417	177.0	92.17	32.83	14.33	12.000	6.500	18.500	0.630
50	142.72	24.09	72.27	24.723	167.7	81.00	23.00	10.50	7.330	5.330	12.670	0.880

Correlation analysis

The correlation analysis of 12 quantitative characters of *brassica campestris* revealed that following characters have positive but highly significant association with one another such is leaf length with internodes length and petiole length, petiole with internodes, plant height with plant biomass and pod weight, pod mass with plant bio mass, stem length with internodes length, leaf length and petiole length, seed mass with pod weight and plant bio mass, seedpod mass with plant length, pod weight per plant and Bio mass of plant, stem mass with siliqua weight, Bio mass and length of plant, 1000 grains weight with height of plant which were given in (Table 5).

SDS-PAGE analysis

All the genotypes were tested through proteomic assay, in order to estimate the picture of genetic

diversity in seed storage protein. The banding patterns of *Brassica campestris* are shown in Fig. 1. Electrophoregram show that out of total6 polypeptide bands, only band-1 is monomorphic because present in all genotypes while the remaining five bands including B-2, B-3, B-4, B-5 and band 6 are polymorphic. To find out the genetic diversity between *Brassica campestris* dendrogram were constructed (Fig 2).

Table 5. Correlation Analysis of *Brassica campestris* genotypes among various quantitative and qualitative parameters.

variable	P/Bio.M	H.D	Int.L	L.L	Pet.L	Plt.H	Pod/w/p	S.L	Sed.w	sed+pod/w	St.w
H.D	-0.058										
Int.L	-0.054	-0.171									
L.L	-0.017	-0.107	0.981***								
Pet.L	0.117	0.002	0.776***	0.806***							
Plt.H	0.723***	0.032	-0.028	0.015	0.139						
Pod/w/p	0.844***	-0.140	-0.058	-0.035	0.084	0.556***					
S.L	0.068	-0.145	0.971***	0.975***	0.857***	0.104	0.023				
Sed.w	0.642***	0.076	-0.106	-0.074	-0.051	0.255	0.646***	-0.049			
seed+pod/w	0.545***	-0.213	-0.068	-0.033	0.179	0.403***	0.613***	-0.016	0.375*		
St.w	0.933***	-0.019	-0.022	0.017	0.149	0.751***	0.615***	0.108	0.420**	0.419**	
Thou g/w	0.203	-0.088	0.066	0.063	0.071	0.270*	0.118	0.071	-0.025	0.097	0.250

Key; Plant biomass (P/Bio.M), days to harvesting (H.D), internodes' length (Int.L), Leaf length (L.L), petiole length (Pet.L), Plant height (Plt.H), pods weight per plant (pod/w/p), Stem length (S.L), Seed weight (Sed.W), Seed and pods weight (Sed+Pod/w), stem weight (St.w), Thousand grains weight (Thou g/w).

Two-ways cluster analysis (TWCA) using Ward's method sorted 50 genotypes into two linkages viz. Linkage-1 and linkage-2.Linkage-1 consists of cluster-1 and cluster-2. Similarly Linkage 2 consists of cluster-3 and cluster-4. Cluster-1 consists of three sub clusters i.e. sub cluster-1, sub cluster-2 and sub cluster-3. Sub cluster-1 consists of genotypes collected from (Khadango, Nimazkot, Bag-dushkhel and Shalkandai). Sub cluster-2 consists of (Ispanr, Pingal, Zaim-dara, and Chatpatpayeen) and sub cluster-3 consists of (Mungaie, Barmkai, Danda,Alimas and Khadango). Cluster-2 consists of two sub cluster i.e. sub cluster-4 and sub cluster-5. Sub cluster-4 consists of genotypes collected from (Barjam-makhai, Sayardara, Binshahi, Beyarai, Tormang and ouch).Sub cluster-5 consists of (Bajauro, kot, Rehanpur, Utala, Shagokas, Khona-T, Saraye-payeen, Zanggain kambat, Bag- dushkhel, Kamr-kotkai). Cluster-3 divided into sub cluster-6 and cluster-7.Sub cluster-6 consists of genotypes collected from (Khagram, Darmal-bala, Deri-T, Gumbat-banda, Lajbok, Laram, Rabat, Gulmuqam, Khal-kandaro, Samerbag and Khadango). Sub cluster-5 consists of (Band-agra, Khal-hayaseri, Luqmanbanda and Munda).Cluster-4 is only consisting of sub cluster 8 which consist of genotypes (Talash-nasfa, Pato, Sangolai and Agra-gulabad). For confirmation of phylogenetic relationship among 50 cultivars of *Brassica campestris* scattered plot were constructed. All the cultivars were clustered in four groupies just like that of phylogenetic tree represented in (Fig.3).

Locus variation detected through SDS-PAGE

During present study intra-specific locus variation among *Brassica* genotypes were also detected. Out of 6 loci the locus-1 (band-1) is importantly monomorphic due to the presence 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% shown in (Table 1).

Yield contributing traits analysis

To identify such high yielding genotypes in *Brassica campestris* the following genotypes were selected as high yielding genotypes. The most high yielding genotypes were BC1and BC41 (collected from nasafa

and korheraipayen)and most low yielding genotypes were BC24and BC17 (collected from sayardara and sangolai) shown in (Fig.4).

Discussion

During the present study 50 genotypes of *Brassica campestris* which were collected from lower Dir Khyber Pukhtunkhwa, Pakistan was analyzed using morphological characterization and proteomic profiling. Morphological analysis revealed that intra-specific variation is present in both quantitative and qualitative traits. Out of total 18 morphological traits, 12 traits were quantitative and 6 traits were qualitative. 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, 100 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. Highly positive significant correlation was found between stem lengths 2nd and Stem length 1st i.e. 0.980.Based on scored morphological traits, dendrogram grouped all genotypes into three clusters.

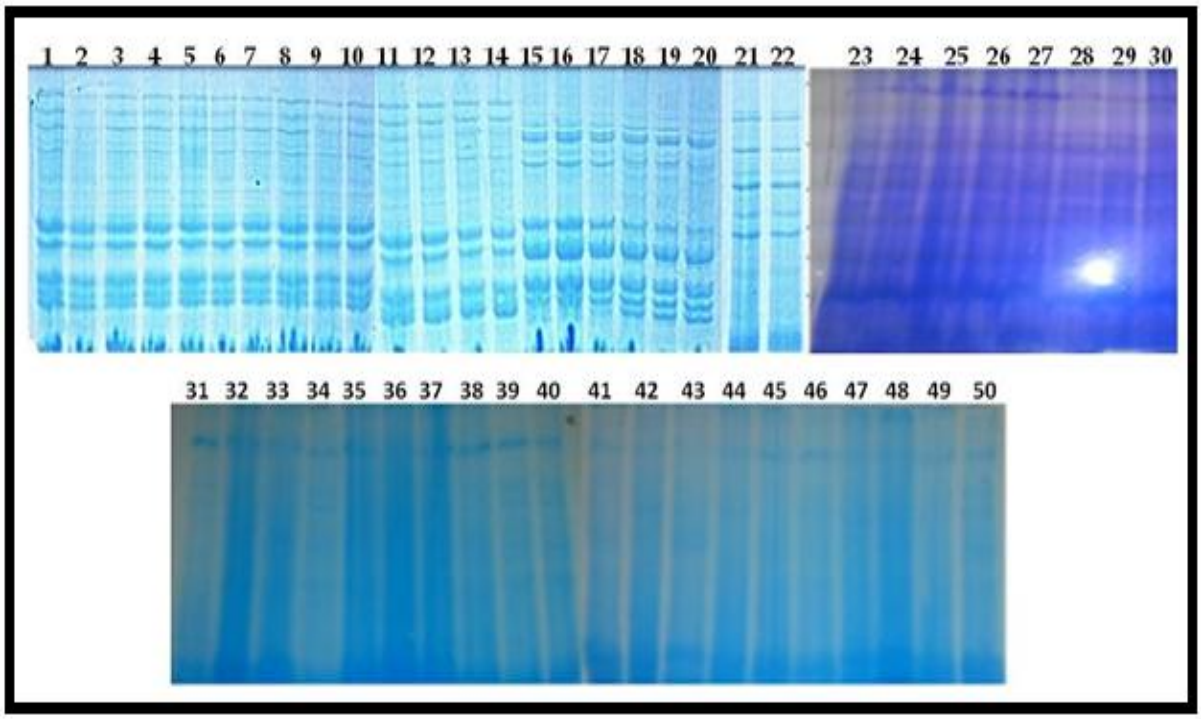

Fig. 1. Electrophoregram showing intra specific variation in 50 different genotypes of *Brassica campestris* collected from lower Dir Khyber Pukhtun Khwa, Pakistan.

The genotypes in the same cluster showed similarity in their morphological traits. Germination and seedling establishment are critical stages in the life cycle of a plant especially under adverse environmental conditions (Ashraf *et al.,* 2004). 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 free of environmental fluctuations (Akhtar, 2001; Rabbani *et al*., 2001). Since a lot of species are genetically intimately related, it is often complicated to morphologically discriminate differences among species. Because SDS-PAGE of seed protein is a simple and reliable method therefore it is extensively used in studies on phenotypically close texa and also used as genetic markers in the study of genetic variation (Sinha *et al.,* 2012).

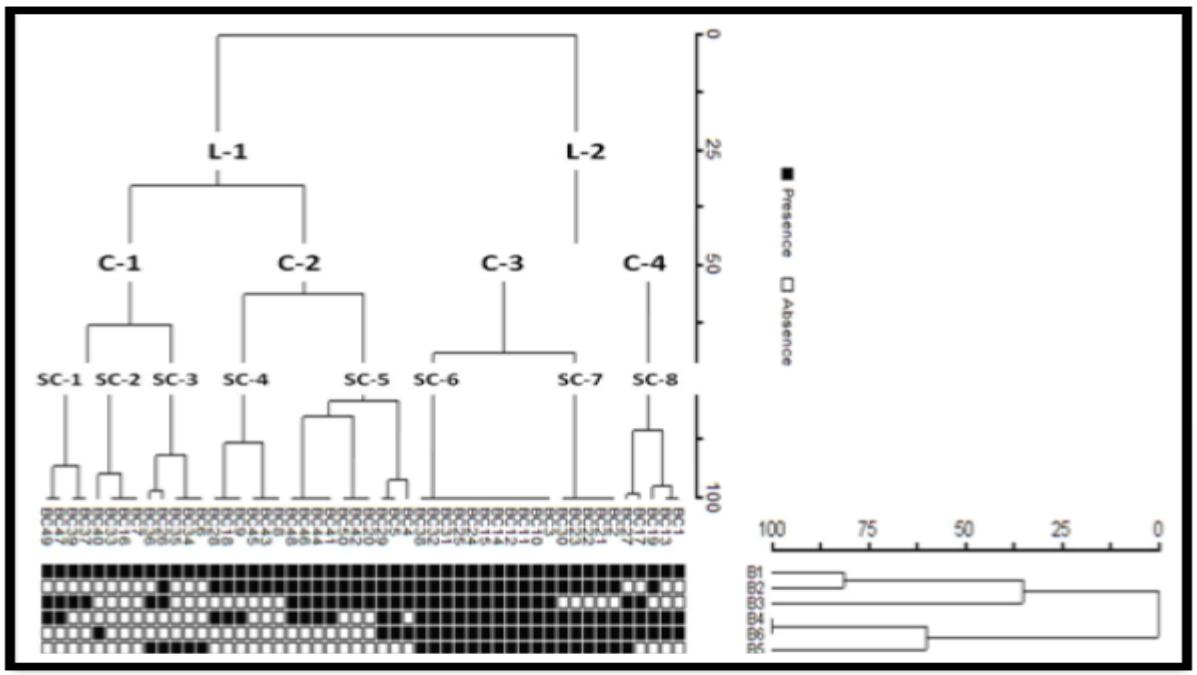

Fig. 2. Phylogenetic relationship based on SDS-PAGE in 50 different genotypes of *Brassica campestris* from lower Dir Khyber Pukhtunkhwa, Pakistan.

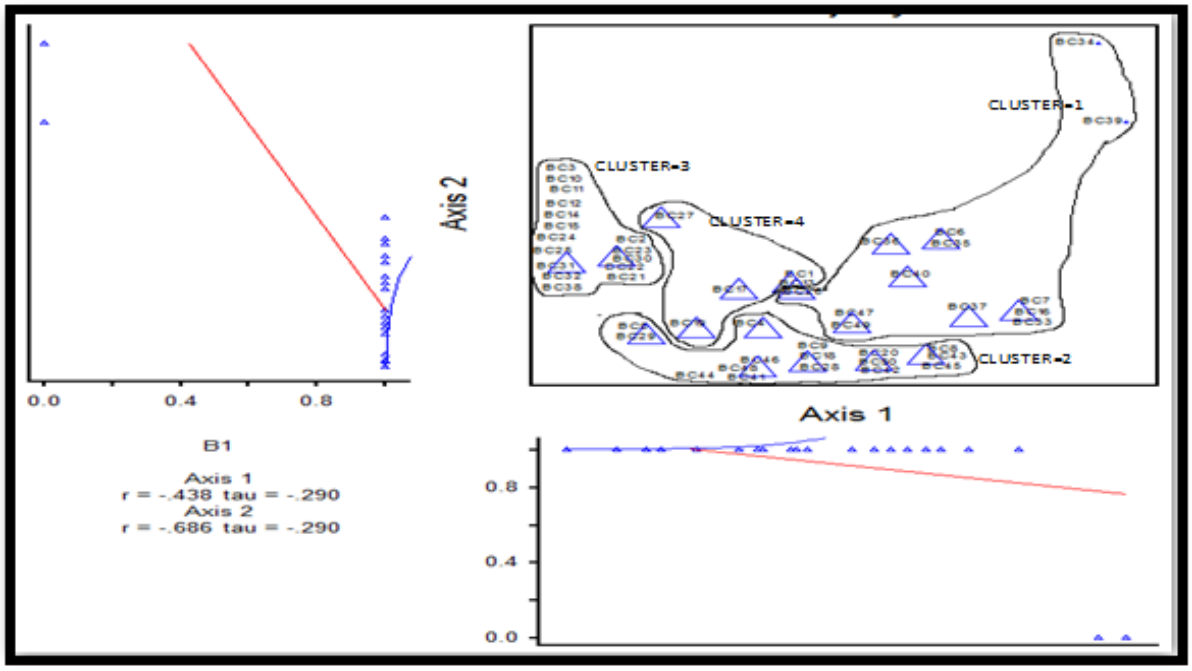

Fig. 3. Conformation of Phylogenetic relationship by scattered plot detected through SDS-PAGE in 50 cultivars of *Brassica campestris*.

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 evaluation of genetic diversity and is particularly considered as a dependable technology because seed storage proteins are highly independent of environmental fluctuation (Javid *et al.*, 2004; Iqbal *et al.*, 2005; Nisar *et al.*, 2007). Since in mature seeds, type and amount of proteins are more constant than other plant tissue (Magni *et al.*, 2007).

Different degrees of genetic diversity were observed in *Brassica campestris* germplasm by using SDS-PAGE.

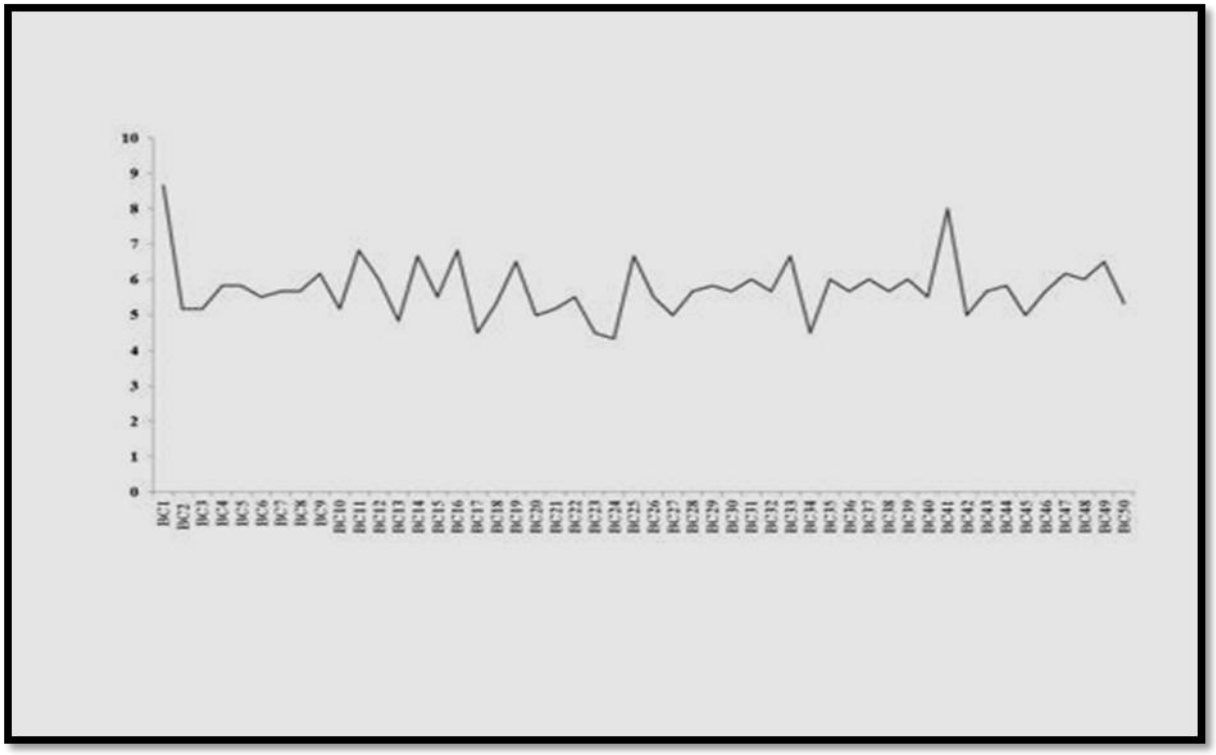

Fig. 4. Graphical representation of high yielding genotypes in 50 genotypes of *Brassica campestris*.

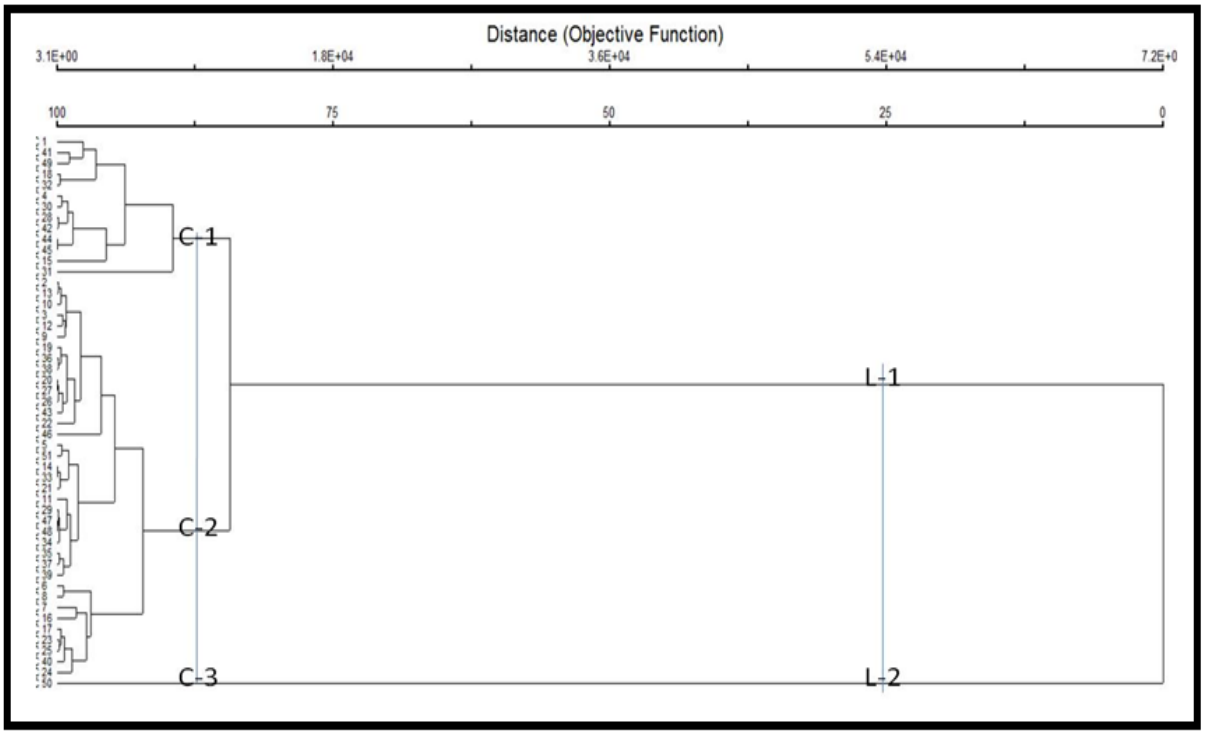

Fig. 5. Intra-species Phylogenetic relationship detected through morphological traits analysis in 51 different genotypes of *Brassica campestris* collected from Dir lower, Khyber Pukhtun Khwa, Pakistan.

All the genotypes and cultivars were tested through proteomic assay, in order to estimate the picture of genetic diversity in seed storage 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*. Due to the absence of some Protein poly

peptide 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 and cluster plotting sorted all the accessions into four clusters. The accessions in one cluster are mostly identical in their protein profile.

Conclusion

Fifty genotypes of *Brassica campestris* seeds were collected from different region of Dir lower Khyber Pukhtun Khwa, Pakistan. These genotypes were grown in Botanical Gardan Department of Botany, University of Malak and Khyber Pukhtun Khwa, Pakistan, for the estimation of genetic diversity using the morphological traits and Biochemical marker. A total of 18 morphological parameters were scored for estimation of genetic diversity and phylogenetic relationship through cluster plotting. Similarly, six loci (bands) were detected in the collected genotypes, out of them locus-1 (band-1) contain 100% protein bands and marked as monomorphic locus.

The remaining loci i.e. 2,3,4,5 and 6 were polymorphic and show 28%, 42%, 36%, 56% and 25% variation respectively. Inter specific locus contribution toward genetic diversity (LCTGD) was 83.33% in the collected genotypes. SDS-PAGE profiling based on Two-way cluster plotting successfully resolved the collected genotypes into 4 clusters. The present result shows that SDS PAGE is a powerful technique for the estimation of intra-specific Genetic diversity.

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