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ESTIMATION OF VARIABILITY AMONG INDIGENOUS *Brassica juncea* L. ACCESSIONS BASED ON MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS

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Genetic diversity was studied in 39 indigenous *Brassica juncea* accessions along with a check for 10 morphological and 4 biochemical traits under field conditions by cluster and principal component analyses. Each accession was sown in 2 rows of 4 meter length. Row to row and plant to plant spacing was kept at 45 and 10 cm respectively. Cluster analysis divided the total 39 accessions into five and seven major groups during 2007 and 2008, respectively. Data based on first and second five PCs with > 1 contributed 73.30% and 64.45% of the variability amongst accessions, respectively. Scatter plot and tree diagrams demonstrated sufficient diversity among the *Brassica juncea* accessions for various morphological and biochemical traits and some extent of association between different clusters. The results concluded that morpho-biochemical diversity in the studied material is structured by genotypes and this diversity could be utilized for future cultivar breeding and germplasm conservation programs.

Keywords: Genetic diversity, *Brassica juncea*, accessions, morphology, biochemical attributes

INTRODUCTION

In Pakistan traditional (rapeseed-mustard, groundnut and sesame) and non-traditional (sunflower, safflower and soybean) oil seed crops are grown to fulfill its edible requirements (Bakht *et al*., 2010a and b; MINFAL, 2012; Siddique et al., 2012; Shafi *et al*., 2013; Taran *et al*., 2013). Mustard (*Brassica juncea* (L.) is one of the most important species in the genus *Brassica*. It is predominant cruciferous species in Indian subcontinent and has been widely grown throughout Pakistan for hundreds of years as an oilseed crop. Based on the patterns of morphology and crop use, it contains a number of variables but inter fertile subspecies. This morphological variation is the result of long term selection with varying objectives in different parts of the world where the species was originally domesticated. *B. juncea* has a great potential for semiarid conditions and is known to be more drought tolerant and shattering resistant than *B. napus* and *B. campestris* (Woods *et al*., 1991; Getinet *et al*., 1996). The performance of the species outside South Asia has been studied by Kirk and Oram (1978) in Australia, Woods *et al*. (1991) in Canada and Kjellstrom (1993) in Sweden.

Estimates of genetic diversity and relationships between germplasm collections are very useful for facilitating efficient germplasm collection and management (Bakht *et al*., 2011a and b; 2012; 2103; Farhad *et al*., 2011; Khan et al., 2011; Siddique *et al*., 2013). Many tools are now available for studying variability and the relationships among accessions including total seed protein, isozymes and various types of molecular markers (Tommasini *et al*., 2003; Yu *et al*., 2005; Hasan *et al*., 2006; Hasan *et al*., 2008; Fu *et al*., 2006; Ali *et al*., 2007; Liu et al., 2008; Ana *et al*., 2009; Krstkowiak *et al*., 2009; Hartings *et al*., 2009; Iniguez-Luy *et al*., 2009; Trick *et al*., 2009; Cheng *et al*., 2009; Allender and King, 2010). However, morphological characterization is the first step in the description and classification of germplasm (Smith and Smith, 1989; Smykal *et al*., 2008). Various numerical taxonomic techniques have been successfully used to classify and measure the patterns of phenotypic diversity in the relationships of species and germplasm collections of variety of crops (Takahata and Hinata, 1986; Gupta *et al*., 1991; Perry and McIntosh, 1991; Dias *et al*., 1993; Amurrio *et al*., 1995; Li *et al*., 1995; Revilla and Tracy, 1995; Smith *et al*., 1995; Tatineni *et al*., 1996; Gomez Campo, 1999). In plant breeding program, several characters are simultaneously considered making it feasible to approximate the genetic divergence using multivariate techniques. These multivariate techniques include principal component and cluster analysis which have analogous efficacy to establish the most suitable cross combinations (Machado *et al*., 2000). In past, multivariate analysis had been used to assess and differentiate the genotypes for various morphological traits in sorghum (Teshome *et al*., 1997; Ayana and Bekele, 1999; Hasanuzzaman *et al*., 2002; Tesso *et al*., 2005; Chozin,

2007; Aruna and Audilakshm, 2008; Mujaju and Chakuya, 2008; Bib *et al*., 2010) and wheat (Ahlawat *et al*., 2008; Golabadi *et al*., 2006. The objective of the present study was to characterize and classify the phenotypic variation and affinities among the different mustard germplasms from Pakistan using multivariate analysis.

MATERIALS AND METHODS

***Experimental lay out*:** Thirty nine accessions of Indian mustard (*Brassica juncea* L.) along with a check were evaluated for 14 morphological biochemical traits under field conditions, during the years 2007 and 2008 at the Institute of Agricultural Biotechnology and Genetics Resource (IABGR) National Agriculture Research Center (NARC), Islamabad, Pakistan (Table 1). Each accession was sown in 2 rows of 4 meter length. Row to row and plant to plant spacing was kept at 45 and 10 cm respectively. For seed bed preparation pre-sowing irrigation was applied and sowing was carried out under optimum moisture conditions. Recommended doses of all essential fertilizers were applied at the time of land preparation. Planting of the experiments was done with hand drill and thinning was done two weeks after germination to maintain optimum plant population. Weeds were controlled manually. The data were recorded on five randomly sampled plants from each accession for days

Table 1. Accessions number and locations of collected *Brassica juncea* germplasm used in the study.

S. No	Accession No	Collection Place	S. No	Accession No	Collection Place
1	(600)Check 1	Canola Mustard	21	625	Islamabad
2	601	Islamabad	22	626	Chakwal
3	602	Sarghoda	23	630	Haripur
4	603	Kohat	24	638	Mianwali
5	604	Chakwal	25	639	Faisalabad
6	605	D.M.Jamali	26	640	Mansehra
7	606	Gilgit	27	641	Bhawalpur
8	607	Faisalabad	28	642	Faisalabad
9	608	Islamabad	29	643	Pakpattan
10	609	Mianwali	30	644	Lodhran
11	610	Rajanpur	31	649	Panu Aqil
12	611	Rahim Yar Khan	32	657	Khuzdar
13	612	Fateh jang	33	658	Rawlakot
14	613	Rawalkot	34	659	Kohat
15	614	Rahim Yar Khan	35	660	Naseerabad
16	615	Islamabad	36	672	Charsada
17	616	Islamabad	37	673	Bunner
18	617	D.G.Khan	38	674	Khairabad
19	618	Islamabad	39	675	Swabi
20	619	Lasbella	40	676	Bunner

Table 2. Morphological traits recorded in the 40 accessions of *Brassica juncea*.

Trait designation	Code	Description of the trait
Days to flower initiation	DFI	Number of days from seed sowing to the appearance of first open flower
Days to 50% flower initiation	50% DFI	Number of days from seed sowing to the appearance of 50% open flower
Plant height (cm)	PH	The height of plants was recorded from the ground level to the tip of plant with the help of a meter rod of five randomly selected plants
Primary Branches/plant	PBPP	Primary branches were counted from ground level to the base of main raceme of each randomly selected plant in each genotype
Length of main inflorescence (cm)	LMI	For this trait data were recorded on the central inflorescence emerging from the basal node of main shoot towards top
Siliquae main/inflorescence	SPMI	Number of siliqua main-inflorescence^{-1} was recorded by counting total siliqua formed from base to the tip of main inflorescence
D. Maturity stage		
Days to maturity	DM	Days taken from seed sowing to the physiological maturity of the crop
Seeds/siliqua	SPS	Counted as total number of seeds of same pod used for seeds/siliqua
1000-seed weight (g)	SW	Weight of 1000 dry seeds

Table 3. Basic statistics of all accessions of *Brassica juncea* used in the study.

Parameters	Mean	Minimum	Maximum	Std. Dev.
50% DFI	66.2	54.0	78.0	5.5
DM	130.3	121.0	144.0	5.7
LPP	17.2	13.0	27.0	2.8
PH	170.9	136.7	198.3	15.4
PBPP	8.4	6.0	12.0	1.5
LMI	55.6	35.3	69.7	6.9
SPMI	46.2	32.0	62.0	6.5
SPS	18.5	12.0	27.0	3.2
YPP	41.5	28.2	66.7	8.4
1000-SW	3.3	2.2	4.2	0.5
OC	45.4	41.5	49.6	1.5
PC	25.2	21.4	28.6	1.5
GSL	85.9	57.2	104.6	14.1
EA	41.0	28.9	59.3	8.9

Legends: DFI= Days to Flower Initiation; DM= Days to Maturity; LPP= Leaves Per Plant; PH= Plant Height; PBPP= Primary Branch Per Plant; LMI= Length of Main Inflorescence; SPMI= Seed Per Main Inflorescence; SPS=Seeds Per Siliqua; YPP= Yield Per Plant; 1000-SD= 1000 Seed Weight; OC= Oil Content; PC= Protein Content; GSL= Glucosinolate Content; EA= Erucic Acid

to 50% flower initiation, days to maturity, plant height (cm), primary branches plant^{-1}, length of main inflorescence (cm), siliquae, inflorescence^{-1}, seeds siliquae^{-1}, 1000-seed weight (g), seed yield plant^{-1} (g), oil, protein, glucosinolates (μMg^{-1}) and erucic acid contents (%). Biochemical analysis of seed for various fatty acids was determined by Near Infrared-Reflectance Spectroscopy (NIRS) at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan (Table 2).

***Statistical analysis*:** Basic statistical analysis of different morphological and biochemical characters was carried out according to Gomez and Gomez (1984). Cluster and principal component analyses were carried out on ten morphological and four biochemical traits for the years 2007 and 2008 separately using NTSYS-pc, version 2.1 and STATISTICA-6 programme as described by Sneath and Sokal (1973). Basic statistics of all accessions of *Brassica juncea* used in the present study is shown in Table 3.

RESULTS AND DISCUSSION

To conserve and utilize germplasm efficiently, it is essential to investigate the extent of diversity available. Morphological characterization is an essential step in the characterization and classification of crop germplasm because a breeding program mainly relies on the magnitude of morpho-phenological variability (Mohamed et al., 2012). The morphological traits used in this study showed a pronounced variation among accessions. The coefficients defining five principal components of the data for *B. juncea* in 2007 are given in (Table 4; Fig. 1). The first principal component had 22.3% of the total variation in the morphological and biochemical characters. PC1 showed primarily the variations in 50% flower initiation (0.14), days to maturity (0.14), leaves plant^{-1} (0.45), plant height (0.32), primary branches plant^{-1} (0.41), length of main inflorescence

Table 4. Principal components for morphological and biochemical characters in 40 accessions of *Brassica juncea* L. during 2007.

	PC_1	PC_2	PC_3	PC_4	PC_5
Eigenvalue	3.11	2.05	1.60	1.49	1.16
Cumulative eigenvalues	3.12	5.17	6.77	8.27	9.42
Proportion of variance	22.27	14.65	11.41	10.67	8.30
Cumulative variance	22.27	36.93	48.34	59.01	73.30
Traits					
Days to 50% flower initiation	0.14	0.15	0.16	0.50	0.36
Days to maturity	0.14	-0.06	0.45	-0.18	0.51
Leaves plant^{-1}	0.45	0.06	-0.08	0.22	-0.23
Plant height	0.32	0.02	0.27	0.22	0.09
Primary branches plant^{-1}	0.41	0.19	-0.10	0.26	-0.14
Main raceme length	0.32	-0.01	-0.20	-0.53	0.16
Siliquae main raceme^{-1}	0.43	0.06	-0.08	-0.18	-0.13
Seed siliqua^{-1}	-0.04	0.36	-0.38	0.13	0.44
Seed yield plant^{-1}	0.04	0.30	0.33	-0.43	-0.09
1000 seed weight	0.05	-0.40	0.40	0.10	-0.05
Oil content	-0.03	0.60	0.07	-0.11	0.08
Protein content	0.02	-0.18	-0.37	0.01	-0.12
Glucosinolates content	-0.42	0.09	-0.01	0.08	0.09
Erucic acid content	-0.16	0.40	0.29	0.09	-0.51

(0.32), seed main inflorescence^{-1} (0.43), seed yield plant^{-1} (0.04), 1000-seed weight (0.05) and protein content (0.02) (Fig.1). Second principle component contributed a total of

14.7%, having 50% flower initiation (0.14), leaves plant^{-1} (0.06), plant height (0.02), primary branches plant^{-1}(0.19), seed main inflorescence^{-1} (0.06), seed plant^{-1} (0.36), seed yield plant^{-1} (0.30), oil content (0.60), glucosinolates content (0.09) and erucic acid (0.40). The component 3 explained 11.4% of the total variation and was contributed by 50% flower initiation (0.16), days to maturity (0.45), plant height (0.27), seed yield plant^{-1} (0.33), 1000-seed weight (0.40), oil content (0.07) and erucic acid (0.29. The principal component four accounted for 10.8% of divergence, and among those values recorded for 50% flower initiation(0.50), leaves plant^{-1} (0.22), plant height (0.22), primary branches plant^{-1} (0.26), seed siliqua^{-1} (0.13), 1000-seed weight (0.10), protein content (0.01), glucosinolate content (0.08) and erucic acid (0.09). The total contribution of fifth component were 8.3 %, having 50% flower initiation (0.36), days to maturity (0.51), plant height (0.09), length of main inflorescence (0.16), seed siliquae^{-1} (0.44), oil content (0.08) and glucosinolates content(0.09 (Table 4, Fig. 1). Similar results were also reported by Padilla *et al*. (2005), Padilla *et al*. (2007), Cartea *et al*. (2008), Soenags *et al*. (2008), Kim *et al*. (2010) in *Brassica rapa*; Teshome *et al*. (1997); Ayana and Bekele (1999); Hasanuzzaman *et al*. (2002); Tesso *et al*. (2005); Chozin, (2007); Aruna and Audilakshm (20080; Mujaju and Chakuya (2008); Bib *et al*. (2010) and Ali *et al*. (2011) in sorghum; Ahlawat *et al*., (2008) and Golabadi *et al*. (2006) in wheat.

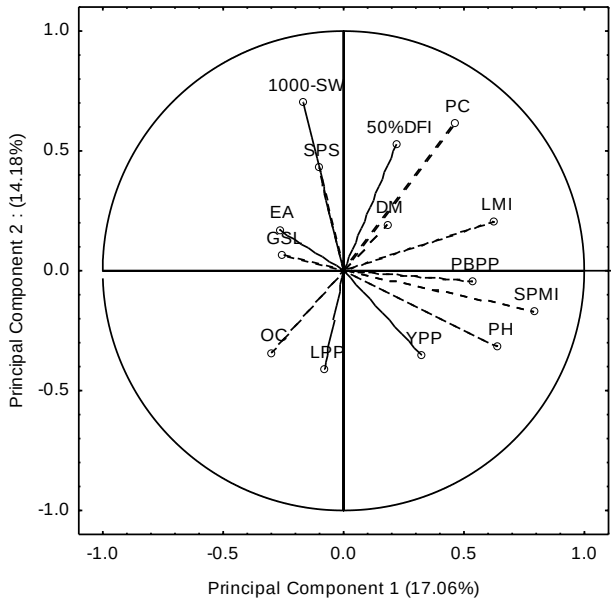

Figure 1. Scatter diagram (traits) of 1st and 2nd PCs for morphological and biochemical traits in Indian mustard genotypes during 2007.

The coefficients defining five principal components of the data for *B. juncea* in 2008 is indicated in Table 5 (Fig. 2). The first principal component had 16.0% of the total variation in the morphological and biochemical characters. PC1 showed primarily variations in leaves plant^{-1} (0.35), plant height (0.20), primary branches plant^{-1} (0.23), length of main inflorescence (0.06) and seed main inflorescence^{-1} (0.32). Second principle component contributed 14.8% of the total variation, having 50% flower initiation (0.14), seed main inflorescence^{-1} (0.32), length of main inflorescence (0.32), seed yield plant^{-1} (0.36), 1000-seed weight (0.05) and oil content (0.30). The component three explained 13.9% of the total variation and was associated with 50% flower initiation (0.45), days to maturity (0.16), plant height (0.49), primary branches plant^{-1} (0.14), length of main inflorescence (0.14) and seed siliqua^{-1} (0.07). The principal component four accounted for 10.5% of divergence, having 50% flower initiation (0.16), days to maturity (0.25), plant height (0.06), seed main inflorescence^{-1} (0.08), 1000-seed weight (0.67) and erucic acid (0.22). The total contribution of fifth component were 9.3% of the total variation having 50% flower initiation (0.04), days to maturity (0.34), leaves plant^{-1} (0.33), primary branches plant^{-1} (0.23), length of main inflorescence (0.39), seed main inflorescence^{-1} (0.37), 1000 seed weight (0.16), protein content (0.33) and Glucosinolates content (0.24). These results are in agreement with earlier findings of Islam and Islam (2000) and Rabbani *et al*. (1998) who evaluated a total of 52 mustard germplasm collected from Pakistan and reported comparatively low level of phenotypic variation among them.

Table 5. Principal components for morphological and biochemical characters in 40 accessions of *Brassica juncea* L during 2008.

	PC_1	PC_2	PC_3	PC_4	PC_5
Eigenvalue	2.25	2.08	1.94	1.47	1.29
Cumulative eigenvalues	2.25	4.32	6.26	7.73	9.02
Proportion of variance	16.04	14.83	13.86	10.48	9.25
Cumulative variance	16.04	30.87	44.73	55.20	64.45
Traits					
Days to 50% flower initiation	-0.28	0.14	0.45	0.16	0.04
Days to maturity	-0.46	-0.10	0.16	0.25	0.34
Leaves plant^{-1}	0.35	-0.04	-0.16	-0.13	0.33
Plant height	0.20	-0.21	0.49	0.06	-0.05
Primary branches plant^{-1}	0.23	-0.24	0.14	-0.02	0.23
Main raceme length	0.06	0.32	0.14	-0.16	0.39
Siliquae main raceme^{-1}	0.32	0.32	-0.21	0.08	0.37
Seed siliqua^{-1}	-0.33	-0.03	0.07	-0.55	-0.05
Seed yield plant^{-1}	-0.24	0.36	-0.05	-0.18	0.40
1000 seed weight	-0.14	0.05	-0.07	0.67	0.16
Oil content	-0.35	0.30	-0.26	-0.12	-0.19
Protein content	-0.23	-0.43	-0.12	-0.04	0.33
Glucosinolates content	-0.13	-0.50	-0.25	-0.15	0.24
Erucic acid content	-0.07	-0.04	-0.51	0.22	-0.17

The dendrogram constructed on the basis of *Brassica juncea* divided the total accessions into five major groups (Fig. 5). Group I had three accessions with early flowering behavior. Group II was the largest and consists of 11 clusters.

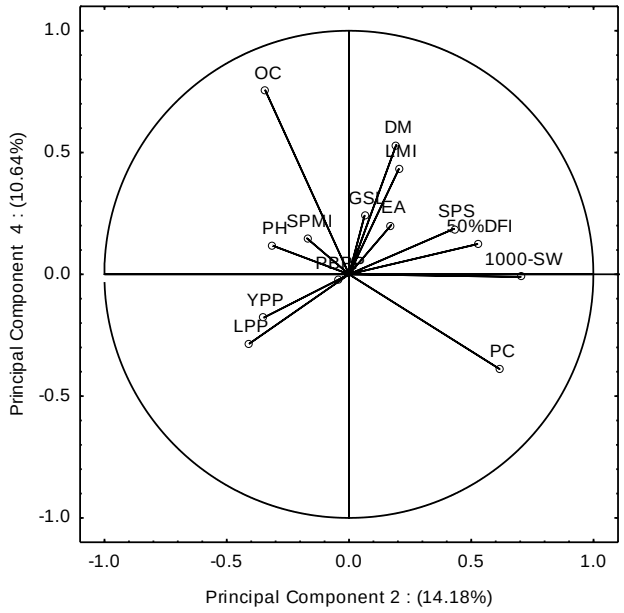

Figure 2. Scatter diagram (traits) of 2nd and 3rd PCs for morphological and biochemical traits in Indian mustard genotypes during 2008.

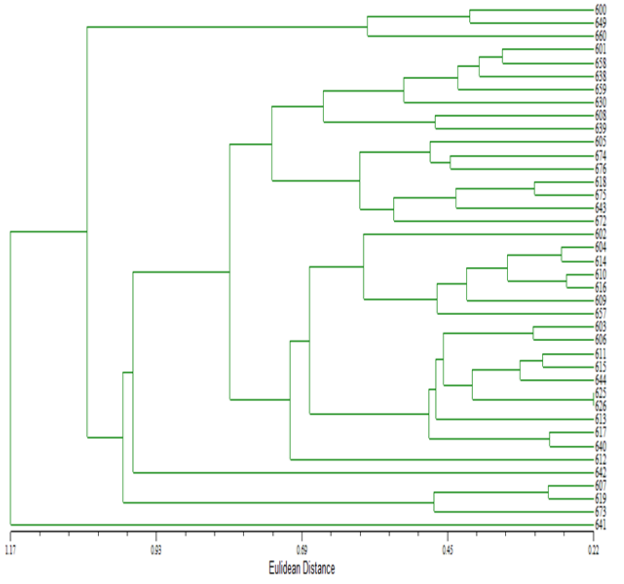

Figure 5. Dendrogram presenting the genetic relationship among different *Brassica juncea* genotypes used in the study during 2007.

Cluster one and eight had five accessions, clusters two and ten had two accessions each, clusters three, four, five, six, seven, nine and eleven had 3, 4, 1, 6, 2, 1 and 1 accession respectively. The accessions part of that group had more yield and branches. Group III was composed of one cluster containing one accession which was different in plant stature from the rest of the accessions. Group IV was divided into two clusters. Cluster one had two accessions and cluster two had only one accession. The accessions in this group were late in maturity and also had low yield. Group V had one cluster and one accession. The accession in this group had more raceme length and also had more siliquaes. Dendrogram constructed on the basis of *Brassica juncea* during 2008, revealed seven major groups (Fig. 6).

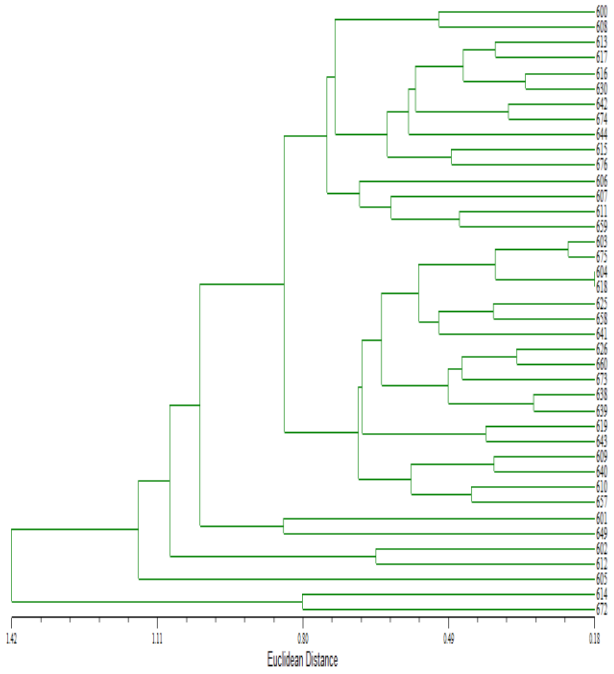

Figure 6. Dendrogram presenting the genetic relationship among different *Brassica juncea* genotypes used in the study during 2008.

Group I had one cluster and two accessions. The accessions in this group were resistant to various abiotic stresses. Group II had four clusters where clusters one and three had two accessions each, cluster two had five accessions, and cluster four had one accessions. Accessions in this group were late maturity and low yielding. Group III, the largest one was divided into ten clusters. Clusters one, two, three, five, seven, eight and nine had two accessions each, clusters four and ten had one accession each and cluster six had four accessions, the accessions in this category were medium maturity and also had high yield. Group IV had one cluster containing one accession. Group V had two clusters where cluster one had two accessions and cluster two had one accession. The accessions in these two groups had more

branches. Similarly, group VI had two clusters where one had one accession and cluster two had two accessions. The accessions in this group were early maturity. Group VII had only one cluster with one accession. The accession in this group was different in pod shape. These results confirmed the clustering pattern of genotypes obtained through principal component analysis. Our findings were supported by the earlier findings of Aytacand Kinaci (2009) and Alemayehu (2001). Similar results are also reported by Jagadev *et al*. (1991), Elizabeth*et al*. (2001), Choudhry and Joshi (2001), Elizabeth *et al*. (2001), Balkaya*et al*. (2005) and Belete (2011) who evaluated different brassica accessions and concluded that groups were primarily associated with morphological differences not its geographically affinity. Our findings are in line with the previous study of Rodriguez *et al*. (2005) who reported genetic diversity among *Brassica napus* genotypes using cluster analysis based on agro morphological traits and they identified 4 groups. Similarly, Wu *et al*. (2007) reported that accessions having high genetic variations for various physio-morphological and seed traits were grouped together. It is also reported that different morphological and yield characters contribute towards genetic divergence in various entries of *Brasica sp*. (Choudhry and Joshi, 2001). Alamayeha and Becker (2002) also suggested that principal component analysis disclosed complex relationships among the accessions and traits, and that yield components and length of growing period contributed maximum towards genetic variation in 36 Ethiopian mustard accessions. The clustering pattern of the genotypes used in the present study showed that collection of the same location can also group into different clusters. The genotypes belonging to different locations were grouped in the same cluster. This shows that geographic diversity was not related to genetic diversity of the genotypes. Our findings were supported by the earlier results of Jahan *et al*. (2013).

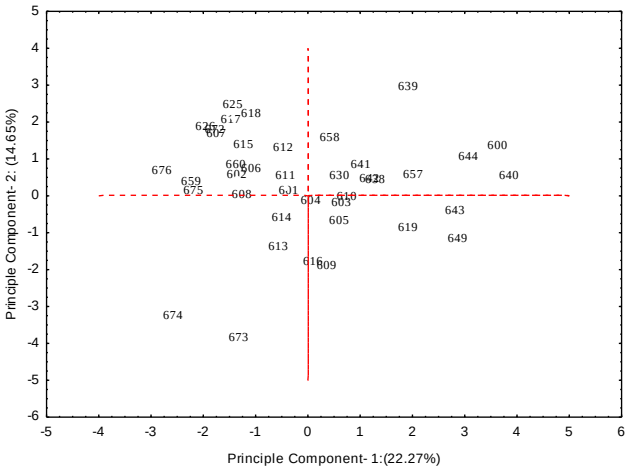

Figure 3. Scatter diagram of first two PCs for morphological and biochemical traits in Indian mustard accessions during 2007.

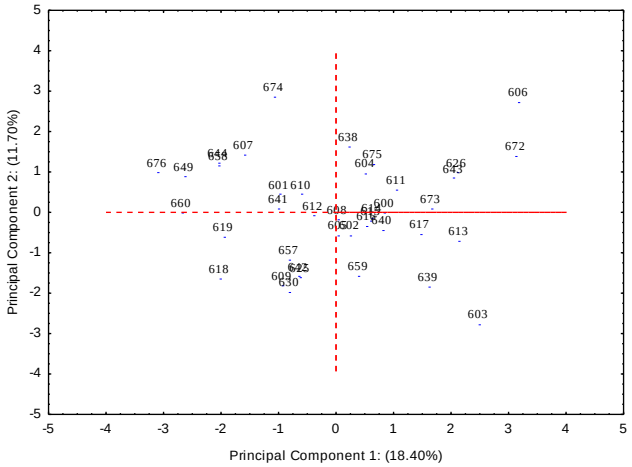

Figure 4. Scatter diagram of 1st and 3rd PCs for morphological and biochemical traits in Indian mustard genotypes during 2008.

The configurations of the 40 *B. juncea* accessions along with the scattered principal component analysis (PCs) axes are shown in Figs. 3 and 4. This study differentiated all the accessions of *B. juncea* studied, and identified 14 morphological traits which were significantly different among the accessions and can be used for further characterization of Brassica accessions. It also identified 4 highly diverse accessions, providing opportunities for optimizing parental sources in future breeding programs to develop new or more productive Brassica varieties. Morphological traits proved useful in assessing the diversity and relationships of *B. juncea* germplasm. On the basis of the overall results it can be concluded that genotypes 603, 639, 673 and 674 were found to be the most distinct genotypes for the studied characters.

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