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Full Length Article

Genetic Diversity in Indigenous Landraces of *Brassica napus* based on Morphological and Biochemical Characteristics using Multivariate Techniques

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Abstract

In order to assess genetic diversity of *Brassica napus* L., the seventy indigenous landraces along with two check cultivars (Shiralee and Pakola) were collected from different regions of Pakistan. The said germplasm was evaluated for 25 morphological and biochemical traits under field conditions during 2012 and 2013 at University of Haripur, Pakistan. Significant genotypic variability was observed among the tested landraces of *B. napus* for majority of traits during both years of studies. Among *B. napus* germplasm, the foremost genetic variability producing traits were, plant height and maturity, while adequate variations were recorded for flower initiation and completion, main raceme length and silique per main raceme and glucosinolate content. During 2012, cluster analysis divided the total 70 landraces of *B. napus* into nine main groups and contribution of five principal components (PCs) was 14.13, 13.11, 10.55, 9.85 and 8.25%, respectively. However, during 2013, the genotypes were categorized in seven main groups and the contribution of five PCs was 22.54, 16.69, 7.91, 6.54 and 6.11%, respectively. Based on two year studies, genotypes i.e., 24876, 24864, 24866 and 24901 were found promising with maximum genetic diversity and may be selected for future breeding program.

Keywords: *Brassica*; Cluster analysis; Earliness; Genetic diversity; Principal component analysis

Introduction

Germplasm of a specific crop collected from the diverse sources offers greater genetic diversity and may furnish useful traits to widen the genetic base of the crop species. The success in the improvement of crops both qualitatively and quantitatively and the development of a crop species requires the availability and accessibility of genetic diversity. Knowing of duplicates, organization of core collection of a particular population and the selection of parental lines for development of new cultivars are directly related to the genetic diversity (Jatoi *et al*., 2011; Yousaf *et al*., 2011; Zada *et al*., 2013).

The family Brassicaceae contains about 350 genera and 3500 species, and is one of the 10 most economically important plant families with a wide range of agronomic traits (Rich, 1991; Love *et al*., 2005). *Brassica napus* L. (AACC, 2n = 38)is a comparatively new species that originated in a limited geographic region through spontaneous hybridizations between turnip rape (*B. rapa* L. AA, 2n = 20) and cabbage (*B. oleracea* L. CC, 2n = 18) genotypes (Kimber and McGregor, 1995). Composition of oil and protein in the seed of mustard (*B. juncea* L. AABB, 2n = 36) and rapeseed (*B. rapa* L. and *B. napus* L.) is 42% and 25%, respectively.

Brassica oil is composed of unsaturated fatty acids and contains fat-soluble vitamins A,D, E and K. After extraction of oil, the oilcake contains proteins of high biological value and applicable quantities of calcium and phosphorus, which is a good source for animal feeding and fertilizer for crops. Among oilseed crops, rapeseed and mustard ranks third in terms of area and production after soybean and cotton (Islam and Islam, 2000; Ali *et al*., 2015). Today, oilseed rape is the most important source of vegetable oil in Europe and the second most important oilseed crop in the world after soybean. However, its limited geographic range and intensive breeding has led to a relatively narrow genetic base. The gene pool of elite oilseed rape breeding material has been further eroded by an emphasis on specific oil and seed quality traits. Consequently, genetic variability in this important crop is restricted with regard to many characters of value for breeding purposes (Khan *et al*., 2013).

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Due to the presence of undesirable long chain fatty acids like erucic acid in the seed oil, it becomes unfavorable to human health. Erucic acid increases blood cholesterol, interferes in myocardial conductance and shortens coagulation time. European economic committee has restricted cultivation of Brassica crop that contains more than 5% erucic acid content in their oil (Zada *et al*., 2013). Several plant breeders throughout the world introduced canola type cultivars by using selection, mutation, conventional breeding and modern biotechnological techniques (Chen *et al*., 1988). Both linoleic and linolenic acids are essential fatty acids; however, less than 3% linolenic acid is preferred for oil stability.

Like any other crop species, the first step in Brassica improvement is evaluation of all the available landraces, including collection, evaluation and molecular characterization of germplasm lines. On average, the locally collected brassica genotypes are more resistant to various biotic and abiotic stresses than already released improved cultivars (Zada *et al*., 2013). Knowledge about germplasm diversity and genetic relationship could be a valuable aid in crop improvement strategies.

Presently, several important methods are used to assess genetic variability in various crop populations. These methods rely on the data of pedigree, agronomic, morphological, and biochemical traits and more recently the molecular data (Mohammadi and Prasanna, 2003). Precise evaluation of the patterns of genetic diversity can be invaluable in crop breeding for diverse applications such as analysis of genetic variability in cultivars (Turi *et al*., 2012), recognizing different parental combinations and introgression of desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998). Genetic distance among canola cultivars was estimated through multivariate analysis, and 30 cultivars from various sources were analyzed and clustered based upon five morphological and yield traits i.e., crown diameter, branches per plant, pods per plant, seeds per pod and yield per plant and placed in three distinct clusters (Ali *et al*., 1995). Keeping in view the importance of *B. napus* L., an attempt was made to determine the genetic diversity in indigenous landraces based on morphological and biochemical characteristics using multivariate techniques.

Materials and Methods

Germplasm and Experimental Procedure

Seventy genotypes including 68 indigenous landraces and two check cultivars i.e., Shiralee and Pakola of *Brassica napus* L. were collected from different regions of four provinces, FATA and Gilgit - Baltistan, Pakistan (Table 1). The said germplasm was evaluated for 25 morphological and biochemical traits under field conditions during 2012 and 2013 at University of Haripur, -Pakistan. Each accession was sown in 2 rows of 5 m length in the 3rd week of September during 2012 and 2013. Row to row and plant to plant spacing was kept at 45 and 10 cm, respectively. Planting of the experiments was done with hand drill and thinning was done after two weeks of germination to maintain optimum plant population. Weeds were controlled manually. Recommended cultural practices and inputs were applied uniformly to all entries during both growing seasons to minimize the field environmental variations. In both seasons, the harvesting was made during first week of March.

Traits Measurement

Data were recorded on 10 randomly selected plant for various traits i.e., days to flower initiation, days to 50% flowering, days to flower completion, days to maturity, leaf petiole length, leaf length, leaf width, leaf width length ratio, leaves per plant, plant height, length of main racemes, silique main per raceme, silique length, silique width, seeds per silique, 1000 seed weight and seed yield per plant. Trait selection and measurement techniques were based on IPGRI descriptors of *Brassica* and *Raphanus* (Table 2). Bulked seed samples from each accession were analyzed to determine fatty acids composition using Near-Infrared Spectroscopy (NIRS) (FOSS NIR Systems Model 6500) equipped with ISI version 1.02 a software of Infra Soft International according to the manufacturer's protocol. A sample size of 2 to 5 g seed was used for biochemical analysis. Analysis of oil content (%), protein content (%), glucosinolates content ($\mu M\ g^{-1}$), oleic acid (%), linolenic acid (%) and erucic acid content (%) was carried out at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan.

Statistical Analysis

Data on various morphological and biochemical traits were subjected to basic statistical analysis to measure the genetic variation in the collected landraces of *B. napus* L. according to Gomez and Gomez (1984). Cluster and principal component analyses were carried out on ten morphological and four biochemical traits for the years 2012 and 2013 separately using NTSYS-pc, version 2.1 (Rohlf, 2002) and STATISTICA-6 program as described by Sneath and Sokal (1973). Basic statistics of all accessions of *B. napus* L. used in the present study is shown in Tables 3 and 4.

Results

Descriptive statistical analysis showed significant differences among the landraces for majority of the studied traits (Tables 3 and 4). During 2012 and 2013, among landraces of *B. napus* L., the maximum genetic variation was recorded for plant height [205.7 cm (ranging from 186.0 to 230.0 cm) and 225.9 cm (ranging from 173.0 to 263.0 cm)],

Table 1: Germplasm (including 68 accessions and two cultivars) of *Brassica napus* used in the present study

Accession #	Location of collection	Plant Type	Maturity	Aphids (Resistant /Susceptible)	Accession #	Location of collection	Plant Type	Maturity	Aphids (Resistant /Susceptible)
24842	Khanpur (KPP)	Erect	Medium	Resistant	24876	Sundas (NA)	Erect	Early	Susceptible
24843	Okara (PP)	Erect	Medium	Susceptible	24877	Shakarghar (PP)	Erect	Early	Susceptible
24844	Dara (KPP)	Erect	Medium	Susceptible	24878	Ghizer (NA)	Erect	Medium	Susceptible
24845	Swabi (KPP)	Erect	Medium	Susceptible	24879	Mardan (KPP)	Erect	Medium	Susceptible
24846	Rawalpindi (PP)	Erect	Medium	Resistant	24880	Attock (PP)	Erect	Medium	Susceptible
24847	Tank (KPP)	Erect	Medium	Susceptible	24881	Khairabad (KPP)	Erect	Medium	Resistant
24848	Islamabad (C)	Erect	Medium	Susceptible	24882	Mattani (KPP)	Erect	Early	Resistant
24849	Haripur West (KPP)	Erect	Medium	Susceptible	24884	Bunner (KPP)	Erect	Early	Susceptible
24850	Nowshera (KPP)	Erect	Medium	Resistant	24885	Takhtbhai (KPP)	Erect	Medium	Susceptible
24851	Batgram (KPP)	Erect	Medium	Susceptible	24886	Pabbi (KPP)	Erect	Medium	Susceptible
24852	Chirat (KPP)	Erect	Medium	Susceptible	24887	Taxila (PP)	Erect	Medium	Susceptible
24853	Mianwali (PP)	Erect	Medium	Susceptible	24888	Sarghoda (PP)	Erect	Early	Susceptible
24854	Bannu (KPP)	Erect	Early	Susceptible	24889	Murree (PP)	Erect	Medium	Susceptible
24855	Pannian (KPP)	Bushy	Early	Resistant	24890	PindiBhatian (PP)	Erect	Medium	Susceptible
24856	Mansehra(KPP)	Erect	Late	Susceptible	24891	Ghizer (NA)	Erect	Medium	Susceptible
24857	Charsada (KPP)	Erect	Medium	Susceptible	24892	Swat (KPP)	Bushy	Medium	Resistant
24858	Shiekhupura (PP)	Erect	Medium	Resistant	24893	Haripur (KPP)	Erect	Early	Susceptible
24859	Batgram (KPP)	Erect	Late	Susceptible	24894	Sheikhan (KPP)	Erect	Early	Susceptible
24860	Rahim Yar Khan (PP)	Erect	Medium	Susceptible	24895	Batgram (KPP)	Erect	Medium	Susceptible
24861	Rsialpur (KPP)	Bushy	Medium	Resistant	24896	Balakot (KPP)	Erect	Medium	Susceptible
24862	Chamkani (KPP)	Erect	Late	Susceptible	24897	Miandam (KPP)	Erect	Medium	Susceptible
24863	Tarnul (PP)	Erect	Medium	Susceptible	24898	Batkhalla (KPP)	Erect	Medium	Resistant
24863	Taxila (PP)	Erect	Early	Susceptible	24899	Shangla (KPP)	Erect	Late	Susceptible
24864	Shabqadar (KPP)	Erect	Medium	Resistant	24900	Azakhail (KPP)	Erect	Medium	Susceptible
24865	Khairabad (KPP)	Erect	Medium	Susceptible	24901	Abakhel (KPP)	Erect	Medium	Susceptible
24866	Kurram Agency (FATA)	Erect	Late	Susceptible	24902	Shahbazkhel (KPP)	Erect	Medium	Susceptible
24867	Mahandri (KPP)	Bushy	Medium	Resistant	24903	Peharkhel 01 (KPP)	Erect	Medium	Susceptible
24868	Kohat (KPP)	Erect	Medium	Susceptible	24904	Rawat (PP)	Erect	Medium	Resistant
24869	Faisalabad (PP)	Erect	Late	Susceptible	24905	Jaglot (NA)	Erect	Late	Susceptible
24870	Miandam (KPP)	Erect	Medium	Susceptible	24906	Sultan abad (NA)	Bushy	Medium	Susceptible
24871	Fateh Jang (PP)	Erect	Medium	Susceptible	24907	Havellain (KPP)	Erect	Medium	Susceptible
24872	Swabi (KPP)	Erect	Medium	Susceptible	24908	Abbottabad (KPP)	Erect	Early	Susceptible
24873	Peshawar (KPP)	Erect	Medium	Susceptible	24909	Dargai (KPP)	Erect	Medium	Resistant
24874	Jhelum (PP)	Erect	Early	Susceptible	Shiralee	NARC, Islamabad	Erect	Medium	Resistant
24875	Rawlakot (AJK)	Erect	Medium	Susceptible	Pakola	NARC, Islamabad	Erect	Medium	Resistant

KPP = Khyber Pakhtunkhwa Province, PP = Punjab Province, C = Capital, FATA = Federally Administered Tribal Areas, AJK = Azad Jammu and Kashmir, NA = Northern Areas

Table 2: Classification of earliness, morphological and yield related traits in *B. napus* L. during 2012 and 2013

Trait designation	Traits description
C. Flowering stage	
Days to flower Initiation	Number of days from seed sowing to the appearance of first open flower
Days to 50% flowering	Days from seed sowing until 50% of plants have the first flower in each accession
Days to flower completion	Number of days taken from date of sowing to the date at which about 95% population in an accession showed bloom.
Leaf petiole length (cm)	Length from the stem to the lamina base including lobes of largest leaf
Leaf length (cm)	Length of largest leaf from the stem to the apex of leaf blade including petiole
Leaf width (cm)	Lamina width across the widest portion of the same leaf used for leaf length
Leaves per plant	Total number of developed leaves or leaf scars on the main stem
Plant height	Plant height was measured in centimeters from the ground level to the tip of the plant with the help of a meter rod for each selected plant
Primary branches per plant	Number of primary branches per plant was recorded from the ground level to the base of main raceme of each selected plant in each accession
Siliqua per main inflorescence	Number of silique main inflorescence^{-1} was obtained by counting the total silique formed from the base to the tip of the main inflorescence
Length of main inflorescence	For this trait data were recorded on middle one inflorescence emerging from main shoot towards top
D. Maturity stage	
Days to maturity	Days taken from seed sowing to the physiological maturity of the crop
Siliqua length (mm)	Measured from the base to the tip of the silique
Siliqua width (mm)	Measured at the thickest portion of the same silique used for SL
Seeds per silique	Counted as total number of seeds of same pod used for SL
Seed yield per plant	Seed yield per plant was recorded by threshing all silique from the selected plants and weighing them on single plant basis using electronic balance in each accession
1000-seed weight (g)	Weight of 1000 dry seeds

followed by days to flowering [120.8 days (ranging from 99.0 to 133.0 days) and 101.2 days (ranging from 70.0 to 131.0 days)] and days to maturity [166.8 days (ranging from 142.0 to 181.0 days) and 165.2 days (ranging from 158.0 to 179.8 days)], respectively. However, low variation was noticed for rest of the traits particularly

seed oil quality traits. Generally, accessions varied for several traits of economic importance, but also showed dissimilar pattern of variation for various morphological and yield traits.

During 2012, cluster constructed for 70 genotypes (including two check cultivars i.e., Pakola and Shiralee) of *B. napus* L. was further divided into nine main groups (Fig. 1). Each group was further subdivided into clusters. Group-I had three sub-clusters and comprised of 25 genotypes. The genotypes included in this group were early in maturity, and have long main raceme length, moderate plant height, long silique length and moderate oil and protein content. Group-II was subdivided into two clusters, and cluster one comprised of four genotypes, while cluster two had 14 genotypes. These genotypes were having moderate maturity and plant height, long silique length, more seeds per silique, bold seeds and moderate oil and protein contents (Table 8).

Group-III comprised of four clusters, and among them cluster one had two genotypes, cluster two had seven genotypes, and clusters three and four owned two and eight genotypes, respectively (Fig. 1). Features of the genotypes in this group were; tall plants, more branches, long main raceme length, more silique per plant, long silique length, more seeds per silique and seed yield, high oil and oleic acid content (Table 8). Group-IV and V contained one each cluster and four and three genotypes, respectively, and distinguishing traits of these genotypes were late maturity, more branches, silique per main raceme, seeds per silique, bold seeds, and more seed yield, while low glucosinolates, high erucic acid and oleic acid (Table 8). Groups VI, VII and IX had one each cluster and one genotype, while Group VIII had one cluster and two genotypes. The genotypes included in these groups were late in maturity, with dwarf plant height, low seed yield, and high protein and glucosinolate contents.

During 2013, the composition of cluster constructed for *B. napus* L. 70 genotypes including two check cultivars revealed seven main groups (Fig. 2). Group-I was further divided into four sub-clusters and among them cluster one has four genotypes, cluster two has two, three has six and cluster four has four genotypes and these genotypes were had early maturity, long raceme length, more seeds per silique, high glucosinolate content and moderate seed yield, oil and protein content (Table 9). Group-II has one cluster and five genotypes which were moderate for plant height, long main raceme length, moderate silique per main raceme, bold seeds, high glucosinolate and erucic contents, moderate oil and protein contents. Genotypes falling in group-III, were early maturing, tall, more branches, long siliques, more seeds per silique, high seed yield and protein (Table 9).Genotypes in group-IV were having more branches, long raceme length, bold seed, high oil and oleic acids, while low glucosinolate and linoleic acid. Group-V comprised of four sub-clusters, and six genotypes were placed in cluster one,

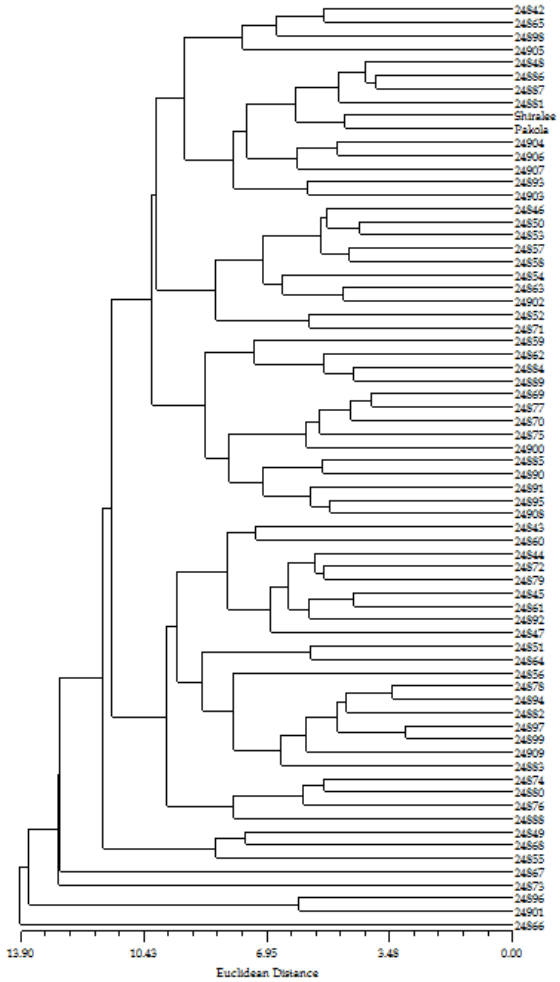

Fig. 1: Dendrogram presenting genetic relationship among 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. during 2012

cluster two and three each had four genotypes, while cluster four had three genotypes and distinguishing features were late maturity, tall plants, long main raceme length and silique length, more seeds per silique, high oil and oleic acid (Table 9). Group-VI was having four sub-clusters, and among them, he cluster one had four genotypes, cluster two had two genotypes, while clusters three and four had three each genotypes. Genotypes included in this group revealed long silique length, moderate seeds per silique and seed yield, high oil and oleic acid contents. Group-VII had four clusters, cluster one had one genotype, clusters two and three had four each genotypes, and cluster four had two genotypes with late maturity, high erucic acid and low oleic acid content.

Principal component analysis (PCA) is a data analysis tool usually used to reduce the dimensionality (number of variables) of a large number of inter-related variables, while retaining as much of the information (variation) as possible.

Table 3: Means and variances for earliness, morphological, yield and biochemical traits in *B. napus* L. during 2012

Traits	Mean	Minimum	Maximum	Variance	SD	CV (%)
Days to flower initiation	65.3	49.0	89.0	10.0	15.4	17.4
Days to 50% flowering	97.4	65.0	112.0	8.3	8.5	14.5
Days to flower completion	120.8	99.0	133.0	7.9	6.6	17.2
Days to maturity	166.8	142.0	181.0	8.3	5.0	3.1
Leaf petiole length	15.0	3.9	23.2	4.9	32.6	29.7
Leaf length	25.2	13.9	34.0	3.8	15.3	23.5
Leaf width	10.5	4.3	16.0	2.5	23.6	39.6
Leaf length/width ratio	2.5	1.2	4.5	0.6	25.3	24.2
Leaves/plant	20.5	14.0	28.0	3.0	14.8	13.2
Plant height	205.7	186.0	230.0	11.1	5.4	6.5
Primary branches/plant	11.4	8.0	14.0	1.7	15.0	23.2
Main raceme length	65.1	39.0	81.0	11.2	17.1	17.4
Silique/main raceme	58.1	29.0	83.0	12.0	20.6	21.3
Silique length	7.5	6.8	8.5	0.4	5.8	16.3
Silique width	0.6	0.2	0.9	0.2	29.1	14.4
Siliqua length/width ratio	14.6	7.6	37.0	5.2	35.9	19.3
Seeds/silique	26.2	20.0	32.0	3.4	13.1	23.2
Seed yield/plant	45.7	22.5	103.7	14.9	32.7	30.4
1000-seed weight	3.5	3.2	4.1	0.2	5.3	26.8
Oil content	45.5	29.8	51.9	4.0	8.8	6.1
Protein content	25.6	21.2	30.3	2.4	9.2	7.3
Glucosinolates	100.8	12.7	163.6	27.1	26.9	22.3
Oleic acid	42.8	26.8	56.3	7.5	17.4	15.7
Linoleic acid	11.4	9.5	15.2	1.5	12.7	14.3
Erucic acid	53.8	21.6	60.9	8.5	15.7	16.3

Table 4: Means and variances for earliness, morphological, yield and biochemical traits in *B. napus* L. during 2013

Traits	Mean	Minimum	Maximum	Variance	SD	CV (%)
Days to flower initiation	68.6	47.0	93.0	164.3	12.8	18.7
Days to 50% flowering	86.4	59.0	120.0	186.2	13.6	15.8
Days to flower completion	101.2	70.0	131.0	230.8	15.2	15.0
Days to maturity	165.2	158.0	179.8	18.0	4.2	2.6
Leaf petiole length	17.9	6.9	33.3	35.0	5.9	33.0
Leaf length	41.9	13.6	61.5	109.3	10.5	25.0
Leaf width	14.8	6.7	61.2	39.2	6.3	42.1
Leaf length per width ratio	2.9	0.7	4.6	0.4	0.7	22.3
Leaves per plant	20.0	12.0	28.0	10.7	3.3	16.4
Plant height	225.9	173.0	263.0	343.8	18.5	8.2
Primary branches per plant	11.7	6.0	18.0	6.1	2.5	21.1
Main raceme length	76.3	46.0	96.0	157.1	12.5	16.4
Siliqua per main raceme	73.8	28.0	102.0	195.1	14.0	18.9
Siliqua length	6.2	2.7	9.2	1.1	1.1	17.2
Siliqua width	0.5	0.3	0.6	0.0	0.1	11.9
Siliqua length per width ratio	13.4	8.9	18.8	4.3	2.1	15.4
Seeds per siliqua	25.1	4.6	34.0	26.3	5.1	20.4
Seed yield per plant	50.7	25.0	108.7	277.4	16.7	32.8
1000-seed weight	3.2	1.8	5.1	0.8	0.9	28.2
Oil content	46.4	39.9	53.3	6.3	2.5	5.4
Protein content	25.0	19.2	31.9	4.4	2.1	8.4
Glucosinolates	96.9	49.2	152.0	564.6	23.8	24.5
Oleic acid	46.6	29.0	60.1	48.2	6.9	14.9
Linoleic acid	9.3	6.7	14.8	2.3	1.5	16.3
Erucic acid	49.3	15.3	74.2	93.7	9.7	19.6

Moreover, PCA calculates an unrelated set of variables (factors or principal components) and gives supplementary information on usefulness of characters for definition of groups. When the PCA is run on correlations, one rule-of-thumb is to retain those factors whose eigenvalues are greater than one.

Principal component analysis constructed based on various quantitative traits in *B. napus* L. produced very informative outcome of the studied genotypes (Fig. 3 and 4). The coefficients defining five principal components for *B. napus* L. grown during 2012 are given in Table 5. These coefficients were scaled to observe correlation between observed variables and derived components. The first principal component had 14.13% of the total variation in morphological and biochemical characters.

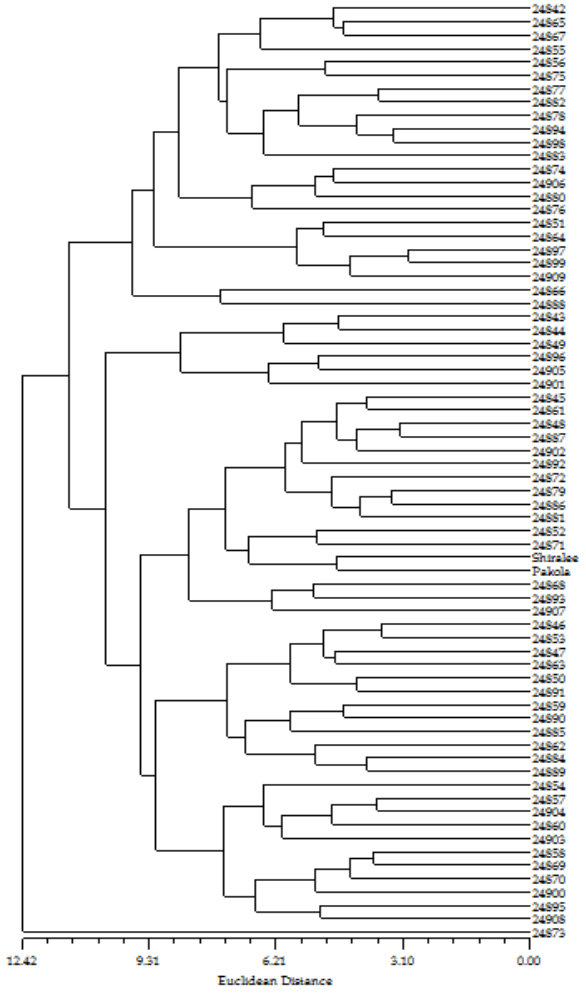

Fig. 2: Dendrogram presenting genetic relationship among 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. during 2013

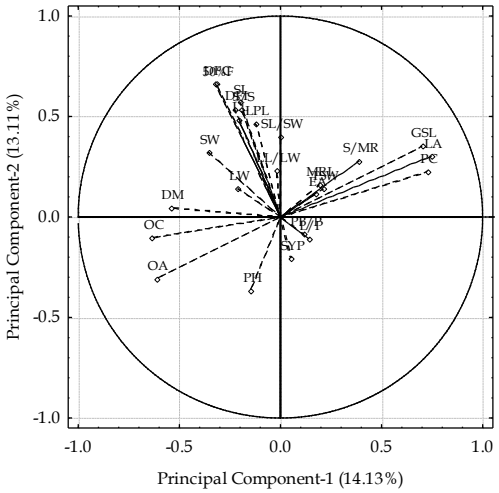

Fig. 3: Contribution of quantitative and qualitative traits in 1st and 2nd PCs in 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. during 2012

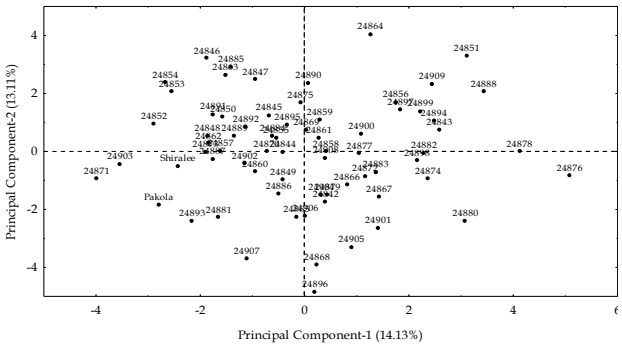

Fig. 4: Scatter diagram for 1st and 2nd PC for 25 earliness, morphological, yield and biochemical traits in 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. during 2012

In PC1, demonstrated mainly the variations in linoleic acid (0.40), protein (0.39) and glucosinolates (0.37) contributed positively. Contributions of leaf length/leaf width ratio (-0.01), leaf petiole length (-0.06) and plant height (-0.08) were negative (Table5).

The PC2 accounted for an additional13.1%, having 50% flowering (0.37). Flower completion (0.37) and silique length (0.32), while primary branches (-0.05), oil content (-0.06) and oleic acid (-0.17) contributed negatively (Table 5). The variation in PC3 was mainly attributed to10.55% of the total variation and comprised of silique length (0.36), silique length per width ratio (0.33) and seeds per silique (0.33), whereas glucosinolates (-0.02), oil content (-0.03) and flower initiation (-0.04) had negative contribution (Table 5).

The fourth principal component accounted for 9.85% of divergence, having flower initiation (0.44), 50% flowering (0.39) and days to flower completion (0.32) but inversely with linoleic acid (-0.01), silique width (-0.06) and silique main per raceme (-0.08)(Table 5). The PC5 accounted for an additional 8.25% of the total variation having glucosinolates (0.31), oil content (0.28) and erucic acid (0.23). On the other hand, seed per silique (-0.01), plant height (-0.02) and 50% flowering (-0.03) had negative values (Table 5).

The coefficients defining five principal components for *B. napus* L. genotypes grown during second growing season 2013 are given in Table 6 (Fig. 5 and 6). These coefficients were scaled, to observe correlations between observed variables and derived components. The PC1 had 22.54% of the total variation in morphological and biochemical characters.

In PC1, flower completion (0.32), 50% flowering (0.28) and plant height (0.27) contributed positively, while protein content (-0.01), leaf length per leaf width ratio (-0.04) and 1000-seed weight (-0.05) contributed negatively (Table 6).

Table 5: Principal components analysis for earliness, morphological, yield and biochemical traits in *B. napus* L. during 2012

	PC1	PC2	PC3	PC4	PC5
Eigenvalues	3.53	3.28	2.64	2.46	2.06
Cumulative eigenvalues	3.53	6.81	9.45	11.91	13.97
Variance %	14.13	13.11	10.55	9.85	8.25
Cumulative variance	14.13	27.25	37.80	47.65	55.90
Traits	Eigenvectors				
Days to flower initiation	-0.12	0.30	-0.04	0.44	0.09
Days to 50% flowering	-0.17	0.37	0.02	0.39	-0.03
Days to flower completion	-0.17	0.37	0.03	0.32	-0.07
Days to maturity	-0.29	0.03	0.01	-0.03	0.14
Leaf petiole length	-0.06	0.26	-0.31	-0.21	-0.15
Leaf length	-0.11	0.27	-0.41	-0.20	-0.12
Leaf width	-0.11	0.08	0.03	-0.24	-0.31
Leaf length per width ratio	-0.01	0.13	-0.39	0.00	0.12
Leaves per plant	0.08	-0.06	0.29	-0.05	-0.17
Plant height	-0.08	-0.20	0.06	-0.02	-0.02
Primary branches per plant	0.06	-0.05	0.02	0.19	-0.29
Main raceme length	0.10	0.09	-0.20	-0.09	-0.36
Siliqua per main raceme	0.21	0.16	-0.10	-0.08	-0.38
Siliqua length	-0.11	0.32	0.36	-0.26	0.02
Siliqua width	-0.19	0.18	0.11	-0.06	0.17
Siliqua length per width ratio	0.00	0.22	0.33	-0.25	-0.10
Seeds per siliqua	-0.10	0.30	0.33	-0.18	-0.01
Seed yield per plant	0.03	-0.11	0.19	0.31	-0.06
1000-seed weight	0.11	0.08	-0.11	0.12	0.14
Oil content	-0.34	-0.06	-0.03	-0.18	0.28
Protein content	0.39	0.13	0.11	0.13	-0.12
Glucosinolates	0.37	0.20	0.01	-0.04	0.31
Oleic acid	-0.33	-0.17	-0.02	0.12	-0.33
Linoleic acid	0.40	0.17	0.03	-0.01	0.10
Erucic acid	0.09	0.06	-0.15	-0.16	0.23

Table 6: Principal components analysis for earliness, morphological, yield and biochemical traits in *B. napus* L. during 2013

	PC1	PC2	PC3	PC4	PC5
Eigenvalues	5.63	4.17	1.98	1.63	1.53
Cumulative eigenvalues	5.63	9.81	11.78	13.42	14.95
Variance %	22.54	16.69	7.91	6.54	6.11
Cumulative variance	22.54	39.23	47.14	53.67	59.79
Traits	Eigenvectors				
Days to flower initiation	0.21	0.34	-0.14	-0.14	0.05
Days to 50% flowering	0.28	0.29	-0.10	-0.14	0.06
Days to flower completion	0.32	0.25	-0.07	-0.11	0.05
Days to maturity	0.28	0.21	0.15	0.13	-0.10
Leaf petiole length	0.25	-0.14	0.19	-0.14	-0.27
Leaf length	0.19	-0.10	0.20	-0.10	-0.33
Leaf width	0.15	0.11	0.06	0.39	0.25
Leaf length per width ratio	-0.04	-0.22	0.01	-0.46	-0.48
Leaves per plant	0.24	0.11	-0.19	-0.11	0.05
Plant height	0.27	0.12	0.08	0.18	-0.14
Primary branches per plant	0.14	-0.07	-0.21	-0.13	0.03
Main raceme length	0.12	-0.18	0.22	0.37	-0.21
Siliqua per main raceme	0.17	-0.22	0.20	0.35	-0.09
Siliqua length	0.24	-0.20	0.26	-0.18	0.33
Siliqua width	0.21	-0.12	0.28	-0.16	0.13
Siliqua length per width ratio	0.14	-0.16	0.06	-0.11	0.34
Seeds per siliqua	0.22	-0.24	0.25	-0.16	0.28
Seed yield per plant	0.18	0.20	-0.06	0.02	-0.08
1000-seed weight	-0.05	0.09	0.17	0.26	-0.12
Oil content	-0.04	-0.30	-0.14	0.02	0.19
Protein content	-0.01	0.29	0.29	-0.16	-0.08
Glucosinolates	-0.22	0.20	0.42	-0.12	0.06
Oleic acid	0.27	-0.17	-0.27	0.10	-0.14
Linoleic acid	-0.15	0.26	0.31	-0.02	-0.02
Erucic acid	-0.19	0.03	0.11	-0.15	0.15

Second principal component contributed variation of 16.69%, having flower initiation (0.34), 50% flowering (0.29), protein content (0.29), while primary branches (-0.07), leaf petiole length (-0.14) and siliqua length per width ratio (-0.16) had contributed negatively. The PC3resulted with 7.91% of the total variation and comprised of glucosinolates content (0.42), linoleic acid (0.31) and silique width (0.28), whereas seed yield per plant (-0.06), flower completion (-0.07) and 50% flowering (-0.10) had negative contribution (Table 6).

The PC4 accounted for 6.54% of divergence, having leaf width (0.39), main raceme length (0.37) and silique main per raceme (0.35) but inversely with linoleic acid (-0.02), leaf length (-0.10) and flower completion (-0.11). The total contribution of fifth component was 6.11% of the total variation having silique length per width ratio (0.34), silique length (0.33) and seeds per silique (0.28). On the other hand, the linolenic acid (-0.02), protein content (-0.08) and seed yield per plant (-0.08) had negative values (Table 6).

Principal component analysis based on *B. napus* L. genotypes showed different grouping pattern, indirectly supporting cluster analysis (Fig. 4 and 6). The separation based on PC1 and PC2 revealed that the genotypes were distributed in all the directions, which clearly identified the diversity of the experimental materials (Fig. 4 and 6). In the first and second principal components, the genotypes 24864, 24851, 24888, 24876, 24878, 24880, 24896, 24907 and 24871 were the most variable ones among the studied genotypes. In the first and third principal components, genotypes 24866, 24876, 24878, 24901, 24871 and 24903 showed greater genetic diversity. The determined variability can be used in the in the future breeding programs to preserve the precious germplasm from genetic erosion. Promising genotypes (24847, 24866, 24850, 24877 and 24843) were identified with potential genes of interest to improve earliness, yield components and oil content (Table 7).

Overall, the results revealed that significant differences were observed among the 70 landraces with considerable level of genetic variability for majority of the traits. Major variability producing traits were plant height and time to maturity, while adequate variations were recorded for flower completion, 50% flower initiation, glucosinolate content, main raceme length and silique per main raceme.

During 2012, cluster analysis divided the total 70 landraces into nine main groups and contributions of five principal components. In 2013, cluster for above genotypes was categorized into seven main groups and the contributions of five principal components. Accessions used in the present study produced a reasonable level of variations for various traits.

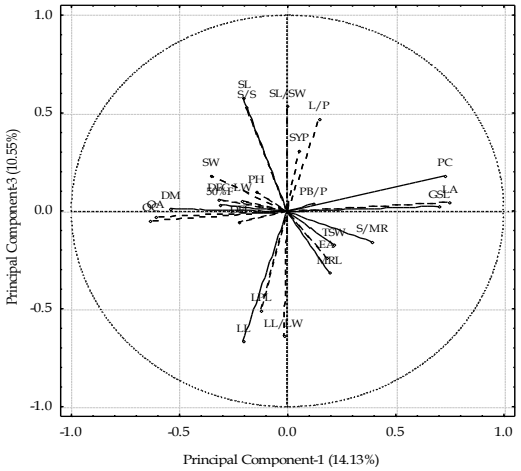

Fig. 5: Contribution of quantitative and qualitative traits in 1st and 2nd PCs in 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. during 2013

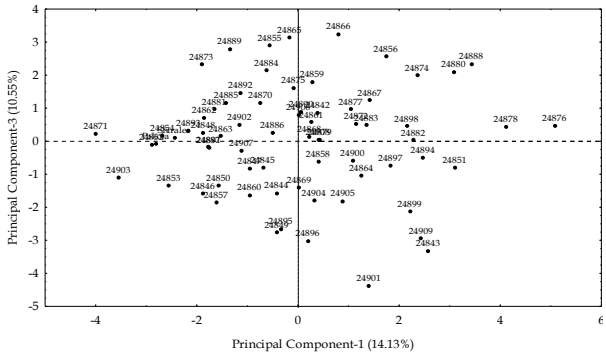

Fig. 6: Scatter diagram for 1st and 2nd PC for earliness, morphological, yield and biochemical traits in 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. during 2013

Discussion

Morphological characterization is the first step in description and classification of genetic resources (Arslanoglu *et al*., 2011). Landraces are open pollinated populations whereas commercial cultivars are hybrids selected for uniformity and production synchrony, and been improved using only primitive mass selection. In order to choose the best genotypes for the inclusion in breeding programs, the landraces with significant genetic variations should be selected. However, for characters related to crop uniformity in brassica, the commercial cultivars and hybrids did better than landraces of brassica (Padilla *et al*., 2007; Ali *et al*., 2015). For centuries, farmers through mass selection have improved populations/landraces; however, their cultivation is reducing than entire crop area. Compared with commercial hybrids, the local populations of brassica are less productive and lack uniformity and field durability however, these brassica populations are valuable genetic resources and should be released as commercial cultivars after evaluation, and to be conserved in the gene bank for future breeding programs (Balkaya *et al*., 2005).

In present studies, significant variations were observed in indigenous landraces of *B. napus* L. for plant height, days to flower completion and maturity followed by yield related traits and oil quality parameters. Variations for seed yield followed by plant height, glucosinolates content, main raceme length, and silique per main raceme were observed in various populations of *B. carinata* L. (Zada *et al*., 2013). Similarly, some past studies revealed significant variation among various landraces of *B. campestris* L. (Yousaf *et al*., 2011) and *S. indicum* L. (Akbar *et al*., 2011) for seed yield and oil quality traits. The 509 *B. napus* L. inbred lines genotyped with 89 genome-specific SSR primer combinations, and principal coordinate analysis and software STRUCTURE revealed that genetic diversity of winter oilseed rape was lower than the diversity found in other germplasm types (Bus *et al*., 2011).

During growing seasons of 2012 and 2013, cluster constructed for *B. napus* L. 70 genotypes revealed nine and seven main major groups, respectively. These groups further sub-divided in different sub-clusters/groups and showed ample genetic variability through different traits i.e., plant height, main raceme length, silique per main raceme, seed size, oil, protein, glucosinolate and erucic acid content. In accessions of *B. juncea* L., scattered plot and tree diagrams demonstrated sufficient diversity for various morphological and biochemical traits and some extent of association was reported between different clusters (Ali *et al*., 2015).A set of 96 *B. napus* L. genotypes was characterized using SSR markers, and allelic information from 30 SSR primer combinations amplifying 220 alleles at 51 polymorphic loci and provided unique genetic fingerprints for all genotypes (Hasan *et al*., 2006).

In past findings, maximum genetic variation was observed in various genotypes of Indian mustard (*B. juncea* L.) for morphological, seed yield and oil quality traits (Rabbani *et al*., 1998a). From hierarchical cluster analysis, it was authenticated that genotypes collected from the same origin were not necessarily falling in the same cluster and had low diversity; rather the cluster analysis was more influenced by morphological traits than accession origin. Present findings also revealed that geographic origin of the *B. napus* accessions had no significant effect on varied grouping of the genotypes. Therefore, cluster is mostly made on traits basis and not based on origin in various Brassica populations (Gupta and Pratap, 2007; Hu *et al*., 2007; Rabbani *et al*., 1998b; Dhillon *et al*., 1999). However, it is also not necessary that the accessions/genotypes grouping always made because of their morphological traits, though some researchers were inconsistent with this opinion. Dias *et al.* (1993) grouped populations of Portuguese cabbage (*B. oleraceaacephala*) and Galega kale (*B. oleracea* var. acephala cv. Galega) based on their geographical origin and morphological differences.

Table 7: Main characteristics of the 70 genotypes (including 68 indigenous landraces and two cultivars) of *B. napus* L. belonging to different clusters during 2012 and 2013

Trait of interest	Range	Accessions identified
Days to maturity	<160	24876, 27877, 24882, 24894, 24908, 24874, 24854, 24869 and 24888
Primary branches	≥ 15	24847, 24849, 24859, 24872, 24879, 24878, 24891, 24848, 24860, 24865 and 24900
Main raceme length	≥ 90	24851, 24863, 24872, 24878, 24880, 24885, 24905, 24906 and Shiralee
Silique per main raceme	≥90	24843, 24860, 24909, 24895, 24844, 24877, 24884, 24898 and Pakola
Silique length	≥7	24847, 24848, 24892, 24890, 24888, 24877, 24874, 24873, 24870 and Pakola
Seed per silique	≥28	24844, 24845, 24851, 24854, 24855, 24856, 24859, 24885, 24888 and 24892
Seed yield per plant	≥80	24866, 24868, 24882, 24889, 24896, 24883, 24880, 24870 and 24859
1000-ssed weight	≥4	24843, 24846, 24849, 24850, 24864, 24868, 24869, 24876, 24877, 24891, 24898 and Pakola
Oil content	≥50	24850, 24852, 24857, 24871, 24893, 24882, 24897 and Shiralee
Protein content	≥27	24843, 24851, 24874, 24880, 24884, 24888, 24908 and 24872
Oleic Acid	≥50	24844, 24849, 24854, 24861, 24868, 24870, 24881, 24890, 24891, 24907, 24908, Pakola and Shiralee

Table 8: Mean values and standard deviation (SD) of six clusters based on earliness, morphological, yield and biochemical traits in *B. napus* L. during 2012

Traits	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days to flower initiation	66.84	10.29	63.21	8.09	83.00	16.09	82.00	19.40	82.00	17.37	67.60	12.70
Days to 50% flowering	96.60	6.54	97.93	5.14	111.00	7.91	104.00	8.62	104.00	8.80	99.00	8.40
Days to flower completion	119.72	8.22	120.86	5.83	133.00	5.09	127.00	5.00	127.00	3.88	123.20	4.60
Days to maturity	166.24	9.04	168.93	6.16	181.00	4.16	177.00	6.43	177.00	4.35	170.80	1.64
Leaf petiole length	15.65	5.62	15.11	4.66	23.00	46.44	17.00	29.55	14.90	25.44	12.82	4.25
Leaf length	25.88	3.62	24.94	3.35	32.10	17.92	29.90	18.34	29.90	20.48	23.64	2.53
Leaf width	10.09	1.99	10.31	1.98	14.00	25.48	12.80	26.06	8.80	4.92	11.70	4.64
Leaf length per width ratio	2.65	0.57	2.51	0.58	3.81	25.66	2.20	2.71	2.20	13.23	2.29	0.85
Leaves per plant	19.92	2.75	21.50	3.01	28.00	16.41	21.00	3.67	21.90	4.74	18.20	4.60
Plant height	205.12	11.84	205.21	8.36	216.00	4.92	213.00	13.48	214.00	207.84	198.60	15.60
Primary branches per plant	11.08	1.53	11.71	1.68	14.00	16.17	17.00	4.68	15.00	3.83	11.80	2.68
Main raceme length	65.16	8.23	58.21	15.06	80.00	13.73	58.00	1.75	62.00	4.48	63.80	17.38
Siliqua per main raceme	56.64	10.64	58.29	14.69	83.00	22.99	87.50	2.86	74.40	2.11	53.80	6.76
Siliqua length	7.50	0.46	7.40	0.38	8.00	5.94	8.40	15.75	7.60	35.25	7.56	0.26
Siliqua width	0.54	0.17	0.63	0.16	0.90	0.72	23.67	0.28	0.23	31.48	0.48	0.08
Siliqua length per width ratio	15.65	6.10	12.85	4.82	26.67	38.98	29.00	13.68	29.00	5.52	16.06	2.29
Seeds per siliqua	25.68	33.44	50.59	20.92	30.00	11.39	33.40	1.19	35.40	3.31	26.60	4.10
Seed yield per plant	40.23	10.26	43.51	0.17	75.80	28.55	73.50	2.94	79.0	8.57	49.88	23.15
1000-seed weight	3.49	0.17	4.16	3.45	3.90	4.29	4.30	2.39	4.10	3.70	3.34	0.15
Oil content	44.99	4.71	46.34	2.71	46.40	9.23	41.40	1.46	45.80	4.64	46.90	3.91
Protein content	25.86	1.97	27.79	27.12	23.20	20.87	27.90	6.47	31.90	19.24	24.12	1.73
Glucosinolates	96.84	29.02	69.17	6.84	56.30	11.74	54.50	3.83	54.50	10.60	97.10	25.45
Oleic acid	43.28	7.42	41.84	1.66	51.90	8.09	47.80	10.84	51.80	12.33	40.72	7.60
Linoleic acid	11.33	1.42	14.74	7.30	20.90	18.52	14.20	21.34	19.10	24.59	11.02	1.14
Erucic acid	52.68	9.83	63.21	8.09	83.00	16.09	82.00	19.40	82.00	17.37	57.04	2.64

There were certain relationships between geographical origin of germplasm collections of *B. rapa* subsp. *rapa* L. from northwestern Spain and the groups formed on basis of origin (Padilla *et al*., 2005). Similarly, Cartea *et al*. (2003) reported that landraces of *B. oleracea* have stronger relation by geographical origin rather than with morphological differences.

Grouping of some of the genotypes based on various ecological regions exhibited the association between morphological characters and geographical origin, indicated regional similarities in selection criterion, easy exchange of germplasm between neighboring regions and perhaps identical ancestors within these regions. Similarly, Abideen *et al.* (2013) and Rabbani *et al.* (1998a) findings revealed that cluster/grouping of *B. napus* L. and *B. juncea* L. genotypes was always made on traits basis, and which verified and strengthen the present phenomenon. Similarly, Rodriguez *et al.* (2005) also did classification based on characters and not based on their origin in 36 populations of *B. napus*L. var. *pabularia* crop. Balkaya *et al*. (2005) also concluded that there was no relationship between the morphological characters and geographical origin of the white head cabbage (*B. oleracea* L.) genetic resources from Turkey. They reported that clustering of the different genotypes was based on their morphological values of hidden characters. Present results were further supported by the past findings about genetic diversity in *B. campestris* L., and *B. napus* L. (Chauhan *et al*., 2008). Amurrio *et al.* (1995) using Iberian pea landraces based on quantitative and qualitative characters, concluded that cluster groups were not related to the geographical origin of pea accessions.

Table 9: Mean values and standard deviation (SD) of six clusters based on earliness, morphological, yield and biochemical traits in *B. napus* L. during 2013

Traits	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days to flower initiation	62.38	10.51	74.40	12.92	61.00	0.00	55.67	10.11	63.11	8.08	74.43	13.64	77.21	15.30
Days to 50% flowering	78.50	9.39	91.20	11.95	90.00	2.83	74.50	10.78	79.70	8.52	94.29	14.12	110.00	14.10
Days to flower completion	90.25	10.37	106.80	12.07	116.00	1.41	90.33	14.22	94.83	11.82	108.52	15.04	122.11	15.00
Days to maturity	163.25	3.61	165.20	3.27	163.50	3.54	165.67	2.94	168.27	4.69	165.71	3.87	188.20	13.21
Leaf petiole length	13.49	4.21	21.34	2.79	19.45	0.78	20.08	9.78	17.66	4.99	20.70	5.45	14.01	12.22
Leaf length	34.66	10.28	52.56	5.20	31.10	17.54	48.75	11.62	44.12	8.58	44.73	8.28	39.22	13.21
Leaf width	15.59	12.30	14.74	1.78	14.45	1.91	13.92	4.51	15.17	3.84	17.09	10.36	17.78	14.44
Leaf length per width ratio	2.56	0.76	3.58	0.34	2.09	0.94	3.67	0.72	3.02	0.60	2.93	0.73	2.44	0.73
Leaves per plant	21.19	3.10	17.80	3.27	24.00	1.41	18.50	4.23	20.20	3.53	18.86	2.63	24.6	13.29
Plant height	225.31	17.36	201.80	8.32	237.00	5.66	227.33	16.60	228.95	15.44	228.71	21.05	250.1	17.77
Primary branches per plant	11.00	2.16	11.40	1.82	13.00	1.41	13.33	1.63	12.39	2.33	11.57	2.58	10.90	2.22
Main raceme length	73.69	15.93	81.60	7.50	73.50	14.85	79.33	7.79	78.70	8.49	71.95	14.97	69.32	14.87
Siliqua per main raceme	76.00	10.84	79.60	9.21	92.00	4.24	79.67	20.34	70.45	13.52	72.76	17.43	64.45	11.21
Siliqua length	6.36	1.10	6.18	0.79	6.55	0.92	5.20	0.97	6.27	0.88	6.28	0.68	6.23	2.11
Siliqua width	0.47	0.05	0.46	0.04	0.45	0.01	0.47	0.08	0.47	0.07	0.49	0.05	0.44	0.03
Siliqua length per width ratio	13.69	2.31	13.69	2.85	14.59	2.50	11.09	1.71	13.57	2.18	12.98	1.97	11.78	2.98
Seeds per siliqua	26.25	3.42	25.00	1.58	29.00	1.41	22.33	7.71	26.30	3.25	25.24	2.77	23.22	3.80
Seed yield per plant	48.96	17.68	44.24	9.51	90.05	26.38	48.75	15.37	49.97	12.01	53.39	16.64	46.99	18.11
1000-seed weight	3.10	0.82	3.96	0.68	2.50	0.42	3.38	1.07	2.98	0.81	3.19	1.05	2.90	0.80
Oil content	45.89	2.46	45.94	1.16	42.35	2.33	46.73	1.82	47.31	3.00	46.93	2.06	41.0	21.34
Protein content	26.00	2.33	26.08	0.80	27.35	1.91	24.15	2.13	23.93	2.24	24.49	1.80	22.10	20.00
Glucosinolates	107.33	23.34	143.66	5.35	97.00	20.36	79.97	19.79	83.53	22.40	89.92	14.07	118.00	9.91
Oleic acid	42.23	7.08	34.00	1.97	42.00	1.84	52.90	4.39	50.34	6.06	48.22	5.68	39.43	5.99
Linoleic acid	10.21	1.26	11.24	1.11	12.10	3.82	8.42	0.87	8.28	1.39	8.80	1.01	11.26	1.00
Erucic acid	51.81	7.25	59.90	0.61	31.35	22.70	48.00	16.06	48.22	8.15	48.90	9.59	55.45	8.90

Clusters (genotypes from those clusters) can only be related to geographical origin if their natural habitats differ so that the selective pressure forced populations to adapt in different directions. A total of 169 *B. napus* L. lines were genotyped with 84 SSR markers, and Nei's unbiased genetic diversity and Shannon's information index showed that genetic diversity was highest among lines from Europe followed by South Korea, Japan, China and Pakistan while lines from Australia and Canada had the lowest diversity (Gyawali *et al*., 2013; Jankulovska *et al*., 2014).

In present studies, principal component analysis of *B. napus* L. accessions revealed grouping relationship differently, and indirectly supported cluster analysis. The separation of genotypes based on PC1 and PC2 revealed that the populations were distributed in all the directions, which clearly recognized the diversification in indigenous landraces of *B. napus* L. Previous data based on first and second five PCs with > 1 contributed 73.30% and 64.45% of the genetic variability, respectively among various accessions of *B. juncea* L. (Ali *et al*., 2015). Past researchers have made divergence studies of morphological and seed attributes using principal component and cluster analyses in Brassica species (Takahata and Hinata, 1986), *B. napus* L. (Bus *et al*., 2011; Gyawali *et al*., 2013), Indian mustard (Dias *et al*., 1993; Rabbani *et al*., 1998b), Ethiopian mustard (Alemayehu and Becker, 2002; Genet *et al*., 2005; Warwick *et al*., 2006), and white head cabbage (Balkaya *et al*., 2005). However, the present two methods i.e., cluster and principal component analyses were found more appropriate and can better dig out the relationship between the genotypes of assorted origins.

Conclusion

Seventy landraces of *B. napus* L. revealed significant genetic variability for majority traits during both years of studies. Among *B. napus* L. germplasm, the prime genetic variability producing traits were, plant height and time to maturity, while sufficient variations were recorded for flower initiation and completion, main raceme length and silique per main raceme and glucosinolate content. During 2012 and 2013, cluster analysis divided the total 70 landraces of *B. napus* into nine and seven main groups, respectively and contribution of five principal components.

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