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ASSESSMENT OF GENETIC VARIATION IN ETHIOPIAN MUSTARD (*BRASSICA CARINATA* A. BRAUN) GERMPLASM USING MULTIVARIATE TECHNIQUES

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Abstract

The study aims to determine the extent of genetic variability and relationships among the *Brassica carinata* germplasm using agro-morphological characters. The germplasm assayed comprised 134 accessions acquired from abroad and collected locally from diverse ecologies of Pakistan. All the genotypes were characterized for 33 agro-morphological characters ranging from seedling emergence to crop maturity. The data were analyzed by numerical taxonomic techniques using 2 complementary procedures: cluster and principal component analyses. A considerable level of variability was noticed for a number of agro-morphological traits. The largest variation was observed in seed yield (kg/ha) whereas a moderate variability was observed in plant height, main raceme length, silique/main raceme, glucosinolate contents and erucic acid. Hierarchical cluster analysis categorized the 134 accessions into seven main clusters. First three principal components (PCs) accounted for a total of 39.03% of variability among the accessions using agro-morphological traits. PC1 had 17.79% of total variation in agro-morphological traits; PC2 depicted 11.45% of total morphological variability, while PC3 accounted for 9.80% of the total variation. On the basis of greater yield potential, seed yield per plant, 1000-seed weight, oil contents, protein contents and oleic acid four promising genotypes (25939, 25942, 25994 and 26190) have been identified for future breeding and variety development programs.

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 crop species. The successes in the improvement of crop both qualitatively and quantitatively and the development of a species requires the availability and accessibility of genetic diversity. Knowing of duplicates, organization of core collection of a particular population and the selection of parents for the development of new cultivars are directly related to the genetic diversity (Jatoi *et al*., 2010; Pervaiz *et al.,* 2010; Turi et al. 2012). The family Brassicaceae, containing about 350 genera and 3500 species, is one of the 10 most economically important plant families with a wide range of agronomic traits (Rich, 1991; Christopher *et al*., 2005). It has 34 chromosomes with genome composition BBCC, and is thought to result from an ancestral hybridization event between *B. nigra* (genome composition BB) and *B. oleracea* (genome composition CC) (Prakash & Hinata, 1980). Although *B. carinata* is cultivated as an oilseed crop in Ethiopia (Alemayehu & Becker, 2002), it has generally high levels of undesirable glucosinolates and erucic acid (Getinet *et al*., 1997), making it a poor choice for general cultivation as an oilseed crop in comparison to the closely related *B. napus* (canola). The plant is also grown as a leaf vegetable, with a mild flavor. It is known as *yabesha gomen* in Amharic. Named varieties include Texsel, which is particularly adapted to temperate climates.

Ethiopian mustard (*B. carinata*) is currently being evaluated as an option to the traditional canola / mustard cultivation, especially for low rainfall areas of the world. In its area of adoption, the crop has been shown to possess acceptable yield levels, as well as resistance to various biotic and abiotic stresses (Getinet *et al*., 1996). In spite of these strong positive attributes, the crop suffers from several agronomic limitations like longer crop duration, poor harvest index, low oil content and long plant stature. Restricted level of natural variability for specified traits has greatly constrained the breeding programmes aimed at overcoming these limitations (Hirano *et al*., 2009; Prakash & Hinata, 1980). Several breeding options like varietal hybridization, induced mutagenesis and to a limited extent artificial resynthesis from the progenitor diploid species have been explored in the past with poor selection advances for yield and component traits. Induced mutagenesis has however, helped significantly to improve seed quality profile (Barro *et al*., 2003). *B. carinata* possesses many desirable agronomic characteristics that are rare in the other Brassica oil crops: heat and drought tolerance, disease and pest resistance, and a yellow-seeded germplasm (Gugel *et al*., 1990). Its importance to breeders has increased because of rapid improvement in its seed quality (Teklewold & Becker 2005). As a result, its genetic diversity has prompted considerable interest among Brassica breeders.

Like any other crop species the first step in Brassica improvement is full assessment of all materials, including collection, evaluation and molecular characterization of germplasm lines. Often, local varieties of oilseed crops are of excellent quality and flavors have a good level of resistance to pests and diseases and may be superior to exotic materials (Williams *et al*., 1991). Knowledge about germplasm diversity and genetic relationship among breeding material could be an invaluable aid in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and segregating populations. These methods have relied on pedigree data, morphological data, agronomic performance data, biochemical data and more recently molecular (DNA-based) data (Mohammadi & Prasanna, 2003). Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including (i) analysis of genetic variability in

cultivars (Smith, 1984), (ii) identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and (iii) introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998).

Due to the presence of undesirable long chain fatty acids like erucic acid (50%) in the seed oil, it becomes detrimental 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 (Dhillon *et al.,* 1992). Several works through selection, mutation as well as conventional breeding and modern biotechnological techniques have been reported for developing mustard variety containing low erucic acid (18: 2) (Chen *et al*., 1988). Both linoleic and linolenic acids are essential fatty acids; however, less than 3% linolenic acid is preferred for oil stability. To evaluate germplasm diversity various markers are used (Yousuf *et al*., 2006; Akbar *et al*., 2011). The main objective of this study was to characterize and classify the phenotypic variation among the Ethiopian mustard (*B. carinata*) germplasm from Pakistan and abroad using multivariate analyses.

Materials and Methods

The research was carried out in the field area of Institute of Agri-Biotechnology & Genetic Resources (IABGR), National Agricultural Research Centre, Islamabad, Pakistan (33° 33' N and 73° 06'E) during the year 2010-2011. Annual average rainfall in this region ranges from 500-900mm with 70% in summer and 30% in winter. A total of 132 *Brassica carinata* accessions were used as experimental material. The material was provided by National Gene bank, Institute of Agri-Biotechnology & Genetic Resources (IABGR), National Agricultural Research Centre Islamabad, Pakistan (Table 1). The accessions were sown on silty clay loam soils with a pH 7.5. The experiment was laid out in an augmented design. Each plot has a size of 0.6 x 2 m^2 with 2 lines per accession. Length of the row was 2 m, path between beds was 60cm and row to row distance was kept as 30cm. Two improved cultivars Chakwal-raya and Peela-raya were repeated as checks after every 30 accessions. For seed bed preparation presowing irrigation was applied to plant experiment under optimum moisture condition. Planting of the experiment was done with hand drill. Thinning was done to maintain most favorable plant population. Weeds were discarded by hand once 30 days after planting. Qualitative observations were recorded for leaf shape (LS), leaf margins (LM), leaf incision (LI), leaf color (LC), flower color (FC) and seed color (SC). Quantitative observations were recorded for days to flowering initiation (DFI), days to 50% flowering (50%DF), days to flowering completion (DFC), days to maturity, leaf petiole length (cm), leaf length (cm), leaf width (cm), leaf length/width ratio (cm), number of leaves/plant, plant height, primary branches/plant, main raceme length, silique/main raceme, silique length, silique width, silique length/width ratio, seeds/silique, seed yield/ plant and 1000-seed weight (Table 2). Trait selection and measurement techniques were based on IPGRI Descriptors of *Brassica* and *Raphanus*.

Table 1. List of *B. carinata* accessions used during present study at NARC, Islamabad.

No.	Source of acquisition/collection	No. of accessions
1.	Centre for Genetic Resources (CGN), Wageningen, The Netherlands	114
2.	Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany	10
3.	Local collections	8
4.	Check cultivars	2
	Total	134

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 1.02a software of Infra Soft International according to the manufacturer's protocol. A sample size of 2 to 5g seed was used for analysis. Biochemical analyses were carried out at Biochemical Lab, Crop Breeding Section, Nuclear Institute for Food and Agriculture (NIFA), Peshawar for: Oil content (%), Protein content (%), Glucosinolates content (μM g^{-1}), Oleic acid (%), Linolenic acid (%) and Erucic acid content (%).

Data were subjected to simple statistical analysis for all the quantitative traits to assess the amount of genetic variation. Thirty three recorded agro-morphological and seed quality traits were also analyzed by numerical taxonomic techniques using 2 complementary procedures: cluster and principal component analyses (Sneath & Sokal, 1973). The means of each trait were standardized prior to cluster and principal component analyses using Z-scores. Estimates of Euclidean distance coefficients were made between all pairs of accessions. The resulting Euclidean dissimilarity coefficients were used to evaluate the relationships between accessions with cluster analysis using NTSYSpc version 2.01 (Roulf, 2002). Agro-morphological characters were also used to perform principal component analysis. Scattered plots of first three principal components were produced to provide a graphical representation of the pattern of variation among all the genotypes (Statistica, version 6.0).

Results and Discussion

The descriptive statistics revealed considerable level of variability for a number of agro-morphological traits among different accessions *B. carinata* in this study

(Table 3). The largest variation was found for seed yield/ha followed by plant height, glucosinolate contents, main raceme length, silique/main raceme and erucic acid. The variance for said traits was observed as 145801, 331.7, 167.7, 62.4, 31.5 and 23.3, respectively. A low variation was noticed for rest of the agro-morphological parameter particularly seed quality traits. Generally accessions varied in several traits of economic importance and dissimilar pattern of variation among them was noticed for various agro-morphological traits. Muthone (2010) also observed considerable variability for most the traits in *B. carinata*. Similarly, findings of Yousuf *et al*., (2011) and Akbar *et al*. (2012) for days to flower initiation, days to maturity, plant height, days to flower completion are in strong association with the present data; however values for silique per main raceme, seeds per silique and seed yield per plant do not match with our results. However, plant height and leaf length recorded in this study found to be less as compared to reported by Muthone (2010). Moreover, the genotypes assayed were also found to be late maturing. This pattern may be attributed to the genotypic differences and the environment, in which these accessions were investigated.

Table 2. Agro-morphological and seed traits recorded for *B. carinata* accessions at NARC, Islamabad.

No.	Trait	Scale	Description of the trait
A. Qualitative Traits:			
1.	Leaf shape (LS)	-	1.Lanceolate, 3.Spatulate, 5.Obovate, 7.Broad-elliptic, 9.Broad-circular
2.	Leaf margins (LM)	-	1.Entire, 3.Lobed, 5.Cleft, 7.Parted, 9.Sect.
3.	Leaf incision (LI)	-	0.Entire, 1.Creenate, 2.Dentate, 3.Serrate, 4.Undulate, 5.Double-dentate.
4.	Leaf color (LC)	-	1.Yellow-green, 2.Light-green, 3.Green, 4.Dark-green, 5.Purple-green
5.	Flower color (FC)	-	1.White, 2.Cream. 3.Yellow
6.	Seed color (SC)	-	1.Yellow, 2.Yellow-brown, 3.Light-brown, 4.Brown, 5.Dark-brown, 6.Red-brown, 7.Red, 8.Blue-black, 9.Grey-black, 10.Others
B. Quantitative Traits:			
7.	Days to flower initiation	(No.)	Number of days from seed sowing until 5% of plants have first flower in each accession.
8.	Days to 50% flowering	(No.)	Numbers of days from seed sowing until 50% plants have at least one flower in each accession.
9.	Days to flower completion	(No.)	Numbers of days from seed sowing until (DFC) 95% plants showed bloom.
10.	Period of flowering	(No.)	Days from beginning of flowering until end of flowering.
11.	Days to maturity	(No.)	Days from seed sowing until 75% plants of the accession have dry or yellowish silique and mature seeds.
12.	Leaf petiole length	(cm)	Petiole length of the largest leaf where petiole intercepts the stem and leaf blade. Recorded at full bloom stage.
13.	Leaf length	(cm)	Actual measurements of the largest leaf from the base to the apex of leaf blade excluding petiole. Recorded at full bloom stage.
14.	Leaf width	(cm)	Actual measurements across the widest portion/section of the same leaf used for LL. Recorded at full bloom stage.
15.	Leaves/plant	(No.)	Average number of intact leaves or leaf scars per plant at flowering stage.
16.	Plant height	(cm)	Distance from the soil surface to the top of plant.
17.	Primary branches/ plant	(No.)	Total number of branches originating from main stem which gives rise to other silique-bearing branches.
18.	Main raceme length	(cm)	Actual measurement of uppermost inflorescence Emerging from main shoot of the randomly selected plants.
19.	Silique/main raceme	(No.)	Total number of silique on main raceme for the plant used for counting primary branches.
20.	Silique length	(mm)	Distance from the base to the tip of individual silique.
21.	Silique width	(mm)	Distance across the widest point of the same silique used for length.
22.	Seeds/silique	(No.)	Average number of seeds per silique.
23.	Thousand seed weight	(g)	Weight of 100 random dried seeds was calculated and then converted to 1000-seed weight by multiplying 10.
24.	Seed yield/plant	(g)	Average seed weight of five randomly selected plants for each accession was recorded at 13% moisture content.
25.	Seed yield/ha	(g)	Total seed weight of individual accession at 13% moisture content.

Hierarchical cluster analysis based on agro-morphological traits divided 134 accessions into 7 main clusters and 13 sub-clusters. Maximum number of accessions (51) was present in group II, followed group III (29), group I (23), group V (22), group VI (7) and group IV (1) and group VII (1) (Fig. 1; Table 4). Group 1 genotypes were characterized as very early in flowering and maturity, with medium number of leaves per plant, medium plant height, less number of branches per plant, medium number of siliqua per main raceme. Very low number of seed per silique, medium seed yield per plant and medium 1000-seed weight, more oil content, medium protein content and oleic acid. More content of glucosinolates, linolenic acid, and erucic acid were observed in group I (Table 5). Group II was the largest group comprising accessions with medium days to flowering, plant height, seed yield per plant and maturity, maximum number of silique length and width, 1000-seed weight and oil contents, silique length, 1000-seed weight. Lower number of branches per plant, protein contents and glucosilulates were observed in group II. Group III genotypes showed maximum number of days to maturity, days to flowering and 1000 seed weight. Medium silique per main raceme, medium silique length, silique width, branches per plant, seed yield per plant, medium contents of oil, protein, glucosinolates and erucic acid. Lower number of seeds per silique was observed in group III. Group IV was the smallest of all groups with distinctive morphological features. Genotypes of group IV were featured by medium number of days to maturity, plant height, branches per plant and glucosinolates contents. Maximum silique length and width, seeds per silique and erucic acid contents was observed in group IV, whereas minimum ratio between leaf length/leaf width and protein contents. Group V accessions depicted medium number of days to flowering and maturity, silique length, seed yield per plant and oil contents, maximum plant height, minimum leaf length, 1000-seed weight, protein contents, glucosinolates and erucic acid was observed in group V. Group VI genotypes were characterized as very late in flowering and maturity, high 1000-seed weight, maximum seed yield per plant, leaves per plant and glucosinolates, medium oil, protein contents and erucic acid was observed in group VI. Group VII genotypes were characterized as late in flowering and maturity, maximum plant height, seed yield per plant, protein contents and glucosinolates, medium seed yield per plant and number of branches per plant, whereas minimum 1000 seed weight, oil contents and erucic acid (Table 5).

Table 3. Descriptive statistics of agro-morphological and seed quality traits in *B. carinata* accessions.

Trait	Mean	Minimum	Maximum	SD	CV (%)	Variance
Days to flower initiation	124.0	116	133	3.4	2.8	11.7
Days to 50% flowering	132.1	124	140	3.6	2.7	13.2
Days to flower completion	141.9	132	150	4.1	2.9	16.5
Flowering period	17.9	7.0	26.0	2.7	15.0	7.2
Days to maturity	177.0	170.0	185.0	3.5	2.0	12.0
Leaf petiole length	8.8	3.5	16.6	2.6	29.4	6.7
Leaf length	15.6	8.7	25.7	3.6	23.1	13.0
Leaf width	9.2	5.3	13.9	1.7	19.0	3.0
Leaf length/width ratio	1.7	1.1	3.1	0.3	18.9	0.1
Leaves/plant	19.4	13.4	25.8	2.7	14.0	7.4
Plant height	190.8	149.6	242.2	18.2	9.6	331.7
Primary branches/plant	13.1	7.6	22.8	3.0	23.1	9.2
Main raceme length	40.8	22.8	64.2	7.9	19.4	62.4
Siliqua/main raceme	21.8	13.6	40.2	5.6	25.7	31.5
Silique length	48.0	39.6	56.6	4.1	8.5	16.7
Silique width	4.8	4.1	6.2	0.4	7.5	0.1
Silique length/width ratio	10.1	7.4	11.9	0.8	8.0	0.6
Seeds/silique	17.1	14.2	30.9	1.7	9.7	2.8
Seed yield/plant	9.9	3.2	18.6	3.0	30.0	8.8
Thousand seed weight	3.4	2.9	3.6	0.1	2.7	0.0
Seed yield/ha	1068.2	450.0	2779.2	381.8	35.7	145801.1
Moisture contents	4.2	2.7	5.8	0.6	13.7	0.3
Oil contents	45.5	35.3	51.6	2.5	5.6	6.4
Protein contents	22.1	18.7	30.2	2.0	8.9	3.9
Glucosinolates	104.8	66.5	136.3	12.9	12.4	167.7
Oleic acid	25.5	15.0	37.1	3.7	14.4	13.5
Linoleic acid	10.2	4.9	13.0	1.4	13.4	1.9
Erucic acid	38.1	16.0	47.9	4.8	12.7	23.3

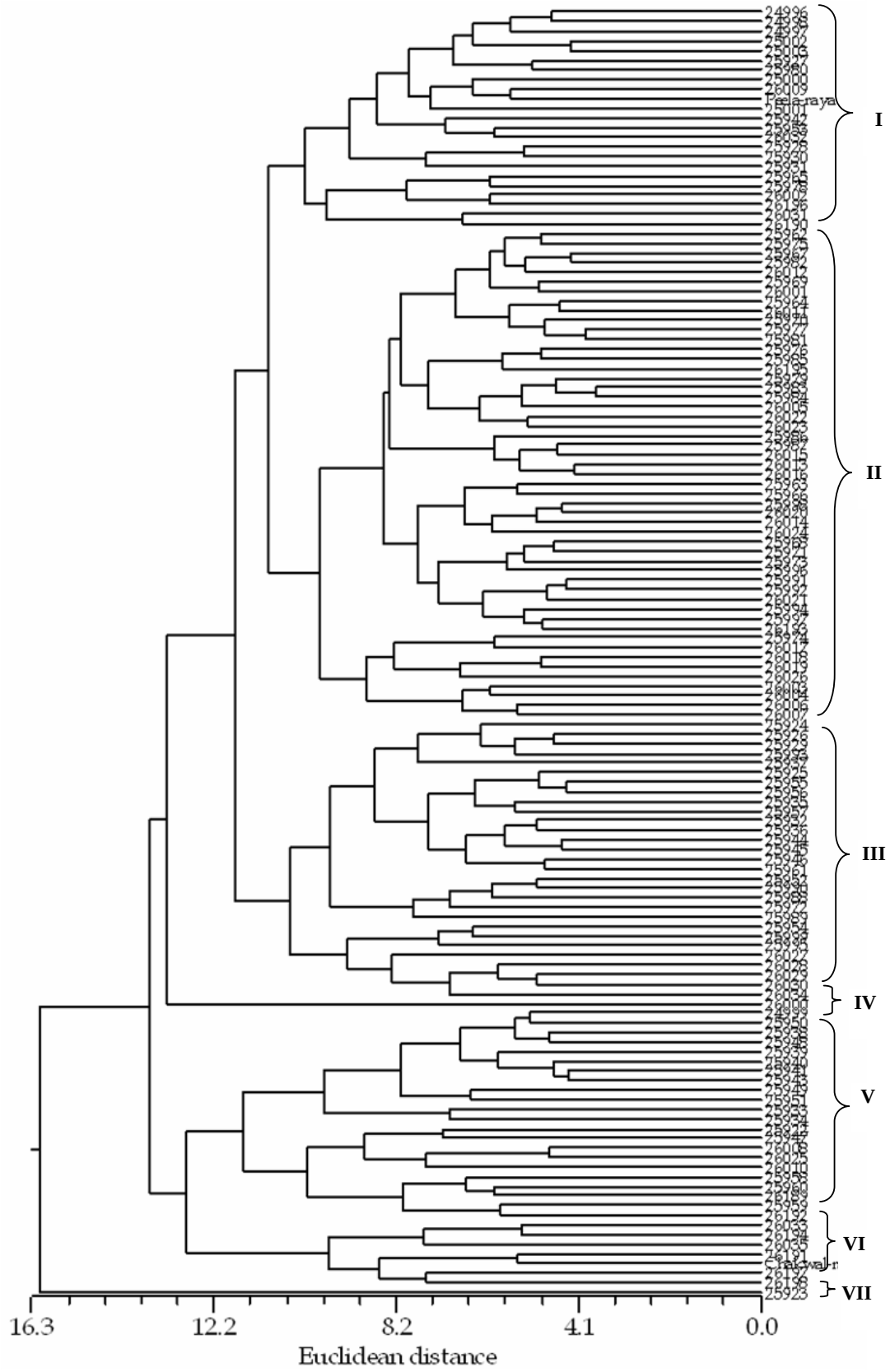

Fig. 1. Dendrogram showing the relationship among 134 accessions of *B. carinata* accessions based on five qualitative and 28 quantitative traits recorded at NARC, Islamabad.

Table 4. Mean±SD of quantitative traits for each of 7 clusters in *B. carinata* accessions at NARC, Islamabad.

Traits	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII
DFI	121.8±1.9	123.0±2.5	127.4±2.2	123.0±2.5	124.3±2.2	125.5±2.3	124.0±2.1
50%DF	129.5±2.3	131.0±2. 7	135.0±3.3	134.0±3.7	132.8±3.7	134.5±2.1	137.0±2.7
DFC	138.8±3.9	141.0±3.1	144.7±2.8	146.0±3.2	142.3±4.7	144.6±2.8	147.0±2.4
PF	17.0±2.6	18.0±3.4	17.0±2.6	23.0±2.8	17.9±3.3	19.1±2.2	23.0±2.5
DM	175.1±2.7	177.0±1.5	178.5±3.3	182.0±2.3	176.4±3.5	177.6±4.2	180.0±3.7
LPL	9.8±2.0	7.3±0.3	9.7±2.7	9.4±2.1	10.5±2.5	7.9±2.1	14.6±2.2
LL	15.3±1.5	14.8±2.5	17.1±3.2	14.0±3.4	14.4±2.5	21.3±3.4	20.2±3.7
LW	9.4±1.0	8.2±1.7	9.6±1.7	8.4±2.4	10.1±1.5	11.2±1.1	13.1±1.3
LL/LW	1.7±0.2	1.8±2.5	1.8±0.4	1.7±0.5	1.4±0.2	1.9±0.3	1.7±0.4
L/P	19.4±2.1	19.2±6.7	19.4±2.6	18.4±5.4	18.3±2.8	22.8±1.9	23.0±1.6
PH	188.7±1.9	187.4±5.4	192.9±21.8	173.6±8.2	200.8±18.7	193.7±18.4	215.8±17.2
PB/P	12.7±10.1	12.1±2.8	12.9±2.5	13.0±2.6	14.2±2.9	19.1±3.6	14.8±3.2
MRL	43.4±4.6	40.5±6.7	39.1±8.7	57.8±5.6	41.7±6.4	38.2±3.1	30.6±3.4
S/MR	22.9±5.2	21.4±5.4	19.5±4.5	27.2±4.7	22.3±5.9	27.4±7.0	26.2±5.2
SL	46.3±0.2	50.8±2.8	46.6±3.4	52.3±3.8	44.8±3.3	48.2±2.4	41.6±2.8
SW	4.5±0.7	4.9±0.3	4.7±0.3	4.9±0.4	4.6±0.3	5.2±0.5	4.2±0.8
SL/SW	10.3±1.1	10.4±0.8	9.9±0.6	10.6±0.7	9.6±0.6	9.4±1.1	10.0±0.8
S/S	17.1±3.2	16.8±1.3	17.0±1.2	30.9±1.4	17.4±1.1	16.9±0.8	17.2±0.7
SY/P	9.9±0.1	9.5±3.0	10.3±2.6	8.1±2.2	10.3±3.0	9.8±3.6	12.6±3.2
1000SW	3.4±0.6	3.4±0.1	3.4±0.1	3.4±0.5	3.3±0.1	3.4±0.1	2.9±0.2
MC	4.3±314.8	4.0±252.0	4.3±328.2	4.3±225.0	4.3±613.0	4.6±401.7	4.7±371.0
OC	45.7±2.7	46.4±0.5	45.9±0.6	46.6±0.8	44.3±0.5	42.0±0.3	35.3±0.4
PC	23.1±2.1	21.3±1.9	22.3±1.9	21.6±1.7	21.4±2.6	25.3±1.9	27.3±1.8
GSL	107.0±2.7	102.4±1.3	111.2±1.4	104.5±1.6	98.0±1.8	114.8±2.6	13.5±2.4
OA	23.8±13.5	24.1±8.3	25.4±13.4	23.6±7.5	29.2±14.7	28.3±17.5	32.3±15.2
LA	10.9±0.9	10.6±3.0	10.3±2.4	11.1±2.8	8.7±4.3	10.0±3.0	4.9±2.8
EA	39.3±4.4	39.8±0.9	39.2±0.8	41.5±0.7	32.8±4.3	35.5±1.8	27.9±1.3

Table 5. Main characteristics of *B. carinata* accessions belonging to different clusters.

Main group	Sub-clusters	No. of lines	%age of lines	Main features
I	2	23	17.2%	Very early in flowering and maturity; medium number of leaves/plant, medium plant height; less number of branches/plant; very low number of seeds/silique; higher linolenic acid and erucic acid contents.
II	3	51	38.05%	Medium days to flowering, plant height, seed yield/plant and maturity; maximum 1000-seed weight and oil contents; lower number of branches/plant, protein contents and glucosilulates.
III	3	29	21.64%	Maximum number of days to flowering, maturity and 1000-seed weight; medium branches/plant, seed yield/plant; medium contents of oil, protein, glucosinolates and erucic acid and lower number of seeds/silique.
IV	1	1	0.74%	Medium plant height, branches/plant and glucosinolates contents; maximum number of days to maturity; minimum ratio between leaf length & width and protein contents.
V	2	22	16.41%	Medium number of days to flowering and maturity, seed yield/plant and oil contents; maximum plant height; minimum leaf length, 1000-seed weight, protein contents, glucosinolates and erucic acid.
VI	1	7	5.22%	Very late in flowering and maturity, higher 1000-seed weight, maximum seed yield/plant, leaves/plant and glucosinolates; medium oil and protein contents and erucic acid.
VII	1	1	0.74%	Late in maturity; maximum plant height, seed yield/plant, protein and glucosinolate contents; medium seed yield/plant; minimum 1000-seed weight, oil contents and erucic acid.

Hierarchical cluster analysis showed that some of accessions collected from various regions were grouped into the same cluster, while many others fell into different clusters. Grouping was not associated with the geographic distribution instead accessions were mainly grouped due to their morphological differences. Thus, it cannot be generalized that all the accessions having same origin would always have low diversity among them. Results of the present investigation agreed with previous studies using morphological data to characterize germplasm of Ethiopian mustard (*B. carinata*) in which geographic origin of the collected material had no effect on grouping of the accessions/varieties (Dhillon *et al*., 1999). Grouping pattern in their studies indicated that the clusters were heterogeneous with regard to the geographical origin of genotypes included. Lines from different geographic regions were pooled in the same cluster. Results of the present work also coincide with the classification of 36 populations of nabicol crop (Rodriguez *et al*., 2005) where most of the populations were grouped mainly due to their earliness and not related to their geographic origin. There were certain relationships between the geographical origin of germplasm collections of *B. rapa* subsp *rapa* L. from northwestern Spain and the groups formed (Padilla *et al*., 2005). The work done by all these researchers agree to the present outcome that this method can clear complex relationships between populations of diverse origins in a more simplified way. By hierarchical cluster analysis, present study revealed that some of the accessions collected from various geographical regions were grouped into the same cluster, while some other accessions fell into different clusters. Present findings for group-I are in accordance with the results of Morris (2010) that these clusters are likely to define accession groups with similar geographic origins. However for rest of the groups it can be generalized that grouping on cluster basis is not always associated with the geographical distribution instead accessions are grouped on the basis of their morphological differences.

Principal component analysis based on quantitative traits observed in Ethiopian mustard yielded informative outcome (Fig. 2). The cumulative contribution of the first 3 principal components accounted for 39.03% variability (Table 6). PC1 depicted 17.79% of total variation among various traits. Days to flower initiation (0.259), days to 50% flowering (0.269), days to flower completion (0.231), leaf length (0.217), leaf width (0.278) and oleic acid (0.303) contributed positively to PC1. In contrast, silique length (-0.182), silique length/width ration (-0.182), 1000 seed weight (-0.131), oleic acid (-0.294), linolenic acid (-0.295) and erucic acid (-0.294) contributed negatively to PC1 (Table 6). Second principal component (PC2) accounted for 11.45% of the total variation and illustrated primarily the variation in days to flower initiation (0.268), days to 50% flowering (0.297), days to flower completion (0.322), days to maturity (0.213), leaf length/width ratio (0.391), silique length (0.299), silique width (0.301) and 1000-seed weight (0.210). Conversely, leaf petiole length (-0.291), leaf width (-0.150), plant height (-0.151) and seed yield ha/ha (-0.164) were negatively associated with PC2. PC3 explained an additional 9.80% of the total variation among various traits and was positively associated with days to flower initiation (0.241), days to maturity (0.214), leaf petiole length (0.244), plant height (0.228), oil content (0.244), glucosinolates (0.422), linolenic acid (0.275) and erucic acid (0.333), whereas main raceme length (-0.121), silique width (-0.230), siliqua per main raceme (-0.134) silique length (-0.232), silique width (-0.230) and oleic acid (-0.304) had relatively large negative weights on PC3 (Table 6).

Principal component analysis of the *B. carinata* accessions revealed diverse grouping pattern, which, in general supported cluster analysis (Fig. 3). The first 3 principal components were plotted to observe the relationships between the accessions. The separation on the basis of PC1 and PC2 revealed that the genotypes were scattered in all the quarters, which show the high level of genetic diversity in the evaluated genotypes (Fig. 3). Among the 134 accessions were found diversely scattered on the scattered plot. In the first and second principal components 25923, 25947, 25003, 26192, 25959, 26035, 26034, 26197, 25995, 26003, 26006, 25977 and 25001 showed greater genetic diversity based on accessions numbers. In the first and third principal components, and 26051, 26010, 26192, 25923, 25960, 25924, 25989, 25930, 25001, 25977, 26006, 26023 and 25974 showed greater genetic diversity based on accessions numbers. Although principal component analysis grouped accessions together with more morphological similarities, the clusters did not necessarily include all the accessions from the same or nearby sites. Diversity of populations within a geographical origin and similarity of populations beyond geographical limits have also been reported in *B. carinata* genotypes (Alemayehu & Becker, 2002).

Divergence studies of morphological and seed attributes using principal component and cluster analyses have been made by different researchers (Balkaya *et al*., 2005; Warwick *et al*., 2006; Dawood *et al.,* 2009; Jatoi *et al.,* 2011) which were in support of present investigation that both cluster and principal component analyses disclosed complex relationship among the accessions in a more understandable way. The variability among the accessions from diverse origin could be related primarily to their morphological differences and secondly to horticultural use. Current study explored a considerable range of genetic diversity in *B. carinata* germplasm. Important agro-morphological traits like early maturity, plant height, branches/ plant, seeds/silique, silique/plant, seed yield, oil contents and fatty acids serve as a criterion to select promising *B. carinata* genotypes. Accessions evaluated in this work exhibited a reasonable level of diversity for some of the traits of economic significance providing a resource for future crop improvement. Promising accessions with potential genes of interest to improve earliness, yield components, higher oil contents and better quality four accessions (25939, 25942, 25994 and 26190) have been identified (Table 7). For the improvement of cultivated *B. carinata*, it would be desirable to use diverse collections with more variability for use in future breeding programs.

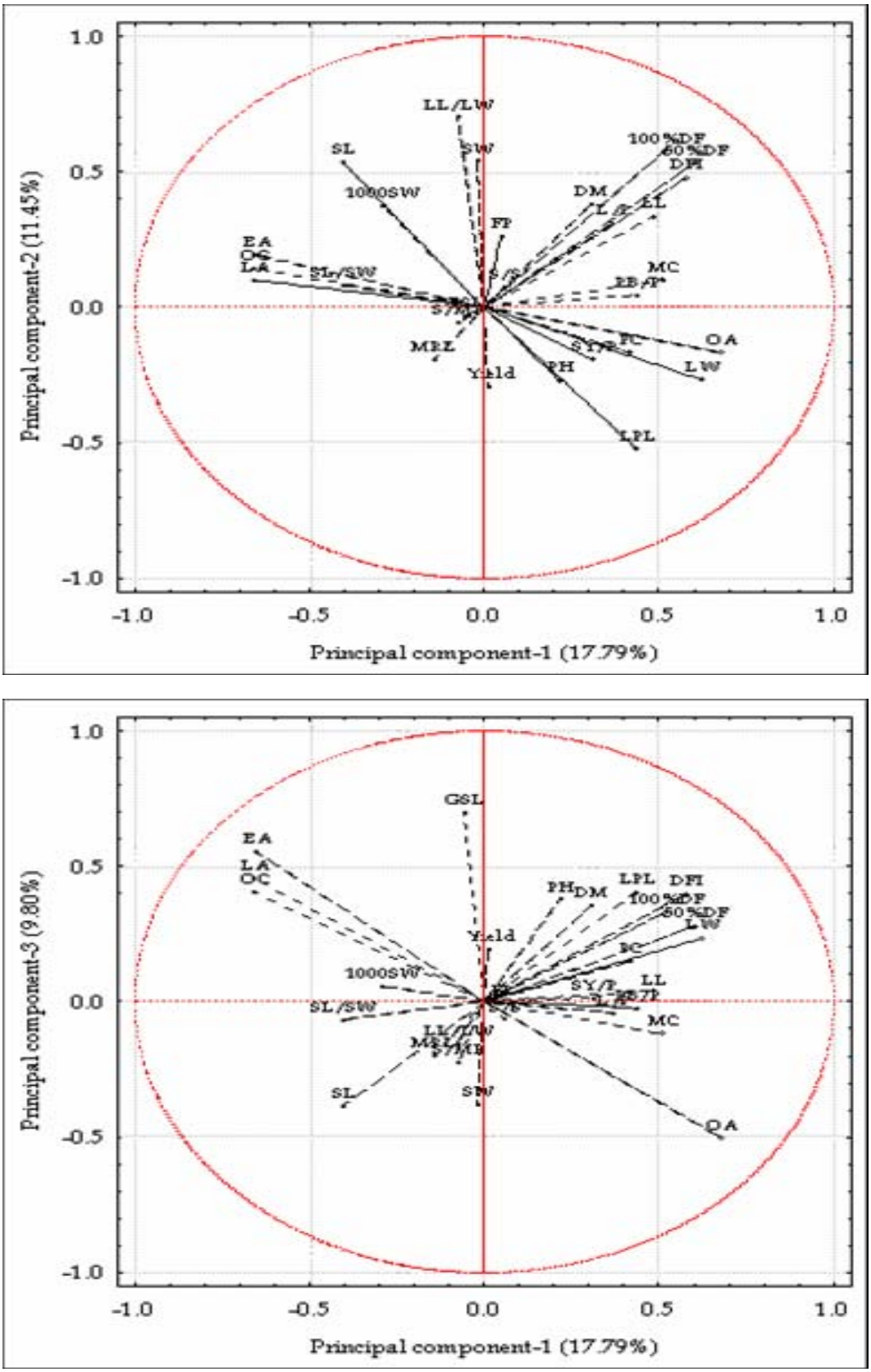

Fig. 2. Principal components analysis plot showing the contribution of 28 traits to total variation in first three PCs in a collection of *B. carinata* accessions.

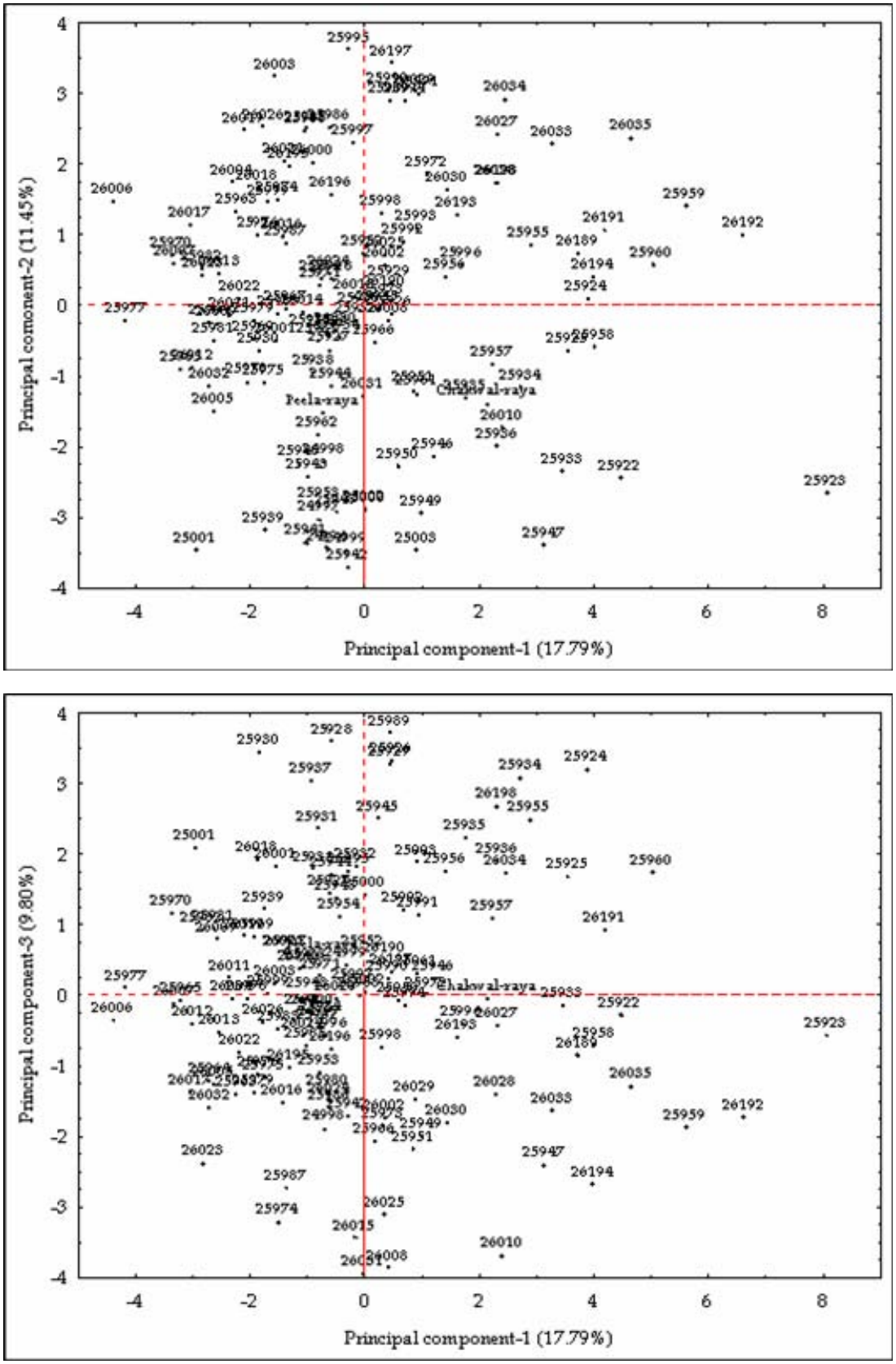

Fig. 3. Two dimensional scatter plot of the genetic relationship among 134 *B. carinata* accessions as revealed by first three principal components.

Table 6. Eigenvectors, Eigen values, total variance, and cumulative variance for 134 accessions based on 28 agro-morphological traits.

Traits	Eigenvectors		
	PC1	PC2	PC3
Days to flower initiation	0.259	0.268	0.241
Days to 50% flowering	0.269	0.297	0.168
Days to flower completion	0.231	0.322	0.199
Flowering period	0.021	0.147	-0.005
Days to maturity	0.137	0.213	0.214
Leaf petiole length	0.195	-0.291	0.244
Leaf length	0.217	0.187	0.021
Leaf width	0.278	-0.150	0.142
Leaf length/width ratio	-0.035	0.391	-0.095
Leaves/plant	0.165	0.175	-0.024
Plant height	0.096	-0.151	0.228
Primary branches/plant	0.196	0.025	-0.015
Main raceme length	-0.065	-0.106	-0.121
Silique/main raceme	-0.034	-0.030	-0.134
Silique length	-0.182	0.299	-0.232
Silique width	-0.009	0.301	-0.230
Silique length/width ratio	-0.182	0.047	-0.041
Seeds/silique	0.025	0.043	-0.039
Seed yield/plant	0.140	-0.108	0.008
Thousand seed weight	-0.131	0.210	0.033
Seed yield/ha	0.006	-0.164	0.115
Moisture contents	0.230	0.056	-0.071
Oil contents	-0.294	0.080	0.244
Protein contents	0.187	-0.093	0.090
Glucosinolates	-0.026	-0.018	0.422
Oleic acid	0.303	-0.092	-0.304
Linoleic acid	-0.295	0.054	0.275
Erucic acid	-0.294	0.109	0.333
Eigenvalues	4.98	3.21	2.74
Total variance (%)	17.79	11.45	9.80
Cumulative variance (%)	17.79	29.23	39.03

Table 7. Elite accessions identified on the basis of important agro-morphological traits for future use.

Trait of interest	Range	Accessions identified
Days to maturity (No.)	<172	25949, 25963, 25978, 26009, 26031 and 26032.
Plant height (cm)	≥118	25923, 25938, 25957, 25958, 25961, 25968, 25969, 25978 and 26001.
Branches/plant (No.)	>16	25941, 25942, 25959, 25997, 26011, 26034 26189 and 26192.
Silique length (mm)	>53	25963, 25965, 25970, 25976, 25985, 25986, 25994, 26012 and 26195.
Silique width (mm)	>5.19	25972, 25974, 25998, 26008, 26016, 26019, 26020, 26021, 26024 and 26025.
Seeds/silique (No.)	>18	25939, 25943, 25971, 25988, 26000, 26024, 26031 and 26034.
Seed yield/plant (g)	>15	25934, 25942, 25966, 25975, 25994, 26008, 26031 and 26190.
1000-seed weight (g)	≥3.23	25928, 25937, 25963, 25981, 25997, 25999, 26006, 26029, 26190 and 26193.
Oil content (%)	>50	25925, 25928, 25931, 25944, 25962, 25970, 25977, 26007 and 26026.
Protein content (%)	>26	25923, 26010, 26022, 26035, 26191, 26198, Chakwal-raya and Peela-raya.
Oleic acid (%)	>32	25923, 25933, 25947, 25949, 25951, 25959 and 26191.

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References

Akbar F., M.A. Rabbani, Z.K. Shinwari and S.J. Khan. 2011. Genetic divergence in sesame (*Sesamum indicum* L.) landraces based on qualitative and quantitative traits *Pak. J. Bot*., 43: 2737-2744

Akbar, F., N. Yousaf, M.A. Rabbani, Z.K. Shinwari and M.S. Masood. 2012. Study of total seed proteins pattern of sesame (*Sesamum indicum* L.) landraces via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). *Pak. J. Bot*., 44: 2009-2014

Alemayehu, N. and H. Becker. 2002. Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). *Genet. Resour. Crop Evol*., 49: 573-582.

Balkaya, A., R.Yanmaz, A. Apaydýn and H. Kar. 2005. Morphological characterization of the whitehead cabbage (*Brassica oleracea* var. *capitata* subvar. alba) populations in Turkey. *NZ J. Crop Hort. Sci*., 33: 333-341.

Barro, F., E.J. Fernandez, DeLa M. Vega and A. Martin. 2003. Modification of glucosinolate and erucic acid contents in doubled haploid lines of *Brassica carinata* by UV treatment of isolated microspores. *Euphytica*, 129: 1-6.

Chen, B.Y., W.K. Heneen and R. Jonsson. 1988. Independent inheritance of erucic acid content and flower color in the C-genome of *Brassica napus* L. *Plant Breed*., 100: 147-149.

Christopher, G.L., J.R. Andrew, A.C.L. Geraldine, J.H. Clare, B. Jacqueline, B. Gary, C.S. German and E. David. 2005. Brassica ASTRA: an integrated database for Brassica genomic research. *Nucleic Acids Res*., 1; 33: 656-659.

Dawood, A., A. Kikuchi, S.A. Jatoi, F. Kiuchi and K. N. Watanabe. 2009. Genetic variation in the chloroplast DNA (cpDNA) of Zingiberaceae taxa from Myanmar envisaged by the PCR-RFLP analysis. *Ann. Appl. Biol*. 155: 91-101

Dhillon, S.S., S.S. Kumar and N. Gupta. 1992. Breeding objectives and methodology. In: *Breeding Oilseed Brassicas*, (Eds.): K.S. Labana, S.S. Banga and S.K. Banga. Narosa Publishing House New Delhi, pp. 10-17.

Dhillon, S.S., K. Singh and K.S. Barar. 1999. Diversity analysis of highly selected genotypes in Indian mustard (*Brassica juncea* Czern & Coss). 10th Int. Rapeseed Cong., Canberra, Australia; September 26-29, 1999.

Getinet, A., G. Rakow, J.P. Raney and R.K. Downey. 1996. Development of zero erucic acid Ethiopian mustard through an inter-specific cross with zero erucic acid Oriental mustard. *Can. J. Plant Sci*., 74: 793-795.

Getinet, A., G. Rakow, J.P. Raney and R.K. Downey. 1997. Glucosinolate content in interspecific crosses of *Brassica carinata* with *B. juncea* and *B. napus*. *Plant Breed*., 116: 39-46.

Gugel, R.K., G. Sguin-Swartz and A. Petrie. 1990. Pathogenicity of three isolates of *Leptosphaeria maculans* on *Brassica* species and other crucifers. *Can. J. Plant Path*., 12: 75-82.

Hirano, R., S.A. Jatoi, M. Kawase, A.Kikuchi and K. N. Watanabe. 2009. Consequences of ex situ conservation on the genetic integrity of germplasm held at different genebanks: a case study of bread wheat collected in Pakistan. *Crop Sci.* 49: 2160-2166.

Jatoi, S.A., A. Javaid, M. Iqbal, O.U. Sayal, M.S. Masood and S.U. Siddiqui. 2011. Genetic diversity in radish germplasm for morphological traits and seed storage proteins. *Pak. J. Bot*., 43: 2507-2512.

Jatoi, S.A., A. Kikuchi, D. Ahmad and K.N. Watanabe. 2010. Characterization of genetic structure of mango ginger (*Curcuma amada* Roxb.) from Myanmar in farm and genebank collection by the neutral and functional genomic markers. *Elect. J. Biotech*., 13: 6.

Mohammadi, S.A. and B.M. Prasanna. 2003. Analysis of genetic diversity in crop plants salient statistical tools and considerations. *Crop Sci*., 43: 1235-1248.

Morris, J.B. 2010. Morphological and reproductive characterization of guar (*Cyamopsis tetragonoloba*) genetic resources regenerated in Georgia, USA. *Genet. Resour. Crop. Evol*., 57: 985-993.

Muthoni, J. 2010. Characterization of Ethiopian mustard (*Brassica carinata* A. Braun) lines for vegetative agromorphological traits at Arusha, Tanzania. *J. Hort. For*., 2: 1-6.

Nagaharu, U. 1935. Genome analysis in brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization." *Jap. J. Bot.* 7: 389-452.

Padilla, G., M.E. Cartea, V.M. Rodrıguez and A. Ordas. 2005. Genetic diversity in a germplasm collection of *Brassica rapa* subsp *rapa* L. from northwestern Spain. *Euphytica*, 145: 171-180.

Pervaiz, Z.H., M.A. Rabbani, I. Khaliq, S.R. Pearce and S.A. Malik. 2010. Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. *Elect. J. Biotech*., 13: 1-12.

Prakash, S. and Hinata, K. 1980. Taxonomy, cytogenetics and origin of crop Brassicas, a review. *Opera Botanica*, 55: 1-57.

Rodriguez, E., C.S. Morris, J.E. Belz, E.C. Chapin, J.M. Martin, W. Daffer and S. Hensley. 2005. *An assessment of the SRTM topographic products*, Technical Report JPL D-31639. Pasadena, California: Jet Propulsion Laboratory.

Roulf, F.J. 2002. *NTSYS–pc: Numerical Taxonomy and Multivariate Analysis System*. Version 2.01. Exeter Publishing Ltd., Setauket, NY.

Smith, J.S.C. 1984. Genetic variability within U.S. hybrid maize: Multivariate analysis of isozyme data. *Crop Sci*., 24: 1041-1046.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical taxonomy -the principles and practice of numerical classification. (W. H. Freeman: San Francisco.)

Teklewold, A. and H.C. Becker. 2005. Comparison of phenotypic and molecular distances to predict heterosis and F1 performance in Ethiopian mustard (*Brassica carinata* A. Braun). *Theor. Appl. Genet*., 112: 752-759.

Thompson, J.A., R.L. Nelson and L.O. Vodkin. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Sci*., 38: 1348-1355.

Warwick, S.I., R.K. Gugel, T. Mcdonald and K.C. Falk. 2006. Genetic variation of Ethiopian mustard (*Brassica carinata*) germplasm in western Canada. *Genet. Resour. Crop Evol*., 53: 297-312.

Williams, C.N., J.O. Uzo and W.T.H. Peregrine. 1991. Vegetable production in the tropics. In: *Intermediate Tropical Agriculture Series*, Longman Scientific and Technical, Essex.

Turi, N.A., Farhatullah, M.A. Rabbani and Z.K. Shinwari. 2012. Genetic diversity in the locally collected *Brassica* species of Pakistan based on microsatellite markers". *Pak. J. Bot*., 44: 1029-1035.

Yousuf, Z., S. Masood, Z.K. Shinwari, M.A. Khan and A. Rabbani. 2006. Evaluation of taxonomic status of medicinal species of the genus *Solanum* and *Capsicum* based on poly acrylamide gel electrophoresis. *Pak. J. Bot*., 38: 99-106.

Yousuf, M., S.U. Ajmal, M. Munir and A. Ghafoor. 2011. Genetic diversity analysis for agro-morphological and seed quality traits in rapeseed (*Brassica campestris* L.). *Pak. J. Bot*., 43: 1195-1203.

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