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Morphological, Biochemical, and Molecular Characterization of Exotic *Brassica* Germplasm

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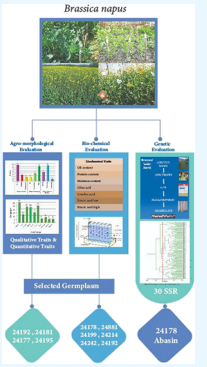
ABSTRACT: Oilseed rape (*Brassica napus* L.) is an important oilseed crop. We examined the diversity of germplasm expressed at three distinct levels (i.e., morphological, biochemical, and DNA levels). In this study, 150 *B. napus* L. accessions with three check varieties were provided by Bioresources Conservation Institute. The germplasm was grown in field conditions for data collection of 15 quantitative and nine qualitative agro-morphological traits. The result indicated that for 15 quantitative agro-morphological traits, the highest coefficient of variation was recorded for plant height and days to flowering initiation. For nine qualitative traits, most of the accessions have a spatulate leaf, brown color seeds, yellow flowers, and erect silique attitude. The best adoptable genetically diverse exotic *Brassica* germplasms were selected, i.e., accessions 24178, 24881, 24199, 24214, 24242, and 24192. Based on biochemical analysis for high oil content and high oleic acid content, chakwal sarsoon and accession 24192 were selected. For high oleic and linoleic acids, accession 24181 performed best, for low erucic acid accessions 24177 and 24195. Based on molecular (SSR) markers, the top 50 selected genotypes were evaluated with 30 SSR markers. The 47 genotypes with three check varieties were clustered in six major groups; the coefficient of similarity ranged between 0.18 and 1.00. Based on SSR data, the germplasms accession 24178 and Abasin were the most diverse genotypes. These genotypes have the capacity and could be used in future breeding programs. High genetic variations were investigated through the SSR among the studied genotypes of *Brassica napus* L. The present study also concluded that SSR is a better technique for intraspecific genetic diversity. Other modern techniques should be applied such as SNIP for the investigation of a high level of genetic diversity among crop plants in the future.



1. INTRODUCTION

Oilseed rape (*Brassica napus* L.) is an important oilseed crop grown in the world and especially in Asian countries.[1] The harvested area for collecting crops has been estimated to be 23 million ha in Asian countries like Pakistan, and a part of that area which equals 0.807 million ha consists of oilseed crops that approximately constitute 3% of the total harvested area. The requirement for edible oil in Pakistan is increasing day by day due to the ever-increasing population. A large amount of foreign exchange is being spent on edible oil imports every year.[2] *B. napus* L. is being considered to have a huge popularity in terms of being widely grown in Europe and Canada.[3] To ensure efficient rapeseed production, breeders have aimed to produce high-yielding and high-quality cultivars. A promising crop enhancement approach utilizing the genetic diversity of the crop has been executed to reach goals of formation of high-yield varieties.[4] We can demonstrate genetic diversity through several ways such as biochemical diversity, morphological diversity, and DNA diversity. Morphological characteristics are vastly beneficial to classify and give details of the germplasm, and the probability of successful execution of the crop enhancement approach depends on the degree of genetic variation.[5] The environment is one of the major causes that affect the morphological trait diversity, and we need to observe the growth of plants until maturity to obtain desirable variants.[6] The resemblance of several characters among many obtained new varieties makes the biochemical techniques and electrophoresis techniques required to differentiate various varieties.[7] Characterization of proteins and fats and selection of desirable ones are of great importance for rapeseed breeders.[8] With the recent development of molecular techniques, estimation of genetic diversity has become even faster and more reliable.[9] Many types of molecular markers are available and have been used to study genetic diversity in *Brassica* and other crop species.[10] These markers include RAPD, AFLP, and SSR. Microsatellites or simple sequence repeats (SSRs) are widely dispersed in all eukaryotic genomes. SSRs are

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Table 1. *Brassica napus* L. Germplasm Accessions Acquired from the Gene Bank, NARC

s. no.	accession	s. no.	accession	s. no.	accession	s. no.	accession	s. no.	accession
1	24177	31	24207	61	24243	91	24855	121	24886
2	24178	32	24208	62	24244	92	24856	122	24887
3	24179	33	24209	63	24245	93	24857	123	24888
4	24180	34	24210	64	24246	94	24858	124	24889
5	24181	35	24211	65	24247	95	24859	125	24891
6	24182	36	24212	66	24248	96	24860	126	24892
7	24183	37	24213	67	24249	97	24861	127	24893
8	24184	38	24214	68	24250	98	24862	128	24894
9	24185	39	24215	69	24251	99	24863	129	24895
10	24186	40	24216	70	24252	100	24864	130	24896
11	24187	41	24217	71	24253	101	24865	131	24897
12	24188	42	24218	72	24254	102	24866	132	24898
13	24189	43	24219	73	24255	103	24867	133	24899
14	24190	44	24220	74	24256	104	24868	134	24900
15	24191	45	24221	75	24257	105	24869	135	24901
16	24192	46	24222	76	24258	106	24870	136	24902
17	24193	47	24223	77	24259	107	24871	137	24903
18	24194	48	24224	78	24842	108	24872	138	24904
19	24195	49	24225	79	24843	109	24873	139	24905
20	24196	50	24226	80	24844	110	24874	140	24906
21	24197	51	24227	81	24845	111	24875	141	24907
22	24198	52	24228	82	24846	112	24876	142	24908
23	24199	53	24229	83	24847	113	24877	143	24909
24	24200	54	24230	84	24848	114	24878	144	26095
25	24201	55	24237	85	24849	115	24879	145	26337
26	24202	56	24238	86	24850	116	24880	146	26347
27	24203	57	24239	87	24851	117	24881	147	26352
28	24204	58	24240	88	24852	118	24882	148	26357
29	24205	59	24241	89	24853	119	24883	149	26362
30	24206	60	24242	90	24854	120	24884	150	26373
check 1	22851	chakwal sarson		check 2	22853	Takwara	check 3	22855	Abasin-95

characterized as codominant, highly polymorphic, abundant, and randomly distributed markers in genomes. SSRs have been used for studies of parentage, genetic mapping, and genetic diversity.[11] All obtained details about the genetic diversity of the germplasm are taken into consideration during the present investigation. An assessment to eight germplasms of *B. napus* L. has been done to investigate the genetic variation and relationship in these different genotypes in Peshawar K.P.K. through RCBD experimental design.[12] They registered the data on agronomic parameters such as plant height, primary branches per plant, pods per main raceme length, pods per plant, pod length, seed per pod, 1000-seed weight, and seed yield per plant. High genetic variations were investigated for these germplasms in almost all the agronomic parameters. Plant heights of these genotypes showed high genetic variations ($P < 0.01$). Less genetic variations were found for the primary branches per plant in these genotypes ($P > 0.05$). Other traits like pod per the main raceme, pod length, seed yield per plant, 1000-seed weight, and seed per pod were also highly significant differences. They found a highly significant and positive correlation of plant height with pod per the main raceme ($r = 0.77$) and pod length ($r = 0.71$). They also investigated a significant positive correlation for seed yields with pods per plant ($r = 0.71$). A lot of researchers have studied 134 cultivars for genetic diversity with the help of agro-morphological markers.[13] They collected the data of 31 quantitative and qualitative traits and investigated high differences among the genotypes of seed yield per hectare. The variance value for these seed yields per hectare genotypes was 145801. Plant height also showed maximum genetic variation in these genotypes with a 331.7 value of variance. The variance values of glucosinolate content, main raceme length, pods per the main raceme, and erucic acid were 167.7, 62.4, 31.5, and 23.3, respectively. A low level of genetic variation was found for seed quality and other traits. The genetic variations by morphological traits of 39.03% were investigated in the first three PCs, which were collected 17.79% in PCs, 11.45% in principal component 2, and 9.80% of genetic variations were investigated in principal component 3 for agro-morphological traits of these genotypes. Several studies have performed cluster and principal analysis for the study of genetic variations among 114 germplasms of *B. rapa* L. in 2005 to 2006.[14] By cluster analysis, they investigated high genetic variations among the genotypes because of differences found in agro-morphological and seed quality traits in their studied genotypes. From the study that was carried out in 2005, they concluded that seven out of 21 principal components with an eigenvalue higher than 1, contributed 74.09% of the whole genetic differences among these 114 genotypes. They also concluded from the study of 2006 that five out of 21 principal components contributed 66.08% with an eigenvalue higher than 1.

2. MATERIALS AND METHODS

2.1. Experimental Material. The experimental material comprised 150 accessions with three check varieties of

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chakwal-sarson, Takwara, and Abasin-95 of *B. napus* accessions, which were obtained from the National Gene Bank. The germplasm list is provided in Table 1.

2.2. Experimental Site. The field experiment was conducted during *Rabi* 2019 and molecular evaluation through markers in 2020 at the Central Lab for Biological Sciences.

2.3. Field Plot Techniques. The experimental material of 150 accessions with three check varieties, chakwal-sarson, Takwara, and Abasin-95 of *B. napus*, was laid out in augmented design. The germplasm was sown in six rows each of 5.0 m length with a spacing of 30 cm and inter accession distance kept at 60 cm. Recommended practices of irrigations and fertilizer application were also performed. A hand drill was used for planting the experiment at a depth of 3 to 4 cm. For optimum plant, population thinning was carried out. For weed control, hand weeding was done.

2.4. Data Collection. The qualitative and quantitative observations were recorded for five randomly selected plants for different traits in each accession. A standard descriptor of the International Board of Plant Genetic Resources, (IBPGR), for *Brassica* and *Raphanus,* was used for data collection (Tables 2 and 3).

Table 2. Qualitative Traits

s. no.	qualitative traits	parameters
1	leaf shape	1 = lanceolate; 3 = spatulate; 5 = obovate; 7 = broad-elliptic; 9 = broad circular)
2	leaf margin	(1 = entire; 3 = lobed; 5 = cleft; 7 = parted; 9 = sect
3	leaf incision	0 = entire; 1 = crenate; 2 = dentate; 3 = serrate; 4 = undulate; 5 = doubly dentate).
4	leaf color	1 = yellow green; 2 = light green; 3 = green; 4 = dark green; 5 = purple green; 6 = purple; 7 = other)
5	flower color	1 = white; 2 = cream; 3 = yellow
6	seed color	1 = yellow; 2 = yellow brown; 3 = light brown; 4 = brown; 5 = dark brown; 6 = red brown; 7 = red; 8 = blue black; 9 = gray black; 10 = other
7	silique surface outline	(3 = smooth; 5 = undulating; 7 = constricted between seeds).
8	silique attitude	1 = erect; 2 = hanging; 3 = pointing down
9	silique shattering	3 = low; 5 = intermediate; 7 = high

Table 3. Quantitative Traits Measured

s. no.	quantitative traits	abbreviations	unit
1	days to flowering initiation	DFI	no
2	days to 50% flowering	DF	no
3	days to flower completion	DFC	no
4	days to maturity	DM	no
5	leaf length	LL	*Cm*
6	leaf width	LW	*Cm*
7	plant height	PH	*Cm*
8	primary branches per plant	PB/P	no
9	main raceme length	MRL	*Cm*
10	stem thickness	ST	Mm
11	pods/main raceme (no.)	P/MR	no
12	pod length (cm)	PL	*Cm*
13	pod width mm	PW	Mm
14	seeds per pod (SS)	S/P	no
15	1000-seed weight (TSW)	1000 SW	G

2.5. Qualitative Traits. 2.6. Quantitative Traits. 2.7. Biochemical Analysis. The following biochemical traits were recorded for oil quality of each accession by near-infrared reflection spectroscopy (NIRS) at The Nuclear Institute for Food and Agriculture (NIFA), as shown in Table 4.

Table 4. Biochemical Traits Analyzed

s. no.	biochemical traits
1	oil content
2	protein content
3	glucosinolate content
4	oleic acid content
5	linoleic acid
6	erucic acid content

2.8. Statistical Analysis. *2.8.1. Data Analysis for Morphological and Seed Quality Traits.* For basic statistics, the data recorded were averaged and cultivar means were analyzed such as range, mean, variance, and coefficient of variation while all qualitative characters were classified into distinct groups, and their frequency percentage values were calculated. According to other studies, the data collected from quantitative traits and cluster analysis was performed.[15] Before cluster analysis, the mean of each trait was standardized so that the effect due to the scaling error could be avoided. For all of the pairs of genotypes, coefficients of Euclidean distance were calculated. To estimate the relationship among the genotypes with a cluster analysis, the Euclidean dissimilarity coefficient matrices were used by applying the complete linkage method (NTSys PC v 2.1 and Statistica v 6.0).

2.8.2. Molecular Analysis. DNA Extraction. The CTAB (cetyltrimethylammonium bromide) method of Murray and Thompson and revised by Doyle was used for DNA extraction during the present work. The extracted DNA was analyzed both quantitatively as well as qualitatively.[16,17]

SSR Analysis. The DNA was then amplified using 30 different SSR primers. The concentrations of PCR reagents for each reaction were optimized for efficient and accurate amplification. All reagents were Invitrogen products (Invitrogen, USA).

Gel Electrophoresis. PCR-amplified products were evaluated through electrophoresis by the following procedure: 1 g of agarose gel was weighed and dissolved in 100 mL of 1× TAE (Tris-acetate EDTA) buffer. Then, it was heated for 2 min until boiling starts. When gel became lukewarm, 50 μL of ethidium bromide was added and shifted to a gel tray to polymerize. PCR products were loaded in wells. The DNA ladder (100 bp) was loaded in the first well of the gel for the quantification of amplified products. After electrophoresis, amplified products were examined beneath UV Tran's illuminator.

Gel Scoring. After amplification and electrophoresis, the gel was scored for positive and negative results. All the visible and unambiguous fragments were scored as scorable fragments.

3. RESULTS

3.1. Investigation of Genetic Diversity through Agro-Morphological Traits. One hundred and 50 accessions of *B. napus* L. with three-check verity were screened during the current research. An IBPGR descriptor was used to record selected agro-morphological parameters at various stages of plant growth. The experimental materials were grown between

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2019 and 2020. In the present study, genetic variations were investigated with the help of nine qualitative and 15 quantitative agro-morphological traits. A considerable amount of genetic diversity was investigated among these genotypes for many traits.

3.2. Agro-Morphological Traits. Basic statistics such as mean, minimum, maximum, standard deviation, variance, and covariance percentage (CV %) were applied for all 15 agro-morphological traits of *B. napus* L., which showed high genetic variations. Notable variations were observed for different traits, where the highest variance was recorded for plant height, followed by days to flower initiation, and then pods per the main raceme, main raceme length, days to 50% flower completion, days to flower completion, and days to maturity. Analysis of variance evaluated had the same or very low value for traits like seeds per pod, stem thickness, leaf length, leaf width, primary branches per plant, 1000 seed weight, pod length, and pod width, as shown in Table 5.

Table 5. Basic Statistic of Agro-Morphological Traits of *Brassica napus* L.

traits	mean	minimum	maximum	SD	variance	CV %
DFI	95.75	65	151	14.62	213.75	15.27
DF 50%	102.38	72	144	12.61	159.11	12.32
DFC	109.68	78	152	12.19	148.48	11.11
DM	170.02	120	204	9.04	81.77	5.32
LL	13.10	6.4	22.68	3.35	11.20	25.55
LW	8.01	3.14	15.6	2.36	5.56	29.44
PH	148.48	81	207.8	30.61	937.05	20.62
PB/P	11.62	6.4	18	2.03	4.11	17.44
MRL	58.63	25	87	12.78	163.37	21.80
P/MR	63.61	38.4	105.4	14.27	203.54	22.43
ST	21.93	13.14	36.82	3.81	14.55	17.39
PL	4.93	2.07	8.52	1.16	1.34	23.49
PW	3.94	2.16	5.76	0.67	0.45	16.95
S/P	19.27	8	30.1	4.38	19.17	22.72
1000 SW	4.62	2.732	9.6	1.56	2.44	33.81

3.3. Qualitative Parameters. *3.3.1. Leaf Shape.* A high level of genetic diversity was identified for the leaf shape in this study, as indicated in Table 5. The highest frequency percentage was shown by spatulate, followed by falcate, cordate, and deltoid, while the lowest frequency percentage was recorded for the obovate and sagittate types of leaves (Figure 1A).

3.3.2. Leaf Margins. Parameter leaf division (margins) showed an elevated level of genetic variability among the studied genotypes of *B. napus* L. Five types of plants were registered based on the leaf margin. Leaf division of most genotypes, i.e., 65 (42.48%), was recorded as crenate-type margins while 45 (29.41%) genotypes were investigated with a dentate type of leaf division. The lowest frequency percentage (2.61) was recorded for dentate margins (Figure 1B).

3.3.3. Leaf Incision. Low variations were recorded for leaf incision among 153 genotypes in this study. The lyrate type of leaf incision was most frequent (62.09%) followed by the entire type (32.68%) while the lowest frequency (5.23%) was recorded for the crenate type of leaf incision, as shown in Figure 1C

3.3.4. Leaf Color. In the present study, three types of leaves were investigated based on leaf color. Ninety accessions out of 153 showed the green color of leaf followed by dark green (46 genotypes). The lowest frequency percentage was recorded for purple-green leaves, as shown in Figure 1C.

3.3.5. Flower Color. During this study, three types of flower color were noticed; among the observed color variants, the highest frequency was recorded for yellow color, followed by cream color, while the lowest frequency was recorded for white-colored flowers (Figure 1C).

3.3.6. Seed Color. Seed diversity was investigated based on their color; the highest frequency was recorded for brown color seeds, followed by dark-brown and gray-black. While the lowest number (4) was recorded for gray black seed (Figure 1D).

3.3.7. Silique Attitude. The erect type of silique attitude (93) was most frequent followed by hanging (36), while the lowest number was recorded for the pointing down silique attitude (Figure 1E).

3.4. Cluster Analysis of Agro-Morphological Traits. A multivariate analysis technique with the help of complementary analysis also called cluster analysis was applied for the analysis of 15 agro-morphological traits. The patterns of genetic association between *B. napus* L. genotypes were studied by using Euclidean dissimilarity coefficient matrices. To find out and evaluate the level of agro-morphological similarity and investigate the genetic association among the studied genotypes, the result is represented in the form of a dendrogram.

3.5. Euclidean Distances. During the present study, a wide range of Euclidean distances was investigated among all studied genotypes of *B. napus* L. The range for Euclidean distances was from 0 (between 24778 and 24873) to 14.24 (between 24177 and 24873).

3.6. Cluster Analysis. In conducting our study, a total number of 153 genotypes of *B. napus* L. were distributed into six main clusters at 7.83 genetic linkages through cluster analysis depending on 15 agro-morphological parameters. Cluster I consisted of 15 accessions. Cluster II was the largest cluster with 74 accessions. Cluster III consisted of 29 accessions followed by cluster IV that was composed of nine accessions; in addition to that, cluster V and cluster VI had 25 and 2 accessions, respectively, as shown in Tables 6 and 7 and Figure 2.

3.7. Correlation Analysis. Pearson correlation was calculated for finding of correlation between quantitative traits. According to the analysis, leaf length was positively and significantly correlated (r = 0.771) to leaf width. Plant height was significantly and positively correlated to (r = 0.367, r = 0.473, r = 0.167, r = 0.228) leaf length, leaf width PW, and 1000 seed weight, respectively. Main raceme length was significantly and positively correlated to days to maturity (r = 0.218) and stem thickness (r = 0.165). Seeds per pod was negatively and significantly correlated to leaf width (r = −0.238). Thousand seed weight was negatively and significantly correlated to days to maturity (r = −0.172) and pods per main raceme (r = −0.269) while 1000 seed weight was positively and significantly correlated to leaf length (r = 0.281) and leaf width (r = 0.21), as shown in Table 8.

3.8. Biochemical Analysis. Edible oil contains essential fatty acids linoleic and linolenic acids. For oil stability, 3% linolenic acid is preferred. Oils containing high amounts of erucic acid are suitable for industrial applications. Therefore, both developments of commercial varieties free of erucic acid content and with high erucic acid content are breeding objectives for *Brassica* oil crops. Other important objectives are

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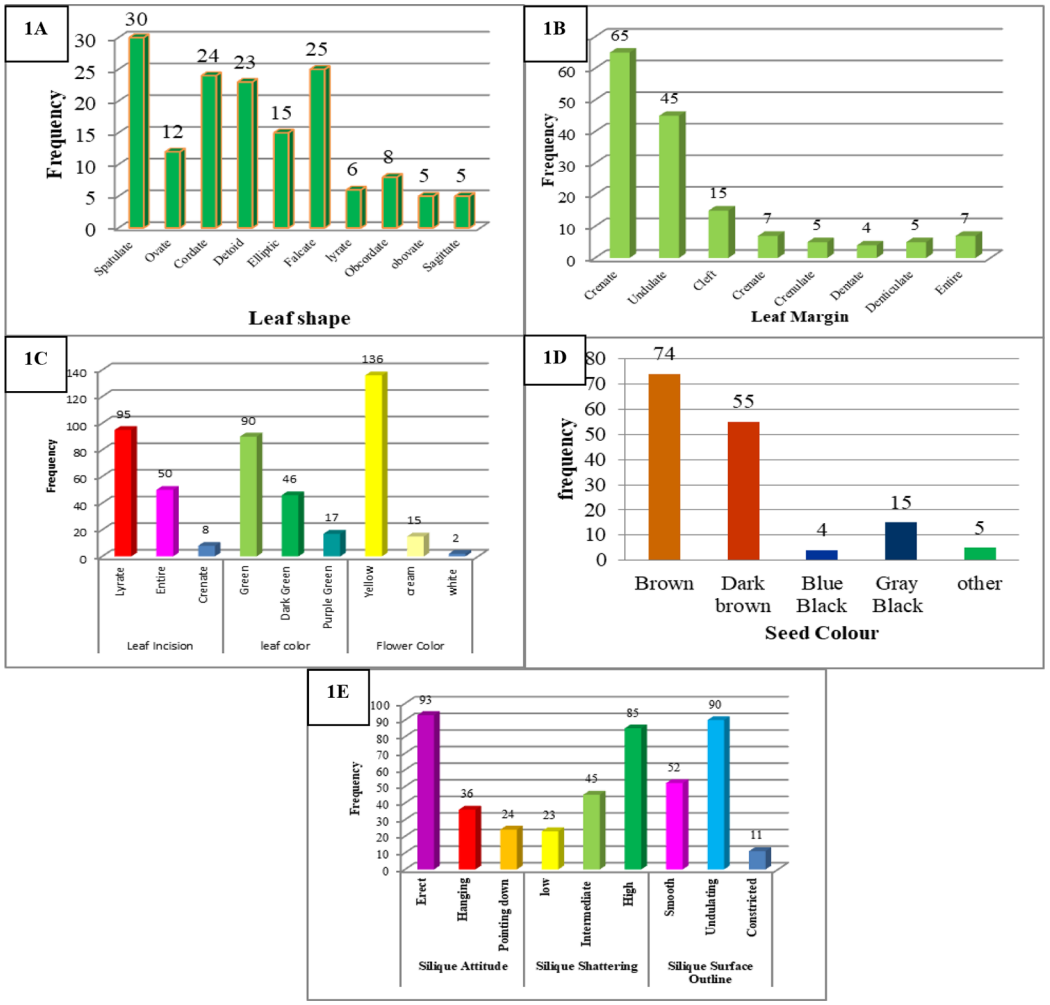

Figure 1. (A–E) Bar charts of frequency distribution for qualitative parameters of the studied genotypes.

Table 6. Cluster Analysis Based on Agro-Morphological Traits

clusters	no.	genotypes
cluster I	15	24177, 24191, 24199, 24180, 24221, 24206, 24207, 24190, 24858, 24224, 24225, 24252, 24194, 24196, 24198
cluster II	73	24179, 24182, 24181, 24186, 24187, 24211, 24845, 24183, 24245, 24861, 24213, 24215, 24239, 24238, 24855, 24856, 24256, 24859, 24853, 24854, 24857, 24865, 24246, 24259, 24249, 24850, 24846, 24257, 24852, 24188, 24240, Abasin, 24251, 24241, 24864, 24253, 24870, 24216, 24899, 24248, 24868, 24250, 24874, 24866, 24872, 24209, 24228, 24218, 24223, 24220, 24212, 24217, 24229, 24219, 24222, 24901, 26352, 24904, 24906, chakwal Sarson, 24362, Takwara, 26373, 24900, 24903, 24902, 24185, 24889, 24230, 24860, 24862, 24258,
cluster III	29	24184, 24204, 24203, 24214, 24227, 24254, 24242, 24189, 24205, 24208, 24843, 24226, 24237, 24844, 24842, 24192, 24255, 24847, 24848, 24849, 24193, 24195, 24247, 24857, 24197, 24200, 24210, 24201, 24202
cluster IV	9	24875, 24882, 24879, 24880, 24876, 24878, 24891, 24881, 24884
cluster V	25	24244, 24909, 24883, 24897, 24863, 24898, 24867, 26095, 26337, 26357, 24869, 24243, 24908, 24907, 24892, 24905, 24893, 24896, 26347, 24877, 24886, 24887, 24888, 24895, 24894
cluster VI	2	24877, 24873

the increase in oleic acid, an increase in linoleic acid, and the reduction of linolenic acid content. The world is positioned to face a serious shortage of edible oil due to an abrupt increase in population and enhanced per capita consumption. The great challenge for the coming decades will be the task of increasing edible oil production with less cropped land, particularly in countries with limited cropped land resources. Improved genotypes with nutritional benefits and high edible oil production are needed to the utmost. Based on biochemical analysis following accessions were selected for high oil content and protein content, low moisture content, high oleic acid, and high linoleic acid, and low and high erucic acid, as shown in Table 9.

3.9. Investigation of Genetic Diversity among the Genotypes of *B. napus* L. through SSR Markers. SSR-based molecular analysis utilizing a total of 30 microsatellite markers has been executed on a group of 50 diverse *B. napus* genotypes; they were collected from all three subspecies, and their selection was dependent on their high-quality agro-morphological performance.

3.9.1. Intraspecific Variations through B. napus L. Genotypes. To observe genomic variability through 50 *B. napus* genotypes, a number of 30 SSR markers have been used, as it is shown in Table 10. The marker size was measured in relation to their known sizes, and the desired amplified fragments have been observed. The number of alleles per locus

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Table 7. Promising Genotypes Based on Agro-Morphological Parameters

parameters	chakwal sarson	Takwara	Abasin	range	genotypes
DM (100%)	176	163	173	≥137 days	24178, 24857, 24873, 24901, 26352, 26357,
LL (cm)	9	10.8	10.84	≥20.2 cm	24194, 24199, 24221, 24258, 24881
LW (cm)	7.2	5.2	5.48	≤13	24191, 24199, 24221, 24842, 24881
PH (cm)	132.8	119.4	136	≥200	24198, 24202, 24208, 24225, 24237
PB/P	12.8	12.4	10	≥17	24230, 24258, 24863, 24866, 24898
MRL (cm)	57.8	44.8	59.2	≥81.8	24193, 24197, 24201, 24876, 24880
P/MR	105.4	96.4	56.2	≥96	24904, 24906, 26362, 26373
ST (mm)	23	19.18	20.138	≥29.9	24212, 24214, 24217, 24220, 24902
PL (cm)	4.29	5.27	4.54	≥7.72	24194, 24200, 24204, 24210, 24197,
PW (mm)	4.5	4.41	3.69	≥5.31	24178, 24222, 24242, 24881, 24907
S/P (no)	20.1	19.6	19.5	≥26	24184, 24214, 24227, 24242, 24250, 24254, 24848
1000SW (g)	3.624	5.24	3.274	≥8.3	24178, 24182, 24187, 24199, 24221

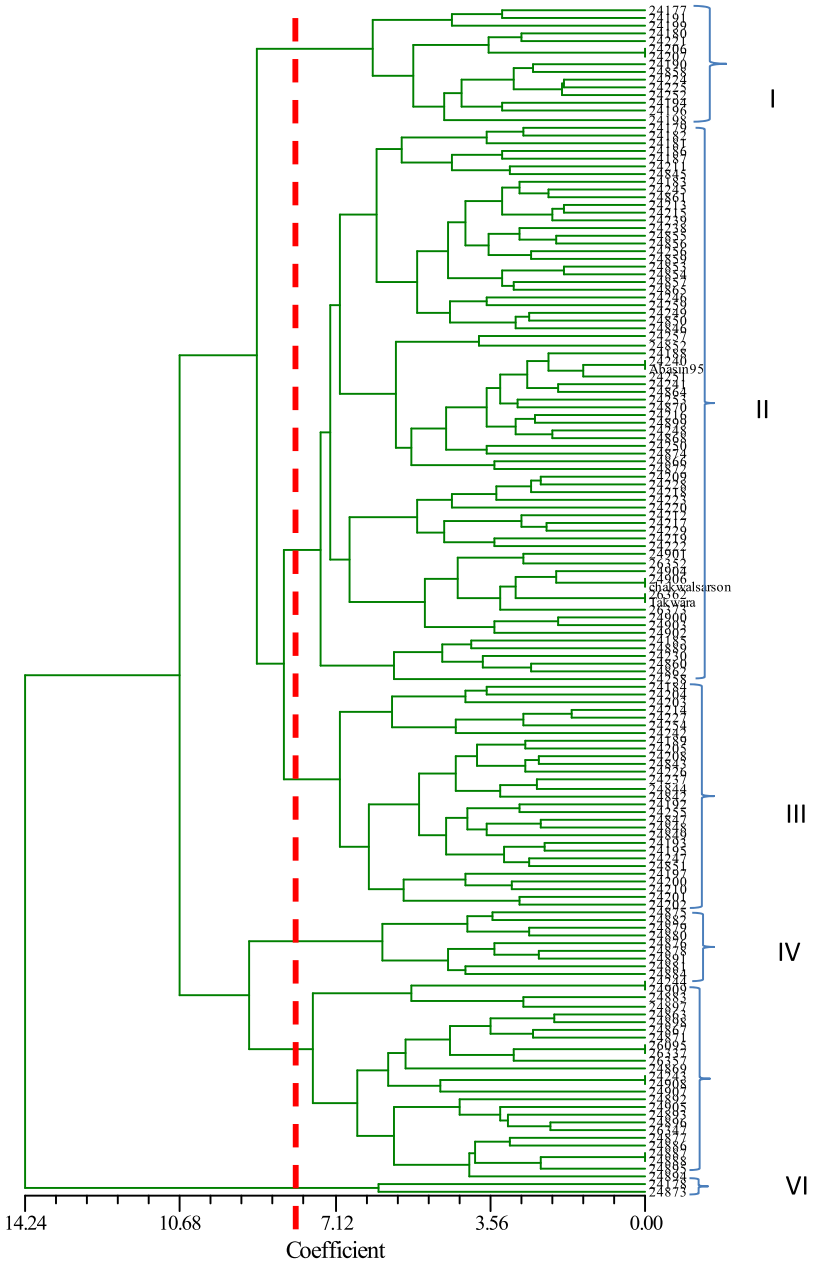

Figure 2. Dendrogram based on agro-morphological traits.

variability was from 1 to 3. Polymorphic banding patterns were revealed by most primers. Most of the amplified fragments observed ranged from 100 to 400 bp. A total of 30 primers (86.15%) were discovered in all genotypes. The other two

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Table 8. Correlation of Quantitative Traits of *Brassica napus* Germplasm

	LL (cm)	LW (cm)	PH (cm)	PB/P	MRL (cm)	P/MR	ST (mm)	PL (cm)	PW (mm)	S/P (no)	1000SW (g)
DM (100%)	−0.0567										
LL (cm)	−0.1263	0.7710*									
LW (cm)	−0.0292	0.3672*	0.4738*								
PH (cm)	0.0035	0.0583	0.0603	0.0608							
PB/P	0.2186*	−0.0004	0.0335	0.1431	0.1134						
MRL (cm)	0.0242	−0.1593	−0.1604	−0.0495	0.0266	−0.0028					
P/MR	0.1353	−0.0784	−0.1407	−0.1004	0.1262	0.0597	0.1655*				
ST (mm)	−0.0001	0.0595	0.0244	−0.0862	−0.1586	0.0229	−0.0301	0.0189			
PL (cm)	−0.0524	0.0585	−0.0072	−0.0327	0.1673*	−0.0179	0.1414	0.0382	0.0138		
PW (mm)	0.0423	−0.1502	−0.2389*	−0.1299	−0.0666	0.0695	0.0232	0.1187	0.1322	−0.0248	
S/P (no)	−0.1762*	0.2810*	0.2151*	0.2288*	−0.0634	−0.2698*	0.0173	−0.0346	0.1205	−0.0035	−0.1078

Table 9. Accessions Selected Based on Biochemical Traits

biochemical traits	range	accessions
oil content	≥48.8	24189, 24192, 24894, 26095, 26357, chakwal sarsoon
protein content	≥32.5	24183, 24191, 24209, 24896, 26347
moisture content	≤6.1	24210, 24230, 24239, 24246, 24250
oleic acid	≥71.3	24181, 24192, 24201, 24204, 24209
linoleic acid	≥13.8	24181, 24195, 24204, 24252, 24856
erucic acid low	≤28.3	24250, 24903, 24907, 26352, 26373, 24230
erucic acid high	≥60	24177, 24195, 24238, 24249, 24252, 24254

primers (6.15%) have the names of Ni2-A12 and RA2-A11 amplifying two alleles for each one of them.

3.9.2. Cluster Analysis. The genetic similarity values ranging from 18 to 100% were calculated among different *B. napus* genotypes. All of the genotypes were classified into six major groups based on the UPGMA similarity method. Cluster I consisted of six genotypes. Cluster II contained 10 genotypes. Cluster III is the largest cluster that contained 31 genotypes, which is further divided into two subclusters (IIIA and IIIB). Cluster IIIA contained 15 genotypes including two check varieties, i.e., chakwal sarson and Takwara. Cluster IIIB contained 16 genotypes. The groups IV, V, and VI contained

Table 10. Details of SSR Markers Used, Indicating *Brassica napus* and Total Number of Alleles

s. no.	primer name	total amplified alleles (a)	polymorphic allele (b)	%age polymorphism ($b \times 100/a$)	size range (bp)	melting temp.
1	Na10-B08	1	1	100	100	59
2	Na10-D03	1	1	100	190−200	58
3	Na10-D09	1	1	100	110, 290	57.5
4	Na10−F06	1	1	100	100−110	59
5	Na10-G08	1	1	100	280	56
6	Na12-A07	1	1	100	210	58
7	Na12B09	1	1	100	210	54
8	Na12-C08	1	1	100	210	59.3
9	Na12-D04	1	1	100	280	58
10	Na14-D07	1	1	100	110	54
11	Ni2-A06	1	1	100	280	54
12	Ni2-A12	1	2	100	110−200	54
13	Ni2-B01	1	1	100	500	54
14	Ni2-C12	1	1	100	140−150	55.7
15	Ni3-G05	1	1	100	200,	54.5
16	Ni4B10	1	1	100	200	58.7
17	Ni4-D09	1	1	100	200	54
18	Ol09-A03	1	1	100	110	54
19	Ol10-B01	1	1	100	200	59
20	Ol10-F11	1	1	100	180−200	54
21	PBCESSRJU1	1	1	100	390	54
22	PBCESSRJU2	1	1	100	120	53
23	PBCESSRJU4	1	1	100	290−300	54
24	PBCESSRJU5	1	1	100	190−200	54
25	PBCESSRJU7	1	1	100	200−220	54
26	PBCESSRJU8	1	1	100	203−228	53
27	PBCESSRJU12	1	1	100	120	54
28	PBCESSRJU14	1	1	100	180	54
29	PBCESSRJU16	1	1	100	250	54
30	PBCESSRNA8	1	1	100	290	54

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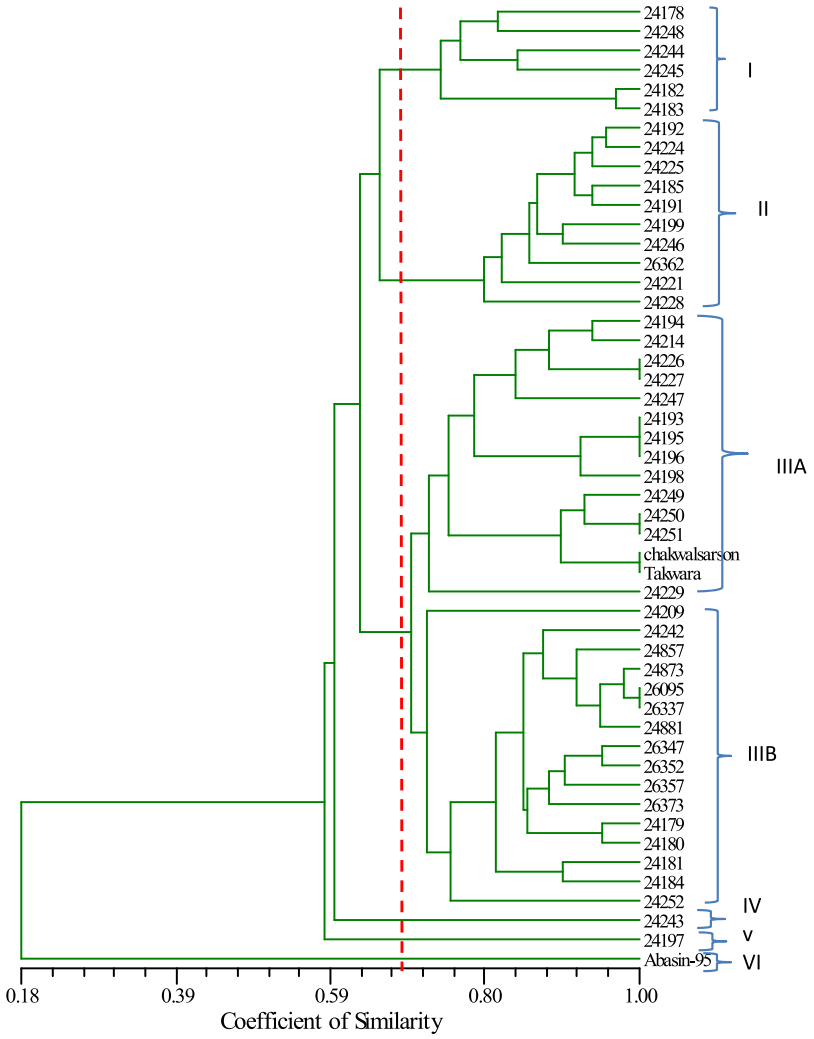

Figure 3. Dendrogram of 50 genotypes of *Brassica napus* L. based on SSR.

only one genotype, i.e., 24243, 24197, and Abasin- 95. In general, the most diverse groups have been noticed. Some groups had a mutual origin. While the origin for other groups was not the same. The research results revealed an elevated degree of intraspecific resemblance through genotypes from various regions. An increased level of genomic diversity has been recognized between exotic accessions. It is assumed to be related to two causes, the diverse geographical origin of accessions or a different genetic origin (Figure 3; Table 11).

4. DISCUSSION

4.1. Investigation of Genetic Diversity through Agro-Morphological Parameters. The investigation of the extent of available diversity is crucial for the efficient utilization and conservation of crop germplasm. Breeders mostly depend upon the extent of morphological variation; thus, the study of morphological parameters is so much necessary for the classification and study of crop germplasm.[18] Therefore, it is very essential to make proper planning for the collection and assessment of germplasms, which are locally found in many areas globally to prevent them from being extinct.[19] The evaluation and investigation of the genetic diversity of crop germplasm are also very useful for future breeding programs.[20] The conservation of genetic diversity is very necessary because after the green revolution due to the high demand for better seed varieties, seed companies focused on the production of high-yielding hybrid seeds with narrow genetic variability putting the genetic diversity under threat. Thus, maintenance of the present genetic diversity of crop germplasms is particularly important. Nowadays, crop germplasms are considered a useful source of genetic diversity, which is used by many plant breeders for the production of a new variety in many breeding programs globally.[21] This study evaluated genetic diversity among the *B. napus* L. genotypes by agro-

Table 11. Distribution of 40 *Brassica napus* L. Genotypes into Different Groups Based on Cluster Analysis

groups	no. of genotypes	accessions
I	6	24178, 24248, 24244, 24245, 24182, 24183
II	10	24192,24224, 24225, 24185, 24191, 24199, 24246, 26362, 24221, 24228.
IIIA	15	24194, 24214, 24226, 24227, 24247, 24193, 24195, 24196, 24198, 24249, 24250, 24251, chakwal Sarson, Takwara, 24229.
IIIB	16	24209, 24857, 14873, 26095, 26337, 24881, 26337, 24881, 26347, 26352, 26357, 26373, 24179, 24180, 24181, 24184, 24252
IV	1	24243
V	1	24197
VI	1	Abasin-95

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morphological parameters, and valuable results were obtained. During the present study, many parameters like plant height, leaf width, seed per pod, seed yield per plant, main raceme length, stem thickness, and flowering related traits showed high variability. A similar study was performed to study *Brassica carinata* L. germplasms and found high genetic variability among the traits of plant height, main raceme length, and silique on main raceme length while another study evaluated *Eruca sativa* L. and found out high genetic variation for the parameters of silique, length, main raceme length, plant height, leaf length, days to flowering completion, and days to 50% flowering.[22,23] Former studies have evaluated *B. napus* L. and identified significant variations for the parameter of grain yield per plant among the studied genotypes.[24] High variations among the *Brassica* studied germplasms for the parameter of seed grain per plant, and thousand-seed weight has been announced before in a lot of studies.[25] The present study was also supported by the studies of others as they observed high genetic variability for the studied parameters.[26] Notable variations were investigated in the present study for the parameters of days to flowering initiation, days to 50% flowering, plant height, seed per pod, seed yield per plant, leaf length, stem thickness, main raceme length, and leaf width. All these parameters would help in future breeding programs for the production of more suitable varieties of *B. napus* L. Very low levels of genetic variations were investigated for some parameters among the studied genotypes of *B. napus* L.; therefore, they were ignored in the current study. It is very important to collect local along with exogenous genotypes for the development of a better breeding plan for the conservation of genotypes for future utilization.[27,28] Collection of samples from different ecological conditions is the only way to produce a high genetic variation for the selection of high-quality and high-yielding cultivars for different agroecological zones.[29,30] These parameters have great value in different breeding programs. Thousand seed weights and grain yields per plant have a great effect on yield. Apart from these, primary branches per plant and the number of seeds per pod have an important role in crop yield.[31]

4.2. Cluster Analysis of the Agro-Morphological Parameters. In the past, many plant breeders and scientists have applied and obtained promising variations for different *Brassica* cultivars in agro-morphological parameters with the help of two main complementary techniques of analysis, principal component analysis and cluster analysis. These analysis techniques were mentioned in former studies to have a successful execution in mustard cultivars.[32−35] These techniques were also stated before in a lot of studies to be found efficiently in white head cabbage and can play a crucial role in the investigation of genetic diversity among diverse genotypes.[36] The distribution of studied *B. napus* L. cultivars into clusters was not due to their local or global origin and divergent habitat; rather, it was their variations at agro-morphological levels. Preceding studies have obtained similar results for qualitative and quantitative parameters of *Iberian pea* cultivars.[37] A considerable level of genetic variation was investigated among the studied *B. napus* L. genotype for different agro-morphological parameters during the present study. In the future, these parameters could play a key role in the production of new *B. napus* L. varieties.

4.3. Genetic Similarity Matrix and Cluster Analysis. In the present study, *B. napus* and L. genotypes were investigated having similarity in the range of 63 to 100%. The dendrogram of the present study was generated through a dissimilarity matrix using UPGMA (unweighted pair groups method with arithmetic averages) and distributed all the studied genotypes into five main groups.

5. CONCLUSIONS

In the present study, valuable genetic diversity was investigated with the help of agro-morphological techniques among the studied genotypes for different parameters. The best adoptable genetically diverse exotic *Brassica* germplasms were selected, i.e., accessions 24178, 24881, 24199, 24214, 24242, and 24192. Based on biochemical analysis, for high oil content and high oleic acid content, chakwal sarsoon and accession 24192, were selected, respectively. For high oleic and linoleic acids, accession 24181 performed best, for low erucic acid accessions 24177 and 24195. These genotypes have great capacity and could be used in future breeding programs. Based on biochemical analysis, for high oil content and high oleic acid content, chakwal sarsoon and accession 24192 were selected, respectively. For high oleic and linoleic acids, accession 24181 performed best, for low erucic acid accessions 24177 and 24195. High genetic variations were investigated through SSR primers among the studied genotypes of *B. napus* L. These variations were not due to their geographical origin as many genotypes belonging to the same geographical location but were found in a different group. Based on SSR data, the germplasms accession 24178 and Abasin were the most diverse genotypes. The present result also provides strength to the past studies that SSR is not influenced through environmental conditions or agro-morphological and biochemical parameters, because a majority of genotypes that were observed in a different group during the agro-morphological study were present within the same group during the SSR study. Thus, the present study revealed that variation investigated at the morphological level among the studied genotypes of *B. napus* L. was more due to environmental conditions. The present study also concluded that SSR is a better technique for the study of intraspecific genetic diversity. Other modern techniques should be applied as SNIP for the investigation of a high level of genetic diversity among crop plants in the future.

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Notes

The authors declare no competing financial interest.
The contributions presented in the study are incorporated in the article; additional questions can be directed to the corresponding author.

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■ REFERENCES

(1) Xuan, L.; Yan, T.; Lu, L.; Zhao, X.; Wu, D.; Hua, S.; Jiang, L. Genome-wide association study reveals new genes involved in leaf trichome formation in polyploid Oilseed rape (Brassica napus L.). *Plant, Cell & Environment* **2020**, *43*, 675−691.
(2) Amjad, M. (2014) Oilseed crops of Pakistan. Pakistan Agricultural Research Council Islamabad,(PARC), 1−59.
(3) Coombs, A. R. (2020) Beyond the Nitrogen Thesis: Temporal and Spatial Patterns of Brassica Napus Oilseed Design Diversification for Crop Rotation, Soil Pest Suppression, Livestock Management, and Convertible Husbandry in the British Agricultural Revolution (s), 1715−1830. Doctoral dissertation, The University of Chicago, ProQuest LLC; Chicago, Illinois DOI: 10.6082/uchicago.2676
(4) Hatzig, S. V.; Nuppenau, J.-N.; Snowdon, R. J.; Schießl, S. V. Drought stress has transgenerational effects on seeds and seedlings in winter Oilseed rape (Brassica napus L.). *BMC Plant Biol.* **2018**, *18*, 297.
(5) Tao, Y.; Zhao, X.; Mace, E.; Henry, R.; Jordan, D. Exploring and exploiting pan-genomics for crop improvement. *Molecular plant* **2019**, *12*, 156−169.
(6) Nunes-Nesi, A.; Nascimento, V. d. L.; de Oliveira Silva, F. M.; Zsögön, A.; Araújo, W. L.; Sulpice, R. Natural genetic variation for morphological and molecular determinants of plant growth and yield. *Journal of experimental botany* **2016**, *67*, 2989−3001.
(7) Silva-Pérez, V.; De Faveri, J.; Molero, G.; Deery, D. M.; Condon, A. G.; Reynolds, M. P.; Evans, J. R.; Furbank, R. T. Genetic variation for photosynthetic capacity and efficiency in spring wheat. *Journal of experimental botany* **2020**, *71*, 2299−2311.
(8) Friedt, W., Tu, J.; Fu, T. Academic and economic importance of Brassica napus rapeseed. In Liu, S.; Chalhoub, B. Eds., *The Brassica napus genome*, 2018 1−20. Springer, Cham DOI: 10.1007/978-3-319-43694-4_1.
(9) Li, C.; Hao, M.; Wang, W.; Wang, H.; Chen, F.; Chu, W.; Zhang, B.; Mei, D.; Cheng, H.; Hu, Q. An efficient CRISPR/Cas9 platform for rapidly generating simultaneous mutagenesis of multiple gene homoeologs in allotetraploid Oilseed rape. *Front. Plant Sci.* **2018**, *9*, 442.
(10) Sandhu, R.; Rai, S.; Bharti, R.; Kour, A.; Gupta, S.; Verma, A. Studies on genetic diversity among various genotypes of Brassica napus L. using morphological markers. *International Journal of Current Microbiology and Applied Science* **2017**, *6*, 469−480.
(11) Gong, Q.; Lian, J.-L.; Li, X.-R.; Huang, Z.; Xu, A.-X.; Dong, J.-G.; Yu, C.-Y. The genetic diversity and heterotic groups of 169 Chinese semi-winter rapeseed (Brassica napus) cultivars and inbred lines. *Mol. Breed.* **2020**, *40*, 1−15.
(12) Ahmad, B.; Mohammad, S.; Azam, F.-I.; Ali, I.; Ali, J.; ur Rehman, S. Studies of genetic variability, heritability and phenotypic correlations of some qualitative traits in advance mutant lines of winter rapeseed (Brassica napus L.). *Am.-Eurasian J. Agric. Environ. Sci.* **2013**, *13*, 531.
(13) Malek, M. A.; Rafii, M. Y.; Afroz, S. S.; Nath, U. K.; Mondal, M. M. A. Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants. *Sci. World J.* **2014**, *2014*, 968796.
(14) Yousuf, M. 2007. *Assessment of Genetic Diversity for agro-morphological and Biochemical Traits, and Inheritance Studies in Rapeseed*. Pir Mehr Ali Shah Arid Agriculture University Rawalpindi: Pakistan.
(15) Sneath, P. H. A.; Sokal, R. R. Numerical taxonomy. In *The principles and practice of numerical classification*. 1973 W.H. Freeman & Co..
(16) Murray, M.; Thompson, W. Protocol of DNA isolation. *Nucleic Acids Res.* **1980**, *8*, 4321−4325.
(17) Doyle, J. DNA protocols for plants. In Hewitt, G. M.; Johnston, A. W. B.; Young, J. P. W. eds., *Molecular techniques in taxonomy*, 1991 57 283−293, NATO ASI, Springer: Berlin, Heidelberg DOI: 10.1007/978-3-642-83962-7_18.
(18) Koffi, K. K.; Gbotto, A. A.; Malice, M.; Dje, Y.; Bertin, P.; Baudoin, J.-P.; Bi, I. A. Z. Morphological and allozyme variation in a collection of Cucumeropsis mannii Naudin (Cucurbitaceae) from Côte d'Ivoire. *Biochem. Syst. Ecol.* **2008**, *36*, 777−789.
(19) Balkaya, A.; Ergün, A. Diversity and use of pinto bean (Phaseolus vulgaris) populations from Samsun, Turkey. *New Zealand Journal of Crop and Horticultural Science* **2008**, *36*, 189−197.
(20) Iannetta, P.; Begg, G.; Hawes, C.; Young, M.; Russell, J.; Squire, G. Variation in Capsella (shepherd's purse): an example of intraspecific functional diversity. *Physiologia Plantarum* **2007**, *129*, 542−554.
(21) Baranger, A.; Aubert, G.; Arnau, G.; Lainé, A.; Deniot, G.; Potier, J.; Weinachter, C.; Lejeune-Henaut, I.; Lallemand, J.; Burstin, J. Genetic diversity within Pisum sativum using protein-and PCR-based markers. *Theor. Appl. Genet.* **2004**, *108*, 1309−1321.
(22) Zada, M.; Shinwari, Z. K.; Zakir, N.; Rabbani, M. A. Study of total seed storage proteins in Ethiopian mustard (Brassica carinata A. Braun) germplasm. *Pak. J. Bot.* **2013**, *45*, 443−448.
(23) Shinwari, S.; Akbar, F.; Rabbani, M. A.; Mumtaz, A. S.; Shinwari, Z. K. Evaluation of genetic diversity in different genotypes of Eruca sativa from Pakistan by SDS-PAGE analysis. *Pak. J. Bot.* **2013**, *45*, 1235−1240.
(24) Ali, N.; Javidfar, F.; Elmira, J. Y.; Mirza, M. Y. Relationship among yield components and selection criteria for yield improvement in winter rapeseed (Brassica napus L.). *Pak. J. Bot.* **2003**, *35*, 167−174.
(25) Abideen, S. N. U.; Nadeem, F.; Abideen, S. A. Genetic variability and correlation studies in Brassica napus L. genotypes. *Int. J. Innovation Appl. Stud.* **2013**, *2*, 574−581.
(26) Rameeh, V. Multivariate analysis of some important quantitative traits in rapeseed (Brassica napus) advanced lines. *J. Oilseed Brassica* **2016**, *1*, 162−169.

(27) Jain, S.; Qualset, C.; Bhatt, G.; Wu, K. Geographical patterns of phenotypic diversity in a world collection of durum wheats 1. *Crop Science* **1975**, *15*, 700−704.
(28) Ghafoor, A.; Zubair, M.; Malik, B. A.; Iqbal, S. M. Evaluation of selected germplasm of mungbean (Vigna radiata (L.) Wilczek). *Pak. J. Bot.* **1992**, *24*, 112−118.
(29) Brown, A. Isozymes, plant population genetic structure and genetic conservation. *Theor. Appl. Genet.* **1978**, *52*, 145−157.
(30) Laghetti, G.; Pienaar, B.; Padulosi, S.; Perrino, P.; Ecogeographical distribution of Vigna savi in southern Africa and some areas of the Mediterranean basin. Plant Genetic Resources Newsletter (IPGRI/FAO), Bulletin des Ressources Phytogenetiques (IPGRI/FAO). Noticiario de Recursos Fitogeneticos (IPGRI/FAO) 1998.
(31) Murty, B. R.; Qadri, M. I. Analysis of divergence in some self-compatible forms of Brassica campestris var. brown sarson. *Indian J. Genet. Plant Breed.* **1966**, *26*, 45−58.
(32) Rabbani, M. A.; Iwabuchi, A.; Murakami, Y.; Suzuki, T.; Takayanagi, K. Phenotypic variation and the relationships among mustard (Brassica juncea L.) germplasm from Pakistan. *Euphytica* **1998**, *101*, 357−366.
(33) Gupta, V. P.; Sekhon, M. S.; Satija, D. R. Studies on genetic diversity, heterosis and combining ability in Indian mustard [Brassica juncea L. (Czern & Coss)]. *Indian J. Genet.* **1991**, *51*, 448−453.
(34) Genet, T.; Labuschagne, M. T.; Hugo, A. Genetic relationships among Ethiopian mustard genotypes based on oil content and fatty acid composition. *African J. Biotechnol.*, **2015** *4* 1256.
(35) Alemayehu, N.; Becker, H. Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (Brassica carinata A. Braun). *Genetic Resources and Crop Evolution* **2002**, *49*, 573−582.
(36) Balkaya, A.; Yanmaz, R.; Apaydin, A.; Kar, H. Morphological characterisation of white head cabbage (Brassica oleracea var. capitata subvar. alba) genotypes in Turkey. *New Zealand Journal of Crop and Horticultural Science* **2005**, *33*, 333−341.
(37) Amurrio, J. M.; De Ron, A.; Zeven, A. Numerical taxonomy of Iberian pea landraces based on quantitative and qualitative characters. *Euphytica* **1995**, *82*, 195−205.

https://doi.org/10.1021/acsomega.3c05688
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