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GENETIC DIVERGENCE IN *BRASSICA NAPUS* L.GERMPLASM AS DETERMINED BY QUANTITATIVE ATTRIBUTES

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Abstract

Brassica napus L., a candidate with high yield and good quality oil potential was evaluated for genetic divergence for two years on two locations. A collection of 328 lines belonging to various origins along with a check variety Faisal Canola was sown in the field following augmented design and phenotyped for eighteen quantitative traits. The recorded data when statistically analyzed inferred that, days to flower initiation, 50 % flowering, flower completion, 50 % maturity were main contributors of variations in the germplasm and were highly related with pod dehiscence and yield. Furthermore, BN328, BN371, BN494, BN618, BN625 and BN627 were found diverse lines in both years. The outcomes from this study are very helpful to proceed for any Oilseed rape breeding programs to improve yield.

Key words: *Brassica napus* L., Pod Shatter, PCA, Yield.

Introduction

Brassica napus, an important cultivated oilseed crop, famous for good quality oil, belongs to oilseed rape of the Brassica family. Oilseed rape, worldwide, was cultivated on an area of 33.74 million hectares, which produced 66.85 million tons of seeds and 26.63 million tons of oil (Anon., 2016). In Pakistan 0.23 million hectares of land produced 0.2 million tons of rapeseed and 0.395 million tons of rapeseed oil (Anon., 2016). In Pakistan, demand of edible oil is very high owing to continually increasing population and improved living standards of masses. There is a serious shortage of edible oil in the country. Every year billions of precious foreign exchange is spent on import for edible oil. During 2014-15, US $ 1.37 billion were spent for import of 1.78 million tons of edible oil (Anon., 2014-15).

Brassica napus L. is an amphidiploid having AACC genome (2n=38), developed by a cross between diploid species *B. rapa* L. having AA genome (2n=20) and *B. oleracea* L. having CC genome (2n=18) (Parakash & Hinata, 1980). Its oil contains low erucic acid content (Aytac *et al.,* 2006). It is an important crop of the *Pothwar* region but faces heavy yield losses due to severe shattering at maturity and during harvesting. The dehiscence of mature, ripe and dry fruit is a natural process by which various plant species spreads their seed in order to survive, but this phenomenon of pod dehiscence results in significant yield loss in agriculture. Moreover, the shattered seed can remain in the soil for almost 10 years, giving rise to weeds in the following crops (Gulden *et al.,* 2003). According to a study the typical yield losses ranges from 10-25% (Price *et al.,* 1996). Seed losses of about 50% of the expected yield have also been reported when harvesting was delayed (Child & Evans, 1989). Although, the agronomic practices like windrowing and spraying of desiccants to reduce pod shatter and to achieve better uniformity of ripening for harvest are being carried out, yet such practices add to the cost of production and also reduce the flexibility in farm operations (Kadkol, 2009).

The solution lie in the enhanced inherent shatter resistance that could provide the option of delaying harvest which will allow more evenly mature seeds and also decrease the occurrence of chlorophyll contamination from immature seeds in the extracted oil (Morgan *et al.,* 1998). Such successful efforts will boost up the local production of edible oil in the country. Various scientists have extensively used morphological traits in their studies to estimate genetic divergence in various crops (Maqbool *et al.,* 2010, Markonato *et al.,* 2016, Naheed *et al.,* 2016) and planned successful breeding programs.

When dealing with a large set of germplasm lines, irrespective of the kind of data; whether it be of morphological, biochemical or molecular markers, multivariate analyses techniques are used for determining the genetic divergence. The most commonly algorithms being used by various researchers for this purpose are principal component analysis (PCA), cluster analysis, principal coordinates analysis (PCoA) and multidimensional scaling (MDS) (Ciancaleoni *et al.,* 2014; Šamec *et al.,* 2016).

The present study was carried out, in view of the importance of genetic divergence for the improvement of crop for better yields and more shatter resistance in *Brassica napus.* The germplasm was evaluated for two consecutive seasons at two locations in order to determine the magnitude of variation due to various traits along with determining diverse and promising lines which could possibly be then used to breed population with improved pod shatter resistance and higher yield potential.

Materials and Methods

The germplasm (Table 1) was studied at two locations, *viz.*, National Agriculture Research Centre, at Islamabad [Location 1] (Latitude=33º - 42' N, Longitude 73º- 10' E and Altitude = 508 m) and Pir Mehr Ali Shah Arid Agriculture University Research Farm, Chakwal

Road at Rawalpindi [Location 2] (Latitude=33°-06'N and Longitude= 73°-00'E and Altitude = 502 m). Average temperatures and rainfall data recorded for both locations during the experiment duration are depicted in figure 9 and 10 (Source: Pakistan Meteorological Department). The experiment was conducted for two consecutive growing seasons of 2013-14 and 2014-15, at both locations following augmented design with 328 entries and a check variety; Faisal Canola, repeated after every 20 entries. Seeds were planted on 10th of October 2013 at location 1 and 15th of October 2013 at location 2 and harvesting were completed on 15th of April 2014 at location 1 and 21st of April 2014 at location 2 for first year and again planted on 13th of October 2014 at location 1 and on 18th of October 2014 at location 2 and harvested on 18th of April 2015 at location 1 and 25th of April 2015 at location 2 for second year. The type of soil at both locations was found to be silt loam (*Ochric andosols* according to FAO system). The plot size for each entry was single row of 5 m length. All the recommended practices were done to raise the crop. Data were recorded starting from when flowering began until the last entry was of fully matured and harvested plants. Practice of selfing at flowering time by using butter paper bags was done to get true to type seeds for next year sowing.

Randomly selected five plants were tagged for data recording. Data for eighteen quantitative traits; *viz.*, days to flower initiation (DFI); days to 50% flowering (DF50%); days to flower completion (DFC); days to 50% maturity (DM50%); days to 100% maturity (DM 100%); shattering percentage after 0, 7, 14 and 21 days of physiological maturity (pod shatter percentage); leaf length (LL) and leaf width (LW); plant height (PH); primary branches/plant (PB/P); main raceme length (MRL); pods/main raceme (P/MR); stem thickness (ST); pod length (PL); pod width (PW); seeds/pod (S/P); 1000-seed weight (TSW) and seed yield/plant (SY/P), were recorded. The data for all these parameters were analyzed for differences based on method provided by Steel *et al.*, (1997) and Principle Component Analysis was performed following Ogunbayo *et al.*, (2005) to determine the contribution of these parameters to diversity and to select diverse lines. For principal component analysis a correlation matrix was used and criterion of Eigen value significance was used to select statistically significant principal components as determined by Kaiser (1960).

Table 1. Origin details of germplasm studied.

Sr. No.	Origin	Number of accessions
1.	Australia	18
2.	Canada	35
3.	China	62
4.	Czech	1
5.	Denmark	1
6.	France	3
7.	Germany	4
8.	Netherlands	1
9.	Pakistan	98
10.	Sweden	5
11.	Unknown	100
12.	Check	1
Total		**329**

Results

Results of analysis of variance, as shown in Table 2, revealed that the lines had significant variation for all the traits for both years and locations. The extent of ranges for various parameters studied during growing season of year 2013-14 and 2014-15 are depicted in Figure 1 and Figure 2, respectively. A similar trend of fluctuation in ranges was observed for both year data, which is evident from the standard deviation values of various parameters studied (Figs. 1 &2).

The first four principal components out of 18, exhibited Eigen value greater than one and significant for the year 2013-14 and 2014-15 (Figs. 3 & 4). The remaining 14 principal components (PCs) showed non-significant variation and were not considered for interpretation. For year 2013-14 and 2014-15 the initial four principal components accounted for 60.72 % and 58.50 % of the total variation, respectively in the whole set germplasm lines. The first principal component showed 26.14 % variation, second 18.30 %, third 9.32 % and forth 6.96 % in the year 2013-14 (Fig. 3). For year 2014-15 the first PC accounted 26.27 % of the total variance, second 15.30 %, third 9.56 % and forth 7.37 % (Fig. 4). According to criterion developed for determination of importance of a trait coefficient for each significant PC by Johnson & Wichem (1988), the first PC was highly related to days to flower initiation, days to 50% flowering, days to flower completion, days to 50% maturity, days to 100% maturity and pod shatter percentage for both years. This suggests that the first PC is a weighted average of these four traits. In the second PC the traits of significant importance were leaf width, leaf length, plant height, main raceme length, number of pods per main raceme and thousand seed weight for the year 2013-14 (Table 3). The important traits in PC2 for the year 2014-15 were leaf length, leaf width, primary branches per plant, main raceme length, number of pods per main raceme and thousand seed weight (Table3). The third PC was related to pod length, pod width and number of seed per pod and PC4 to pod shatter percentage and seed yield per plant for the year 2013-14 (Table 3). The significant trait for year 2014-15 in PC3 was pod length, pod width and number of seed per pod and significant traits in the forth PC were pod shatter percentage and seed yield per plant (Table 3).

The projection of parameters on the first and second PCs revealed that leaf width, main raceme length and thousand seed weight were positively related to seed yield per plant in both years where as days to 50% maturity, days to 100% maturity, number of pods per main raceme, leaf length and plant height were positively related to pod shatter percentage in both years. Only the stem thickness behaved conversely for all other parameters on PC1 in both years. Therefore, it had negative correlation with all other parameters (Figs. 5 & 6). The projection of the parameters on PC1 and PC2 for both years depicted that main parameters contributing to yield were Leaf width, Main raceme length and thousand seed weight, whereas the parameters responsible for pod shatter percentage were days to 50% maturity, days to 100% maturity, number of pods per main raceme, leaf length and plant height.

Table 2. Mean square of various traits for 2013-14 and 2014-15 years.

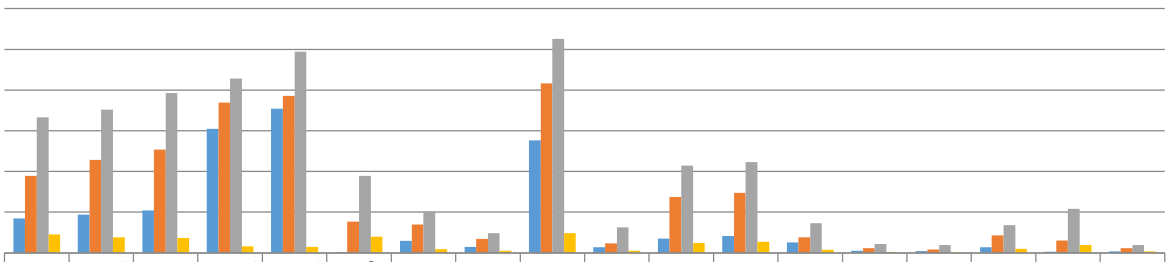
SoV	Year 2013-14									
	DF	**DFI**	**DF50**	**DFC**	**DM50**	**DM100**	**Pod Shtr**	**LL**	**LW**	**PH**
Acc.	328	1020.9**	732.5**	658.1**	133.3**	110.1**	795.9**	41.7**	12.6**	1188.5**
Locs.	1	432.2**	294.6**	74.2**	412.4**	230.9**	102.1**	99.3**	715**	2116**
Err.	329	1.94	0.50	1.13	0.04	16.69	6.01	1.65	0.03	1.97
Acc×Locs	328	1.94	0.50	1.13	0.04	16.69	6.012	1.65	0.03	1.97
Total	657	511.3	366.4	329.2	67.2	63.7	400.5	21.8	7.42	597.6
SoV	**DF**	**PB/P**	**MRL**	**P/MR**	**ST**	**PL**	**PW**	**S/P**	**SY/P**	**TSW**
Acc.	328	12.2**	291.7**	373.4**	26.5**	2.8**	1.07**	50.9**	180.5**	5.4**
Locs.	1	2.6**	12.5**	18.3**	10.7**	42.1**	35.8**	270.8**	481.3**	166.6**
Err.	329	0.17	1.21	2.76	0.80	0.01	0.009	0.21	0.17	0.006
Acc×Locs	328	0.17	1.21	2.76	0.80	0.01	0.009	0.21	0.17	0.006
Total	**657**	**6.22**	**146.2**	**187.8**	**13.65**	**1.50**	**0.59**	**25.9**	**90.9**	**2.97**
	Year 2014-15									
SoV	**DF**	**DFI**	**DF50**	**DFC**	**DM50**	**DM100**	**PodShtr**	**LL**	**LW**	**PH**
Acc.	328	940.7**	727.9**	670.8**	105.2**	96.9**	741.6**	40.3**	13.03**	1181**
Locs.	1	895.2**	1619.2**	1421**	3131.9**	946.8**	475.3**	975.9**	241**	3355**
Err.	329	0.18	0.18	0.01	0.28	0.22	0.10	0.14	0.05	0.26
Acc×Locs	328	0.18	0.18	0.01	0.28	0.22	0.10	0.14	0.05	0.26
Total	657	471.1	365.9	0.03	57.4	49.9	371.03	21.6	6.9	595.06
SoV	**DF**	**PB/P**	**MRL**	**P/MR**	**ST**	**PL**	**PW**	**S/P**	**SY/P**	**TSW**
Acc.	328	13.68**	295.6**	391.1**	28.31**	2.77**	1.08**	51.05**	185.04**	5.6**
Locs.	1	736**	1346**	1180**	359.2**	108.4**	27.6**	1322**	1169**	22.27**
Err.	329	0.19	0.23	0.02	0.13	0.04	0.005	0.07	0.03	0.01
Acc×Locs	328	0.19	0.23	0.02	0.13	0.04	0.005	0.07	0.03	0.01
Total	**657**	**8.06**	**149.7**	**0.01**	**14.7**	**1.5**	**0.5**	**27.5**	**94.1**	**2.8**

**= Highly significant, SoV= Source of variance, DF= Degree of freedom, DFI= Days to flower initiation, DF50= Days to 50% flowering, DFC= Days to flower completion, DM50= Days to 50% maturity, DM100= Days to 100% maturity, PodShtr= Pod shattering, LL= Leaf length, LW= Leaf width, PH= Plant height, PB/P= Primary branches per plant, MRL= Main raceme length, P/MR= Pods per main raceme, ST= Stem thickness, PL=Pod length, PW= Pod width, S/P= Seeds per pod, SY/P= Seed yield per plant, TSW= Thousand seed weight

Table 3. Eigen values for first four PCs.

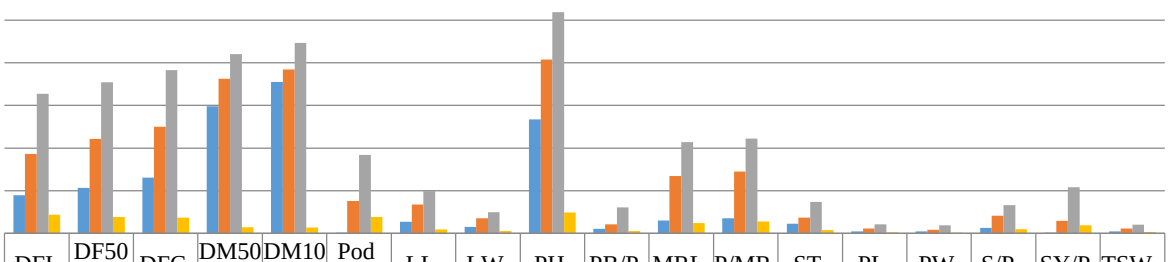
Variables	2013-14				2014-15			
	Eigen values				Eigen values			
	PC1	**PC2**	**PC3**	**PC4**	**PC1**	**PC2**	**PC3**	**PC4**
DFI	**0.89**	-0.06	-0.01	-0.08	**0.89**	-0.082	-0.001	-0.13
DF50	**0.93**	-0.0007	0.01	-0.09	**0.93**	-0.009	-0.03	-0.13
DFC	**0.92**	0.006	0.01	-0.11	**0.92**	0.013	-0.02	-0.16
DM50	**0.76**	0.29	-0.14	-0.30	**0.80**	0.24	0.0004	-0.24
DM100	**0.78**	0.19	-0.22	-0.32	**0.81**	0.21	-0.007	-0.24
PodShtr	**0.54**	0.18	0.26	**0.42**	**0.44**	-0.23	-0.07	**0.61**
LL	0.24	**0.58**	-0.08	0.24	0.40	**0.52**	0.18	0.23
LW	-0.09	**0.74**	0.04	0.12	-0.01	**0.63**	0.11	0.14
PH	0.11	**0.67**	-0.09	0.21	0.25	**0.42**	0.05	0.27
PB/P	-0.31	**0.42**	-0.20	-0.36	-0.23	**0.57**	0.06	-0.26
MRL	-0.40	**0.60**	-0.25	0.08	-0.30	**0.61**	0.21	0.22
P/MR	0.12	**0.61**	-0.46	0.26	0.24	**0.60**	0.26	0.35
ST	-0.13	-0.10	0.16	-0.41	-0.11	0.06	-0.02	-0.29
PL	0.08	0.24	**0.66**	0.09	0.07	0.04	-0.75	0.20
PW	0.03	0.38	**0.55**	-0.08	-0.01	0.26	-0.62	0.09
S/P	0.05	0.38	**0.57**	-0.05	0.03	0.26	-0.69	0.04
SY/P	-0.39	**0.46**	-0.10	-0.43	-0.40	**0.44**	-0.01	-0.40
TSW	-0.32	**0.51**	0.23	-0.35	-0.28	**0.54**	-0.29	-0.23

DFI= Days to flower initiation, DF50= Days to 50% Flowering, DFC= Days to flower completion, DM50= Days to 50% maturity, DM100= Days to 100% maturity, PodShtr= Pod shattering, LL= Leaf length, LW= Leaf width, PH= Plant height, PB/P= Primary branches per plant, MRL= Main raceme length, P/MR= Pods per main raceme, ST= Stem thickness, PL=Pod length, PW= Pod width, S/P= Seeds per pod, SY/P= Seed yield per plant, TSW= Thousand seed weight



	DFI	DF50 %	DFC	DM50 %	DM100%	Pod Shtr	LL	LW	PH	PB/P	MRL	P/MR	ST	PL	PW	S/P	SY/P	TSW
Min	42.5	47	52	152.2	177	0.5	14.82	7.167	138.1	6.9	17.5	20.5	13	2.54	2.195	6.75	1.397	2.01
Mean	94.52	114.4	127	184.5	192.8	38.25	34.67	17.33	208.3	11.79	68.57	73.7	19.19	5.787	4.201	21.61	15.31	5.668
Max	166.5	176	196.5	214	247	94.5	50.8	24.4	262.9	31.2	107.2	111.3	36.27	11.03	9.51	33.88	54.25	9.61
SD	22.59	19.14	18.14	8.164	7.422	19.95	4.57	2.513	24.38	2.478	12.08	13.66	3.642	1.2	0.733	5.049	9.5	1.648

Fig. 1. Descriptive statistics of various traits for cropping season 2013-14.
where, DFI= Days to flower initiation, DF50= Days to 50% flowering, DFC= Days to flower completion, DM50= Days to 50% maturity, DM100= Days to 100% maturity, PodShtr= Pod shattering, LL= Leaf length, LW= Leaf width, PH= Plant height, PB/P= Primary branches per plant, MRL= Main raceme length, P/MR= Pods per main raceme, ST= Stem thickness, PL=Pod length, PW= Pod width, S/P= Seeds per pod, SY/P= Seed yield per plant, TSW= Thousand seed weight



	DFI	DF50 %	DFC	DM50 %	DM100%	Pod Shtr	LL	LW	PH	PB/P	MRL	P/MR	ST	PL	PW	S/P	SY/P	TSW
Min	44.75	53.5	65.4	149.1	177.4	0.9	13.5	7.767	133.8	5.3	15.1	17.75	11.3	2.49	2.27	6.55	0.848	2.2
Mean	93.21	110.7	125.1	181.2	192.2	37.96	33.84	17.77	203.9	10.79	67.28	72.52	18.32	5.633	4.23	20.83	14.83	5.911
Max	163.5	177	191.6	210.2	223.2	92.1	49.45	25	259.4	30.4	107.2	111.1	36.8	10.7	9.51	33.15	53.95	10.1
SD	21.69	19.08	18.31	7.253	6.962	19.26	4.487	2.553	24.3	2.616	12.16	13.98	3.763	1.177	0.735	5.053	9.619	1.68

Fig. 2. Descriptive statistics of various traits for cropping season 2014-15.
where, DFI= Days to flower initiation, DF50= Days to 50% flowering, DFC= Days to flower completion, DM50= Days to 50% maturity, DM100= Days to 100% maturity, PodShtr= Pod shattering, LL= Leaf length, LW= Leaf width, PH= Plant height, PB/P= Primary branches per plant, MRL= Main raceme length, P/MR= Pods per main raceme, ST= Stem thickness, PL= Pod length, PW= Pod width, S/P= Seeds per pod, SY/P= Seed yield per plant, TSW= Thousand seed weight

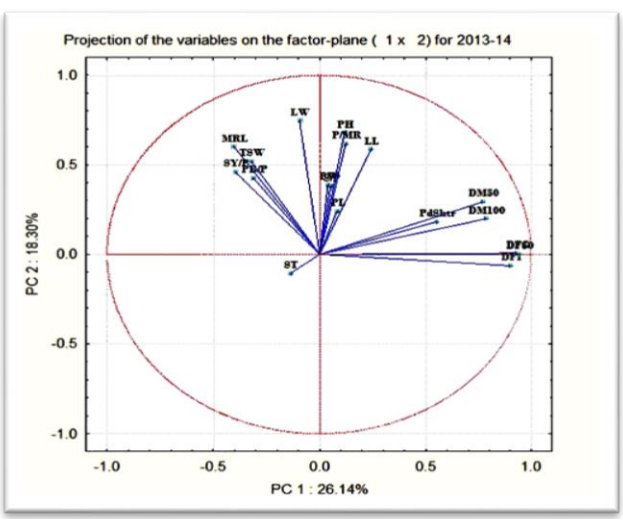


Fig 3. Scree plot between eigen values and number of PCs for the year 2013-14.

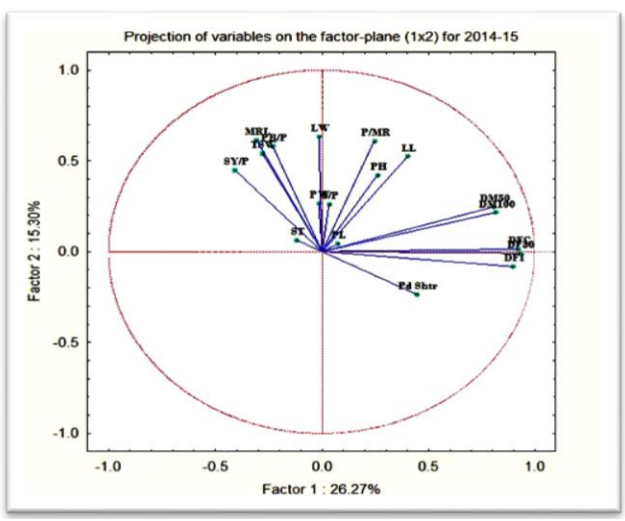


Fig. 4. Scree plot between eigen values and number of PCs for the year 2014-2015.

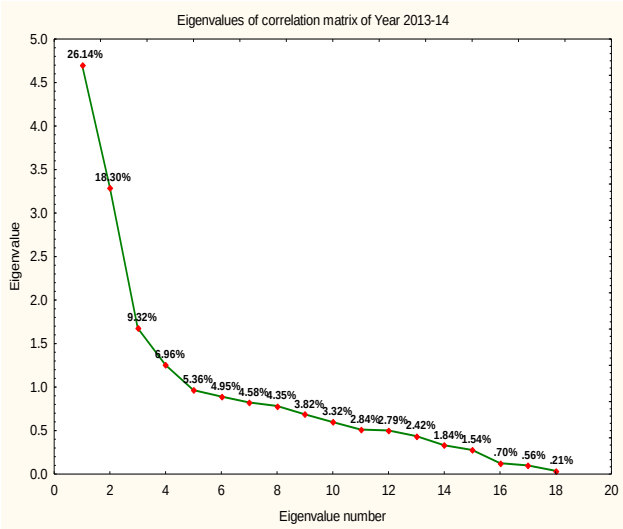


Fig. 5. PCA of traits for year 2013-14.

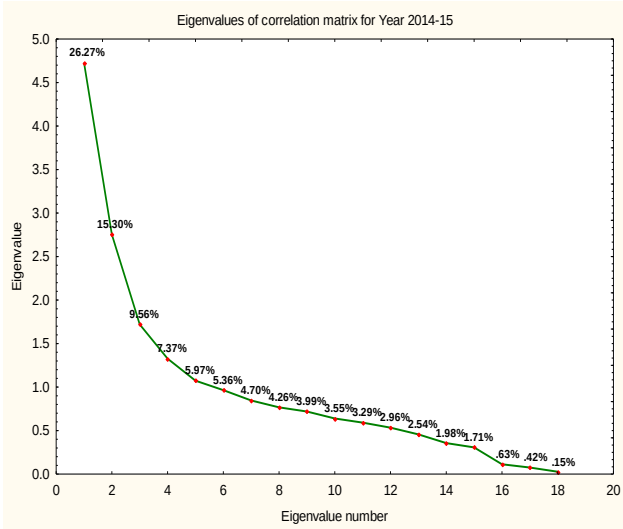


Fig. 6. PCA of traits for year 2014-15.

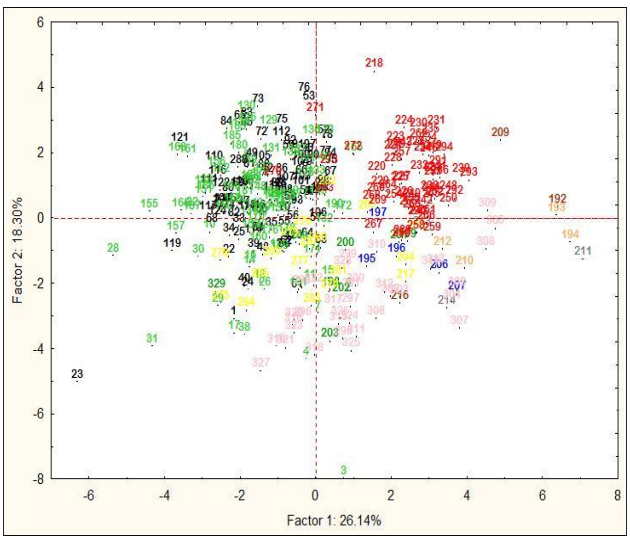


Fig. 7. 2D ordinations of 329 *B. napus* lines on PC1 and PC2 for the year 2013-14.

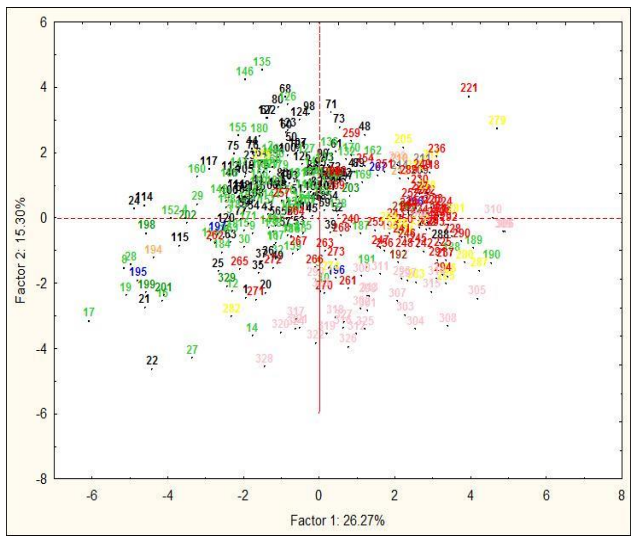


Fig. 8. 2D ordinations of 329 *B. napus* lines on PC1 and PC2 for the year 2014-15.

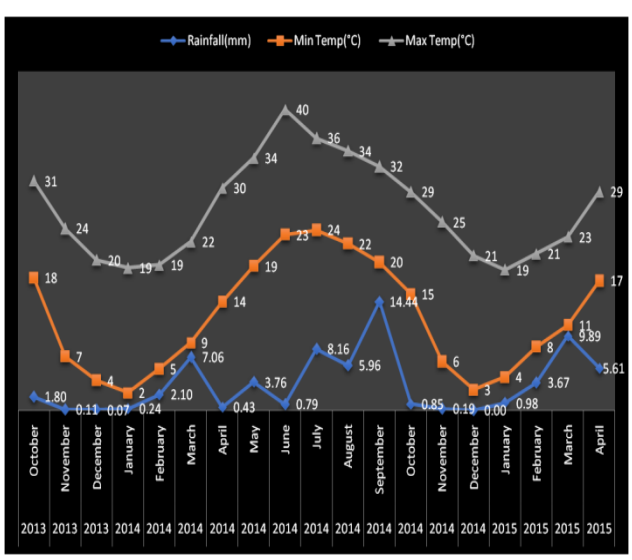


Fig. 9. Average temperatures and Rainfall recorded at NARC (Location 1) during experiment duration.

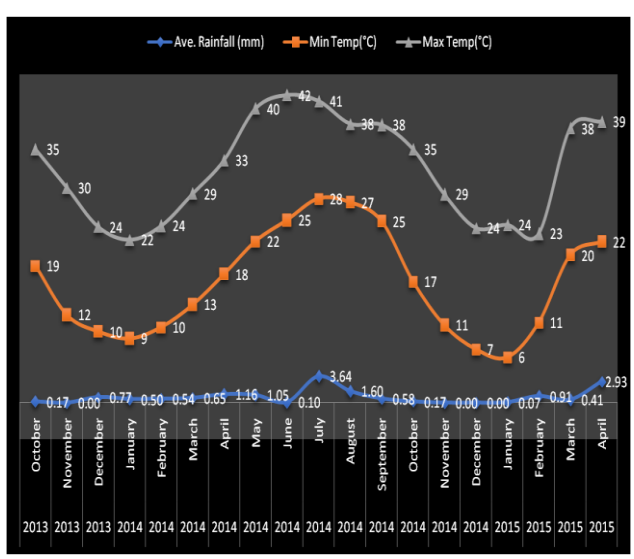


Fig. 10. Average temperatures and Rainfall recorded at University research farm (Location 2) during experiment duration.

The projection of germplasm lines on PC1 and PC2 (Figs. 7 & 8) was helpful to identify diverse lines for further studies.The following most diverse groups of lines were identified;BN328, BN419, BN455 and BN457 were opposite to BN492, BN493, BN494 and BN511. Lines; BN353 BN371, BN376 and BN518 were opposite to BN304, BN618, BN625 and BN627 for year 2013-14 (Fig. 7). The lines BN414, BN324, BN498, BN494, BN328, BN308 were found contrasted to BN610, BN606, BN489, BN587 and BN580 whereas, the lines BN435, BN368, BN426, BN380, BN398, BN371 and BN373 had maximum diversity from BN622, BN626, BN627, BN625, BN628, BN618 and BN619 for year 2014-15 (Fig. 8).

Discussion

Various morpho-biocehmical methods are used to study the genetic divergence in different crop plants (Nasim *et al.*, 2017; Jan *et al.*, 2016; Khan *et al.*, 2016). In our study, although, the two locations, were significantly different but there was no significant interaction between location and accessions for both years of evaluation. This depicts that location had no affect on the lines studied. This may help in the development of such breeding population which has wider adaptability. The range of each parameter gives an immediate extent of diversity among the populations. The range for days to flower completion was higher for both years (52-196.5 and 65.4-191.6 days). Range for pod shatter and seed yield per plant were nearly same for both years (0.5-94% & 0.9-93%) and (1.397-54.25g & 0.848-53.95g), respectively.

The projection of *Brassica napus* lines on PC1 and PC2 showed some population structure for both years but the lines can broadly be divided into two sub-populations, one which contains lines from Pakistan and of Unknown origin and the other which comprised of exotic lines of *Brassica napus* from China, Australia and Canada. Ali *et al.*, (2015) found maximum contribution of first five PCs group in Indian mustard genotypes. According to them the first PC group contributed 22.21% variation as compared to PC2 (14.65%). Our study is also supported by Yu *et al.*, (2007). According to them the first two PCs groups gave about 60% variability as compared to other three groups. Khan *et al.*, (2014) recorded maximum phenotypic variations among 211 *B. napus* genotypes. Among 21 quantitative traits tested, the pod shattering characters gave maximum variation followed by plant height. The similar findings were also reported by Neeru *et al.*, (2015) for multiple important quantitative characters of *B. juncea*. They also found that first three PCs groups had maximum variability value (45.94%).

Conclusion

In conclusion, the main raceme length, leaf width and thousand seed weight were key parameters for the improvement of seed yield per plant whereas to control pod shatter percentage traits like days to 50% maturity and days to 100% maturity, number of pods per main raceme, leaf length and plant height were found more contributing. Lines such as BN328, BN371, BN494, BN618, BN625 and BN627 were the diverse lines for both years. The results obtained from this study may help in the development of potential high yielding oilseed rape breeding population.

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