PHENOTYPIC DIVERSITY ANALYSIS AND PERFORMANCE OF ELITE LINES OF *BRASSICA NAPUS* L.

MUHAMMAD SAJJAD IQBAL* AND MUHAMMAD AKBAR

Department of Botany, University of Gujrat, Gujrat, Pakistan

Keywords: Oilseed crops, Agronomic traits, Cluster analysis

Abstract

Thirteen elite lines of *Brassica napus* L. were tested. Nine phenotypic traits *viz.*, days to 50% flowering, days to maturity, plant height, branches, pods, pod length, pod width, pod weight and seed yield were studied. ANOVA revealed significant results for all the traits while summary statistics exhibited high level of genetic variability in days to 50% flowering, days to maturity, plant height and number of pods. On the other hand, number of branches, pod length, pod width, pod weight and seed yield need more attention for improvement. Coefficient of correlation revealed significant combinations among various traits which could be utilized directly. Cluster analysis based on linkage distances described grouping pattern into three clusters for dissimilarities. Elite line 24866 of Pakistani origin placed in separate cluster prominently that is due to best performance for maximum traits, hence recommended for farmers' field large scale cultivation.

Introduction

Oilseed rape (*Brassica napus* L.) is being cultivated throughout the world as major oilseed crops. Four continents including America, Europe, Australia and Asia are important for food cultivation as oil and meal while non-food uses are as biofuel or lubricants. It stands second world crop after soybean (Jan *et al.* 2017, Tomkowiak *et al.* 2020). The close relationship between six particularly important species (*Brassica carinata, B. juncea, B. oleracea, B. napus, B. nigra* and *B. rapa*) was described by the triangle of U theory (UN 1935).

It is a major contributor of carbohydrates, proteins and vegetable oil that is essential for human nutrition and absorption of fat-soluble vitamins. In an ideal healthy environment saturated as well as unsaturated fatty acids are very important. Therefore, oils with low saturated fatty acids < 6%, high oleic acid > 50%, moderate linoleic acid < 40% and low linolenic < 14% are considered ideal for edible purposes (Hu *et al.* 2007). Moreover, for combating biofuel consumption and climatic change challenges oilseed rape is getting popularity as a biodiesel crop. It is estimated that substantial demand in oilseed crops would be increased many-folds in near future (FAO 2002, Nikzad *et al.* 2020).

Canola is a term which arose from "Canadian Oil" and is used to represent any variety of rapeseed that contains low levels of erucic acid (EA) and glucosinolates (Canola Council of Canada 2003). *B. napus* is self-fertile; however, outcrossing rates as high as 47% have been reported (Williams *et al.* 1986). Genetic diversity of oilseed rape is considered to be low because of the short cropping history and the strong breeding focus on seed quality characters, i.e. low erucic acid and glycosinolate contents which narrowed down the genetic base (Friedt and Snowdon 2009).

Breeding for agronomic and economic values tends to bias the exploitation of genetic variation (Ali *et al.* 2018). Therefore, it is necessary to continuously integrate diverse germplasm into the breeding process in order to maintain a high level of genetic diversity. Hybrids produced from crosses between genetically diverse resynthesized rapeseed and adopted oilseed types showed a high yield potential (Seyis *et al.* 2005). The latter study demonstrated that crosses

^{*}Author for correspondence: <drsajjad.iqbal@uog.edu.pk>.

among genotypes from genetically divergent clusters tended to show higher levels of heterosis for seed yield and other traits (Chen *et al.* 2020). Thus in the present study attempts were taken to characterize unique generic lines under local environment with best performance to recommend for future breeding programs.

Materials and Methods

Thirteen elite lines of *Brassica napus* obtained from Plant Genetic Resource Program, NARC, Islamabad, Pakistan were studied. Genotype 24866 (Donor#525) belonged to Pakistan, while 7 genotypes *viz.*, 27388 (LMZ 98833) China, 27397 (LMQY 11) China, 27398 (LMQY 11) China, 27406 (LMQY 11) China, 27410 (LMHYZ 4) China, 27425 (LMHYZ 4) China, 27435 (OP-10) China, were originated from China and 5, namely Australia 27440 (Cobbler), Australia 27443 (Marlin), Australia 27444 (Outback), Australia 27445, (Rivette) Australia 27450 (Thunder), Australia represented from Australia. Germplasm was planted at University of Gujrat, Gujrat in RCBD. The phenotypic traits recorded by selecting ten randomly healthy plants were days to 50% flowering, days to maturity, plant height, number of branches, number of pods, length of pods, pod diameter, weight of pods, and seed weight. Statistical analysis as ANOVA was performed by MSTATC computer software. Summary statistics and coefficient of correlation were conducted through MS Excel while cluster analysis was performed by 'Statistica' version 6.02 after constructing phylogenetic tree.

Results and Discussion

High variation was recorded in morpho-physiological traits (Table 1). Days to maturity ranged from 100 to 145 days with mean \pm SE value of 123.94 \pm 0.89 days. Early days to maturity were recorded in 27440 belonged to Australia (100 days) and were followed by 27410 China (110 days) and 27443 Australia (111 days) whereas 27406 China took 146 days to mature. Early maturing genotypes are suggested to use in future crop improvement. Highest plant height 73 cm was recorded in 24866 (Pakistan) followed by 27445 (Australia with 66 cm and 26410 (China) 64 cm. Sharma and Thakur (1993) reported highest plant height with closest row spacing in *B. napus*. The lowest plant height was found for the control treatment. Hu *et al.* (2007) also found significant differences between Chinese and European group in plant height while studying various agronomic traits.

Number of branches recorded in selected plants were 2 to 9 with mean \pm SE. Maximum number of branches recorded in 24866 from Pakistan (9 branches) and varied to 27445 from Australia (8 branches) and 27398 from China (7 branches) while the minimum number of branches were recorded in 27435 from China (2 branches). Present results are in agreement with the findings of the Zebarjadi *et al.* (2011).

Seed weight ranged from 0.27 to 24.09 g with mean value of 3 while the maximum seed weight was recorded in 27435 (China) 24.09 g and varied to 27445 belonging to Australia (17.96 g) and in 27435 China (15.708 g). Minimum seed weight was found in line 27410 belonging to China (0.27 g). These traits need to be further characterized by broadening collections of *Brassica rapa* to obtain desired results. High variability existed in the current set of germplasm with reference to characters; days to flowering, days to maturity, plant height and number of pods indicated potential of current set of genotypes for farmer's field cultivation and genotype by environment trials to explore their possible hidden potential. On the other hand, traits such as number of branches, pod length, pod width, pods weight and seed yield need more attention for improvement. Sharma and Thakur (1993), reported that the highest plant height was observed with the closest row spacing in *Brassica napus*. Plant height is a genetic trait helpful for isolation of

Traits	$Mean \pm SE$	Sd	Variance	Min.	Max.	RSd	DF	SS	MS	Line	Error	F value	CV%
Days to 50% flowering	56.16 ± 0.85	3.08	9.5	46.85	58.97	5.49	13	4.27	2.13	30.58	1.11	1.91**	1.89
Days to maturity	123.94 ± 0.89	3.23	10.41	113.95	127.21	2.6	13	1.78	0.89	31.49	0.75	1.18^{**}	0.7
Plant height	44.97 ± 2.06	7.44	55.29	39.33	66.67	16.54	13	0.05	0.02	0.21	0.03	0.7*	5.27
No. of branches	3.56 ± 0.07	0.26	0.07	3.36	4.3	7.4	13	109.49	54.74	395.61	89.25	0.61^{*}	8.53
No. of pods	110.67 ± 3.39	12.22	149.32	99.66	146.04	11.04	13	3.4	1.7	155.15	2.52	0.67*	3.51
Pod length	10.7 ± 0.4	1.44	2.08	8.98	12.4	13.48	13	0.08	0.04	5.72	0.02	1.72^{**}	1.49
Pod width	2.94 ± 0.05	0.16	0.03	2.58	313	5.59	13	0.01	0.005	0.09	0.01	7.27**	0.94
Pod weight	7.72 ± 0.27	0.96	0.93	5.86	8.85	12.48	13	0.58	0.29	2.83	0.16	1.76^{**}	5.33
Seed yield	3.38 ± 0.14	0.52	0.27	2.13	3.99	15.43	13	0.18	0.09	0.91	0.03	2.95**	5.33

2	
2	
1	
11	
-	
3	
.2	
3	
a	
~	
B	
÷	
•	
\$	
Ĕ	
-=	
_	
e	
-	
e	
3	
1	
-=	
\$	
i:	
2	
5	
-	
5	
<u> </u>	
0.0	
0	
6	
Ē	
d	
-	
2	
8	
-	
Ĕ	
•=	
=	
1	
.9	
-	
<	
\geq	
6	
\mathbf{a}	
4	
<	
-	
3	
5	
÷Ē	
S	
ti.	
ta	
S	
>	
5	
3	
Ξ	
ā	
2	
5	
Η.	
1	
Ĩ	
q	
~	

superior genotypes. Ahmad *et al.* (2007) and Hu *et al.* (2007), advocated that genetically distinct lines should be used in breeding programs for improvement of *Brassica napus*.

Analysis of variance revealed that all of the traits were significant. High coefficient of variance (CV) for number of branches, pod weight and seed yield indicated the high potential of germplasm for these characters. The mean square value for number of pods was 54.74 that was highest, and its CV was 8.53% followed by 2.13 for days to 50% flowering with CV 1.89% whereas the lowest mean square value was 0.005 for pod width with CV 0.94%. Highest CV was 8.53% for number of pods (NP) while lowest CV 0.7% was recorded in days to maturity. It is assumed that high variability lies for high CV whereas genotypes with lowest CV considered as more stable with highest genetic purity. ANOVA depicts significant results for all of the characters under study. Ali *et al.* (2002), also found significant result for days to flowering and maturity. Days to maturity were found significant in all of the genotypes (Ali 2018).

Coefficient of correlation showed that days to 50% flowering were positively correlated with days to maturity, plants height, number of branches, number of pods, pods length, pod width, pods weight and seed yield (Table 2). Positive association showed that these traits could be used for crop improvement. Quality traits evaluation is very important for selecting elite lines in *Brassica napus* as reported in 488 global collections (Chen *et al.* 2018). Number of branches and number of pods were found to be positively associated with plant height, this indicated that as plant height increases it increases the branches and pods per plant ultimately it increases grain yield. Seed yield found to be positively associated with days to 50% flowering, days to maturity, pods length, pods width and pods weight while significant but negative association with plant height, number of branches and number of pods. These traits need to be incorporated with improved ones. Azadgoleh *et al.* (2009), highlighted the importance of cultural practices including water, fertilizer, variety selection, seed rates, seed depths, weeds and pest control to get successful high yielding crop.

Traits	D50% F	DM	PH	NB	NP	PL	PW	Pod wt.
DM	0.98**							
PH	-0.96**	-0.91**						
NB	-0.9**	-0.85**	0.96**					
NP	-0.85**	-0.82**	0.92**	0.98**				
PL	0.61**	0.43*	-0.71**	-0.64**	-0.54**			
PW	0.85**	0.76**	-0.93**	-0.88**	-0.79**	0.83**		
Pod wt.	0.73**	0.66**	-0.78**	-0.64**	-0.52**	0.74**	0.89**	
SY	0.91**	0.85**	-0.91**	-0.77**	-0.67**	0.73**	0.92**	0.93**

Table 2. Correlation of coefficient for 9 morphological traits in 13 elite lines of Brassica napus.

**Highly significant, *significant at 0.05 and 0.01%. D 50% F, days to 50% flowering; DM - days to maturity, PH - plant height, NB - number of branches, NP - number of pods, PL - pod length, PW - pod width, Pod wt. - pod weight and SY - seed yield.

Highest linkage distance of 55.78 was recorded in 24866 (Pakistan) and 27444 (Australia), followed by 52.23 between 24866 (Pakistan) and 27450 (Australia) and then 52.02 in 24866 and 27443. While lowest distance 2.13 recorded in 27443 and 27450 reflected low variability. Genotypes closer to each other had similarities common in various traits while genotypes present at wider distances showed possible genes of interest and probable opportunity for crossing and crop improvement.

Phylogenetic tree based on nine characters for 13 genotypes presented in Fig. 1 was divided in two major groups. Group-I contained one genotype 24866 belonging to Pakistan, while group-II comprised of all remaining 12. Cluster analysis showed that genotype 1 present at 50% linkage distances maintained its uniqueness as local genetic material based on performance as compared to exotic material representing Australia and China. Group-II comprised of following

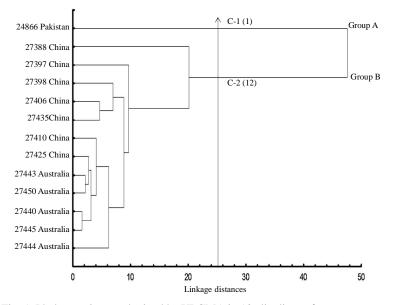


Fig. 1. Phylogenetic tree obtained by UPGMA in 13 elite lines of Brassica napus.

i.e. 27388, 27397, 27398, 27406, 27435, 27410, 27425 originated from China and 27443, 27450, 27440, 27445 and 27440 originated from Australia showed close association. It supports that geographic origin also have more appealing impact than genetic makeup therefore more stable markers like RAPD, SCAR, SSR, suggested to be included in future studies. Further based on cluster pattern phylogenetic relationship advocates genotype 24866 Pakistan was most probably the best due to its adaptability in the local environment and it could be surely recommended for direct cultivation at farmer's field. In another experiment, Hu *et al.* (2007), stated that cluster analysis of 63 accessions based on agronomic characters was consistent with known pedigree information and geographic origin. Genotype 24866 showed excellent performance under local climatic conditions. Therefore, it is recommended for large scale cultivation and to start breeding program that would be helpful towards self-sufficiency in the field of seed oils.

References

- Ahmad MA, Munir I, Ali W, Swati ZA, Khatak MS, Sohail C and Khan I 2007. Assessment of genetic diversity of local and exotic *Brassica napus* germplasm. Pak. J. Biol Sci. 10(15): 2490-2494.
- Ali N, Javidfar F and Attary AA 2002. Genetic variability, correlation and path analysis of yield and its components in winter rapeseed (*Brassica napus* L.). Pak. J. Bot. 34(2): 145-150.
- Ali N, Khan NU, Ullah F, Rabbani MA, Bibi Z, Gul S, Ali S, Khan SM, Khalil IH, and Naveed K 2018. Genetic diversity in indigenous landraces of *Brassica napus* based on morphological and biochemical characteristics using multivariate techniques. Int. J. Agric. Biol. 20(2): 277-287.

- Azadgoleh EMA, Zamani M, Esmaeil and Yasari 2009. Agronomical important traits correction in rapeseed (*Brassica napus* L.) genotypes. J. Agric. Biol. Sci. **5**(5): 798-802.
- Canola Council of Canada 2003. Canola grower's manual: Chapter 2 canola varieties. [Online] Available: http://www.canola-council.org/chapter2.aspx.
- Chen B, Xu K, Li H, Gao G 2018. Evaluation of quality traits and their genetic variation in global collections of *Brassica napus* L. Plant Genet. Res. **16**(2): 146-155.
- Chen R, Shimono A, Aono M, Nakajima N, Ohsawa R and Yoshioka Y 2020. Genetic diversity and population structure of feral rapeseed (*Brassica napus* L.) in Japan. PLoS ONE **15**(1): e0227990. https://doi.org/10.1371/journal.pone.0227990.
- FAO 2002. Protein sources for the animal feed industry. Expert Consultation and Workshop, Bangkok. 29 April - 3 May 2002. Animal production and health, FAO, Rome.
- Friedt W, and Snowdon RJ 2009. Oilseed rape. In: Oil Crops. Handbook of Plant Breeding, Springer-Verlag, Berlin. pp. 91-126.
- Hu S, Yu C, Zhao H, Sun G, Zhao S, Vyvailova M and V. Kucera 2007. Genetic diversity of *Brassica napus* L. germplasm from China and Europe assessed by some agronomically important characters. Euphytica **154**(1): 9-16.
- Jan SA, Shinwari ZK, Rabbani MA, Niaz IA and Shah SH 2017. Assessment of quantitative agromorphological variations among *Brassica rapa* diverse populations. Pak. J. Bot. 49(2): 561-567.
- Nikzad A, Kebede B, Pinzon J, Bhavikkumar J, Wang X, Yang RC and Rahman H 2020. Potential of the C genome of the different variants of *Brassica oleracea* for heterosis in spring *B. napus* Canola. Front. Plant Sci. 10: 1691. doi: 10.3389/fpls.2019.01691.
- Seyis F, Friedt W and Luhs W 2005. Yield of *Brassica napus* L. hybrids developed using resynthesized rapeseed material shown at different locations. Field Crop Res. 96: 176-180.
- Sharma J and Thakur R 1993. Performance of rainfed Argentine rape (*Brassica napus*) under dates of sowing and row spacing in the mid-hills of north-western Himalayas. Indian J. Agron. **38**(20): 254-256.
- Tomkowiak A, Bocianowski J, Kwiatek M and Kowalczewski PL. 2020. Dependence of the heterosis effect on genetic distance, determined using various molecular markers. Open Life Sci. **15**(1): https://doi. org/10.1515/biol-2020-0001.
- UN 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.
- Williams IH, Martin AO and White RP. 1986. The pollination requirements of oil-seed rape (Brassica napus). J. Agric. Sci. 106(1): 27-30.
- Zebarjadi A, Kakaei M and Mostafaie A 2011. Genetic variability of some traits in rapeseed (*Brassica napus* L.) under drought stress and non-stress conditions. Biharean Biologist. **5**(2): 127-131.

(Manuscript received on 20 February, 2020; revised on 5 May, 2020)