

## Research Article

# Genetic Variability among Rapeseed (*B. napus* L.) Genotypes for Seed-Yield and Seed-Quality Traits

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**Abstract** | *Brassica napus* L. is one of the essential oilseed crops playing a significant role in commercial edible oil production. To determine diversity within this specie, ten different genotypes of *B. napus* L. including Chinese (CA-2, CA-4, CA-5) and doubled haploid lines from a population were field-tested at the University of Agriculture, Peshawar (UAP). Genotypes were examined for seven morphological traits including days to flowering, primary branches mainstem<sup>-1</sup>, plant height, main raceme length, pods mainraceme<sup>-1</sup>, pod length, 1000-seed weight and three seed quality traits; oil content, glucosinolate and erucic acid. The analysis of variance (ANOVA) found significant variation among *Brassica napus* L. lines for all the studied morphological and seed quality traits. Furthermore, analysis of correlation revealed that 1000 seed weight was significantly correlated with primary branches on main stem, main raceme length, pods mainraceme<sup>-1</sup>, and pod length. Two traits erucic acid and pod length had significant correlation with oil content. Among the studied lines, DH-3 had high oil content, DH-2 had low glucosinolate and CA-2 had low erucic acid content. Similarly, CA-2 and CA-5 performed better for seed-related traits. Based on the results of current study, genetic variation was observed in studied lines. Also, CA-2 and CA-5 were identified as superior genotypes for seed yield and hence were the best genetic resources to increase seed yield in rapeseed. Traits including primary branches on main raceme, pods mainraceme<sup>-1</sup>, pod length and erucic acid were important while making indirect selection to improve seed weight and oil content in rapeseed.

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## Introduction

*Brassica napus* L. is an essential edible oilseed crop that produces multiple products such as food, biofuels, lubricants, and surfactants. Spontaneous interspecific hybridization of two progenitors, *B. rapa* L. (AA, 2n = 20) and *B. oleracea* L. (CC, 2n = 18) leads to origination of an amphidiploid *B. napus* L. (AACC, 2n = 38) (Allender and King, 2010). The *B. napus* L. is assumed to have originated mainly in

Southern Europe (Downey, 1983). In the mid-20<sup>th</sup> century, *B. napus* species of Australia and China was believed to have been derived from the same origin, however, traces of introduction from Canada, Europe and Japan were also detected (Sun, 1948; Liu, 1985; Chen et al., 2008).

Leading producing countries of rapeseed in 2019/2020 are Canada, the European Union, and China (Statista 2019-20). Approximately 80% of

the total production area in China is grown with *B. napus* L. (Hu *et al.*, 2007). Globally, it is the third most essential consuming oilseed crop (Statista 2019-20). Hence, concerning the growing population, it has a significant role in global food security and vegetable oil production (Shahin and Valiollah, 2009; El-Hamid and Zaher, 2018). Several *B. napus* L. varieties have been identified such as canola possessing a definite limit of glucosinolates (<30  $\mu\text{mol/g}$ ) and erucic acid (<2% in oil) in the toasted oil-free meal (Raymer, 2002). Canola in China, has been used as a parent to develop a couple of new varieties (Friedt *et al.*, 2018).

In Pakistan, breeders are interested in producing high yielding and high-quality cultivars for which screening of existing germplasm based on genetic diversity, genetic advance, and heritability is required (Aytac and Kinaci, 2009). Oil content in *B. napus* L. is generally believed as highly heritable (Zhao *et al.*, 2006). Understanding genetic diversity in *B. napus* L. has a significant role in selecting the best parent to produce improved offspring (Fayyaz *et al.*, 2014a, b; Marwede *et al.*, 2004). Therefore, studies have been conducted on genetic diversity among Chinese germplasm and European accession for effective selection (Zhao *et al.*, 2006; Hu *et al.*, 2007).

The conventional breeding program is vigorous, laborious and time-consuming. A lot of efforts have been placed for the promptness of rapeseed breeding. The genomes that have been sequenced and published till now are of *B. rapa* L., *B. oleracea* L., *B. nigra* L., *B. napus* L., and *B. juncea* L. (Wang *et al.*, 2011; Chalhoub *et al.*, 2014; Liu *et al.*, 2014; Parkin *et al.*, 2014; Yang *et al.*, 2016). Several methods have been developed to boost breeding in *B. napus* L. by focusing on morphological and biochemical traits. Microspore culture technique in *B. napus* L. was first reported by Lichter (1982) that has been widely used for generating haploid and doubled haploids (DH) in many plant species (Zhang *et al.*, 2006; Zhao *et al.*, 2008). The genetic basis of morphological traits such as primary branch length, primary branches, inflorescence length, silique length, plant height, seeds per silique, silique density, and seed weight has been studied by quantitative trait loci (QTLs) and genome-wide association (Chen *et al.*, 2007; Cai *et al.* 2014; Li *et al.*, 2016). Moreover, QTL of oil content, glucosinolate, and fatty acid composition has been identified in *B. napus* L. (Zhao *et al.*, 2006; Zhao *et al.*, 2008; Zou *et al.*, 2010a; Bagheri *et al.*, 2013; Hirani *et al.*, 2016).

Considering the significance of the *B. napus*, the present study was conducted to evaluate genetic diversity among ten selected *B. napus* L. genotypes based on morphological and seed-quality traits. Evidence on superior genotypes will help in developing new varieties, with better characteristics.

## Materials and Methods

### *Experimental design and layout*

The experimental material comprised of three *Brassica napus* L. Chinese lines; CA-2, CA-4, CA-5 (used as testers), and seven already developed doubled haploid lines; DH-2, DH-3, DH-4, DH-5, DH-6, DH-7, DH-8. The experiment was conducted in randomized complete block design with three replications at the experimental fields of the University of Agriculture, Peshawar, Pakistan. Each genotype was grown in three rows with 60 cm distance between rows and 20 cm between plants.

The data on the studied parameters were recorded according to the descriptor for Brassica and Raphanus, International Board for Plant Genetic resources, (IBPGR), Rome Italy. Data was recorded on ten randomly sampled plants from each plot to evaluate seven yield-related traits. These parameters were days to 50% flowering (DF), the number (No.) of primary branches on the main stem (PrBr), main raceme length (RL), pod per main raceme (PR), plant height (PIH), pod length (PoL), and 1000 seeds weight (TSW). Thousand seeds weight was recorded by bulking seeds from ten selected plants. Moreover, three seed-quality traits such as oil content (%), glucosinolate ( $\mu\text{molg}^{-1}$ ), and erucic acid (%) were investigated by using Near-Infrared Reflectance Spectroscopy (NIR) System following manufacturer's protocol (Gan *et al.*, 2003) at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan.

### *Statistical analysis*

**Analysis of variance:** Analysis of variance (ANOVA) and mean evaluation of parameters were performed using the computer software (MSTAT-C, 1991). For the significant result from ANOVA, the least significance difference (LSD) at 5% level of significance ( $P \leq 0.05$ ) was calculated for means comparison.

**Correlation analysis:** Analysis of correlation for various traits was performed using computer software (Statistix 8.1, 2008).

## Results and Discussion

### Days to flowering

The ANOVA showed significant variation ( $P \leq 0.05$ ) among the rapeseed genotypes for days to flowering (Table 1). The highest days to flowering was recorded for CA-4 (120 days) whereas lowest days to flowering was recorded for DH-7 (109 days) (Table 2). Days to flowering had significantly ( $P \leq 0.05$ ) negative correlation with glucosinolate ( $-0.494^{**}$ ) (Table 3). [Sabaghnia et al. \(2010\)](#) also found a significantly negative correlation of days to flowering with seed yield while studying 49 canola genotypes in non-stressed environment.

### Primary branches on main stem

Genotypes were significantly ( $P \leq 0.05$ ) different for primary branches on main stem (Table 1). Primary branches on the main stem ranged from four to nine branches (genotype DH7, CA-5) (Table 2). Primary branches on the main stem exhibited a significant correlation with plant height ( $0.651^{**}$ ), main raceme length ( $0.687^{**}$ ), pod per main raceme ( $0.583^{**}$ ), and 1000 seeds weight ( $0.439$ ) (Table 3). [Gangapur et al. \(2009\)](#) also reported that the number of primary and secondary branches plant<sup>-1</sup> had significant ( $P \leq 0.05$ ) correlation with plant height and main raceme length. Similarly, [Aftab et al. \(2020\)](#) evaluated ten *Brassica napus* genotypes for traits association and reported significantly positive correlation of primary branches plant<sup>-1</sup> with days to flowering and maturity.

**Table 1:** Mean squares for different seed-yield and seed-quality traits of ten *Brassica napus* L. genotypes.

Traits	Rep (df=2)	Genotype (df=9)	Error (df=18)	CV%
DF	2.84	23.17*	8.76	2.56
PrBr	6.26	4.94*	1.62	22.66
PIH	1641.80	455.47*	143.26	6.15
MRL	74.22	291.13**	25.08	6.73
PR	372.27	398.81**	71.71	12.54
PoL	0.23	1.15*	0.47	9.05
TSW	1.73	0.78**	0.15	5.15
OIL	3.82	6.23ns	2.82	3.24
GSL	4.00	328.52**	69.52	11.45
EA	0.79	179.73**	2.73	2.83

Days to flowering (DF), number of primary branches on main stem (PrBr), plant height (PIH), main raceme length (MRL), pods per main raceme (PR), pod length (PoL), 1000 seeds weight (TSW), Oil content (oil), glucosinolate (GSL), and erucic acid (EA)

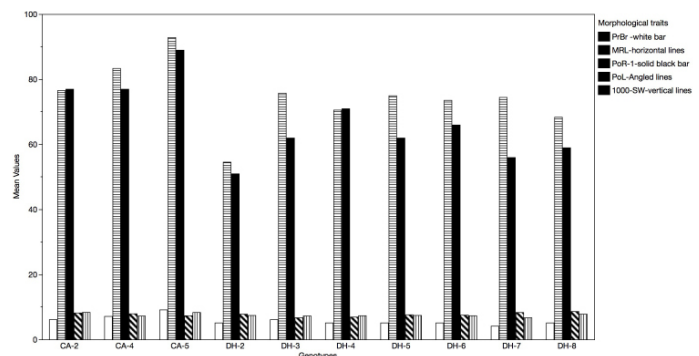
\*, \*\*=Significant at 5% and 1% level of probability, respectively.

### Plant height

Genotypes of rapeseed were significantly ( $P \leq 0.05$ ) different for plant height (Table 1). Mean data for plant height ranged from 179.27cm (DH-2) to 224.47cm (CA-5) (Table 2). A number of variables viz., main raceme length ( $0.591^{**}$ ), primary branches ( $0.651^{**}$ ), and pod per main raceme ( $0.444^*$ ) were significantly ( $P \leq 0.05$ ) correlated with plant height. [Marwede et al. \(2004\)](#) and [Sabaghnia et al. \(2010\)](#) reported significant positive correlation of plant height with primary branches, main raceme length and pods mainraceme<sup>-1</sup>.

### Main raceme length

Mean squares were significantly ( $P \leq 0.01$ ) different among ten genotypes for the main raceme length (Table 1). Genotypes CA-5 had the highest main raceme length (92.73cm), and DH-2 exhibited the lowest value for the main raceme length (54.4cm) (Figure 1). [Ali et al. \(2014\)](#) also described significant variation among rapeseed lines for the main raceme length. All the studied traits were significantly ( $P \leq 0.05$ ) correlated with main raceme length except days to flowering ( $0.08^{ns}$ ), pod length ( $-0.11^{ns}$ ), oil contents ( $-0.36^{ns}$ ), and glucosinolate ( $-0.25^{ns}$ ) (Table 3). [Zhang et al. \(2006\)](#) also reported a positive correlation of main raceme length with pod per main raceme and glucosinolate in *Brassica napus* genotypes.



**Figure 1:** Mean values of morphological traits of ten *B. napus* L. genotypes; number of primary branches on main stem (PrBr), main raceme length (MRL), pods per main raceme ( $PoR^{-1}$ ), pod length (PoL), 1000 and seeds weight (TSW).

### Pods per main raceme

Significant variances ( $P \leq 0.01$ ) among rapeseed genotypes were detected for pods per main raceme (Table 1). Genotype CA-5 produced more pods per main raceme (89.0) whereas DH-2 exhibited the lowest (51.0) pods per main raceme (Table 2). Pods per main raceme had a significant positive correlation with all the traits except for days to flowering

**Table 2:** Mean values for different seed–yield and seed–quality traits of ten *Brassica napus* L. genotypes.

Genotypes	DF (No.)	PrBr (No.)	PIH (cm)	MRL (cm)	PR (No.)	PoL (cm)	TSW (g)	Oil (%)	GSL (µmole/g)	EA (%)
CA-2	117ab	6bc	182.13bc	76.47bc	77abc	8.06ab	8.23a	50.45c	59.23de	29.22f
CA-4	120a	7ab	192.0bc	83.2b	77ab	7.79abc	7.13bc	49.80c	63.58cde	43.85cd
CA-5	116ab	9a	224.47a	92.73a	89a	7.15bcd	8.21a	51.06bc	84.51a	36.34e
DH-2	116ab	5bc	179.27c	54.4d	51f	7.66abcd	7.29b	52.62abc	56.09e	43.25cd
DH-3	114b	6bc	197.93bc	75.6bc	62cdef	6.59d	7.15bc	54.55a	76.16abc	53.76a
DH-4	114bc	5bc	194.87bc	70.47c	71bcd	6.81cd	7.21bc	52.59abc	80.87ab	56.19a
DH-5	117ab	5bc	200.6b	74.8bc	62cdef	7.45abcd	7.296b	51.24bc	82.13ab	43d
DH-6	115ab	5bc	193.47bc	73.47c	66bcde	7.36bcd	7.21bc	53.39ab	69.71bcde	46.83b
DH-7	109c	4c	193.27bc	74.33c	56ef	8.24ab	6.58c	51.02bc	84.01ab	46.75b
DH-8	114b	5bc	189.4bc	68.27c	59def	8.536a	7.70ab	51.59bc	71.656abcd	45.93bc
LSD <sub>(0.05)</sub>	5.078	2.1815	20.531	8.591	14.526	1.174	0.6537	2.8799	14.303	2.8345

Days to 50% flowering (DF), number of primary branches on main stem (PrBr), plant height (PIH), main raceme length (MRL), pods per main raceme (PR), pod length (PoL), 1000 seeds weight (1000SW), Oil content (oil), glucosinolate (GSL), and erucic acid (EA).

(0.20<sup>ns</sup>), pod length (-0.22<sup>ns</sup>), oil content (-0.35<sup>ns</sup>), glucosinolate (-0.18<sup>ns</sup>) and erucic acid (-0.32<sup>ns</sup>) (Table 3). Zou *et al.* (2010b) also reported significant correlation of pods per main raceme with morpho–yield traits such as 1000–seed weight.

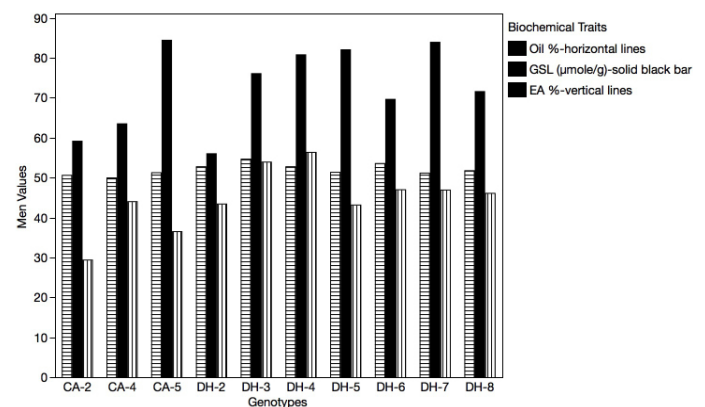
*Pod length*

Mean squares were significantly (P≤0.05) different among rapeseed genotypes for pod length (Table 1). Mean data for pod length ranged between 6.59 cm (DH-3) and 8.536 cm (DH-8) (Table 2). Aytac and Kinaci (2009) also reported highly significant variation for important yield and yield associated traits among brassica cultivars. Pod length exhibited a significant positive correlation with the 1000 seeds weight (0.04\*) whereas highly significant negative association with oil content (-0.478\*\*) while the non-significant correlation with all the other traits studied (Table 3). Our results of a significant positive correlation of pod length with 1000 seeds weight are also supported by Aytac and Kinaci (2009).

*1000 seeds weight*

The rapeseed genotypes studied for 1000–seed weight were significantly (P≤0.01) different (Table 1). Genotype CA-2 produced heaviest seeds (8.23 g) as compared to DH-7 which exhibited lowest weight (6.58g) for 1000 seeds (Table 2). Correlation of 1000 seeds weight was significant with all the traits except days to flowering (0.12<sup>ns</sup>), plant height (0.22<sup>ns</sup>), oil (-0.16<sup>ns</sup>), and glucosinolate (-0.16<sup>ns</sup>) (Table 3). Zhang *et al.* (2017) also identified genetic diversity in *B. carinata* through seed–yield related traits such

as flowering time, length of the main inflorescence, pod on the main inflorescence, pod length, pod width, seed per pod and seed weight.



**Figure 2:** Mean values of ten *B. napus* L. genotypes for oil content (oil %), glucosinolate (GSL), and erucic acid (EA%) of ten *B. napus* genotypes.

*Oil content*

Non-significant differences were observed for oil content among ten genotypes (Table 1). Mean data for oil content ranged from 49.80% for genotype CA-4 to 54.55% for DH-3 (Table 2, Figure 2). Significantly negative correlation of oil content was observed with pod length (-0.478\*\*) however the association was positive (0.454\*) with erucic acid (Table 3). Aytac and Kinaci (2009) also described significant correlation of oil yield with erucic acid. Therefore, it can be speculated that brassica genotypes having higher oil content may also have higher concentration of erucic acid. Moreover, the erucic acid content varies between species (Bhardwaj and Hamama, 2000). Moreover, Alemayehu and Becker (2001) and Zhang *et al.* (2017)

**Table 3:** Correlation coefficients among seed yield related and seed quality traits in Chinese and the DH population of *Brassica napus* L.

Traits	PrBr (No.)	PIH (cm)	MRL (cm)	PR (No.)	PoL (cm)	TSW (g)	OIL (%)	GSL (µmole/g)	EA (%)
DF	0.14 <sup>ns</sup>	-0.14 <sup>ns</sup>	0.08 <sup>ns</sup>	0.20 <sup>ns</sup>	-0.03 <sup>ns</sup>	0.12 <sup>ns</sup>	-0.22 <sup>ns</sup>	-0.494 <sup>**</sup>	-0.33 <sup>ns</sup>
PrBr		0.651 <sup>**</sup>	0.687 <sup>**</sup>	0.583 <sup>**</sup>	-0.20 <sup>ns</sup>	0.439 <sup>*</sup>	-0.22 <sup>ns</sup>	0.10 <sup>ns</sup>	-0.29 <sup>ns</sup>
PIH			0.591 <sup>**</sup>	0.444 <sup>*</sup>	-0.22 <sup>ns</sup>	0.22 <sup>ns</sup>	-0.18 <sup>ns</sup>	0.30 <sup>ns</sup>	-0.02 <sup>ns</sup>
MRL				0.788 <sup>**</sup>	-0.11 <sup>ns</sup>	0.391 <sup>*</sup>	-0.36 <sup>ns</sup>	0.391 <sup>*</sup>	-0.25 <sup>ns</sup>
PoR <sup>-1</sup>					-0.22 <sup>ns</sup>	0.628 <sup>**</sup>	-0.35 <sup>ns</sup>	0.18 <sup>ns</sup>	-0.32 <sup>ns</sup>
PoL						0.04 <sup>*</sup>	-0.478 <sup>**</sup>	-0.22 <sup>ns</sup>	-0.34 <sup>ns</sup>
1000SW							-0.16 <sup>ns</sup>	-0.07 <sup>ns</sup>	-0.509 <sup>**</sup>
OIL								0.02 <sup>ns</sup>	0.454 <sup>*</sup>
GSL									0.30 <sup>ns</sup>

days to 50% flowering (DF), number of primary branches on main stem (PrBr), plant height (PIH), main raceme length (MRL), pods per main raceme (PR), pod length (PoL), 1000 seeds weight (TSW), oil content (oil %), glucosinolate (GSL), and erucic acid (EA%).

\*, \*\*=Significant at 5% and 1% level of probability, respectively.

also reported 28–41% erucic acid content in *B. carinata* germplasm and they also observed genetic variation in six seed quality traits; oil content, protein content, linolenic acid, erucic acid and oleic acid.

#### Glucosinolate

significantly variation ( $P \leq 0.01$ ) existed among genotypes for glucosinolate (Table 1). The genotype CA-5 had the highest glucosinolate content ( $84.51 \mu\text{molg}^{-1}$ ) whereas DH-2 had a lowest glucosinolate ( $56.09 \mu\text{molg}^{-1}$ ) (Table 2, Figure 2). The recommended glucosinolate concentration is  $<30 \mu\text{molg}^{-1}$  in defatted seed meal. However, higher concentration of glucosinolate ( $120 \mu\text{mole/g}$  defatted seed meal) was also found by Kumar *et al.* (2016) that support our findings. Glucosinolate content was significantly correlated with main raceme length ( $0.391^*$ ) and negatively correlated with days to flowering ( $-0.494^{**}$ ) (Table 3). Khan *et al.* (2006) also reported a significant correlation of glucosinolate with main raceme length.

#### Erucic acid

Among the studied genotypes, significant variation ( $P \leq 0.01$ ) existed for erucic acid (Table 1). The mean value for erucic acid was 44.52%. The genotype CA-2 exhibited a lowest (29.22%) whereas DH-4 had the highest value (56.19%) for erucic acid content (Table 2, Figure 2). Erucic acid content was significantly correlated with 1000SW ( $-0.509^{**}$ ) and oil content ( $0.454^*$ ) (Table 3). This suggested that the fatty acid components are significantly correlated but show different correlations with the varied genetic back-

ground. However, Zhao *et al.* (2006) and Zhang *et al.* (2006) reported that oil content had significantly negative association with erucic acid.

### Conclusions and Recommendations

Genetic diversity for morphological traits in the studied genotypes was assessed. A wide range of variation was observed for seven seed-yield and three seed quality traits. Higher oil content was observed in DH lines than in the Chinese genotypes. Moreover, the genotype DH-3 had high oil content, DH-2 had low glucosinolate and CA-2 had low erucic acid content. Among all the genotypes studied, CA-2 and CA-5 performed better for seed yield-related traits.

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### Novelty Statement

*Brassica napus* is an important oilseed crop in Pakistan which could save valuable foreign exchange by reducing import bill. Genetic variability in rapeseed genotypes is important to improve various plant attributes. Sufficient genetic variability in *Brassica napus* could

not only ensure improving oil content but also reducing undesirable chemical constituents. Identification of superior brassica lines for various morpho-agronomic traits including seed yield, oil content and desirable concentration of other chemical constituents would help plant breeders to introgress desirable traits into their progeny.

### Author's Contribution

**Amir Muhammad Khan:** Conceptualized the idea for this research and conducted the experimental design.

**Laila Fayyaz:** Helped in writing this article and also contributed to the review of relevant literature.

**Raziuddin:** Conceived the idea and supervised the research.

**Farhatullah, Sajid Ali, Israr ud Din and Sheraz Ahmad:** Helped in data analysis and critical peer review of the article.

**Haidar Ali and Ijaz Ahmad:** Both helped in data collection and compilation.

### Conflict of interest

The authors declare no conflict of interest.

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