

## GENETIC ANALYSIS OF QUANTITATIVE AND QUALITATIVE TRAITS IN *BRASSICA NAPUS* L. USING F<sub>2</sub> PROGENIES OF DIALLEL CROSSES THROUGH HAYMAN'S APPROACH

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### Abstract

This study was aimed at assessing a detailed genetic analysis of five parental populations of *Brassica napus* L. along with its F<sub>2</sub> population using Hayman's approach. A significant contribution of F<sub>2</sub> crosses was noted for important traits, including BKUC7 x BKUC8 for days to 50% flowering (67 days), BKUC3 x BKUC7 for maximum plant height (105.5 cm) and siliqua plant<sup>-1</sup> (86), BKUC3xBKUC8 for highest oil content (42.6%) and BKUC4 x BKUC8 for high protein content (27.4%). The results showed that self-combination of BKUC4 and BKUC8 had the least glucosinolate concentration (52.2%) while the highest level was of oleic acid (55.0%). Likewise, the lowest erucic acid concentration (41.7%) was recorded for BKUC2xBKUC4. A negative inbreeding depression for days to 50% flowering initiation (-2.48%) and glucosinolate (-17.79%) and linoleic acid (-43.13%) concentrations was recorded for P<sub>4</sub>, while that of erucic acid (-27.31%) was noted for P<sub>1</sub>. BKUC2 x BKUC3 has shown a desired negative heterosis of -14.86% and -19.43% for days to 50% flowering and erucic acid concentration, respectively. Similarly, BKUC2 x BKUC8 has shown a positive heterosis for oil content (13.18%). Protein content (4.83%) and oleic acid concentration (3.15%) were evident for BKUC4 x BKUC8, while BKUC2 x BKUC7 displayed a desired negative heterosis up to -21.14% for GSL. Statistical analysis has shown a significant role of GCA and SCA for all traits except siliqua plant<sup>-1</sup>, which validates the active role of additive and non-additive genetics. Over-dominance and partial dominance gene action were mainly observed for all traits in this study. Overall, a high heritability and desirable positive and negative heterosis for most important traits, like oil content, erucic acid and glucosinolate concentrations, protein content, and days to 50% flowering were observed in our study. In conclusion, our findings provide new insights for future breeding studies on the F<sub>2</sub> progenies of diallel crosses of *B. napus* L.

**Key words:** Combining ability, Heterosis study, Gene action, Heritability, Inbreeding depression.

### Introduction

*Brassica napus* (L.; Brassicaceae) is cultivated globally and most renowned for its production of edible oil. The spontaneous hybridization of *B. rapa* L. and *B. oleracea* L., which have the genetic composition of diploid AA 20 and CC 18, respectively, produced an amphidiploid species *B. napus*, which has diploid AACC 38 genomes. (Iniguez-Luy & Federico, 2011). Because of its two main groups, mustard, and rapeseed, the importance of the *Brassica* genus has increased (Warwick *et al.*, 2006). For many decades, *B. campestris*, *B. juncea*, and *Taramira* (*Eruca sativa*) have been grown in Pakistan for oil purposes (Turi *et al.*, 2012). Overall, rapeseed has been ranked as the 3rd oil-producing crop and second to soybean worldwide. Likewise, the availability of protein rich rapeseed is an attractive and good source of animal feed containing 35 to 45% of protein and 0.35 % of phosphorus (Newkirk, 2009). Rapeseed oil is pivotal for manufacturing industries, being used in paints, inks, nylon making, high-pressure grease, and coating (Aukema & Campbell, 2011).

Utilization of modern molecular breeding techniques are considered important to face the challenges of low yield and oil content in plants. There is much possibility that the genus *Brassica* can bridge this low yielding gap. Since 1980s, due to substantial efforts of plant breeding scientists, a little increase has been seen in quality oil production (Bacha *et al.*, 2015). To make rapeseed oil more

appealing, both the yield and quality are needed to be improved (Rameah *et al.*, 2003). The obvious solution to the overwhelming requirement of the time is to use heterosis breeding in conjunction with conventional breeding. Combining phenotypic analysis between parents and F<sub>1</sub>s with gene function studies allows for the ideal plant crossings to be utilized as future breeding material (Sabaghina *et al.*, 2010). Combining ability studies show yield associated parameters are strongly influenced by dominant gene activity, and it also aids in the selection of parents that will be good candidates for the next generation of breeding projects (Kang *et al.*, 2013).

To improve outcomes relating to quality and production, numerous researchers have studied heterosis and combining ability in *Brassica*. In *Brassica rapa*, positive heterosis (MP and BP) has been studied for yield and yield-related characteristics. Several researchers have examined and reported the same results in additional *Brassica* species (Sincik *et al.*, 2011). Understanding both additive and non-additive gene function types is essential for breeding populations. It aids in understanding the procedure for breeding that enhances the performance of desired features (Dudley & Moll, 1969). Once the variation is evident, the procedure for determining the heritable proportion of variability is critical to enhance the genetic gain. Thus, improvement in quantitative and quality parameters can be achieved by genetic diversity. Therefore, the focus of the current study was to evaluate the heritability and variability in F<sub>2</sub> segregating populations in addition to parents and checks.

Keeping in view this scenario, an investigation was conducted to interrogate combining ability, heterosis, heterobeltiosis (MP and BP), inbreeding depression, and heritability among *B. napus* genotypes (F2 generation). At the same time, quality-related traits were also investigated to advance the breeding program for future variety development.

## Material and Methods

**Experimental site and genetic material:** The present study was conducted at Bacha Khan Agriculture Research Farm (BARF) Charsadda, Pakistan (34.1369° N, 71.8382° E). Genetic material constituted a set of 5 (five) rapeseed genotypes viz., 2702, 2722, Dunkled, P-801 and P1-119.

**Development of F<sub>1</sub> hybrids:** At BARF, 5 distinct genotypes of adopted rapeseed gathered from the public sector were sown from 2017-18. A 5 x 5 diallel pattern was created using 10 direct crosses and 5 self's. For every cross, at least 5 flowers were pollinated randomly in selected plants in order to ensure an adequate number of seeds. The seeds were carefully separated and kept until the next year for planting.

**Development of F<sub>2</sub> genotypes:** Ten F<sub>1</sub>s, 5 self-genotypes, and 5 parental lines were sown at BARF in 2018-19 in order to assess inbreeding depression. Row length of two meter were kept for each entry. While 75 cm space between rows and plants were kept. Natural environment was provided throughout till harvest. Quantitative data was collected randomly, and 20-gram seed samples from each entry were analyzed at NIFA, Peshawar. The remaining seeds were preserved for sowing in the next growing season. In 2019-20, RCB (Randomized Complete Block) design was used to produce F<sub>2</sub> populations of the aforementioned material at the same location alongside parental lines and three checks.

Data was recorded on the following agronomic (Days to 50% flowering, Number of Siliqua plant<sup>-1</sup>, Plant height) and quality-related traits (oil content (%), protein content (%), glucosinolate concentration (%), oleic acid (%), linoleic acid (%) and erucic Acid (%))

Twenty grams (20) of seeds were packed and preserved with proper tags from each plot. The preserved seeds were subjected to qualitative traits analysis at NIFA (Peshawar) and quality traits were determined using Near Infra-Red Spectroscopy (NIRS). A list of the parental genotypes, checks, self's, and F<sub>2</sub> population are given in Table 1.

**Statistical analysis:** The data were organized and presented for ANOVA (analysis of variance) using Statistix 8.1 software. Parameters that revealed significant F-value were further subjected to diallel analyses, using the DIALL98 software followed by model I method II as previously reported Model (Hayman, 1954). Inbreeding depression (ID) was calculated using the following formula:

$$ID\% \text{ in } F_2 = [Sp - Si] \times 100 / Sp$$

where, Sp = Average mean of a parent. Si = Average mean of self's.

Heterosis (Best parent and Mid parent) was elaborated using (Falconer, 1996) method.

## Results

**ANOVA of mean values of different traits:** Mean sum of square of different parameters studies are presented in Table 2. The occurrence of variability in the examined material (F<sub>2</sub> and parents with check) is shown in Table 3. The very first possible flowering and maturity gave the seed the best chance to develop into a larger, higher-quality seed at the right moment. When compared to checks, both parents and F<sub>2</sub>s (on average) have demonstrated earliness (Table 3). Checks for plant height performed better than those performed by parents. However, compared to checks, the majority of crosses had higher pH values. The number of siliqua plant<sup>-1</sup> increased, which in turn increased the rapeseed yield. With few exceptions, the checks' performance was significantly worse than that of the parents and F<sub>2</sub>, hence displayed relatively lowest values for the relevant parameter.

In comparison to checks, F<sub>2</sub>s and parents performed better in terms of oil content. The availability of a respectable genetic variation for protein content between parents and F<sub>2</sub>s was shown by analysis. In contrast, F<sub>2</sub>'s performance was recorded as having a higher protein content than checks. GSL content was a determinant of rapeseed quality. Thus, a minimum GSL % was required. The checks performed better for GSL than the majority of the crosses and parents. Fewer crosses, however, showed noticeably better results. The concentration of oleic acid mostly determines the stability of oil. Except a few crosses that were found to perform better than checks, the average performance of checks for oleic acid was better than that of F<sub>2</sub>s and parents.

Lower levels of linoleic acid in oil were associated with better oil quality retention during cooking and storage. The examination of the parents and F<sub>2</sub>s with checks shows that, overall, they all responded similarly to the concentration of linoleic acid; nevertheless, a small number of crosses showed values that were lower or higher than what the checks performed. Because erucic acid causes fat to build in blood vessels and the heart, it is the most unwanted fatty acid in brassica oil and needs to be minimized. While the performance of a few crossings was closer to the values of the checks, the relative performance of checks was better than that of parents and F<sub>2</sub>s for EA. (see Table 3).

**Combining ability analysis:** Diallel crosses exhibited highly significant differences for both additive (a) and non-additive (b) components, indicating the significance of both gene action types for days to 50% flowering. When comparing days to 50% flowering, a greater value for "a" (GCA) (18.97) than "b" (SCA) (3.98) suggested that additive effects are more advantageous. The frequency of dominant genes is shown by the non-significant value of b1 (1.25) and the significant values of b2 (122.95) & b3 (70.75) for siliqua plant<sup>-1</sup>. The fact that b2 (3.50) has a greater value than b1 (1.76) and b3 (0.32) for protein content, indicates that dominant alleles play a role in regulating characteristic expression. The estimates of genetic factors showed that genotypes for glucosinolate, oleic acid, linoleic acid, erucic acid, plant height, siliqua plant<sup>-1</sup>, oil content, and protein content were highly significant.

**Table 1. List of the parental genotypes, checks, self's and F2 population.**

S. No.	Parents	S.No	F <sub>2</sub> population	S.No.	F <sub>2</sub> population
1.	BKUC2 (2702)	1.	BKUC2 x BKUC2 (self)	10.	BKUC4 x BKUC4 (self)
2.	BKUC3 (2722)	2.	BKUC2 x BKUC3	11.	BKUC4 x BKUC7
3.	BKUC4 (Dunkled)	3.	BKUC2 x BKUC4	12.	BKUC4 x BKUC8
4.	BKUC7 (P-801)	4.	BKUC2 x BKUC7	13.	BKUC7x BKUC7 (self)
5.	BKUC8 (P1-119)	5.	BKUC2 x BKUC8	14.	BKUC7 x BKUC8
<b>Checks</b>					
1.	Check 1	6.	BKUC3 x BKUC3(self)	15.	BKUC8 x BKUC8 (self)
2.	Check 2	7.	BKUC3 x BKUC4		
3.	Check 3	8.	BKUC3 x BKUC7		
		9.	BKUC3 x BKUC8		

**Table 2. Mean sum of square for different quality-related characters and CV (%) in parental and F<sub>2</sub> diallel population of *Brassica napus* L.**

SOV	Days to 50% flowering	Plant height (cm)	Siliqua plant <sup>-1</sup>	Oil%	Protein%	GSL	Oleic acid	Linoleic acid	Erucic acid
<b>Genotypes</b>	10.231**	169.031**	106.8**	8.71**	2.976**	158.81**	32.505**	3.417**	57.687**
<b>CV</b>	1.35	8.71	5.29	1.69	1.20	2.74	0.27	0.79	0.19

**Table 3. Mean performance of different genotypes, selfed lines, parental lines, and checks for days to 50% flowering, plant height (cm), Siliqua per plant, oil content (%), protein content (%), glucosinolate (GSL) content (%), oleic acid (%), linoleic acid (%), and erucic acid (%) in *B. napus***

Genotypes	Days to 50% flowering	Plant height	Siliqua plant <sup>-1</sup>	Oil content	Protein content	GSL content	Oleic acid	Linoleic acid	Erucic acid
BKUC2x BKUC2	70.0±0.47 <sup>cd</sup>	73.6±3.68 <sup>hi</sup>	75.6±3.33 <sup>cdefghi</sup>	41.3±0.21 <sup>d</sup>	23.8±0.13 <sup>o</sup>	63.0±1.08 <sup>ijkl</sup>	55.4±0.29 <sup>a</sup>	8.7±0.08 <sup>cd</sup>	39.6±0.13 <sup>r</sup>
BKUC2x BKUC3	68.7±0.94 <sup>efgh</sup>	68.8±1.99 <sup>i</sup>	70.4±4.00 <sup>hijklmn</sup>	40.1±0.66 <sup>fg</sup>	24.7±0.08 <sup>ijkl</sup>	61.4±0.31 <sup>klm</sup>	52.7±0.08 <sup>e</sup>	8.5±0.12 <sup>ef</sup>	45.3±0.05 <sup>j</sup>
BKUC2x BKUC4	67.7±0.47 <sup>hij</sup>	84.5±9.91 <sup>cdefgh</sup>	80.9±7.15 <sup>abcd</sup>	41.6±0.21 <sup>d</sup>	25.5±0.05 <sup>efg</sup>	65.2±0.17 <sup>ghi</sup>	50.3±0.21 <sup>ij</sup>	8.4±0.00 <sup>fgh</sup>	41.7±0.08 <sup>p</sup>
BKUC2x BKUC7	67.3±0.47 <sup>hij</sup>	81.6±9.23 <sup>cdefgh</sup>	79.5±4.90 <sup>bcdef</sup>	40.3±0.57 <sup>fg</sup>	25.4±0.05 <sup>efg</sup>	52.6±0.16 <sup>q</sup>	51.3±0.20 <sup>b</sup>	7.4±0.05 <sup>kl</sup>	47.6±0.05 <sup>g</sup>
BKUC2x BKUC8	67.3±0.94 <sup>hij</sup>	79.9±5.2 <sup>defgh</sup>	80.4±2.39 <sup>abcde</sup>	39.9±0.33 <sup>g</sup>	26.2±0.08 <sup>bc</sup>	62.0±0.13 <sup>klm</sup>	51.4±0.29 <sup>b</sup>	6.9±0.05 <sup>o</sup>	47.2±0.08 <sup>h</sup>
<b>BKUC2 (P1)</b>	<b>70.3±0.47<sup>cd</sup></b>	<b>76.3±5.9<sup>ghi</sup></b>	<b>70.7±0.89<sup>hijklmn</sup></b>	<b>41.4±0.21<sup>d</sup></b>	<b>24.3±0.05<sup>klmn</sup></b>	<b>66.8±6.53<sup>fgh</sup></b>	<b>55.5±0.28<sup>a</sup></b>	<b>8.8±0.08<sup>c</sup></b>	<b>39.5±0.13<sup>r</sup></b>
BKUC3x BKUC3	70.0±0.81 <sup>cde</sup>	87.8±4.5 <sup>bcdef</sup>	79.6±2.99 <sup>bcdef</sup>	42.4±0.42 <sup>c</sup>	25.3±0.30 <sup>efgh</sup>	71.3±0.45 <sup>cd</sup>	53.4±0.05 <sup>d</sup>	6.3±0.09 <sup>q</sup>	44.2±0.08 <sup>k</sup>
BKUC3x BKUC4	71.3±0.47 <sup>bc</sup>	78.7±3.44 <sup>efghi</sup>	85.3±1.47 <sup>ab</sup>	40.7±0.25 <sup>ef</sup>	24.5±0.08 <sup>ijklm</sup>	71.6±0.14 <sup>c</sup>	51.4±0.17 <sup>b</sup>	8.6±0.08 <sup>de</sup>	43.2±0.14 <sup>n</sup>
BKUC3x BKUC7	<b>74.0±0.81<sup>a</sup></b>	<b>105.5±2.5<sup>a</sup></b>	<b>86.4±5.86<sup>a</sup></b>	40.6±0.34 <sup>ef</sup>	25.6±0.09 <sup>def</sup>	<b>81.0±0.22<sup>a</sup></b>	<b>37.7±0.16<sup>o</sup></b>	<b>10.8±0.08<sup>a</sup></b>	47.9±0.05 <sup>f</sup>
BKUC3x BKUC8	67.7±0.47 <sup>hij</sup>	91.6±4.97 <sup>bc</sup>	68.4±1.67 <sup>klmn</sup>	42.6±0.24 <sup>c</sup>	24.6±0.08 <sup>ijklm</sup>	64.6±0.21 <sup>hij</sup>	52.8±0.26 <sup>e</sup>	8.5±0.05 <sup>ef</sup>	42.7±0.14 <sup>o</sup>
<b>BKUC3 (P2)</b>	<b>70.0±0.81<sup>cde</sup></b>	<b>85.4±10.54<sup>bcdefg</sup></b>	<b>74.3±1.76<sup>efghijkl</sup></b>	<b>42.6±0.16<sup>c</sup></b>	<b>25.5±0.08<sup>efg</sup></b>	<b>71.3±0.45<sup>cd</sup></b>	<b>53.4±0.28<sup>d</sup></b>	<b>6.2±0.05<sup>q</sup></b>	<b>44.2±0.08<sup>k</sup></b>
BKUC4x BKUC4	68.7±0.98 <sup>efgh</sup>	95.8±2.24 <sup>ab</sup>	74.1±1.99 <sup>efghijk</sup>	40.2±0.21 <sup>fg</sup>	25.6±0.39 <sup>efg</sup>	<b>52.2±0.63<sup>q</sup></b>	50.2±0.17 <sup>ij</sup>	7.4±0.14 <sup>kl</sup>	49.8±0.13 <sup>cd</sup>
BKUC4x BKUC7	68.7±0.47 <sup>efgh</sup>	90.1±4.69 <sup>bcd</sup>	78.5±3.56 <sup>cdefg</sup>	38.9±0.10 <sup>h</sup>	26.4±0.09 <sup>b</sup>	62.9±0.09 <sup>ijkl</sup>	51.8±0.05 <sup>s</sup>	<b>6.8±0.08<sup>o</sup></b>	42.7±0.05 <sup>o</sup>
BKUC4x BKUC8	67.7±0.47 <sup>hij</sup>	80.1±4.82 <sup>defgh</sup>	80.7±1.07 <sup>abcd</sup>	38.0±0.08 <sup>i</sup>	<b>27.4±0.04<sup>a</sup></b>	61.3±1.94 <sup>klm</sup>	52.3±0.04 <sup>f</sup>	9.7±0.05 <sup>b</sup>	41.9±0.05 <sup>p</sup>
<b>BKUC4 (P3)</b>	<b>69.3±0.94<sup>defg</sup></b>	<b>74.1±4.04<sup>hi</sup></b>	<b>74.1±1.99<sup>efghijk</sup></b>	<b>40.2±0.21<sup>fg</sup></b>	<b>26.3±0.95<sup>bc</sup></b>	<b>70.1±0.29<sup>cde</sup></b>	<b>50.2±0.26<sup>ij</sup></b>	<b>7.3±0.05<sup>lm</sup></b>	<b>49.9±0.05<sup>c</sup></b>
BKUC7x BKUC7	68.7±1.69 <sup>efgh</sup>	83.8±3.6 <sup>cdefgh</sup>	75.0±2.81 <sup>cdefghij</sup>	39.9±0.19 <sup>g</sup>	26.2±0.23 <sup>bc</sup>	67.9±1.46 <sup>ef</sup>	52.7±0.04 <sup>e</sup>	6.7±0.05 <sup>p</sup>	46.1±0.09 <sup>i</sup>
BKUC7x BKUC8	67.0±0.00 <sup>ij</sup>	79.3±4.92 <sup>defghi</sup>	67.5±1.56 <sup>lmn</sup>	42.3±0.19 <sup>c</sup>	25.2±0.09 <sup>fghi</sup>	75.2±0.82 <sup>b</sup>	47.2±0.08 <sup>m</sup>	7.5±0.05 <sup>k</sup>	49.7±0.05 <sup>d</sup>
<b>BKUC7 (P4)</b>	<b>67.0±0.81<sup>ij</sup></b>	<b>88.0±4.57<sup>bcdef</sup></b>	<b>81.1±2.37<sup>abc</sup></b>	<b>39.9±0.18<sup>g</sup></b>	<b>26.5±0.09<sup>b</sup></b>	<b>66.7±0.31<sup>fgh</sup></b>	<b>52.7±0.05<sup>e</sup></b>	<b>6.6±0.05<sup>p</sup></b>	<b>46.0±0.09<sup>i</sup></b>
BKUC8x BKUC8	70.0±0.8 <sup>cde</sup>	79.3±3.09 <sup>defghi</sup>	73.6±2.69 <sup>fghijkl</sup>	41.2±0.17 <sup>de</sup>	25.8±0.21 <sup>cde</sup>	60.4±0.37 <sup>lmn</sup>	<b>55.0±0.13<sup>b</sup></b>	7.2±0.00 <sup>m</sup>	43.8±0.09 <sup>l</sup>
<b>BKUC8 (P5)</b>	<b>68.3±0.47<sup>fghi</sup></b>	<b>82.5±2.63<sup>cdefgh</sup></b>	<b>72.8±1.73<sup>ghijklm</sup></b>	<b>41.1±0.05<sup>de</sup></b>	<b>26.2±0.09<sup>bcd</sup></b>	<b>60.6±0.29<sup>lmn</sup></b>	<b>51.3±0.08<sup>h</sup></b>	<b>7.2±0.00<sup>m</sup></b>	<b>43.9±0.05<sup>l</sup></b>
Check-1	72.7±1.24 <sup>ab</sup>	88.1±4.36 <sup>bcde</sup>	71.7±0.97 <sup>hijklm</sup>	40.6±0.81 <sup>ef</sup>	24.1±0.13 <sup>lmno</sup>	63.9±0.13 <sup>ijk</sup>	51.4±0.04 <sup>b</sup>	8.5±0.05 <sup>efg</sup>	40.0±0.13 <sup>q</sup>
Check-2	70.7±0.47 <sup>cd</sup>	89.5±1.87 <sup>bcde</sup>	69.7±0.69 <sup>ijklmn</sup>	42.5±0.29 <sup>c</sup>	23.9±0.04 <sup>no</sup>	58.6±0.49 <sup>no</sup>	54.7±0.05 <sup>b</sup>	7.8±0.05 <sup>j</sup>	34.6±0.13 <sup>s</sup>
Check-3	71.3±0.94 <sup>bc</sup>	80.6±5.43 <sup>cdefgh</sup>	71.4±1.72 <sup>hijklmn</sup>	39.8±0.24 <sup>g</sup>	24.2±0.05 <sup>lmno</sup>	59.9±0.33 <sup>mn</sup>	54.8±0.08 <sup>b</sup>	8.4±0.05 <sup>sh</sup>	33.7±0.08 <sup>t</sup>

Letters indicating the significant difference among all genotypes for different traits. Values are means ± standard deviation.

**Table 4. Estimates of genetic parameters for days to 50% flowering, plant height (cm), siliqua plant<sup>-1</sup>, oil content, protein content, GSL content, oleic acid, linoleic acid, and erucic acid.**

SOV	Genotypes	GCA(a)	SCA(b)	b1	b2	b3	Error
DF	24	4	10	1	4	5	48
Days to 50% flowering	10.53**	18.976**	3.981**	0.653	7.762**	1.622*	0.768
Plant height	161.613**	228.268*	121.227*	83.424	102.655	143.646	54.890
Siliqua plant <sup>-1</sup>	131.373**	79.618	84.543**	1.254	122.595**	70.758*	17.535
Oil content	10.141**	9.808**	6.308**	0.740*	7.241**	6.676**	0.110
Protein content	2.867**	2.435**	1.740**	1.763	3.508**	0.321*	0.112
Glucosinolate	151.434**	208.675**	159.648**	23.019	150.560**	194.244**	4.237
Oleic acid	36.587**	37.37**	36.38**	73.30**	41.954**	24.47**	0.021
Linoleic acid	4.194**	1.461**	6.336**	16.240**	4.113**	6.135**	0.005
Erucic acid	36.451**	42.921**	35.154**	21.600**	73.089**	7.516**	0.006

Single \* and double \*\* represent significant ( $p < 0.05$ ) and highly significant ( $p < 0.01$ ) results for combining ability, respectively. GCA (General combining ability), SCA (Specific combining ability), and b1 b2, and b3 are the various components of specific combining ability

**Table 5. Inbreeding depression (%) for days to 50% flowering, plant height, siliqua plant<sup>-1</sup>, oil content, protein content, GSL content, oleic acid, linoleic acid and erucic acid.**

Parameters	Genotypes				
	P1 (self)	P2 (self)	P3 (self)	P4 (self)	P5 (self)
Days to 50% Flowering	0.473	0	0.960	-2.488	-2.439
Plant height	3.500	-2.809	-29.325	17.872	7.270
Siliqua plant <sup>-1</sup>	-6.876	-7.130	-3.959	13.064	-0.733
Oil content	-3.060	3.755	-1.409	-9.446	-1.217
Protein content	2.326	3.266	8.375	4.533	5.480
Glucosinolate	5.735	17.118	25.534	-17.79	-11.28
Oleic acid	7.512	2.869	-0.667	7.206	-7.276
Linoleic acid	10.602	-43.08	-7.788	-43.13	-6.013
Erucic acid	-27.318	-8.522	8.939	-3.766	0.608

P1, P2, P3, P4 and P5 represent BKUC2, BKUC3, BKUC4, BKUC7 and BKUC8 self's, respectively. Values represent positive and negative inbreeding depression percentages of different parameters for five parents

Except siliqua plant<sup>-1</sup>, significant differences were seen for variables "a" and "b," indicating the significance of both types of genes for all the understudied features. Siliqua plant<sup>-1</sup> showed a relatively significant difference for SCA (85.54), suggesting the involvement of certain genes. The higher value of "a" compared to "b" indicated that an additive type of gene activity was more actively involved in the regulation of plant height, oil and protein content, glucosinolate, oleic acid, and erucic acid.

A variation in the levels of dominance components b1, b2, and b3 was observed for each attribute that was understudied. The difference in values between b3, b1 and b2, suggested that particular genes are involved in the expression of glucosinolate and plant height.

After a diallel cross analysis, highly significant differences were found for all genetic parameters (a GCA), b (SCA), b2, and b3 (dominant components), except b1 for oil content which showed relatively significance. The b1 and b3 components were less than the b2, indicating that some genes are more active when the erucic acid parameter, oil content, protein content, and siliqua plant<sup>-1</sup> are expressed (Table 4).

**Inbreeding depression:** Regarding Brassica, we find that certain characteristics—such as days to 50% flowering, which confirm early maturity—benefit greatly from inbreeding depression, whereas erucic acid, which improves oil quality, is the most undesirable. The inbreeding values for P4 and P3 (Self's) varied from -2.48 to 0.96 for days to 50 % flowering (Table 5). Self-P4 had the highest positive

inbreeding value (17.87) for plant height. For siliqua plant<sup>-1</sup>, positive inbreeding is preferred. P4 (self) demonstrated a positive inbreeding (13.06) for siliqua plant<sup>-1</sup> (Table 5). For oil content, negative inbreeding is undesirable. Positive inbreeding was seen in our study for P2 (self) for oil content (3.75%). (see Table 5)

Practically all oil-producing crops have a sufficient protein content, a positive inbreeding depression is essential. All of the self's showed positive inbreeding during the investigation; however, self (P3) had the highest value of 8.37%, followed by P5 (5.48). For this trait, negative inbreeding is required to improve the quality of Brassica oil (GSL). For self P4, the greatest negative value of -17.79% was clearly seen (Table 5).

For oleic acid, the inbreeding depression varied from 7.51 to -7.27. P1 (self) gave a positive response (7.51), and P4 (7.20) gave a positive response (Table 4). For self-P1 in the linoleic acid case, a positive result (10.60) was recorded. In this experiment, a significant negative inbreeding for undesired erucic acid was observed, with self P1 showing a range of (-27.31). (see Table 5).

**Heterosis study:** For days to 50% flowering, breeders are usually more interested in negative heterosis. In this study, a maximum MPH (mid parent heterosis) (-14.86) was disclosed by BKUC2xBKUC3, while maximum BPH (best parent heterosis or heterobeltiosis) (-2.40) was shown by BKUC2xBKUC4 (Table 6). For plant height, best parent (15.00) heterosis values were evident for BKUC3xBKUC4. Positive heterosis favored mankind for siliqua plant<sup>-1</sup>

because it enhanced yield directly. Maximum best parent heterosis (6.18) was revealed for BKUC7xBKUC8. A combination of BKUC2xBKUC8 revealed the maximum BP heterosis for oil content (13.18%). For protein content, positive heterosis is essential because it enhances oil quality. The BKUC4xBKUC8 cross discloses BPH up to 4.83%. The presence of GSL content in *B. napus* oil can affect the oil's quality adversely. So, for GSL content breeders sift out negative heterosis. As evident from Table 6, the most negative BPH and MPH up to -21.05% and -21.14% were noted for BKUC2xBKUC7, respectively. The maximum value of heterosis for oleic acid (3.18) was reported for cross between BKUC3xBKUC8. The positive heterobeltiosis (68.75) for linoleic acid was confirmed for BKUC3xBKUC7. Negative heterosis for erucic acid is valuable to ensure the quality of oil. Negative heterobeltiosis (-19.43) was recorded for BKUC2xBKUC3 (Table 6).

**Heritability estimates and Gene action:** High  $h^2$ , was calculated for days to 50% flowering. Compared to narrow sense heritability (0.50), broad sense  $h^2$  (0.64) was higher. It represents the percentage of phenotypic variance in days that results from additive genes for 50% of the flowering trait. The variation between the two measurements implies that the attribute is also influenced by non-additive genetic

factors. The broad sense heritability was bigger than the narrow sense for these parameters, indicating that the non-additive gene action was dominant in oil content, protein content, GSL percent, oleic acid, linoleic acid, and erucic acid. This verified that non-additive gene action contributes to these characteristics (Table 7).

**Graphical representation for quantitative traits**

**Days to 50% flowering:** The presence of over-dominant gene action for a given character was revealed by the interception that happened on the  $W_r$  (covariance) axis below the point of origin on the regression line (Fig. 1).

The regression line indicates that P2 includes the majority of dominant genes and is closer to the origin. In contrast, P4 has more recessive genes and is the farthest distant from the original. Additionally, H1's value is higher than D's, indicating over dominant gene action (Table 8). The over dominant gene activity for the expression of days to flowering initiation in *B. juncea* was also reported by (Khan and Khan 2005). On the other hand (Chowdhury *et al.* 2004) revealed that partial dominant gene action plays a major role in *B. rapa*. Overdominance is a genetic condition where the heterozygote phenotype lies outside the phenotypical range of both homozygous parents.

**Table 6. Best parent heterosis (BPH (%)) and mid parent heterosis (MPH (%)) for days to 50% flowering, plant height, siliqua plant<sup>-1</sup>, oil content, protein content, GSL content, oleic acid, linoleic acid and erucic acid in 5 x 5 F2 diallel direct crosses of *B. napus* L.**

Crosses	Days to 50 % flowering		Plant height		Siliqua plant <sup>-1</sup>		Oil content		Protein content		GSL content		Oleic acid		Linoleic acid		Erucic acid	
	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH
BKUC2 BKUC3	-1.90	<b>-14.86</b>	-5.29	-2.96	-5.94	2.41	8.12	-4.56	-3.13	-0.86	-13.79	-11.03	-1.37	-3.21	36.90	13.52	-2.13	<b>-19.43</b>
BKUC2 BKUC4	<b>-2.40</b>	12.38	9.26	11.75	3.31	-16.54	-6.81	1.87	-2.79	0.92	-7.03	-4.81	0.46	-4.70	15.59	4.56	<b>-3.10</b>	14.03
BKUC2 BKUC7	0.49	-0.69	-1.97	4.71	1.08	3.33	11.18	-0.78	-3.77	0.26	<b>-21.05</b>	<b>-21.14</b>	-2.65	-5.11	12.18	-4.11	-1.94	-7.34
BKUC2 BKUC8	-1.46	0.71	10.34	11.93	-2.92	7.59	<b>13.18</b>	-3.27	0.12	3.76	2.42	-2.61	0.19	-3.71	<b>-3.70</b>	<b>-13.33</b>	-2.88	-3.10
BKUC3 BKUC4	2.88	-1.24	<b>15.21</b>	<b>15.00</b>	1.07	<b>-13.67</b>	-8.39	-1.80	<b>-6.72</b>	<b>-5.34</b>	2.13	1.29	3.06	-0.32	18.34	27.40	2.39	6.30
BKUC3 BKUC7	<b>10.44</b>	<b>21.64</b>	6.49	11.14	1.75	3.98	6.09	-1.61	-3.15	-1.34	<b>21.49</b>	17.44	<b>-28.44</b>	<b>-28.91</b>	<b>64.45</b>	<b>68.75</b>	<b>8.02</b>	<b>19.82</b>
BKUC3 BKUC8	-0.97	9.10	-6.08	-7.04	3.65	-2.50	-2.87	1.75	-5.98	-4.77	8.36	-0.43	<b>3.18</b>	1.08	18.51	27.04	-2.16	11.02
BKUC4 BKUC7	2.48	11.15	-3.24	1.15	-2.50	-7.16	<b>10.97</b>	-2.95	-0.37	0	-5.69	-8.06	-1.83	0.74	3.54	-1.69	0.73	2.33
BKUC4 BKUC8	-0.97	2.27	10.89	9.96	<b>-7.46</b>	-4.55	<b>10.76</b>	<b>-6.51</b>	<b>4.83</b>	<b>4.63</b>	11.39	3.26	1.88	<b>3.15</b>	35.18	34.55	-1.69	-2.94
BKUC7 BKUC8	0.00	-6.99	<b>-16.76</b>	<b>-12.27</b>	<b>6.18</b>	<b>8.03</b>	10.64	<b>4.61</b>	-4.66	-4.11	14.69	<b>20.19</b>	-10.49	-9.25	13.70	8.47	-0.98	-9.92

**Table 7. Heritability estimates and gene action for days to 50% flowering, siliqua plant<sup>-1</sup>, oil content, protein content, GSL content, oleic acid, linoleic acid and erucic acid.**

Parameters	$h^2B$	$h^2N$	Additive variance	Genotypic variance	Phenotypic variance
Days to 50% flowering	0.64	0.50	1.08	1.35	2.12
Siliqua plant <sup>-1</sup>	0.32	0.11	2.96	8.28	25.81
Oil content	0.94	0.36	0.63	1.63	1.74
Protein content	0.77	0.28	0.14	0.37	0.48
Glucosinolate	0.89	0.31	13.07	37.55	41.79
Oleic acid	0.99	0.29	2.48	8.53	8.55
Linoleic acid	0.99	0.08	0.09	1.15	1.15
Erucic acid	0.99	0.33	2.86	8.72	8.72

Table 8. Components of variance for different parameters.

Parameters	Days to flower initiation	Plant height	Siliqua plant <sup>-1</sup>	Oil content	Protein content	Glucosinolate	Oleic acid	Linoleic acid	Erucic acid
H <sub>1</sub> value	2.209	0	35.29	5.36	1.57	125.53	32.57	5.03	38.04
D value	1.07	0	0	1.03	0.65	13.08	4.28	0.97	14.28

**Plant height:** With additional dominant genes, P5's position on the regression line is close to the origin (Fig. 2). P4's extreme distance from the origin, however, suggests that P4 has more recessive genes related to plant height. Partial dominance is a situation in which a heterozygote shows a phenotype somewhere (but not exactly half-way) intermediate between the corresponding homozygote phenotypes. A partially dominant type of gene action was shown by the regression line, which intersected the  $W_r$  axis above the place of origin. Table 8 indicates that the environment had a higher impact on the plant height, as both H<sub>1</sub> and D (components of variance) values were zero. (Rameeh 2014) reported partial dominance in controlling plant height in rapeseed, while (Farshadfar *et al.* 2013) found over dominance type for plant height in *B. napus*.

**Siliqua plant<sup>-1</sup>:** The  $V_r/W_r$  (variance/covariance) graph showed over dominance for siliqua plant<sup>-1</sup>. With respect to the most prevalent genes, the parental lines P5 and P2 were close to the place of origin. P4, on the other hand, was farthest away from the source and had the greatest recessive genes. The dominant gene action type is revealed in Figure 3. Similarly, D's value was less than dominance variance (H<sub>1</sub>), supporting predominant gene action. (Chowdhury *et al.* 2004; Rameeh 2014) also reported over dominance gene activity for the aforementioned parameter in *B. rapa* and *B. napus*, respectively.

**Oil content:** Over dominant gene action governed the manifestation of the oil content parameter since the interception happened below the origin point (Fig. 4). (Ali *et al.*, 2014; Amiri *et al.*, 2009) both reported identical gene activity for the aforementioned characteristic in rapeseed, further supporting the increased value of H<sub>1</sub> (dominance variance) relative to D (additive variance) showing this occurrence. It was implied that P3 included the most dominant genes because P3 was closer to the origin on the regression line. However, P2 had the greatest recessive gene content since it was located farthest away from the origin on the regression line.

**Protein content:** According to Fig. 5, the P3 genotype, is located nearer the regression line's beginning, has the greatest number of dominant genes, whereas the P1 genotype, which is located further away, has the greatest number of recessive genes. The lower value of D in comparison to H<sub>1</sub> indicates that over dominant gene action was recorded for the current parameter. However, (Singh, 2007) revealed that *B. napus*'s protein content was partially regulated by a partial dominant gene action.

**GSL:** Significant genetic variation among parents for GSL content was shown by the scattering of parental array points on the graph along the regression line (Fig. 6). P5, which was closer to the origin, had more dominant genes, and P4, which was farther away, had more recessive genes, according to the graphical distribution of array points on the regression line. There may be partial dominant gene activity present because the regression line crossed the  $V_r$  axis and above the site of origin. The partial dominant gene action was likewise driven by the positive intercept of the covariance ( $W_r$ ) by variance ( $V_r$ ) regression line. The dominance type of genetic regulation for GSL content was further verified by the higher "H<sub>1</sub>" component than the "D" component. Similarly, (Singh, 2007) reported partial dominance gene action. However, over-dominance's function in controlling the GSL content of Indian mustard was also documented by Thakral *et al.*, (2000).

**Oleic acid:** A partial dominance type of gene action is indicated by the regression line's interception above the place of origin at the  $W_r$  axis (Fig. 7). P2 had more recessive genes than predominant genes since it was farther away from the regression line's origin, but P3, which was closer to the regression line's origin, had more predominant genes. The fact that H<sub>1</sub> is bigger than D indicates that predominant gene action plays a role in the production of the oleic acid trait in *B. napus*. Partial dominant gene action for the present parameter in *B. napus* was verified by (Farshadfar *et al.*, 2013).

The most prevalent genes were found in P1, which were located near the regression line's origin, according to a graphic analysis. With respect to the regression line's origin, parent 4 had the greatest number of recessive genes. Additionally, the greater value of H<sub>1</sub> relative to D in Fig. 8 indicates the partly dominant gene action. Partial dominance genes for the production of the linoleic acid character in *B. napus* were also reported by (Singh, 2007). However, (Thakral *et al.*, 2000) demonstrated that over dominance genes have a role in the expression of linoleic acid traits.

**Erucic acid:** The majority of pre-dominant genes are found in parental line 4, which are close to the regression line's beginning, as shown in Fig. 9. Recessive genes are found in P1, which is away from the regression line's origin. The negative intercept of  $W_r/V_r$  showed that over dominance was present. For the current parameter, a partial dominant type of gene action was demonstrated by the bigger value of H<sub>1</sub> than D, which was also validated over dominance (Ali *et al.*, 2014).

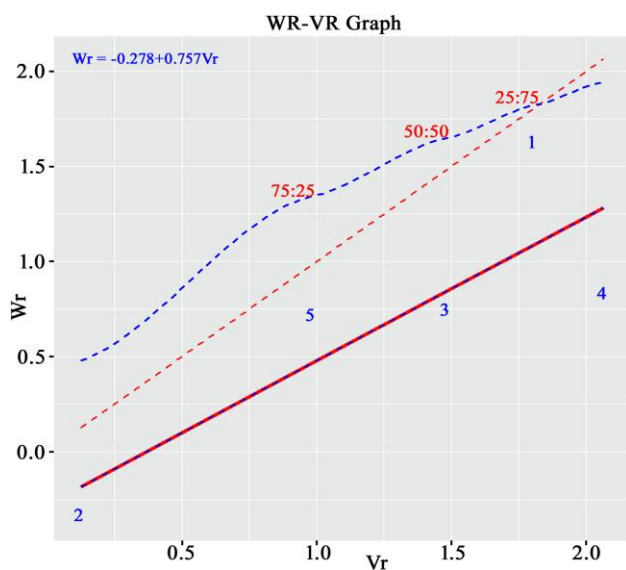


Fig. 1. Vr/ Wr graph for days to 50 % flowers initiation in F2 generation.

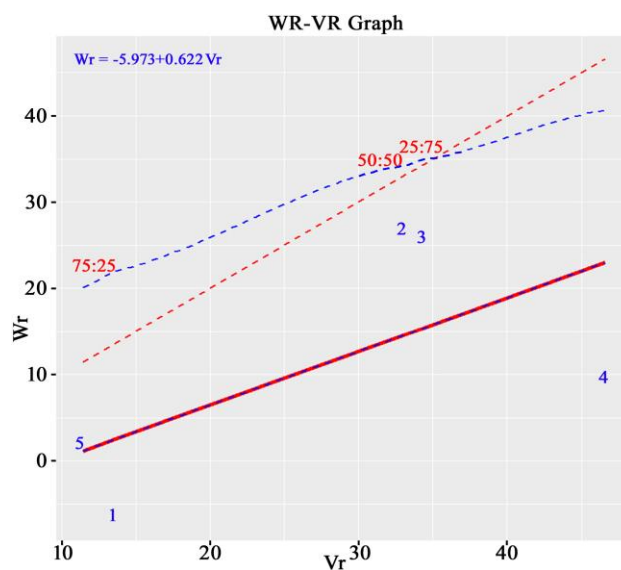


Fig. 2. Vr/ Wr graph for plant height in F2 generation.

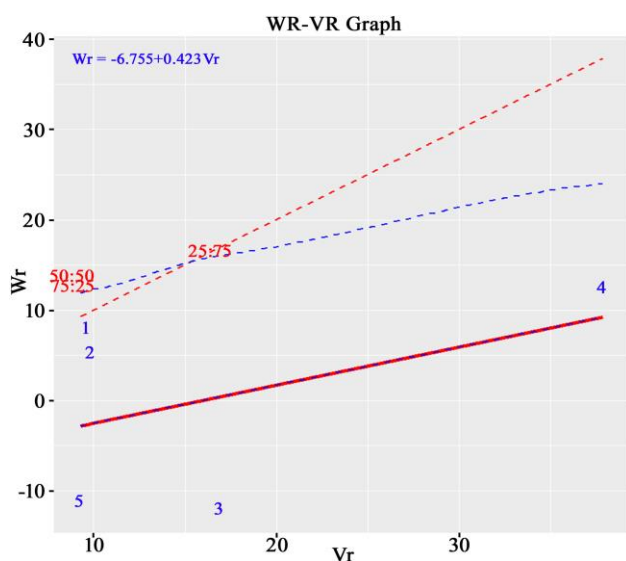


Fig. 3. Vr/ Wr graph for siliqua plant<sup>-1</sup> in F2 generation.

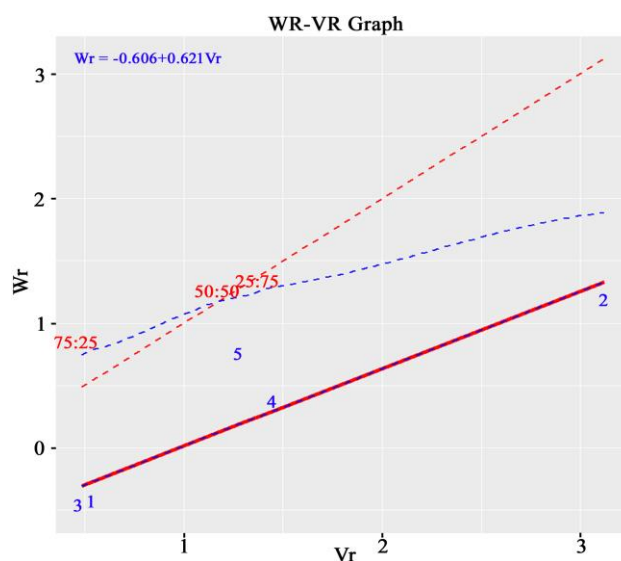


Fig. 4. Vr/ Wr graph for oil content in F2 generation.

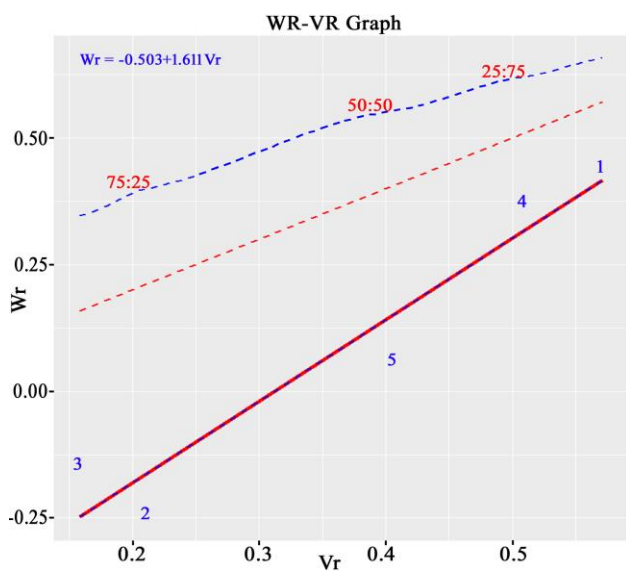


Fig. 5. Vr/ Wr graph for protein content in F2 generation.

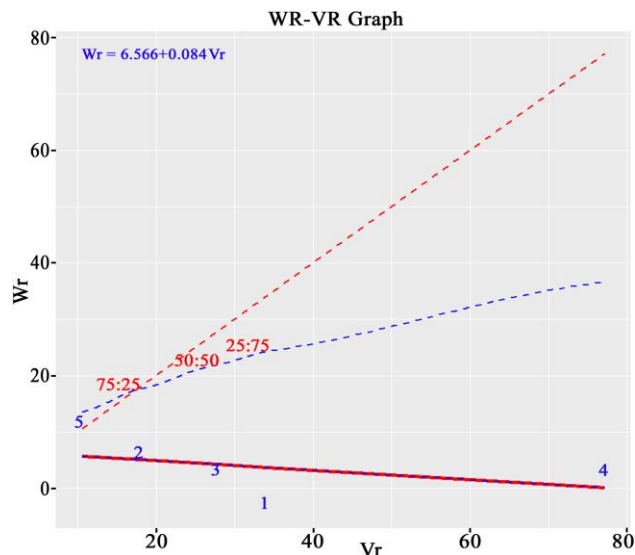


Fig. 6. Vr/ Wr graph for GSL content in F2 generation.

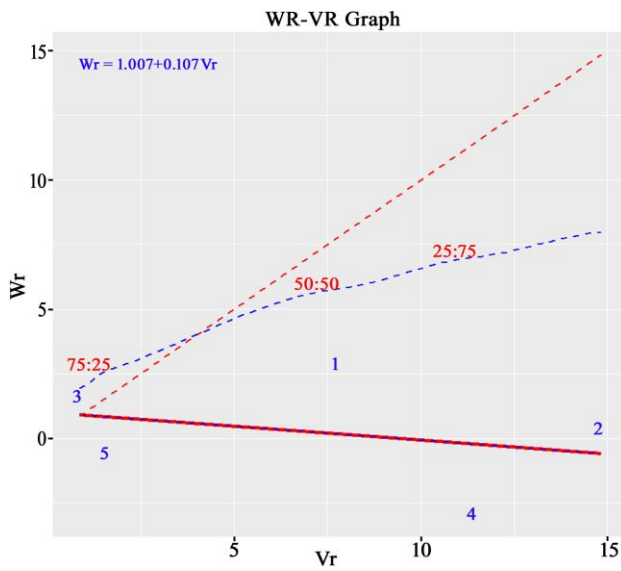


Fig. 7. Vr/ Wr graph for oleic acid in F2 generation.

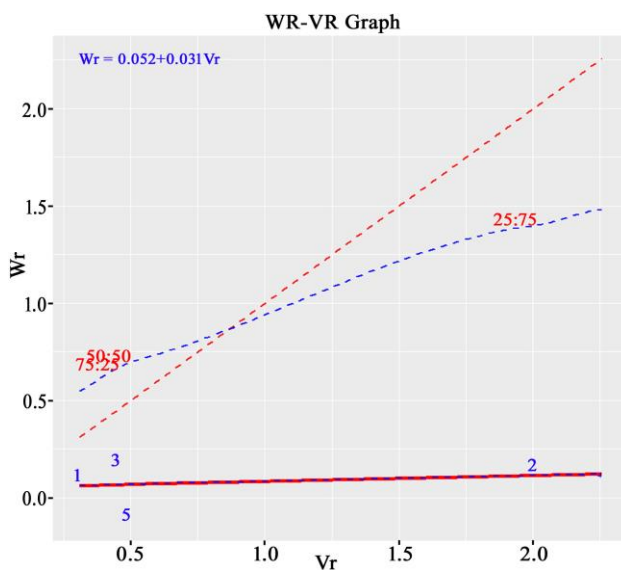


Fig. 8. Vr/ Wr graph for linoleic acid in F2 generation.

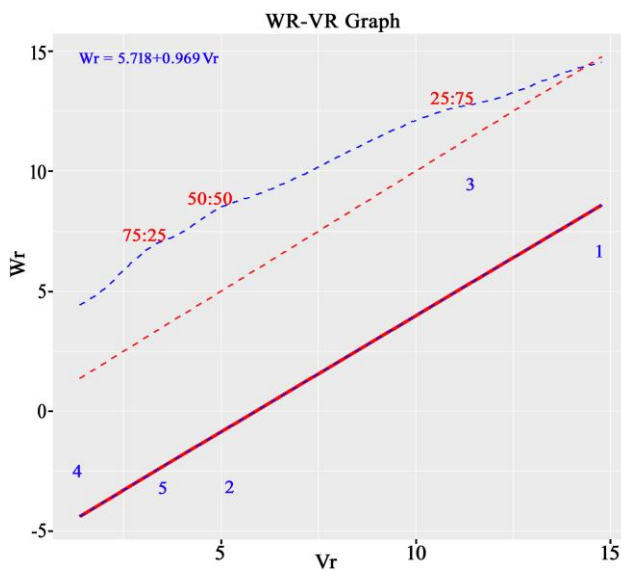


Fig. 9. Vr/ Wr graph for erucic acid in F2 generation.

## Discussion

**Combining ability:** A widely used approach for examining the kind and extent of genetic variation in early generations is Hayman's (1954) additive dominance model. The total of squares is split into two parts in this method: "a" (additive) and "b" (dominance). Three more divisions exist inside the "b" component: b1, b2, and b3. The relevance of genes b1, b2, and b3 demonstrates, in turn, directional dominance, asymmetrical gene distribution, and distinct gene effects. If the (b) dominance component is determined to be significant, the graphic analysis is considered valid.

To ascertain the contribution of additive and non-additive types of gene activity to the transmission of particular traits, the variance attributable to general and specific combining capacity was assessed. For the majority of the features, the very significant differences were shown by the GCA and SCA variances. A higher GCA value compared to SCA indicated that prolonging days to 50% blooming is more advantageous in terms of cumulative effects. The present results for the previously indicated characteristics align with those of Iqbal *et al.*, (2003). The significance of additive gene action for these variations was demonstrated by the considerable GCA observed in plant height. Our study's findings don't line up with those of (Rao & Gulati, 2001; Yadav *et al.*, 2009). In contrast to our results (Ali *et al.*, 2010) discovered a dominant form of gene activity for plant height. According to earlier research on *B. juncea*, populations of the plant exhibited a highly significant non-additive gene action for siliqua plant<sup>1</sup> (Shah *et al.*, 2021). Similar conclusions about the partial dominance of genes were reported by Ali *et al.*, (2010), however, Anantharaju & Muthiah (2008) emphasized the significance of additive genes for siliqua plant<sup>1</sup>.

The parents' effects on F1 hybrids increased with their level of combining ability. Parental influences on their F1s were greater for the traits, primarily controlled by additive gene actions than for the traits controlled by both additive and non-additive gene actions. This indicates that the attributes primarily controlled by additive gene actions are more closely associated with the total GCAs of female and male parents. Thus, when breeding hybrids, the combined GCA of the male and female parents should be taken into account rather than the individual GCAs of the parents, especially for features that are not greatly impacted or controlled by non-additive gene activity (Shen *et al.*, 2005). The amount of seed oil in rape seed has a big impact on its economic value. Parents and hybrids with positive GCA and SCA effects are highly sought after in rapeseed breeding. Additives were discovered to have an impact on *B. napus*'s oil content (Cheema and Sadaqat 2004). The main determinants of oil content, according to Wang *et al.*, (2010) were dominant and additive effects, with the dominant effect being the most important component. According to both studies (Variath *et al.*, 2009; Meena *et al.*, 2014) the main factor influencing oil content was the cytoplasmic genetic influence. There were also notable maternal impacts on the protein content. There were also substantial maternal impacts on the protein content (Nassimi *et al.*, 2006; Abideen *et al.*, 2013) reported that maternal impact was a significant factor in the protein content of F1 hybrid seeds of rape seed (*B. napus* L.). GSL content has been linked to



hemorrhagic liver syndrome and goiter in animals and is thought to be an anti-nutritional component (Salunke *et al.*, 1992). Our results conflict with that of Nasim *et al.*, (2014) who reported non-additive genetic action in *B. rapa*. GSL and oleic acid are the two main components for which the predominance of additive gene activity was previously documented by Rameeh *et al.*, (2018), Vaghela *et al.*, (2011) and Adnan (2013). Selection may be more successful in later segregating generations as a result of the trait being regulated by non-additive gene action, as indicated by the variance caused by GCA and SCA. The predominant gene action for linoleic acid was non-additive, as shown by a comparatively bigger SCA value than GCA value. This proved that cytoplasmic effects were mostly responsible for controlling the trait (Adnan, 2013). Our results refute the claim stated by Gupta *et al.*, (2010) that linoleic acid had additive genetic effects. The higher value of GCA mean squares when compared to SCA mean squares demonstrated the participation of additive gene effects. The study's findings are corroborated by earlier studies conducted by Turi *et al.*, (2010) and Shen *et al.*, (2005).

**Inbreeding depression:** An estimate of the inbreeding depression in F<sub>2</sub>s over the corresponding parental lines, was calculated in terms of values. Positive numbers showed the least amount of inbreeding depression, whereas negative values indicated the greatest. Such self-showing lines may be used in future selection processes because they do not show inbreeding depression and because they demonstrate additive and additive x additive gene interactions. Our results were not in agreement with those of (Singh *et al.*, 2012) who reported negative inbreeding depression for days up to 50% blooming. Cuthbert *et al.*, (2011) and Nasim *et al.*, (2013) reported increased days to bloom start due to inbreeding depression. Early flowering allows seeds enough time to mature and may even improve yield. Like Brassica, where shorter plants and early maturity are desirable to minimize lodging and insect losses, respectively, and eventually to increase seed output (Sincik *et al.*, 2011). Because taller plants (grown for seed yield) in Brassica are more likely to lodge, medium- or short-statured plants are recommended. Additionally, negative inbreeding depression in plant height was found by (Gumber *et al.*, 2006). Since most parental lines displayed negative inbreeding depression whereas, Brandle, (1989) wanted positive inbreeding for siliqua plant<sup>-1</sup>, in contrast to a previous study.

**Heterosis:** Numerous investigations have demonstrated that non-additive effects, such as dominating and epistatic effects are the primary causes of heterosis (Shen *et al.*, 2005). This study showed that both additive and non-additive gene influences were responsible for controlling yield traits. This could be the reason why there was more heterosis for yield than for other qualities. Positive heterosis was noted until the flowering stage. Positive heterosis favors mankind for siliqua plant<sup>-1</sup> since it boosts yield directly. According to earlier research, F<sub>1</sub> hybrids developed through crossings between several types have low heterosis for rapeseed seed oil content and high heterosis for yield-related characteristics (Brandle & McVetty, 1989). Significant levels of heterosis for qualitative features have been seen in rapeseed F<sub>2</sub> hybrids, which is consistent with our findings (Qian *et al.*,

2007; Tian *et al.*, 2017). Hybrid breeding may be one of the improvement techniques under these circumstances. For a successful heterosis breeding program, there must be evidence of the presence of significant heterotic effects in the hybrids which could be utilized for commercial hybrid seed production. The qualitative nature of rapeseed is directly influenced by different characteristics, including oil content, protein content, GSL, oleic acid, and linoleic acid (Ali *et al.*, 2015). In order to develop rapeseed hybrids, favorable heterotic effects are crucial. Variable positive and negative MP and BP heterotic effects were seen in the hybrids for the contributing characteristics. For example, varying amounts of heterosis have been found by several researchers for rapeseed seed quality attributes (Brandle & McVetty, 1989). Contrarily, (Rameeh, 2011; Rameeh, 2012) has also observed a somewhat lower extent of heterosis than the values obtained in our study.

**Heritability estimates:** These main goal of any breeding and bioengineering program is to increase crop output. To derive meaningful conclusions from a given collection of observations, the measurement and evaluation of variability are crucial processes (Marwede *et al.*, 2004). The genetic composition of the breeding materials under study affects the heritability estimates for many traits. Understanding the heritability estimates of the traits is of great interest to the breeders. High heritability estimates suggest that selection for quality traits will be more advantageous because environmental factors will have less impact over it. It has been discovered that heritability estimates help demonstrate the relative importance of selection based on the phenotypic expression of various traits. The presence of h<sup>2</sup>B was also advised by (Singh, 2007) for oil content, while Mahmood *et al.*, (2003) noted that h<sup>2</sup>N played a major impact on this aspect of *B. napus*'s behavior. Rameeh *et al.*, (2018) claimed that non-additive gene action played a significant role in the expression of the GSL phenotype in *Brassica*. Oleic acid levels in *Brassica* were reported to be high (Khan *et al.*, 2008). Similar results were obtained for linoleic acid and erucic acid and discovered the involvement of h<sup>2</sup>B in inheriting the characters (Iqbal *et al.*, 2014).

In nutshell, our study helping us to determine the best genotypes based on combining ability and heterosis. This will help us to make further advancements in the departmental breeding program, ultimately leading us to variety development and registration.

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