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Original Research Article

Heritability and Heterosis Manifestation of *Brassica Rapa* Accessions for Fatty Acids-Related Traits

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Abstract: The emergence of heterosis is a crucial marker of hybrid potential in rapeseed breeding for both hybrid and open-pollinated cultivars. However, testing the combining ability of the parents in the field consumes a great deal of manpower. An efficient way to evaluate heterosis and heritability is to use a line×tester analysis. An experiment was performed in 2022 in the research area of department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Line×tester analysis was performed with ten lines and three testers for the development of thirty F_1 crosses. It was used to estimate heritability and heterosis of various fatty acids related traits as: oil contents, protein contents, glucosinolate contents, and erucic acid content. For this, selected better performer lines (high yield) and poor performer (low-yield) testers from ongoing research were crossed and the data were recorded for different fatty acids-related traits. The recorded data were subjected to Analysis of Variance followed by estimation of heterosis and narrow sense and broad sense heritability. Using three alternative forms of heterosis estimates—heterosis over mid parent, heterosis over better parent, and heterosis over standard check—the thirty F1 hybrids that developed were assessed, along with one check variety (Chakwal-second). Crosses 40980×26240 (for oil), 40979×26240 (for glucosinolate contents), 40978×26240 (for erucic acid contents) had positive and significant heterosis over the commercial hybrids for the studied traits. High narrow sense heritability was observed for erucic acid contents followed by protein contents and glucosinolate contents. The hybrid variety in rapeseed might be developed using the crossings 40978×26240, 40979×26240, and 40980×26240.

Keywords: Mating design, Near Infrared Reflectance Spectroscopy, significant, probability level, genetic gain.

INTRODUCTION

The majority of people in Pakistan are dependent on agriculture since the country is an agricultural one. Several significant crops have been produced and improved in this sector, including wheat, cotton, sugarcane, rice, and maize (Ahsan *et al.*, 2013). Oilseed crops are a vital part of Pakistan's agriculture, and can be grown throughout the year in at least one season. Despite being a significant component of our country's trade and economy, oilseed crops are categorised as "Miner/Marginal crops". The lack of attention paid to oilseed crops and the agronomic sector has caused this status and approach. It is imperative that oilseed crops be improved during the current crisis. The total rapeseed production; seed, oil, and meal have also increased to 6%, 2.5%, and 5%, respectively compared to last year. Since a significant amount is spent on its importation each year, 2.754 million tonnes of edible oil worth Rs 662.657 billion were imported in 2021–2022. The preliminary estimate for the amount of edible oil produced locally during this time is 0.460 million tonnes. An estimated 3.214 million tonnes of edible oil are available annually. Among oilseed crops, cottonseed contributes 54% followed by, rapeseed & mustard 26%, sunflower 10% and canola 6.5%. There is a huge gap between consumption and production of rapeseed. Rapeseed & mustard was cultivated on 0.68 mha that is increased from 0.60 mha from previous year (Govt. of Pakistan, 2021-22). In order to increase the yield of oilseed crops, hybrid seed production is the primary method. It has been proven that hybrid rapeseed tend to produce a higher yield compared to conventional rapeseed, around the world, hybrid rapeseed become

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more popular day by day, and about 60% of crossover crops are grown in China (Raboanatahiry *et al*., 2021). Broader genetic background is often thought to increase heterosis (Seyis *et al*., 2006). The achievement of any plant breeding programme depends on the selection of appropriate parental genotypes. Understanding the various forms of gene action, their proportional contributions to genetic variance, and the calculation of heterosis (mid parent, better parent, and commercial) are essential components of improving the rapeseed crop. In order to further improve the quality of rapeseed, breeders could use this information to select better parental combinations (Sher Aslam Khan *et al*., 2009; Panhwar *et al.*, 2008). Mating designs such as the line×tester design have been used extensively for genetic analysis and estimation of heterosis (Kempthorne, 1957 and Rameeh, 2010). Heterosis; is the result of mating two parents with diverse genetic backgrounds while in comparison to the parents, the hybrid generation frequently exhibits greater vigour, increased disease resistance, improved adaptability to adverse settings, and higher yield (Afrose *et al*., 2019). This study develops *Brassica rapa* F_1 hybrids with different genetic backgrounds using three male testers and ten female lines. The combinations were studied for potential in various plant characters. The degree of heterosis over the mid parent, better parent, and standard check variety and heredity, as well as the narrow or broad sense, were then estimated by evaluating thirty hybrids (F_1) and their parents. The main objectives of the study are to; i. Evaluate lines, testers and their crosses ii. To determine the type of heritability responsible for fatty acids related traits iii. Identification of the best potential cross combinations for more oil content.

MATERIALS AND METHODS

Germplasm consisting of 43 accessions (13 parents and 30 F_1 crosses) of *Brassica rapa* and one check cultivar was collected from different national and international research centres. During the cropping seasons of 2020–21 and 2021–22, the current experiment was conducted in the research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. This location is situated at an altitude of 184 metres, longitude of 73.8˚ East, and latitude of 31.43˚ North. The material was collected from ongoing research (2020-21) for the hybridization of *Brassica rapa* accessions for the improvement of fatty acids. The experiment included 13 parents and 30 F_1 crosses of *Brassica rapa* genotypes, including one check cultivar for improved fatty acid and good yield potential.

Hybridization: The ten selected lines and three testers were hybridized using line×tester mating design. A hybridization experiment with three replications was carried out in the Plant Breeding and Genetics research department at the University of Agriculture Faisalabad.

Data Recording: Ten plants of each accessions per replication were tagged and data were recorded on various parameters.

Oil content (%): Five gram seeds of tagged sample were weighed by electronic balance (Setra, BL-410S) and separated. Then all weighed seeds were analyzed for for oil content through near infrared reflectance spectroscopy.

Protein content (%): Two gram seeds of tagged each sample were weighed by electronic balance (Setra, BL-410S) and separated. Then all weighed seeds were analyzed for protein content through Near Infrared Reflectance Spectroscopy.

Statistical Analysis: In order to predict the hybrid performance of the crosses with ten lines and three testers, Kempthorne's (1957) Line×tester method was used to estimate the Analysis of Variance for hybrid (crosses), line, tester, line× tester, heritability, and other components.

RESULTS

Evaluation of breeding material

Genetic Variability

Analysis of variance of F_1 hybrids of *Brassica rapa* for fatty acid related traits:

All the fatty acid related traits under study had showed highly significant results. Parents showed highly significant variation for all the fatty acids related traits. In terms of oil content, protein content, and erucic acid content, parents vs crosses were significant. Glucosinolate contents showed non-significant differences among parents vs crosses that might be due to the variation of germplasm or environment. For all traits related to fatty acids, F_1 crosses showed highly significant differences. For all characteristics associated with fatty acids, there were highly significant variations in lines and testers. Among line× tester, all the traits showed significant differences. Non-significant differences might be due to different germplasm and environment (Table 1).

Table 1: Mean performance of *Brassica rapa* accessions for yield and fatty acid related traits

SOV	OP	PP	Glu	EA
Replications	54.59**	-40.13**	-228.28**	-84.41**
Accessions	22.04**	24.73**	-319.89**	-13.46**
Parents	11.10**	61.21**	-142.79**	-16.62**
Parents vs crosses	158.25**	183.58**	0.25	-73.69**
Crosses	21.87**	4.15*	404.20**	-10.07**
Lines	10.45**	1.84^{NS}	82.95**	-13.52**

SOV	OP	PP	Glu	EA
Testers	74.15**	17.59**	3790.51**	15.04**
LinesxTesters	21.77**	3.81*	188.56**	-7.80**
Error	14.14	4.62	257.58	13.82
Total	2222.24	1506.77	35528.45	1894.52

**= Highly Significant at 0.01 level of probability, OC= Oil content, PC= Protein content, Glu= Glucosinolate content and EAC= Erucic acid content

Mean comparisons of fatty acid related traits in *Brassica rapa* accessions

Accessions Range	OC (%)	PC (%)	Glu. C (%)	EAC (%)
Accessions	**40981**	**26241**	**26287**	**26262**
Maximum value	51.3	33.9	97.3	50.3
Accessions	**28206**	**26487**	**40982**	**28212**
Minimum value	37	19.9	9.4	37

OC= Oil content, PC, Protein content, Glu= Glucosinolate content and EAC= Erucic acid content

Mean comparisons of fatty acid related traits in *Brassica rapa* accessions

Mean performance of accessions and check cultivar for oil content was observed from 32.6% to 51.3% respectively. Accession 40981 showed maximum oil contents 51.3% while 28206 showed minimum oil content (37%). Accession 26241 had maximum protein content (33.9%) while 26487 showed minimum protein content (19.9%) followed by 25.8% of check cultivar. Glucosinolate contents decreased as compared to check cultivar range from 9.4% to 97.3%, respectively. Maximum glucosinolate contents were observed in 26287 (97.3%) while minimum in 40982 (9.4%). Mean performance of erucic acid content ranged from 37% to 50.3%, respectively. Accession 26262 had maximum erucic acid percentage (50.3%) while 28212 showed minimum erucic acid percentage (37%) followed by 43.2% of check cultivar.

Heterosis manifestation for fatty acids related traits in rapeseed crosses

Oil contents: Table 3 displays the heterosis manifestation for the oil %, which showed varying degrees of magnitude and direction. In 40978×26240 and 40980×26283, positive and highly substantial heterosis over the midparent was found. Cross 40982×26283 had positive and significant mid parent heterosis. While crosses 40975×28215 showed significant and positive mid parent and better parent heterosis.

Protein contents: Table 4 displays the heterosis manifestation for protein contents, which varies in magnitude and direction. In 40977×26283, 40978×26283, and 40980×26283, there was a negative and highly substantial heterosis over the mid parent. Cross 40977×28215 had negative and significant mid parent heterosis. Conversely, crosses 40975×28215 exhibited significant, negative midparent heterosis and better parent heterosis.

Glucosinolate contents: Table 4 displays the heterosis manifestation for the glucosinolate contents. showed varying degrees of magnitude and direction. In 40977×26283, 40979×26283, 40980×26283, 40981×26283, and 40975×28215, negative and highly significant heterosis across the midparent was observed. Cross 40977×28215 had negative and significant mid parent heterosis. A considerable and negative mid parent and better parent heterosis was observed in crosses 40978×28215.

Erucic acid contents: Table 4 displays the heterosis manifestation for erucic acid contents, with varied magnitude and direction indicated. In 40978×26240, 40979×26240, and 40981×26240, there was a negative and highly substantial heterosis over the mid parent. Cross 40977×26283 had negative and significant mid parent heterosis. However, crosses 40979×26283 exhibited better parent and significant negative midparent heterosis.

Heritability: Table 2 lists the broad and narrow sense heritabilities for *Brassica rapa* features associated to fatty acids. The broad sense heritability ranged from 97.40% to -7.76%. The range of narrow sense heritability was 14.00% to 0.34%. High broad sense heritability was observed for erucic acid contents and oil contents under field condition. High narrow sense heritability was observed for erucic acid contents, followed by protein contents and glucosinolate contents.

Table 2: Broad and narrow sense heritability for fatty acid related traits in *Brassica rapa*

Character	Coefficients of variability	Narrow sense heritability (%)	Broad Sense heritability (%)
OC	0.16	1.61	55.57
PC	-0.22	5.16	0.240
Glu. C	0.52	2.44	-44.26
EAC	2.51	8.25	55.97

OC= Oil content, PC= Protein content, Glu= Glucosinolate content and EAC= Erucic acid content

DISCUSSION

Brassica rapa is an important vegetable species. Vegetable oil quality is mostly determined by its fatty acid composition. Vegetable oils with high oleic acid content and low polyunsaturated fatty acid content are valuable resources due to their widespread usage in industrial processes, meals for humans and animals, and other applications. The quality components of forty three entries with various genetic backgrounds were identified in the current study. Developing a low glucosinolate or zero erucic acid variety was one of the main goals of the quality improvement programme. Guo (2015) found significant differences among accessions for all the fatty acid related traits under field conditions Channaoui *et al*., (2017). Tossi *et al*., (2014) found significant differences among genotypes,treatments and their interaction for oil and protein contents. Genetic variability in rapeseed entries for fatty acid related traits has also been reported by Hyder *et al*., (2021a); Rout *et al*., (2018); Hyder *et al*., (2021b); Singh *et al*., (2016); Sohan and Nutan, (2010); Chaurasia and Ram, (2015); Gupta *et al*., (2015) and Sutariya *et al*., (2016). In particular, the increase in oleic acid content within the fatty acid composition of rapeseed oil holds potential as a source of raw materials for biodiesel production. Mean values of fatty acid related traits are considered suitable for the selection of the accessions under the field conditions (Junaid *et al*., 2014).

Since fatty acid composition, both good and bad, play a significant role in human nutrition as health-promoting factors, it would be beneficial to develop new types with higher nutritional content Cartea *et al*., (2010). Our results of heterosis for oil contents were contradictory to Kishor *et al*., (2006), Shen *et al*., (2005), Sood *et al*., (2000) Katiyar *et al*., (2004), who reported standard heterosis 43.38% and better parent heterosis 150.33% for qualitative traits. In addition, results were similiar to Krzymanski *et al*., (1997) who found significant heterosis for oil content and some flowering traits. Fray *et al*., (1997) also reported significant estimates of heterosis for quality related traits. However, Liu (1996) reported heterosis for more oil contents with low protein meal. Similarly, Hu *et al*., (1996) reported significant negative effects of heterosis for protein content. The results of this experiment were in line with previous findings (Cuthbert *et al*., 2011, Akabari *et al*., 2017, Dar *et al*., 2016). Significant heterosis was discovered by Krzymanski *et al*., (1997) for several flowering characteristics, oil content, and seed production. Significant estimations of heterosis for anti-quality components in oil were also reported by Fray *et al*., (1997).

However, Liu (1996) reported heterosis for more branches with greater plant height and lower erucic acid content. Similarly, Hu *et al*., (1996) reported significant positive effects of heterosis for plant height and seed yield/plant. The differences in the results could be due to the differences in genotypes and weather conditions. The results of this experiment were in line with previous findings (McVetty *et al*., 2002 and McVetty *et al*., 2007).

The results were in line with the findings of Sadat *et al*. (2010); Marjanovic *et al*., (2011); Naazar *et al*., (2003); who observed moderate heritability and higher genetic advance for erucic acid contents. Lower broad sense heritability values and lower expected response to selection for protein contents were found by Mahmood *et al*., (2003); Rameeh, (2017); Wang *et al*., (2010) and Evans *et al*., (2018). This suggested that selection can be effective for these traits based on phenotypic expression.

Table 3: Estimation of heterosis over mid parents, better parents and standard check for oil percentage, Protein content, Glucosinolate content and Erucic Acid content in *Brassica rapa*

Sr. No.	Entries	Oil content			Protein content			Glucosinolate content			Erucic Acid content		
	F_1 Hybrids	MPH BPH		CH[1]	MPH BPH		CH[1]	MPH BPH		CH[1]	MPH BPH		CH[1]
1	28244×26240	-10.10	-12.54	-42.2	20.53	16.30	-50.7	4.00	-0.76	2716.44	5.02	2.25	-17.02
2	40961×26240	-11.05	-13.81	-42.5	31.41**	26.33	-46.5	-7.23	-8.23	2459.47	2.38	-3.48**	-26.84
3	40970×26240	-14.92	-16.36	-45.9	42.11**	37.56**	-41.7	-13.24	-14.77	2935.24	1.88**	-0.39	-10.23
4	40975×26240	-14.76	-15.04	-46.57	-2.19	-4.14	-59.41	-4.72	-9.90	2268.5	0.32	-2.91	-57.57
5	40977×26240	-8.00	-8.76	-42.9	-3.18	-6.28	-60.3	-17.62	-21.12	2513.80	5.44**	4.66**	-93.61
6	40978×26240	2.32**	1.06**	-36.8	2.55**	0.82**	-55.8	-14.48	-15.96	3258.2	-6.56**	0.36**	80.11
7	40979×26240	-14.94	-15.15	-46.7	-5.77	-10.27	-62.0	8.90**	6.13**	2205.4	-2.20**	-5.48**	-83.59
8	40980×26240	1.96**	0.16**	-34.9	0.18**	-0.48	-57.8	-22.81	-23.23	2961.6	7.77**	4.45**	-16.10
9	40981×26240	-19.51	-20.03	-49.3	-6.62	-11.47	-62.5	**	1.71**	2842.73	-5.49**	-7.98**	-63.34
10	40982×26240	-6.68	-9.66	-43.5	5.56**	0.97**	-57.2	12.45**	-2.00	3170.1	-7.05	-9.91	-86.65
11	28244×26283	-10.80	-11.74	-40.4	16.10**	13.86**	-53.3	6.76**	-1.07	2380.1	-10.11	-11.14	-46.83
12	40961×26283	-15.90	-16.44	-43.6	4.66**	2.25**	-58.12	-15.78	-19.19	2195.1	-1.04	-3.19	-68.25
13	40970×26283	-13.81	-15.61	-43.0	15.48**	13.61**	-53.47	-19.72	-20.78	2976.3	-5.73	-7.20	-73.77
14	40975×26283	-18.04	-20.83	-46.5	-10.28	-10.61	-63.3	-0.59	-8.67	2485.9	-2.51	-3.03	-42.08
15	40977×26283	-14.35**	-18.18**	-44.78	-11.68**	-13.11**	-64.4	-7.12**	-8.33	2914.6	-0.71**	-5.08**	-28.73
16	40978×26283	-13.93**	-18.11**	-44.7	-4.25**	-7.38**	-59.4	1.66	-3.07	2851.54	4.77	2.39	-65.26

17	40979×26283	-2.75**	-6.14**	-36.6	4.39	1.00	-58.6	-1.37**	-6.73**	2146.6	-2.68**	-9.32**	-12.58
18	40980×26283	4.66**	2.88**	-30.5	-0.62**	-1.59	-58.8	-22.41**	-24.37**	2446.2	8.16	7.42	-43.97
19	40981×26283	-1.25**	-4.32**	-35.4	2.79	-1.00**	-59.4	-12.67**	-15.41**	2458.0	6.36	5.15	-34.30
20	40982×26283	8.60**	14.70**	-42.43	6.62	3.62	-57.5	1.27	-9.32	2430.10	2.67	1.97	-60.42
21	28244×28215	-6.21	-6.50	-38.2	-2.25	-3.30	-61.0	-24.06	-24.66	3112.9	11.58**	11.32**	-42.13
22	40961×28215	-13.03	-13.66	-42.4	5.87**	4.32**	-58.0	-0.02	-4.33	2541.70	-2.70	-6.09	-31.54
23	40970×28215	-22.95	-23.52	-49.7	-0.13	-0.89**	-60.1	-15.54	-21.34	2989.57	6.05	5.84	-18.04
24	40975×28215	28.32**	29.83**	-53.9	-8.72**	-9.18**	-63.0	-8.14**	-8.28	2914.68	-17.53**	-18.22**	-64.21
25	40977×28215	-10.54**	-13.40**	-43.1	-24.46**	-25.03**	-69.83	-1.25**	-10.23**	2753.1	4.50	1.25	-80.01
26	40978×28215	-15.27**	-18.30**	-46.3	43.80	37.92	-39.5	-11.78**	-15.04**	3013.06	-13.69**	-16.78**	-4.75
27	40979×28215	-15.13**	-16.98	-45.5	-25.26**	-27.06**	-70.6	-4.55**	-7.30	2861.82	-3.22**	-8.64	-84.67
28	40980×28215	-24.66	-24.92	-50.7	6.73	4.77	-56.2	-6.40	-11.81	2089.48	-25.44	-25.96	-50
29	40981×28215	-40.84	-41.90	-61.8	-12.23	-14.74	-65.6	-31.24	-34.80	2312.64	-42.04	-42.17	-75.77
30	40982×28215	-47.78	-50.62	-67.5	-27.54	-28.97	-71.4	-13.67	-28.16	2716.4	-50.97	-51.31	-76.74

*=Significant at 5% level of probability, **= Significant at 1% level of probability, **MPH**= Heterosis (Mid Parent), **BPH** = Heterobeltiosis (Better Parent), **CH**= Commercial heterosis over Check1

CONCLUSIONS

Thirty hybrids were evaluated for fatty acid-related characteristics using estimates of three different types of heterosis: heterosis over mid parent (MPH), heterosis over better parent (BPH), and heterosis over standard check (CH); Chakwal-sarson was used as the check. When comparing the better parent and commercial hybrids for characteristics related to fatty acids, there was observed heterosis. Crosses 40980×26240 (for oil), 40979×26240 (for glucosinolate contents), 40978×26240 (for erucic acid contents) had positive and significant heterosis over the commercial hybrids under study. While F_1 hybrids 40979×26240 (for glucosinolate contents), 40978×26240 (for erucic acid contents) had negative and significant heterosis over the commercial hybrids under study. The crosses 40980×26240, 40979×26240 and 40978×26240 could be used for the development of hybrid in rapeseed. High narrow sense heritability was observed for erucic acid contents followed by protein contents and glucosinolate contents. The present study was an attempt to provide a better understanding to breeders about the traits that directly or indirectly influences oil quality and to suggest fatty acids related traits suitable for rapeseed cultivation, different choices of traits, parents and crosses to be selected, in order to maximize the genetic gains through selection for rapeseed breeding.

Conflict of Interest: The Authors declare that there is no conflict of interest.

Authors' Contribution Statements: SEM executed the field research, data analyses, whereas HR and FAK and SHK conceived the idea and supervised the work.

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