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Research Article

Genetic Evaluation among Brassica Advanced Breeding Lines for Qualitative Traits

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ABSTRACT

This research was conducted to assess genetic evaluation between *Brassica* advanced lines for qualitative traits. Twenty-two Brassica genotypes (sixteen advanced lines and six checks) were evaluated in randomized complete block design with three replications at The University of Agriculture, Peshawar during 2021-22 crop growing season. Significant ($p \leq 0.01$) differences among overall genotypes, advance lines, checks and advance lines vs. checks were observed for all studied traits except pod width and protein content. The genotype G06 showed highest value of 48% for oil content, while highest value for protein content of 24% for G20. The Genotype G13 and G19 showed lowest value for glucosinolate content and erucic acid content (47 and 23), respectively. High heritability estimates were observed for erucic acid (0.85), glucosinolate content (0.78), oleic acid (0.77), linolenic acid (0.69), and moisture content (0.61). High genetic advance as a mean percent was manifested for erucic acid (28.0%), glucosinolate content (24.86%) while moderated genetic advance values were recorded for linolenic acid (17.59%) and oleic acid (17.18%). The study also revealed that genetic factors played a significant role in determining glucosinolate content and oleic acid content, making them suitable targets for selective breeding. On the basis of superior performance, G18, G06, G03, G05, G13, G19 and G20 are recommended for growing growers to high yielding Brassica cultivars.

Keywords: Brassica, Genotypes, Biochemical Analysis and Qualitative Traits.

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INTRODUCTION

The genus *Brassica*, encompassing economically important species such as *Brassica juncea*, *B. napus*, and *B. rapa* plays a vital role in global agriculture due to its diverse uses in food, fodder, oilseed, and vegetables. In recent decades, the development of advanced breeding lines has become a cornerstone in *Brassica* crop improvement programs aimed at enhancing traits such as seed quality, disease resistance, and morphological characteristics (Choudhary *et al.,* 2021). In Pakistan, in 2020-21 rapeseed and mustered were sown on 0.22 million hectares, resulting in annual production of 0.30 million metric tons of seed and oil, with 1320 kg ha^{-1} of average yield (USDA, 2020-21). In the cropping year 2019-20, Khyber Pakhtunkhwa achieved a rapeseed production of six thousand tons from a total cultivation area of 13 thousand hectares, attaining an average yield of 418 kg ha^{-1}. The assessment of genetic variability, heritability and genetic advance value stands as essential tools in every breeding program. The population's tendency for diverse genotypes within the group is measured through genetic diversity. Character variability refers to individual's potential to change based on hereditary and environment factors. Heritability estimates offer helpful gaudiness to breeders.

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Additional classifications of genetic variation include additive, dominance and epistasis variance. Broad-sense heritability estimates represent the ratio of total genetic variance (additive, dominance and epistasis) to the overall phenotypic variance. Narrow-sense heritability estimates are the ratio of additive variance to its total phenotypic variance (Falconer and Mackay, 1996). Understanding heritability estimates for a specific trait is crucial for selection as it offers insights into the transmission of genetic traits from parents to offspring (Sabaghnia *et al*, 2010).

Genetic advance measures the improvement in the average genetic makeup and gene distribution of certain traits in a specified direction in the selected populations compared to the parental populations (Allard, 1960). Genetic advance value expected from selection refers to the improvement in genotypic value of characteristics during one cycle of selection at a specific intensity for the new population as compared to the base population. Therefore, the present study was designed to investigate the extent of genetic variation among advanced *Brassica* lines to estimate key genetic parameters such as heritability and genetic advance for important quality traits and ultimately to identify best-performing and genetically uniform genotypes suitable for field cultivation and potential use in future breeding programs.

MATERIALS AND METHODS

Experimental material

This study was conducted to assess the genetic variation among *Brassica* advanced breeding lines for qualitative traits. A total of twenty-two genotypes were used, comprising sixteen advanced breeding lines and six standard check varieties. These genotypes were provided by the Department of Plant Breeding and Genetics**,** The University of Agriculture Peshawar. The experiment was laid out in a (RCB) design with three replications at the Agricultural Research Farm**,** The University of Agriculture Peshawar, during the 2021-22. Standard agronomic practices, including fertilization, irrigation, and pest management, were followed throughout the growing season to maintain optimal plant health.

Research location

Seed samples from selected plant were tested by Infra-Red Spectroscopy at the Biochemical Laboratory, Crop Breeding Section of Nuclear Institute for Food and Agriculture (NIFA), Tarnab Peshawar.

Biochemical traits

Biochemical analysis was the principal parts of this research. Infrared Spectroscopy involves the interaction of infrared radiation with matter. It covers a range of techniques mostly based on absorption spectroscopy. Spectroscopic techniques are used to identify and study the fatty acid profile. The method of infrared spectroscopy is conducted with an instrument called Infrared spectrometer to produce infrared spectrum. Unit of frequency used in Infra-Red Spectroscopy are reciprocal centimeters (cm^{-1}). The data were taken on the following biochemical parameters; Oil content (%), Protein content (%), Glucosinolate contents ($\mu M\ g^{-1}$), Moisture (%), Oleic acid (%), Linolenic acid (%), and Erucic acid (%).

Statistical analysis

Analysis of variance (ANOVA)

The data of all traits was subjected to analysis of variance following (Steel and Torrie, 1980) to study the significant difference in genotypes through biochemical traits. The differences among populations were compared over least significant difference test (LSD) at 5% probability level.

Broad-sense heritability estimates

Heritability is the proportion of genotypic variance to phenotypic variance. Heritability in a broad sense was calculated according to the formula suggested by Singh and Ceccarelli. (1996).

Broad-sense heritability $(h^2) = \frac{V_g}{V_p}$

Where V_p is the phenotypic variance and V_g is the genotypic variance

The components of variance including error variance (V_e), genotypic variance (V_g) and phenotypic variance (V_P), were estimated, according to the following formula:

$$V_P = V_g + V_e$$

$$V_e = EMS$$

$$V_g = \frac{GMS - EMS}{r}$$

According to Stansfield, (1986), heritability values are categorized as, low (<30%), moderate (30-60%) and high (>60%).

Genetic advance (GA)

The genetic advance was estimated following the formula describe by Allard (1960) using 5% selection intensity

G.A. $=(K)(h^2)(\sqrt{V_P})$

Where K is selection differential and at 10% selection intensity (K = 1.755)

$(\sqrt{V_P})$ = standard deviation of phenotypic variance

G.A. as % of mean

G.A. as % of mean = (G.A. / Grand mean) x 100

RESULTS AND DISCUSSION

Oil content

Analysis of variance showed highly significant *($p \le 0.01$)* differences among overall genotypes, advanced lines, checks and advance lines vs. checks for oil content (Table 1). Khan *et al.* (2022) and Sidra *et al.* (2014), who reported significant variations in oil content among advanced *Brassica* lines. Means values among overall genotypes for Oil content ranged from 42 to 48%, with an overall mean of 45%. The genotype G06 showed maximum oil content 48%, followed by G05 (47%) and G13 (47%) whereas minimum oil content was recorded for genotype NARC-sarson (42%). Mean data among advance lines for oil content varied from 42 to 48 %. G03, had minimum oil content (42%), while maximum oil content (48 %) was observed for G06. Mean data among checks for oil content ranged from 42.2 to 47.0%. Minimum oil content (42.2%) were observed for NARC-sarson, while maximum oil content (42.2%) was observed for Husnain (Table 2). Genetic variance for oil content was 1.19 whereas environmental variance was 4.28. This resulted into low heritability (0.22) and genetic advance in percent (1.9%) estimates for oil content in Brassica lines. The low broad-sense heritability estimates suggested that the proportion of phenotypic variation that is due to genetic factors was relatively low which indicated the importance of environmental factors in determining oil content. However, genetic advance value indicating that genetic improvement of oil content is still possible through selective breeding (Table 4). The finding of low heritability estimates and low genetic advance value in oil content is consistent with previous studies by Khali and Ullah (2013) and Sohail *et al.* (2018). This suggests that the genetic control of oil content in *Brassica* is complex and influenced by several of factors, including interactions between genes and the environment.

Protein content

Brassica crops are widely cultivated for their nutritional value, which is characterized by high protein content. The protein content of *Brassica* is influenced by both genetic and environmental factors and understanding the genetic basis of this trait is important for developing new cultivars with improved nutritional quality. Analysis of variance showed non-significant differences among overall genotypes, advanced lines, checks and advance lines vs. checks for protein content (Table 1). These findings contrast with those of Verma *et al.* (2021), who reported significant differences among genotypes for protein content. Mean data among overall genotypes showed a wide range in protein content, from 21 to 24% with an average of 23%. Genotype G20 displayed the highest protein content 24% followed by genotype G04 (24%) and G03 (24%) while the genotype G07 showed the lowest protein content 21%. Mean data among advance lines for protein content varied from 21 to 24%. had minimum protein content (21%), while maximum protein content (21%) was observed for G07. Mean data among checks for protein content ranged from 21.5 to 23.2%. Minimum protein content (21.5%) were observed for Zahoor and BARD-1, while maximum protein content (23.2%) was observed for Husnain (Table 2). The results from the study indicate that there is a negative genetic variance (-0.1) for protein content, which is an unexpected finding since genetic variance is typically positive (Table 4). This suggests that the genetic differences among the genotypes tested are not contributing much to the overall variation in protein content. On the other hand, the environmental variance (2.2) is high, indicating that environmental factors have a greater influence on the variation in protein content. The broad-sense heritability estimates of -0.03 for protein content is also noteworthy which suggests that environmental factors are more important in contributing to the overall variation in the trait than genetic factors. The expected genetic advance as a mean percent of -0.31% for protein content suggests that selecting for genotypes with higher protein content is unlikely to result in much improvement. This is because the expected gain is negative means that selecting the best-performing genotypes will actually lead to a decrease in protein content on average.

Glucosinolate content

The analysis of variance for glucosinolate content revealed significant ($p \le 0.01$) differences among overall genotypes, advanced lines, checks and advance lines vs. checks (Table 1). These findings are in agreement with those reported by Fayyaz and Amin (2015) and Fayyaz and Afzal (2014). Mean value among overall genotypes for glucosinolate content varied between 47 and 90 μ mol g^{-1}, with a mean of 70 μ mol g^{-1}.The highest glucosinolate content was recorded for the genotype G04 (90 μ mol g^{-1}) followed by BRAD-1 (89 μ mol g^{-1}) and Chinese rapa (86 μ mol g^{-1}) while the lowest was observed for Faisal canola (47) . Mean data among advance lines for glucosinolate content varied from 59.6 to 89.6 μ mol g^{-1}. G13, had minimum glucosinolate content (59.6 μ mol g^{-1}), while maximum glucosinolate content (89.6 μ mol g^{-1}) was observed for G04. Mean data among checks for glucosinolate content ranged from 46.7 to 88.5 μ mol g^{-1}. Minimum glucosinolate content (46.7 μ mol g^{-1}) were observed for Faisal canola, while maximum glucosinolate content (47 μ mol g^{-1}) was observed for BARD-1 (Table 2). Genetic variance (126.69) among the *Brassica* advance lines was observed higher in magnitude then the environmental variance (34.82) show high broad sense heritability estimates (0.78) for glucosinolate content. However, high genetic advance value (24.86%) was manifested for glucosinolate content (Table 4). The high genetic variance of suggests that genetic factors contribute significantly to the variation in glucosinolate content. The low environmental variance indicates that the environmental factors that may influence glucosinolate content were relatively constant across the tested genotypes. High heritability estimates indicating that large proportion of the variation in glucosinolate content among the tested genotypes is due to genetic factors. Genetic advance value represents the potential improvement in glucosinolate content that can be achieved through selection, is also relatively high. The results of the present study are in line with the findings of Shaukat *et al.* (2015), who observed moderate to high heritability estimates and high genetic advance value for glucosinolate content in *Brassica* genotypes, suggesting that genetic factors play an important role in determining glucosinolate content in *Brassica*. Overall, these results have important implications for the breeding and selection of *Brassica* genotypes with desirable glucosinolate content.

Moisture content

The analysis of variance revealed significant ($p \leq 0.01$) differences among overall genotypes, advanced lines, checks and advance lines vs. checks for moisture content (Table 1). Mean values among overall genotypes for moisture content ranged from 5.7 to 7.9%, with an average of 7.0%. Genotypes with highest moisture content were Husnain (7.9%) followed by G09 (7.7%) and G06 (7.3%), whereas lowest value of moisture content was observed for NARC-sarson (5.7%). Mean data among advance lines for moisture content varied from 6.6 to 7.7%. G18 had minimum moisture content (6.6%), while maximum moisture content (7.7%) was observed for G09. Mean data among checks for moisture content ranged from 5.7 to 7.9%. Minimum moisture content (5.7%) were observed for NARC-sarson, while maximum moisture content (7.9%) was observed for Husnain (Table 2). Genotypic and environmental variances for moisture content were 0.19 and 0.12 respectively. The high heritability estimates of 0.61 observed for moisture content indicates that a significant proportion of the phenotypic variation in moisture content is due to genetic factors (Table 4). The low genetic advance value of 8.53% indicates that selecting the top 10% of plants with the highest moisture content would lead to an increase in the mean moisture content. The findings of this study are consistent with those reported by Abideen *et al.* (2013), who also reported high heritability estimates and genetic advance value for moisture content. This consistency strengthens the reliability of the findings and supports the potential application of the results in crop improvement programs.

Table 1. Mean squares for biochemical traits of Twenty-two Brassica genotypes (Sixteen advanced lines and six checks).

Parameters	Replications (df = 2)	Genotypes (df = 21)	Error (df = 42)	CV (%)
Oil content	9.88	7.85**	4.28	4.60
Protein content	2.88	2.03	2.21	6.50
Glucosinolate content	17.17	414.90**	34.83	8.39
Moisture content	0.29	0.69**	0.12	5.04
Oleic acid	0.88	95.78**	8.60	6.06
Linolenic acid	0.76	2.90**	0.38	8.20
Erucic acid	56.24	224.71**	12.18	7.18

Oleic acid

The analysis of variance showed significant ($p \leq 0.01$) differences for oleic acid content among overall genotypes, advanced lines, checks and advance lines vs. checks (Table 1). These findings are consistent with those of (Ahmad *et al.*, 2012), who also reported significant variation in oleic acid content among *Brassica* species. among overall genotypes displayed a range of oleic acid content from 37 to 63 %, with an average of 48 %. NARC-sarson holding the highest oleic acid content of 63%, followed by Faisal canola (58 %) and G20 (52 %), whereas BRAD-1 holding the lowest value of oleic acid 37% . Mean data among advance lines for oleic acid varied from 40.0 to 51.8%. G03, had minimum oleic acid (40.0%), while maximum oleic acid (51.8%) was observed for G03. Mean data among checks for oleic acid ranged from 37.0 to 63.1%. Minimum oleic acid (37%) were observed for BRAD-1, while maximum oleic acid (63.1%) was observed for NARC-sarson (Table 3). Genotypic variance and environmental variance were 29.06 and 8.60 respectively. The high heritability estimates estimate (0.77) for oleic acid content indicates that a substantial proportion of the total variation in the trait is attributable to genetic factors. This suggests that selective breeding can be an effective strategy for improving oleic acid content in *Brassica* species. High genetic advance value were observed for oleic acid was 17.18% (Table 4). The results of this study differ from those of Priyamedha *et al.* (2018). They reported moderate to high heritability estimates and low to moderate heritability estimates for oleic acid content in rapeseed genotypes and Indian mustard, respectively.

Table 2. Means performance of Twenty-two Brassica genotypes (Sixteen advanced lines and six checks) for oil content protein content, glucosinolate content and moisture content evaluated during 2021-22.

Genotype (Advanced Lines)	Oil Content (%)	Protein Content (%)	Glucosinolate Content (μ mol g^{-1})	Moisture Content (%)
G01	45	24	77	6.6
G02	45	23	70	7.1
G03	43	24	77	6.8
G04	46	24	90	7.3
G05	47	22	70	6.9
G06	48	22	71	7.3
G07	46	21	66	6.7
G08	46	22	83	6.8
G09	46	23	86	7.7
G13	47	22	60	7.3
G15	45	23	68	7.1
G16	45	23	68	7.1
G17	45	23	62	7.2
G18	43	23	66	6.6
G19	44	23	65	7.0
G20	43	24	77	7.2
Advance lines means	45	23	72.0	7.1
Advance lines $LSD_{(0.05)}$	0.76	0.54	2.17	0.12
Checks				
Faisal canola	44	23	47	7.1
Zahoor	44	23	58	7.2
BARD-1	43	23	89	6.1
NARC- sarson	42	22	50	5.7
Chinese rapa	44	23	86	6.5
Husnain	47	22	64	7.9
Checks means	44	22	65	6.4
Checks $LSD_{(0.05)}$	1.39	1.00	3.96	0.23
Genotypes means	45	23	70	7.0
Genotypes $LSD_{(0.05)}$	3.41	2.45	9.72	0.58

Linolenic acid

Analysis of variance showed significant ($p \leq 0.01$) differences among overall genotypes, advanced lines, checks and advance lines vs. checks for linolenic acid content. Ahmad *et al.*, (2012) also reported significant variation among *Brassica* lines. The mean values among overall genotypes for genotypes ranged from 6.1% to 9.6%, with an overall mean of 7.6% (Table 1). The highest linolenic acid concentration was recorded for BRAD-1 (9.6%), followed by G02 (9.1%) and Faisal canola (8.9%), while the lowest was recorded by genotype G1 (6.1%). Mean data among advance lines for linolenic acid varied from 6.1 to 9.1%. G1 had minimum linolenic acid (6.1%), while maximum linolenic acid (9.1%) was observed for G02. Mean data among checks for linolenic acid ranged from 7.0 to 9.6%. Minimum

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linolenic acid (7.0%) was observed for Zahoor, while maximum linolenic acid (9.6%) was observed for BRAD-1 (Table 3). The genetic variance was recorded to be higher than the environmental variance, with genetic and environmental variance recorded at 0.84 and 0.38, respectively. The broad-sense heritability estimates for linolenic acid was high at 0.69 and the expected genetic advance value was 17.59% (Table 4). These results are in contrast to those of Iqbal *et al.* (2014), who also reported high heritability estimates for linolenic acid content.

Table 3. Means performance of Twenty-two Brassica genotypes (Sixteen advanced lines and six checks) for oleic acid, linolenic acid and erucic acid evaluated during 2021-22.

Genotype (Advanced Lines)	Oleic acid (%)	Linolenic acid (%)	Erucic acid (%)
G01	46	6.1	44
G02	47	9.1	54
G03	52	7.9	44
G04	45	8.0	56
G05	49	6.8	56
G06	49	7.1	54
G07	51	7.1	53
G08	43	8.5	54
G09	40	8.2	55
G13	49	7.2	48
G15	51	6.8	47
G16	51	6.3	48
G17	47	7.9	53
G18	45	7.3	47
G19	51	6.4	44
G20	52	6.2	46
Advance lines means	47.9	7.3	50.1
Advance lines LSD$_{(0.05)}$	1.08	0.22	1.28
Checks			
Faisal canola	58	8.9	30
Zahoor	50	7.0	45
BARD-1	37	9.6	60
NARC- sarson	63	7.5	23
Chinese rapa	41	7.8	58
Husnain	49	8.7	53
Checks means	49.6	8.3	44.6
Checks LSD$_{(0.05)}$	1.97	0.41	2.38
Genotypes means	48	7.6	49
Genotypes LSD$_{(0.05)}$	4.83	1.02	5.75

Erucic acid

The analysis of variance indicated highly significant *($p≤0.01$)* differences for erucic acid content among overall genotypes, advanced lines, checks and advance lines vs. checks (Table 1). Erucic acid levels varied among genotypes from 23 to 60% and an overall mean of 49%. BARD-1 had the highest erucic acid content (60 %) followed by Chinese rapa (58 %) and genotype G04 (56 %), while the lowest was recorded for NARC-sarson (23 %). Mean data among advance lines for erucic acid varied from 43.8% to 55.9%. G20, had minimum erucic acid (43.8%), while maximum erucic acid (55.9%) was observed for G04. Mean data among checks for erucic acid ranged from 23.0 to 59.6%. Minimum erucic acid (23.0%) were observed for NARC-sarson, while maximum erucic acid (59.6%) was observed for BARD-1. Genetic variance (70.84) among the *Brassica* advance lines was observed higher in magnitude then the environmental variance (12.18) resulting high broad-sense heritability estimates (0.85) for erucic acid content. However, high genetic advance value (28.0%) was manifested for erucic acid content (Table 3). Result suggested that erucic acid content is largely determined by genetic factors rather than environmental factors. The high broad-sense heritability estimates of erucic acid concentration indicating that a large proportion of the phenotypic variation in erucic acid content is due to genetic differences among the genotypes. High genetic advance value could be an effective approach for improving erucic acid content in *Brassica* lines (Table 4). The present study are in lune with the findings of Saroj *et al.* (2021) and Shaukat *et al.* (2015). They reported high heritability estimates of erucic acid content among different *Brassica* genotypes.

Table 4. Genetic (Vg), environmental (Ve) and phenotypic (Vp) variances, heritability estimates (h^2), genetic advance value (GA) and genetic advance as a mean percent value (GA %) for studied traits of Twenty-two Brassica genotypes (Sixteen advanced lines and six checks).

Parameters	V_g	V_e	V_p	h^2	GA	GA%
Oil content %	1.19	4.28	5.47	0.22	0.89	1.9
Glucosinolate content (μMg^{-1})	126.69	34.83	161.52	0.78	17.49	24.86
Moisture content %	0.19	0.12	0.31	0.61	0.59	8.53
Oleic acid %	29.06	8.60	37.66	0.77	8.31	17.18
Linolenic acid %	0.84	0.38	1.22	0.69	1.33	17.59
Erucic acid %	70.84	12.18	83.03	0.85	13.65	28.0

CONCLUSION AND RECOMMENDATIONS

Significant ($p<0.01$) differences among overall genotypes, advanced lines, checks and advance lines vs. checks were observed for all studied traits except protein content. Highest oil content was observed for genotype G06, G05 and G13. The Genotype G13 and G19 showed lowest value for glucosinolate content and erucic acid content while the genotype G20 had highest protein content. High heritability coupled with high genetic advance was estimated for erucic acid, glucosinolate content, oleic acid, linolenic acid, and moisture content. High genetic advance as a mean percent were manifested for primary erucic acid and glucosinolate content, while moderated genetic advance values were recorded for linolenic acid and oleic acid. Based on mean performance for various traits, G18, G06, G05, G13, G19 and G20 appeared superior and hence are recommended for further testing to derive high yielding brassica cultivars.

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AUTHOR CONTRIBUTIONS

Muhammad Suliman and **Hassan Mujtaba** contributed to the conceptualization, experimental design, and data collection. **Noshin Shafqat** and **Nadia Jabeen** assisted in data analysis, and manuscript drafting. **Laila Fayyaz** oversaw the research, reviewed the manuscript critically, and provided final approval for publication. All authors read and approved the final manuscript.

COMPETING OF INTEREST

The authors declare no competing interests.

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