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Inheritance Analysis for Quantitative Traits in F_2 Populations of Mustard Genotypes Grown Under Heat Stress Environments

Kamran Hashim Jamali[1,2], Abdul Wahid Baloch[1*], Shah Nawaz Mari[1], Muhammad Ubaidullah Shirazi[3]

[1]Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Pakistan
[2]Department of Plant Breeding and Genetics, Balochistan Agriculture College, Quetta-Pakistan
[3]Nuclear Institute of Agriculture, Tandojam-Pakistan
**Corresponding author's email*: balochbreeders@yahoo.com

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Abstract

Genetic variability is the basic requirement for improving crops which further leads to progress in crop breeding. This study was carried out to determine the inheritance pattern of various agronomically important traits in F_2 populations of mustard genotypes. The objective of this study was to determine the genetic variability for agro-morphological traits under control conditions (CC) and heat stress environments (HS) and provide the future strategies to improve this important oilseed crop. The experiment was conducted at the Botanical Garden, Sindh Agriculture University, Tandojam. During 2022-23, a total of 27 genotypes including nine parental mustard genotypes and eighteen F_2 progenies were grown under control and heat stress conditions. The experiment was laid out in a randomized complete block design with three replications. The analysis of variance for the genotypes, treatments, and F_2 progenies revealed significant differences ($P \leq 0.05$) in most of the studied characters, highlighting the importance of genetic variations in the evaluated breeding materials of mustard for stress breeding. When estimating heritability for different characters, including seed yield plant^{-1}, it was found that F_2 crosses showed varying levels of heritability for different characteristics, ranging from low to moderate and high. This implies that careful selection may be conducted for traits that have low heritability, especially for seed yield.; however, those characters which showed high heritability would be selected at early generation stage to improve the characters.

Keywords: Inheritance, genetic variability, mustard, heat stress, morphological traits

Introduction

Mustard (*Brassica juncea* L. Czern) is a significant oilseed crop that is grown during the winter season of Pakistan. After soybeans, mustard is the second most produced edible oilseed crop in the world (Zandberg *et al.*, 2022). In our country, mustard is cultivated as a winter crop. However, due to changing global climatic conditions, it is increasingly exposed to heat stress, particularly during reproductive stages (Pandey *et al.*, 2024). In 2022-23, a total of 2.681 million tonnes of edible oil (valued at US$ 3.562 billion) was imported. The provisional estimate for local production of edible oil was 0.496 million tonnes. Therefore, the estimated total availability of edible oil, including both imports and local production, is 3.177 million tonnes. This indicates an increase in both the area and production of brassica species. However, despite these figures, current production only meets 12% of Pakistan's edible oil requirements, with the remaining 88% being imported (GoP, 2023).

The yield and stability of Brassica oilseed and mustard crops currently depend on optimizing agronomic practices through enhancement programs, developing new varieties via breeding initiatives, and improving management strategies to prevent disease and pest outbreaks (Zandberg *et al.*, 2022). Environmental conditions are the primary factors influencing biomass accumulation and yield during the growth period (Kirkegaard *et al.*, 2021). Heat stress in plants occurs when the temperature rises above a certain threshold for an extended period of time. This can cause irreversible damage to plant growth and development (Pandey *et al.*, 2024). The flowering stage is particularly vulnerable to high temperatures because it affects pollen development, anthesis, and fertilization, ultimately leading to a decrease in crop yield (Hall and Rao, 1992). In brassica plants, high temperatures hinder their development and can result in flower abortion, causing significant seed yield loss. If the temperature exceeds 30°C during the flowering stage, there can be a drastic decline in seed yield (Nuttal *et al.*, 1992). Mustard crops, which are grown in arid and semi-arid regions during the winter season, are especially susceptible to the detrimental effects of heat stress. Delaying the sowing of mustard crops reduces the vegetative phase, limits dry matter accumulation, and restricts the crop's genetic yield potential. High temperatures during pod formation can also lead to heat blast and increase the gap between pods and grains. Therefore, in order to achieve sustainable seed yield in late-sown Indian mustard, it is essential to minimize the effects of high temperatures during the reproductive stage by utilizing mustard genotypes that are tolerant to heat (Anita, 2023).

The extent of diversity available in the crop decides the success of any crop improvement program with manifested objectives. Creation and assessment of divergence in Indian mustard is highly needed to develop high yielding genotypes with desirable traits (Meena *et al*., 2017). Genetic variability has been the central dogma of plant breeding. An understanding of the genetic behavior of various agro-morphological and yield contributing traits is required for efficient selection of genotypes and breeding for trait specific genotypes. It is highly desirable to study the genetic variability for desirable traits among available promising genotypes and test genotypes to select suitable genotypes and crosses. Since exploitation of genetic differences in traits contributing to yield may be a means of improving the crop for these traits. Based on these considerations, the objective of the current study was to evaluate the genetic variability in mustard genotypes by creating experimental areas with high temperatures

Materials and Methods

Two treatments were used, such as conditions (CC), this refers the crop was grown on normal sowing date (10th October) and, heat stress (HS), this indicates that the terminal heat stress was imposed by growing mustard crops on late sowing date (30th November). The study was conducted at the Botanical Garden, Sindh Agriculture University, Tandojam during the rabi season of 2022-23. For this study, a total of nine mustard genotypes (D-Bahawalpur Raya, HUM-321 (A), S-9, K.J-230, K.J-221, Anmol Raya**,** JS-13, Early Raya and Sindh Raya) were crossed to produce eighteen F_2 progenies. All 18 F_2 progenies, including the parental lines, were grown in three replications during the 2022-23 growing season for genetic analysis conditions total eight characters were studied, such as days to 75% flowering, days to 90% maturity, plant height (cm), branches plant^{-1}, siliquae plant^{-1}, siliquae length (cm), seed yield plant^{-1} (g) and seed index (1000 seed weight, g). Four irrigations were provided at critical stages, including at stem growth stage, flowering stage, siliquae formation stage and maturity stage. The N:P: K (nitrogen, phosphorus, and potassium) was applied at a rate of 40:24:20 kg/acre respectively. This dose was divided into two parts: half was applied before sowing, and the other half was applied at the first irrigation. Weeding was done three times, with two-week intervals, to control the growth of weeds. To control aphids, the Confidor pesticide was used at a rate of 250mL/acre, when the economic threshold was reached. Heritability (broad sense) was calculated using the method by Robinson and Comstock (1949).

$$h^2b\ (\%) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where, h^2 (b) = Heritability in broad sense

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

Table 1. Total rain and temperature recorded in different growing seasons

Months	**Total rain (mm) (mean)**	**Temperature (˚C)**	
		Min (mean)	Max (mean)
October-2022	0.0	19.4	36.9
November-2022	0.0	14.9	32.1
December-2022	0.0	9.5	26.0
January-2023	0.0	5.7	22.8
February-2023	0.0	11.7	31.8
March-2023	0.1	17.1	33.9

Results

Mean squares: The mean squares of sources of variances, including genotypes (G), treatments (T), G × T interaction, parents, F_2 progenies, parents (P) vs. crosses (C), showed significant differences ($p<0.05$) for the majority of the agronomical, and yield characters (Table 2).

Table 2. Mean squares of various traits of F_2 progenies in mustard genotypes

Sources of variances	Replication	Genotype	Treatment	G × T	Parents	F_2 progenies	Parents vs. Crosses	Errors
D.F.	**2**	**26**	**1**	**26**	**8**	**17**	**1**	**106**
Characters								
Days to 75% flowering	1.722	6.504*	249.389**	11.171**	5.250^{ns}	7.157^{ns}	5.443^{ns}	4.150
Days to 90% maturity	9.9321	6.2426*	88.8889**	8.8248**	1.2407^{ns}	7.5256*	24.448^{ns}	3.7560
Plant height	29.75	52.30*	2442.23**	30.75^{ns}	36.935^{ns}	50.71*	202.27^{ns}	31.11
Branches plant^{-1}	0.02080	1.32421**	105932**	0.27868**	1.615**	1.25192**	0.223^{ns}	0.09753
Siliquae plant^{-1}	1812	8761**	156240**	3665**	9272**	3503.2*	94045**	1811
Siliquae length	0.00395	0.06643**	1.12500**	0.01769^{ns}	0.064**	1.6303**	0.42975**	0.01609
Seed yield plant^{-1}	0.177	22.098**	170.263**	9.547**	21.168**	15.8480**	135.8**	0.735
Seed index	0.02507	0.06447*	6.25794**	0.04194^{ns}	0.041^{ns}	0.06178^{ns}	0.29396**	0.03544

**,* significant level at 1 and 5% probably levels respectively, while, ns shows non-significant

Estimation of heritability in broad sense: Different genetic parameters for different characters are given in following paragraphs. The genetic variability was estimated through heritability in broad sense (b.s.).

Days to 75% flowering: For days to 75% flowering (Table 3), the broad sense heritability ranged from 8.14% to 81.77% under CC and 5.26% to 84.21% under HS. The maximum heritability was noted in F_2 crosses like D-Bahawalpur Raya × Early Raya (b.s. 81.77%), followed by Anmol Raya × Early Raya (b.s. 78.95%), D-Bahawalpur Raya x Sindh Raya (b.s. 74.28%), and S-9 x Sindh Raya (b.s. 66.38%) under CC. While K.J-230 × Sindh Raya (b.s. 84.21%), followed by D-Bahawalpur Raya × J.S-13 (b.s. 72.48), D-Bahawalpur Raya x Sindh Raya (b.s. 69.23%), and HUM-321 (A) x JS-13 (b.s. 47.37%) remained on top for heritability estimation under HS. However, the minimum heritability was found in F_2 progenies, such as K.J-221 × Early Raya (b.s. 8.14%), followed by K.J-230 x Early Raya (b.s. 14.05%) under CC, while K.J-221 x Early Raya (b.s. 5.26%) followed by HUM-321 (A) x Early Raya (b.s. 9.09%) under HS.

Days to 90% maturity: For this trait (Table 3), the broad sense heritability ranged from 6.78% to 68.17%, under CC, and 4.61% to 85.19% under HS. The maximum heritability was noted in F_2 populations including Anmol Raya × Early Raya (b.s. 68.17%), followed by D-Bahawalpur Raya × J.S-13 (b.s. 63.71%), S-9 x Early Raya (b.s. 56.76%) and S-9 x JS-13 (b.s. 55.45%) under CC and D-Bahawalpur Raya × Sindh Raya (b.s. 85.19%), followed by HUM-321 (A) × J.S-13 (b.s. 81.55%), HUM-321 (A) × Early Raya (b.s. 74.92%) and D-Bahawalpur Raya × JS-13 (b.s. 64.92%) under HS. Whereas the minimum heritability was noted in HUM-321 (A) × Sindh Raya (b.s. 6.78%), followed by S-9 × Sindh Raya (b.s. 11.90%) under CC, while K.J-221 × Early Raya (b.s. 4.61%) followed by K.J-221 × Sindh Raya (b.s. 10.35%) under HS.

Plant height: The broad sense heritability for plant height ranged from 1.78% to 65.17% under CC and from 1.27% to 77.91% under HS (Table 3). The maximum heritability in broad sense, such as 65.17%, 62.64%, and 24.18% were noted in F_2 progenies D-Bahawalpur Raya × J.S-13, K.J-221 × J.S-13, and K.J-230 × J.S-13 under CC, respectively, while D-Bahawalpur Raya × J.S-13, followed by Anmol Raya × Early Raya and Anmol x J.S-13 reported the highest heritability under HS with values of77.91%, 74.87% and 74.18%, respectively.

Branches plant^{-1}: The F_2 progeny S-9 × J.S-13 showed maximum broad sense (71.68%), followed by S-9 × Sindh Raya (b.s. 51.63%), and HUM-321 (A) × J.S-13 (b.s. 47.40%) under CC, while, D-Bahawalpur Raya × J.S-13 (b.s. 91.05%), followed by K.J-230 × Sindh Raya (b.s. 77.76%) and S-9 x Sindh Raya (b.s. 67.82%) estimated maximum heritability under HS. Whereas the minimum heritability was noted in HUM-321 (A) × Early Raya (b.s. 0.62%), followed by K.J-221 × Sindh Raya (b.s. 1.25%) under CC, however, HUM-321 (A) × Early Raya (b.s. 5.90%) followed by Anmol Raya × Sindh Raya (b.s. 7.09%) under HS (Table 3).

Table 3. Estimation of heritability in broad sense (b.s.) for different characters in F_2 crosses

F_2 progenies	Days of 75 % flowering		Days to 90 % maturity		Plant height		Branches plant^{-1}	
	CC	HS	CC	HS	CC	HS	CC	HS
Bahawalpur Raya × JS-13	44.66	72.48	63.71	64.92	65.17	77.91	19.26	91.05
HUM-321 (A) × JS-13	62.31	47.37	33.29	81.55	6.84	24.77	47.40	47.86
S-9 × JS-13	17.57	29.09	55.45	63.97	18.04	44.59	71.68	17.68
K.J-230 × JS-13	42.60	23.08	42.37	20.84	24.18	34.23	29.63	16.94
K.J-221 × JS-13	18.88	10.00	31.94	19.67	62.64	12.67	23.54	29.86
Anmol Raya × JS-13	35.63	18.75	46.92	42.37	13.48	74.18	17.03	12.97
Bahawalpur Raya × Early Raya	81.77	47.37	54.40	58.62	20.93	10.36	6.99	12.26
HUM-321 (A) × Early Raya	61.42	9.09	20.08	74.92	1.78	15.50	0.62	5.90

S-9 × Early Raya	62.68	29.09	56.76	23.95	5.25	45.83	15.82	13.87
K.J-230 × Early Raya	14.05	11.76	40.54	44.85	7.78	1.27	13.10	15.72
K.J-221 × Early Raya	8.14	5.26	54.60	4.61	6.56	15.79	15.52	11.54
Anmol Raya × Early Raya	78.95	29.09	68.17	23.15	5.58	74.87	3.64	55.07
Bahawalpur Raya × Sindh Raya	74.28	69.23	64.94	85.19	19.30	15.98	9.80	7.96
HUM-321 (A) × Sindh Raya	59.31	25.00	6.78	33.40	3.73	66.58	34.47	10.31
S-9 × Sindh Raya	66.38	25.00	11.90	23.95	10.24	24.43	51.63	67.82
K.J-230 × Sindh Raya	22.34	84.21	42.65	23.57	7.42	43.46	6.35	77.76
K.J-221 × Sindh Raya	19.37	36.84	21.46	10.35	6.16	12.56	1.25	11.17
Anmol Raya × Sindh Raya	51.32	46.15	20.33	44.54	11.74	17.95	7.36	7.09

Siliquae plant^{-1}: The maximum broad sense heritability of 75.35%, 66.80%, 60.10% and 58.66% were estimated in S-9 × J.S-13, K.J-221 × Sindh Raya, HUM-321 (A) × J.S-13 and HUM-321 (A) × Sindh Raya under CC, respectively, while under HS, the maximum heritability of 91.78%, 79.51%, 75.12%, and 74.27% were noted in K.J-221 × Sindh Raya, HUM-321 (A) × Early Raya, S-9 x Early Raya and Anmol Raya x Early, respectively. However, the minimum heritability was found in HUM-321 (A) × Early Raya (b.s. 0.31%), followed by K.J-221 × Sindh Raya (b.s. 1.04%) and K.J-230 × Early Raya (b.s. 1.65%) under CC, however, K.J-230 × Early Raya (b.s. 2.08%), followed by HUM-321 (A) × Sindh Raya (b.s. 3.30%), and D-Bahawalpur Raya × Sindh Raya (b.s. 3.67%) under HS (Table 4).

Siliquae length: For this character (Table 4), heritability in broad sense varied from 5.08% to 82.63% under CC, while from 3.85% to 83.00% was the range under HS. The maximum heritability was noted in Anmol Raya x Early Raya (b.s. 82.63%), followed by Anmol Raya x Sindh Raya (b.s. 70.38%), S-9 x JS-13 (b.s. 64.32%), D-Bahawalpur Raya x Sindh Raya (b.s. 46.02%) and K.J-221 x JS-13 (b.s. 37.30%) under CC. Taking maximum heritability in HS, the crosses D-Bahawalpur Raya x JS-13 (b.s. 83.00%), followed by Anmol Raya x JS-13 (b.s. 82.89%), Anmol Raya x Early Raya (b.s. 79.54%), S-9 x Sindh Raya (b.s. 48.39%) and K.J-221 x JS-13 (b.s. 46.25%) remained on top. However, the minimum heritability was found in F_2 progenies D-Bahawalpur Raya x Early Raya (b.s. 5.08%), followed by K.J-221 x Sindh Raya (b.s. 5.34%) under CC, while HUM-321 (A) x Early Raya (b.s. 3.85%), followed by K.J-230 x Early Raya (b.s. 4.24%) were lowest in heritability under HS.

Seed yield plant^{-1}: For seed yield plant^{-1}, the maximum heritability was noted in HUM-321 (A) × JS-13 (b.s. 84.26%) followed by S-9 × Sindh Raya (b.s. 64.46%), D-Bahawalpur Raya × Early Raya (b.s. 44.15%), and K.J-221 × Early Raya (b.s. 33.88%) under CC, however, D-Bahawalpur Raya × J.S-13 (b.s. 54.77%), followed by K.J-230 × Early Raya (b.s. 45.53%), K.J-221 × Sindh Raya (b.s. 40.74%), and S-9 × JS-13 (b.s. 37.15%) reported the maximum heritability under HS. While the minimum heritability was noted in K.J-221 x JS-13 (b.s. 0.54%), followed by S-9 x Early Raya (b.s. 1.20%) and Anmol Raya x Early Raya (b.s. 2.54%) under CC, whereas, K.J-230 x JS-13 (b.s. 2.59%), followed by K.J-230 x Sindh Raya (b.s. 3.84%) and S-9 x Sindh Raya (b.s. 4.17%) mentioned as lowest in heritability under HS (Table 4).

Seed index: For seed index (Table 4), the maximum heritability in broad sense, such as 40.83%, 24.16%, 22.93%, 22.07% and 21.69% were recorded in F_2 population Anmol Raya x Sindh Raya, S-9 x Early Raya, HUM-321 (A) × Sindh Raya, D-Bahawalpur Raya x Sindh Raya and K.J-221 × Early Raya, respectively under CC. Whereas, D-Bahawalpur Raya x JS-13, S-9 × Sindh Raya, HUM-321 (A) × Sindh Raya, HUM-321 (A) × Early Raya and HUM-321 (A) × JS-13 showed maximum heritability with values of 59.65%, 41.93%, 34.87%, 33.79% and 31.21%, respectively.

Table 4. Heritability estimation in broad sense for different characters in F_2 crosses

F_2 progenies	Siliquae plant^{-1}		Seed index		Siliquae length		Seed yield plant^{-1}	
	CC	HS	CC	HS	CC	HS	CC	HS
Bahawalpur Raya × JS-13	26.93	45.74	11.48	59.65	31.83	83.00	15.33	54.77
HUM-321 (A) × JS-13	60.10	8.44	10.52	31.21	15.45	42.05	84.26	15.29
S-9 × JS-13	75.35	13.92	17.02	14.63	64.32	44.62	24.00	37.15
K.J-230 × JS-13	11.49	59.77	18.51	22.55	19.86	20.50	6.95	2.59
K.J-221 × JS-13	18.77	6.13	13.07	11.17	37.30	46.25	0.54	6.08
Anmol Raya × JS-13	3.89	8.39	15.18	14.08	26.52	82.89	3.61	22.01
Bahawalpur Raya × Early Raya	12.10	26.49	16.27	18.36	5.08	22.02	44.15	8.05
HUM-321 (A) × Early Raya	0.31	79.51	14.08	33.79	9.07	3.85	15.23	24.92
S-9 × Early Raya	3.10	75.12	24.16	18.70	10.03	23.04	1.20	27.05
K.J-230 × Early Raya	1.65	2.08	12.61	18.64	30.08	4.24	3.60	45.53
K.J-221 × Early Raya	1.04	12.03	21.69	16.76	22.92	27.93	33.88	21.75
Anmol Raya × Early Raya	1.78	74.27	15.59	14.50	82.63	79.54	2.54	36.15
Bahawalpur Raya × Sindh Raya	7.36	3.67	22.07	22.95	46.02	38.58	3.11	8.53

HUM-321 (A) × Sindh Raya	58.66	3.30	22.93	34.87	9.07	4.29	4.49	9.98
S-9 × Sindh Raya	2.01	4.15	18.01	41.93	20.32	48.39	64.46	4.17
K.J-230 × Sindh Raya	2.84	3.84	14.93	14.80	7.54	10.84	20.19	3.84
K.J-221 × Sindh Raya	66.80	91.78	17.59	9.90	5.34	16.94	10.79	40.74
Anmol Raya × Sindh Raya	4.97	13.20	40.83	15.63	70.38	44.48	12.46	24.53

Discussion

The breeding program focuses on estimating heritability to identify genotypic variants influenced by phenotypic differences. It provides information about the population's makeup and the proportion of traits passed on from one generation to the next. A higher heritability value improves the selection process and increases the response. Heritability studies are crucial for successful breeding programs as they help plant breeders predict how genes will interact in future generations (Saleem *et al*., 2016). Therefore, the heritability estimates would assist in predicting the potential progress that can be achieved by improving the selection process. For the maturity characters (Days to 75% heading and days to 90% maturity), two F_2 progenies, such as Bahawalpur Raya × JS-13 and Bahawalpur Raya × Sindh Raya showed higher heritability under both CC and HS environments. These results suggest that improvement in maturity is possible through smart selection in early generations. The genetic variability regarding maturity traits in mustard genotypes agrees with those of Gupta *et al*., (2019) and Anjali *et al*., (2022). Plant height is a crucial characteristic that, alongside other traits, determines the yield. The cross Bahawalpur Raya × JS-13 recorded the highest genetic variability for plant height in terms of both environments, including stressful and non-stressful. High heritability suggests the existence of genetic diversity within the F_2 populations in terms of plant height. As a result, various height levels can be selected from these crosses. The current findings support the research conducted by Anjali *et al*., (2022), whereas Evangelin *et al*., (2023) reported a moderate heritability in broad sense for plant height, which is also align to our most of the results observed in number of crosses either in CC or HS.

The genetic variability for yield associated traits (branches plant^{-1}, siliquae plant^{-1}, silique length, seeds siliquae^{-1} and seed index) is also very important. Out of eighteen F_2 progenies, only K.J-221 × Sindh Raya reported high heritability for siliquae plant^{-1} under CC and HS. However, most of the crosses registered high heritability either in CC or HS for above given traits, whereas a number of crosses also reported low and moderate heritability, indicating that yield associated traits are influenced by environmental factors in many crosses under CC and HS. In many previous studies (Gangapur *et al*., 2008; Parsad and Patel., 2018; Parmar *et al*., 2023), it has been reported that most yield associated traits in mustard genotypes have low or moderate heritability values and such characters may be improved through conventional plant breeding methods in later generations. Opposite to our findings, many studies (Alam, 2010; Belete *et al*., 2012; Dawar *et al*., 2018; Evangelin *et al*., 2023; Gupta *et al*., 2019) have also reported high heritability for these yield associated characters.

In order to enhance grain yield, breeders must estimate genetic associations and characterize genetic variability among different genotypes. This is achieved through artificial crosses between parents with dissimilar traits, which leads to significant segregation and the clustering of advantageous alleles (Haydar *et al*., 2020). For seed yield, only two crosses resulted in high heritability estimates under CC, while none of the cross recorded the desired heritability estimation under HS. The majority of the progenies detected low and moderate heritability for seed yield plant^{-1} under CC and HS, exhibiting that this trait is moderately influenced by environment. Early generations can achieve high heritability trait selection. However, if the heritability value is low, the trait must be chosen from the subsequent generation. In a broader context, the value of heritability encompasses the effects of dominance, epistasis, and additive gene activity. If dominant gene activity and epistasis play a more significant role in determining the trait, selection cannot take place in the early generations. Therefore, it must be chosen for the subsequent generation. However, high heritability for seed yield plant^{-1} in mustard was witnessed by Anjali *et al*., (2022), Evangelin *et al*., (2023), Gupta *et al*., (2019), Maurya *et al*., (2018), Raina *et al*., (2023) and Rout *et al*., (2019). Whereas, on the same side, having the low heritability estimation for seed yield plant^{-1}, the studies of Parmar *et al*., (2023) and Prasad and Patel (2018) were found on the side to the current investigation.

Conclusions

The analysis of variance for F_2 generations showed a significant difference in most of the studied characters. In the estimation of heritability, it was observed that F_2 crosses exhibited both low to moderate and high heritability levels for various characteristics. This suggests that careful selection may be conducted for traits that have low heritability.

Authors Contribution

K.H.J: Conducted research experiment and write the manuscript; A.W.B: Conceptualized and designed the experiment; S.N.M: Analyzed the data; M.U.S: Provided the genetic materials

Authors Conflict

The authors declare that they have no conflict of interest

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