www.ajlifesciences.com / ISSN: 2663-1040 AJ Life Sci. 2025, 8 (2): 56-67 Research Article

AJLS

Abasyn Journal of Life Sciences

Open Access

DOI: 10.5281/zenodo.17597237

Estimation of Heterotic Effect of Mustard Genotypes (*Brassica Juncea* L.) Under Water Stress Conditions

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Abstract

The current investigation aims to develop the genetic capability of seed yield and oil content genotypes based on genetic analysis (heterosis) under water-deficient environments. In this manner, 6 mustard cultivars, which were highly water stress-tolerant, like (AARI-Canola, Khanpur Raya, Dhoom-1, Super Raya, Galaxy, and Coral-432) hybridized using a half diallel fashion 6 × 6 and produced 15 F_1 progeny for further genetic analysis. The research was conducted in randomized complete block design having three replications and two treatments, during 2019-20 at SAU, Tandojam. T_1 Well-watered at (stem growth, flowering, silique formation and maturity stages) and T_2 Water stressed at (stem growth and flowering stages), respectively. Total 8 traits were investigated (plant height, branches plant^{-1}, seed yield plant^{-1}, seed index, SPAD chlorophyll, relative water content, oil content and protein content). Mostly studied traits revealed significantly differed ($p \leq 0.05$) for all variances like (mean squares) of genotypes, treatments and genotype×treatment interaction as well as for heterotic effects over better parents under both conditions. The hybrids AARI-Canola×Super Raya, AARI-Canola×Galaxy, AARI-Canola×Coral-432, Khanpur Raya×Coral-432 and Super Raya×Coral-432 demonstrated superior parent heterotic effects for seed yield and its related traits under water deficit. Therefore, these crossing materials are helpful for heterotic breeding on a genetic and water stress basis and could be used for the development of desired genotypes and crosses in future breeding as well.

Keywords: Mustard, water deficit, heterosis, yield traits, oil traits

Article Info:
Received:
September 12, 2025
Received Revised:
November 11, 2025
Accepted:
November 12, 2025
Available online:
November 13, 2025

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1. INTRODUCTION

The *Brassicaceae* family (mustard crop) is a cultivated crop, and its seeds are used as condiments, primarily in sauces and as a flavouring in food or as a component of salad dressings. It is rich in oil and has highly nutritional and economic worth. *Brassica* species are major sources of edible oil worldwide, contributing about 15% of total production vegetable oil[1,2]. In *Brassica* species, the variation in oil yield is influenced by genetic variances, environmental factors and cultivation approaches[3]. Mustard plants cover oil (29 to 40), protein (23 to 30), and carbohydrates (12 to 18) percents respectively[4]. In Pakistan, rape and mustard crops accounts for 2nd most important donors of vegetable oil after cottonseed. During 2022-23, mustard and its related species including rapeseed were grown on 1.332 million acres in Pakistan producing 0.675 million tonnes seed and 0.243 million tonnes of oil[5]. Similarly, during 2023–24, a total of 0.851 million acres were cultivated, producing 0.416 million tonnes of seed and 0.150 million tonnes of oil[6]. Whereas only 14% of the requirement fulfilled of the country by local production, this is insufficient to meet the country's needs[7]. One of the most important aspects of pre-breeding is the assessment of variability. It provides a clear understanding of the genetic structure and diversity within a crop and aids in selecting inherently diverse genotypes for breeding program[8]. Genetic diversity study serves as the foundation for developing novel varieties and is crucial for the assessment, conservation, and use of germplasm resources[9,10]. In Pakistan, the existing mustard varieties are low in seed production. It is necessary to hybridize existing high yielding and suitable breeding strategies to generate useful genetic variations for desired traits[11]. Abiotic stresses negatively impact on plant growth and productivity, often leading to a decline in overall plant health[12]. In Brassica crops, these stresses not only reduce seed yield but also negatively influence oil content and quality. Water stress also has a negative impact on several growth phases during plants lifecycle, which decrease the production of crops[13]. Water stress directly affects flowers, pollinators and eventually reduced seed production in flowering plants[14]. Stress can interrupt the sexual reproduction of plants, like flowers, fertilization, and seed development, which cause yield reductions in crops[15]. Drought causes a significant decrease in the number of flowers in the crops which ultimately reduces number of siliques and consequently yield[16]. Water induced a substantial reduction in yield, physiology, growth and negative effects on production by fluctuating several physiological and yield-related traits[17]. In agriculture heterosis is known as the most effective exploited genetic phenomena, and its application has significantly contributed to improving crop quality and ensuring food security[18]. Plant breeders and farmers exploited heterosis over a century, in open cross-pollinated crops like *Zea mays* are the most successfully commercialized examples, Nevertheless, the full potential inbred of heterosis still poorly understood[19]. Heterosis plays a pivotal role to enable the development of superior hybrids using crossing and selection among diverse parental lines, while a major test for breeders is accurately predicting and efficiently selecting of most capable cultures[20]. Heterosis is the superiority of a hybrid over its parents, often measured against a standard variety under specific conditions. Among the various breeding options, heterosis is a natural phenomenon, by which F_1 hybrids compared with their inbred parents and exploited, extensively used in self-pollinated plants such as mustard[20]. The estimation of heterosis over the mid and better parent is useful in identifying truly heterotic cross combinations; however, such crosses are extremely valuable when they outperform the standard or best-performing variety in region[21].

2. MATERIALS AND METHODS

The existing experiment commenced to assess drought resistant mustard cultivars on genetic basis. Hence, the six screened, high yielding and stress resistant mustard verities such as (AARI-Canola, Khanpur Raya, Dhoom-1, Super Raya, Galaxy and Coral-432) were hybridized half diallel 6 × 6 method of[22]. Then the parents plus their respective fifteen (15) F_1 progeny were cultivated in randomized complete block design having three replications and two treatments at Botanical Garden, SAU, Tandojam. Treatments: T_1 known as Well-watered at (stem growth, flowering, silique formation and maturity stages) and T_2 known as Water stressed at (stem growth and flowering stages), respectively. These agronomical, physiological and oil characters were investigated, like plant height (cm), branches plant^{-1}, seed yield plant^{-1}, seed index (1000 seed weight, g), SPAD chlorophyll, relative water content (%), oil content (%) and protein content (%). For measuring data 10 plants were taken from each replication of the cultivars. Soil and meteorological parameters are also observed in these experiments. All cultural practices also done like spaces between plants and among the rows were kept at 10 and 30 cm, while nitrogen 40, phosphorus 24 and potash 20 kilo gram acre^{-1} was applied respectively. Data were analysed using Statistics 8.1 software and Microsoft office excel 2016.

Table 1. Soil examination of experimental field (botanical garden).

Trials	Depth (Inch)	Soil structure	EC (1:2) dSm^{-1}	pH (1:2)	Biological substance	Water content %	Water holding capacity%
1	0-12	Silty clay loam	0.56	7.8	0.51	21.24	48.8
2	0-12	Silty clay loam	0.80	7.9	0.59	21.66	50.6
3	0-12	Silty clay loam	0.60	8.0	0.42	21.40	49.6

Table 2. Rainfall and temperature data were observed.

Months	Overall rainfall (mm)		Temperature			
	Overall	Avg	Min: ˚C		Max: ˚C	
			Overall	Avg	Overall	Avg
Oct, 2019	0.0	0.0	628.0	20.3	1123.5	36.2
Nov, 2019	0.0	0.0	464.0	15.5	865.0	28.8
Dec, 2019	0.0	0.0	277.0	8.9	730.5	23.6
Jan, 2020	0.0	0.0	202.0	6.5	695.5	22.4
Feb, 2020	0.0	0.0	292.0	10.0	821.5	28.3
Mar, 2020	0.0	0.0	444.5	14.3	1004.0	32.4

2.1 Statistical analysis

Analysis of variance was accomplished according to[23], and the least significant difference (LSD) trial was utilized to evaluate treatment mean differences.

2.2 Heterosis analysis

The heterosis was determined using data set of genotypes and their hybrids from the experiments, which was carried out during the third year of 2019-2020. According to[24], heterosis was estimated for each trait using the standard formulas for mid-parent heterosis (MPH) and better-parent heterosis (BPH), using the following formula as:

$$MPH = [F_1-(P_1+P_2) / 2] / [(P_1 + P_2)] \times 100 \text{ and } BPH = (F_1-HP) / HP \times 100$$

where F_1 represents the hybrid mean, P_1 and P_2 are parental means, and HP denotes the higher parent value.

3. RESULTS AND DISCUSSIONS

3.1 Mean Square

Analysis of variance revealed that all sources of variation (mean squares) genotypes, treatments, crosses, and parents vs. crosses (P vs. C), exhibited significant differences ($p < 0.05$) for most evaluated traits, like plant height (cm), branches plant^{-1}, silique plant^{-1}, seeds silique^{-1}, seed yield plant^{-1}, seed index (1000 seed weight, g), SPAD chlorophyll, relative water content (%), oil content (%) and protein content (%). Moreover, genotype × treatment interactions were also significant differs for most traits. However, the breeding material demonstrated the highest degree of genetic variability (Table 03). Similar results were also reported by[25,26,17,27].

Table 03. Variance analysis of mustard cultivars

Source of variances	Replication	Genotypes	Treatments	G × T	Parents	F_1 hybrids	Parents vs. Crosses	Error
D.F.	03	20	01	20	05	14	01	123
Traits								
Plant height	13.1	596.6**	15502.2**	200.2**	431.22**	503.4**	2832.13**	8.6
Branches plant^{-1}	0.0711	12.7242**	97.2213**	1.4365**	1.1681**	6.9731**	140.121**	0.058
Seed yield plant^{-1}	4.192	130.782**	969.874**	4.453*	84.231**	92.542**	890.212**	2.240
Seed index	0.1160	1.3425**	19.1234**	1.2812**	1.2502**	1.8792**	6.2303**	0.034
SPAD Chlorophyll	7.14	280.11**	3690.35**	11.96*	50.33**	22.89**	5001.21**	4.26
Relative water content	3.75	34.87**	3140.21**	5.98*	18.215**	8.89**	502.23**	1.88
Oil content	1.172	9.621**	112.721**	2.463**	7.4972**	2.2314**	130.123**	0.201
Protein content	1.187	6.241**	961.763**	6.101**	9.241**	4.34**	17.127**	0.407

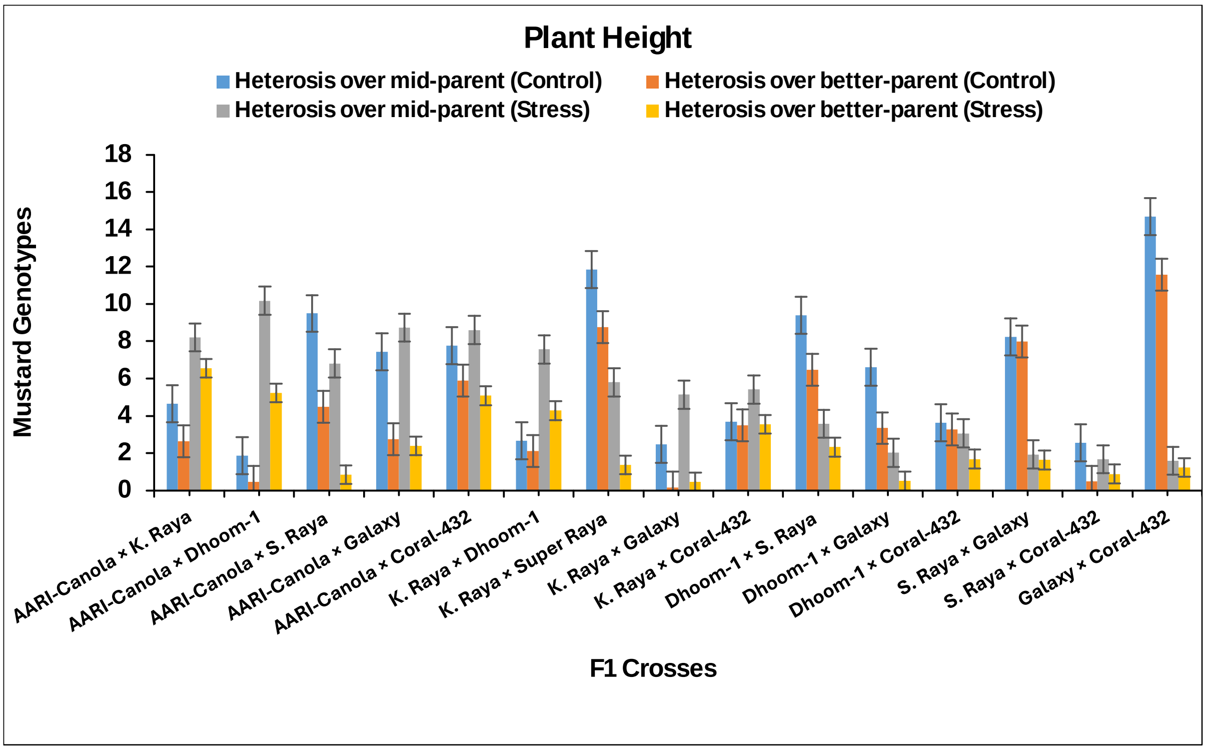
** denotes 1% level of significance; * denotes 5% level of significance; ns shows non significant

3.2 Heterosis estimation

Estimates of heterosis over mid and better parents for various genetic parameters are mentioned in (Figures 1-4).

3.3 Agronomical Traits

The most widely accepted approach for enhancing the productivity of crops is using heterosis, and breeders need to identify the genotype lines exhibit the performance between crosses. In this research, it was shown that there is an extremely high level of heterosis in mustard parental lines that have been observed. Several recent breeders have revealed a wide range of desirable heterotic effects for yield in different field crops[28,29,30,31,32,33,34,35]. In commercially important species ranging from maize to oil palm, hybrid crop variants substantially surpass their inbred parents in performance. While inbred breeding does not need much time or resources, hybrid breeding does take more effort and resources[36]. Plant height is an important agronomic characteristic for crops, especially oilseed crops like mustard. It has an impact on development of seeds, content of oil, and lodging resistant[37]. In mustard, height of plant is preferred middle to heigh, while F_1 crosses effects will be useful in upcoming breeding plans. In crosses, there was a desirable extent of heterosis over the superior parent when it came to plant height under water stress. The crosses under well-watered, Galaxy×Coral-432 (mid parent = 14.70%; better parent = 11.58%), followed by Khanpur Raya×Super Raya (mid parent = 11.85%; better parent = 8.75%) reported maximum heterosis, whereas the highest heterosis exhibited by AARI-Canola×Dhoom-1 (10.17%) and AARI-Canola×Khanpur Raya (6.56%) over mid and better parent, in water scarcity environments, respectively (Fig 1). Previous researchers[38,39,40] had also found substantial positive standard heterotic effects in F_1 combinations of mustard for plant height. Characteristics that contribute to seed yield in mustard are known as yield contributing characters. The improvement of mustard hybrids through favourable heterotic effects has great value in conventional breeding. Under two growing conditions for yield contributing traits branches plant^{-1}, no one hybrid demonstrated negative heterosis over the mid or superior parent. Nevertheless, the higher heterosis over best parent were recorded in AARI-Canola×Coral-432 for mid (50.49%) and better parent (43.47%) under normal conditions, however under water deficit, AARI-Canola×Dhoom-1 and Dhoom-1×Coral-432 reported (49.34%) and (44.76%) (mid and better parent) heterosis. The amount of heterotic over superior parents in this investigation was significantly larger than that reported for branches plant^{-1} by[41,42]. However, two hybrids Dhoom-1×Croral-432 (71.79%) and Galaxy×Coral-432 (108.11%) were found best for better parent heterosis in both conditions respectively. According to recent research on heterosis[39,40], where large number of hybrids (125) were evaluated, however, the amount of superior parent heterosis was lower than that reported in current research. Hence, above mentioned hybrids may prove excellent source to develop hybrid cultivar in mustard.



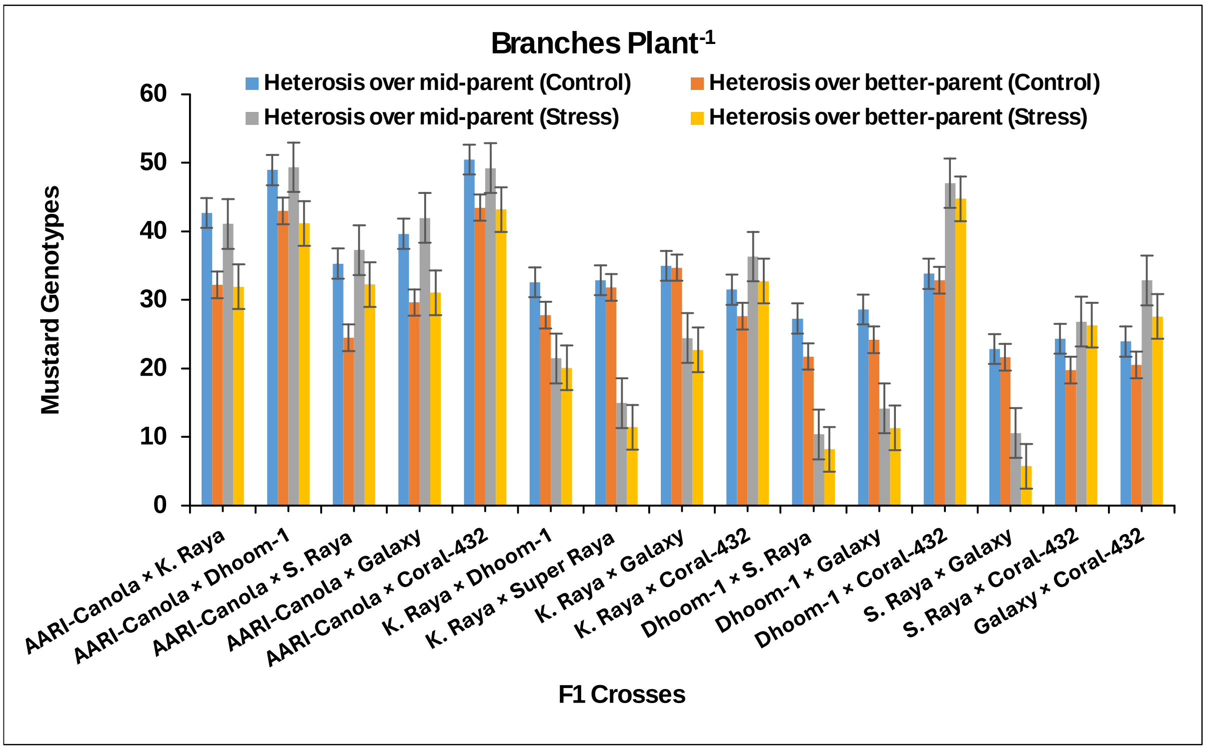
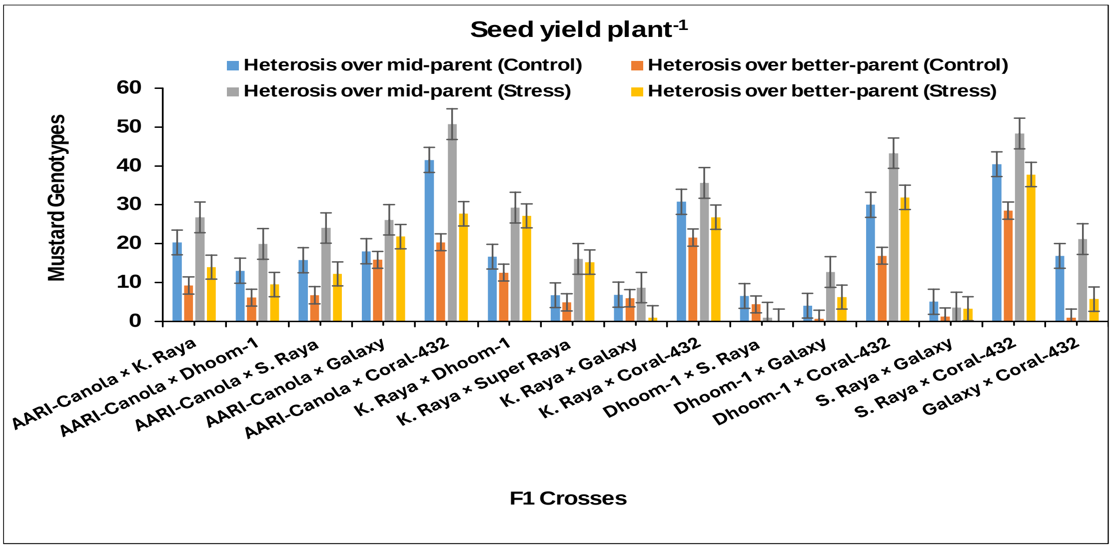


Fig.1. Heterotic effects of F_1 hybrids for plant height and branches plant^{-1} in well watered and water stress environments of mustard.

3.4 Yield Traits

Pertaining the heterosis for seed yield plant^{-1}, the crosses AARI-Canola×Coral-432, Super Raya×Coral-432 (41.55%, 40.47%) and AARI-Canola×Coral-432, Super Raya×Coral-432 (50.75%, 48.36%) depicted maximum heterosis over mid parent in well-watered and water deficit environments, Nevertheless Super Raya×Coral-432, Khanpur Raya×Coral-432 (28.52%, 21.53%) and Super Raya×Coral-432, Dhoom-1×Coral-432 (37.79%, 31.92%) expressed better parent heterosis under both conditions. However for seed index the crosses Super Raya×Coral-432 (15.20%), Khanpur Raya×Coral-432 (10.80%) and Super Raya×Coral-432 (29.14%), Galaxy×Coral-432 (21.59%) reported positive over mid parent under both conditions, while the better parent heterosis shown by Super Raya×Coral-432 (13.99%) and Super Raya×Coral-432 (26.72%) in well-watered and water stress conditions (Fig 2). The above combinations in both conditions expressed a significant degree of heterotic effects over mid and better parent; hence these hybrids may be used on priority level in hybrid breeding programs. More notably, except for two F_1 hybrids, all other hybrids showed positive heterosis in well-watered and water deficit, indicating the genetic capacity of the breeding resources employed in the research. For utilizing these genetic resources for hybrid development, the significant breeding efforts are required. Various researchers reported varying degrees of superior parent heterotic effects for seed yield and seed index. Although, heterotic limit was lower than current results as disclosed by[38,43]. However, researchers like[39,25,44,26,45] reported the greater heterotic amount over better parent for seed yield in mustard.



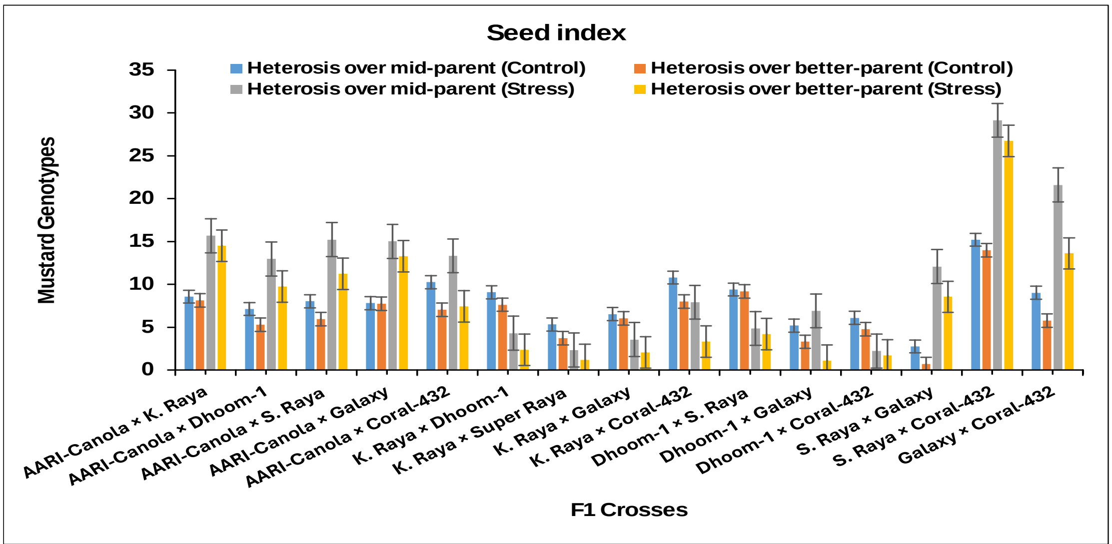


Fig.2. Heterotic effects of F_1 hybrids for seed yield plant^{-1} and seed index in well watered and water stress environments of mustard.

3.5 Physiological Traits

It is critical to assess physiological characteristics before beginning a stress breeding program; thus, the present research focused on 2 crucial traits of stress breeding (relative water content and SPAD chlorophyll). For SPAD chlorophyll in well-watered the maximum positive heterotic effects revealed by AARI-Canola×Super Raya (31.14%) and Super Raya×Galaxy (28.17%), over the mid and better parent. However, in water deficit the hybrid Super Raya×Galaxy (36.51%) and Khanpur Raya×Super Raya (32.93%) expressed the maximum positive heterotic effects over mid and better parent, respectively. Nevertheless, for relative water content the hybrid Super Raya×Galaxy (22.15%) and AARI-Canola×Super Raya (34.65%) witnessed highest positive mid parent heterotic effects in well-watered and water stress, nonetheless, the F_1 crosses expressed highest positive better parent heterosis by Super Raya×Galaxy (7.91%) and AARI-Canola×Super Raya (12.90%) under both conditions (Fig 3). As a result, these hybrids might be used to create vigorous and commercial hybrid genotypes of mustard genotypes, particularly for water stress breeding. In previous stress breeding work, the heterosis for these two parameters was also estimated, such as[46,25,26,17,27,47] reported heterosis in water deficit conditions for both traits (relative water content and chlorophyll), respectively.

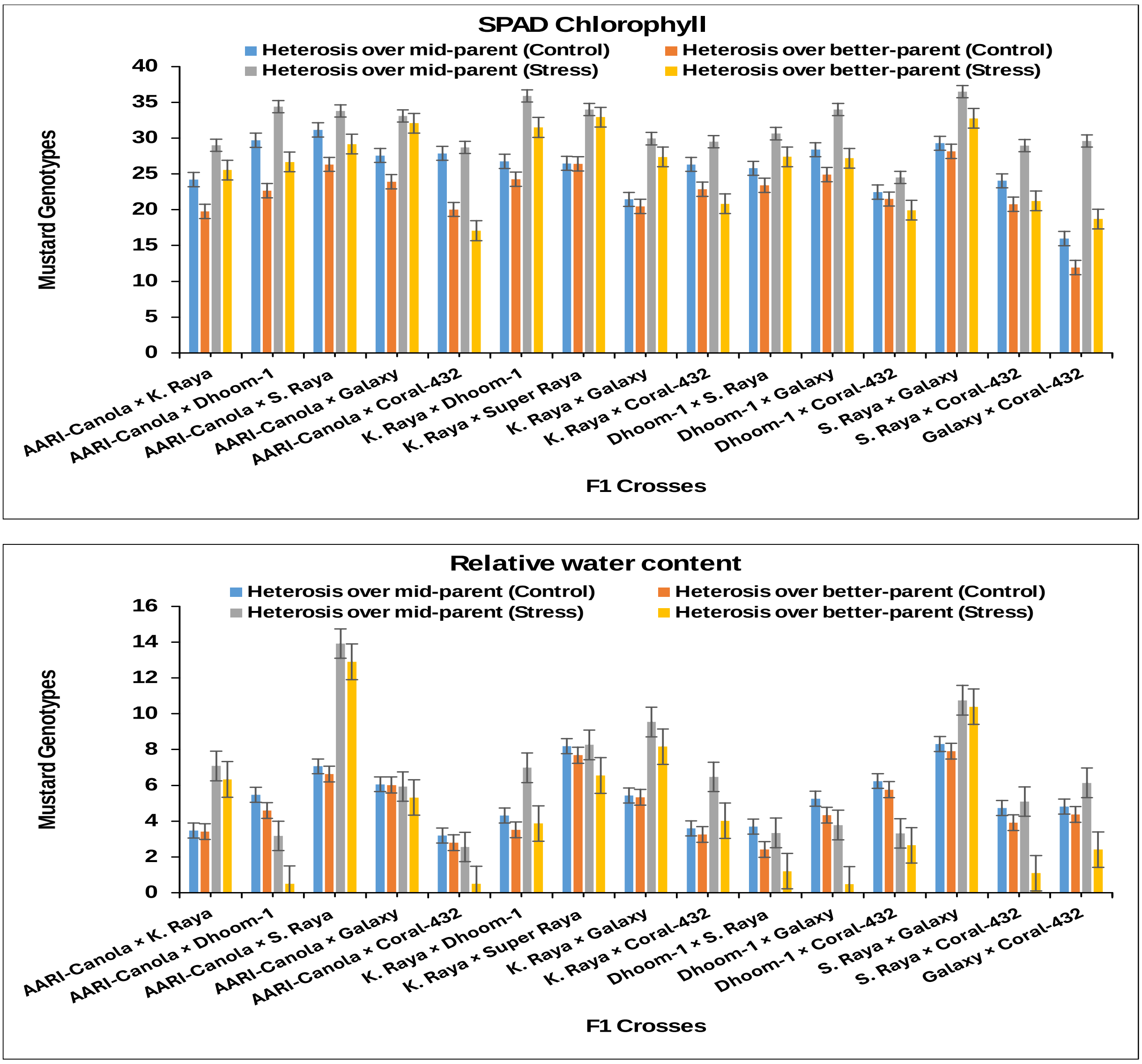
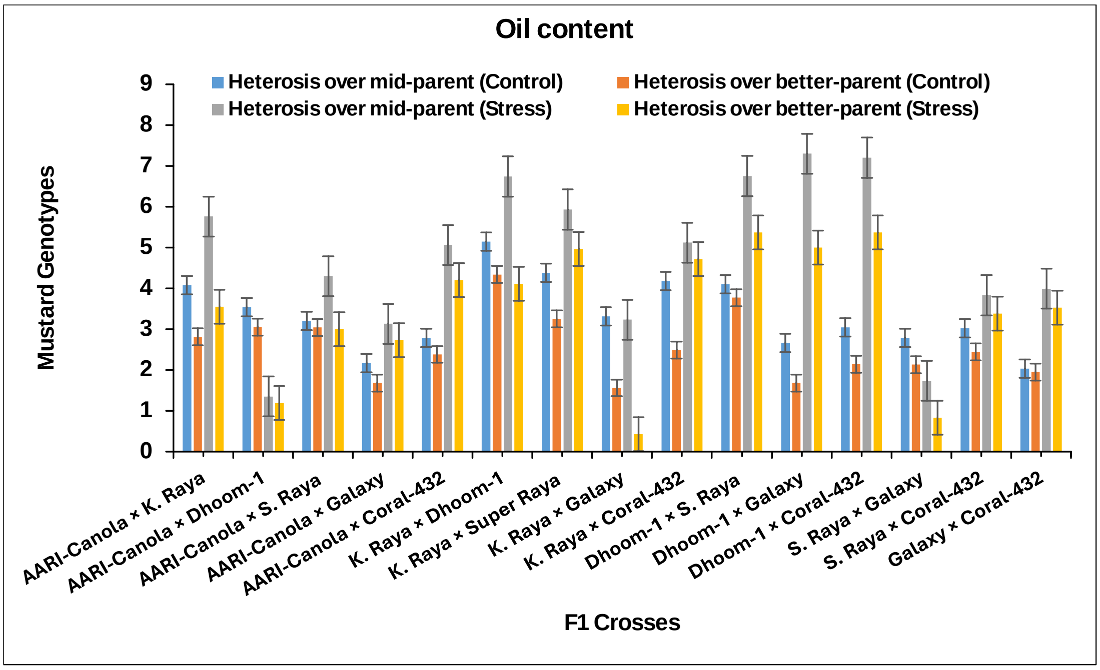


Fig.3. Heterotic effects of F_1 hybrids for SPAD chlorophyll and relative water content in well watered and water stress environments of mustard.

3.6 Oil yield traits

Positive heterosis effects are believed to be beneficial for choosing superior hybrids for oil and protein content since high oil and protein content is one of the most significant goals in mustard breeding. The exceptional effects of heterosis for oil content in well-watered (10.74%) and water stress (5.14%), highest mid parent heterosis observed in Khanpur Raya×Dhoom-1, Nonetheless positive better parent heterosis was estimated in same combination Khanpur Raya×Dhoom-1 (well-watered = 4.34% and water stress = 9.11%). Similarly heterotic effects for protein content the cross AARI-Canola×Super Raya recorded the maximum mid parent heterotic effects in well-watered (12.07%) and water stress conditions (5.37%), While the highest heterotic effects exhibited in Galaxy×Coral-432 (11.62%) and AARI-Canola×Super Raya (2.10%) over better parent, in well-watered and in water stress, respectively (Fig 4). The current result signifying the worth these genotypes tend to have for heterosis breeding. The heterosis for oil and protein content recorded in this study was greater than those previously reported by[38,39,25,48,49,50].



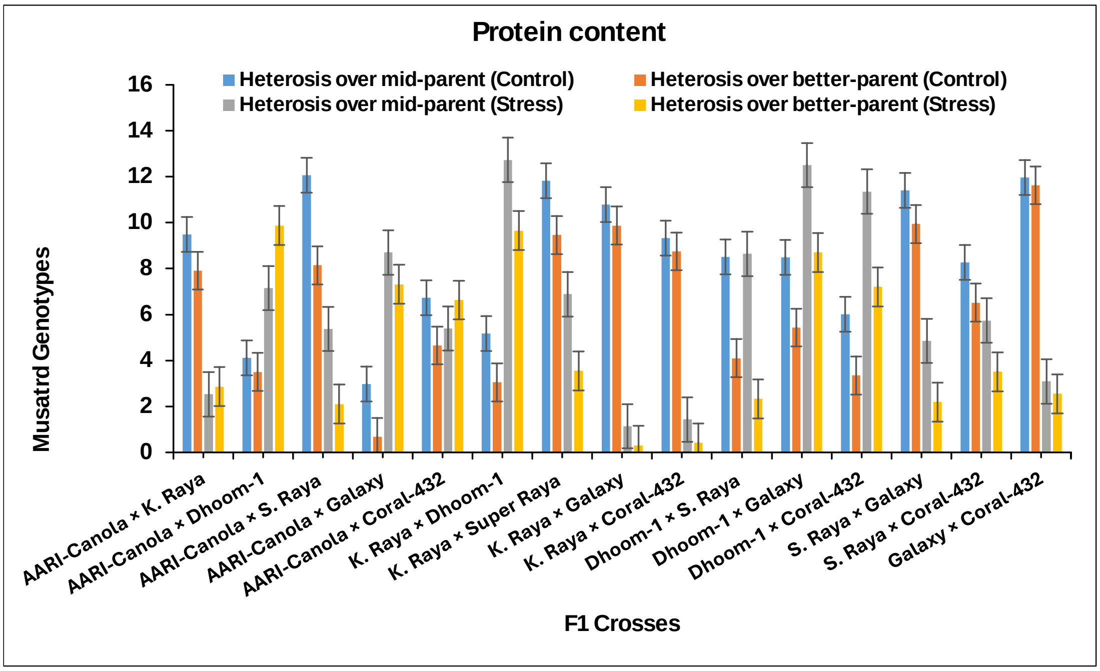


Fig.4. Heterotic effects of F_1 hybrids for oil content and protein content in well watered and water stress environments of mustard.

4. CONCLUSIONS

The present study demonstrated significant heterosis in mustard for agronomic, yield, physiological, and oil traits under both well-watered and water-scarcity environments. Identified crosses, particularly Galaxy × Coral-432 and Super Raya × Coral-432, could serve as base material for developing drought-tolerant mustard hybrids. The consistent expression of positive heterosis across traits highlights the strong genetic potential of the studied parental lines. These results also surpassed many earlier reports, emphasizing the novelty and breeding value of the identified combinations. Particularly, crosses showing high heterosis under stress conditions may serve as valuable genetic resources for developing climate-resilient hybrids. Hence, these combinations could play a vital role in developing high-yielding, climate-resilient mustard cultivars.

ACKNOWLEDGMENTS

I would like to extend my gratitude to all the authors and institutions whose work has significantly contributed to this research.

NOVELTY STATEMENT

This study presents novel findings on heterotic performance of mustard (*Brassica juncea*) hybrids under water stress conditions. It identifies new cross combinations with superior yield and oil traits, offering valuable genetic material for drought-tolerant mustard breeding programs.

AUTHOR'S CONTRIBUTION

Saim Bano conducted all research work and wrote the initial manuscript, Sajid Hussain Rao analysis the data and prepared the final manuscript, Abdul Wahid Baloch designed the experiment, Hajira Imran and Rabab Akram helped in the data collection, Muhammad Arsal and Asadullah checked the grammar of manuscript.

CONFLICT OF INTEREST

It is also declared that this Manuscript has no conflict of interest.

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