

Research Article

To determine the combining ability effects of mustard (*Brassica juncea* L.) genotypes for morphological and physiological characters in water deficit condition

Saima Bano¹, Sajid Hussain Rao^{1*}, Abdul Wahid Baloch², Rabab Akram¹, Hajra Imran¹, Muhammad Aarsal¹, Asadullah³ and Siraj Ahmad Channa²

1. Oilseeds Research Institute, Agriculture Research Sindh, Tandojam, Sindh, Pakistan

2. Department of Plant Breeding & Genetics, Sindh Agriculture University, Tandojam, Pakistan

3. Department of Research and Development, UPL Pakistan (Arysta Life Science) PVT Ltd, Sindh, Pakistan

*Corresponding author's email: sajidrao100100@gmail.com

Citation

Saima Bano, Sajid Hussain Rao, Abdul Wahid Baloch, Rabab Akram, Hajra Imran, Muhammad Aarsal, Asadullah and Siraj Ahmad Channa. To determine the combining ability effects of mustard (*Brassica juncea* L.) genotypes for morphological and physiological characters in water deficit condition. Pure and Applied Biology. Vol. 14, Issue 2, pp650-663. <http://dx.doi.org/10.19045/bspab.2025.140062>

Received: 05/01/2025

Revised: 06/03/2025

Accepted: 12/03/2025

Online First: 17/03/2025

Abstract

The water deficit is an important constraint for agricultural crops. The aim of the study is to elaborate highly genetically potential seeds and oil yield genotypes for water scarcity tolerance to enhance food security and sustainability in limited water resources. In this context, the six highly water stress tolerant mustard genotypes (AARI-Canola, Khanpur Raya, Dhoom-1, Super Raya, Galaxy and Coral-432) and their fifteen F₁ combinations were carried out for genetic analysis in randomized complete block design having three replications and two treatments at Sindh Agriculture University, Tando jam, Pakistan. The T₁ Control: (watered at stem growth, flowering, silique and maturity stages) and T₂ water stress at silique stage: (watered at stem growth and flowering stages) were set to evaluate the genetic traits as (plant height, branches plant⁻¹, silique plant⁻¹, seeds silique⁻¹, seed yield plant⁻¹, seed index, relative water content, chlorophyll, oil content and protein content) for water stress tolerant. The significant variances found are due to General Combining Ability and Specific Combining Ability effects between parents and hybrids. Two parents and five hybrids reported positive and maximum desirable General and specific combining effects for most of the studied characteristics under both conditions. These findings will contribute to the development of more resilient agricultural practices, ensuring that farmers, society and scientific communities can maintain productivity even in challenging environmental conditions by utilizing these parents and crosses in respect to water stress.

Keyword: Brassica juncea; combining ability; oil characters; water deficit; yield characters

Introduction

Mustard is one of the versatile and first domesticated oilseed crops. *Brassica spp.* has the highest commercial and nutritive value, due to its vegetable oil it comes in the world's third-largest position [1]. For decades, it has been used as a vegetable, as well as edible,

cooking, industrial oils and spicy flavor in food. The well-improved mustard has 40 to 45% oil content. Mustard has less than two percent erucic acid, which is acceptable on the international level [2]. The crops of the brassica group are the 2nd prime contributor of vegetable oil in Pakistan after cotton seed.

During 2018-19, 586 million acres cultivated with 337 million tonnes production of mustard and rapeseed [3]. During 2019-20, a total of 860 million acres cultivated with production of 458 million tonnes of mustard and other *Brassica* species, indicating the area production rise in later years. Nevertheless, it fulfills only 12% requirements of Pakistan, remaining 88% of edible oils are imported [4]. Pakistan is unable to produce sufficient edible oil due to increased demand and a fast-growing population [5]. The important aspect of plant breeders is the assessment of variability inherited in a crop and so aids in the selection of genetically varied parents for breeding programs [6]. Divergence in mustard must be created and assessed with respect to obtaining the required features in higher yield varieties [7]. To generate variations with enhanced seed production in Pakistan, necessary to cross existing higher yields and broadly adaptable genotypes [8]. Water scarcity is an essential cause accountable for diminishing agricultural construction because of its associations with other key abiotic factors like salt and heat stresses [9]. A variety of indicators are being employed in research and breeding methods, including leaf wilt, efficiency of water, stress susceptible and index relation [10]. The greater adverse impact on seed yield of water stress occurring at the flowering phases and at other stages of plant growth, it is very sensitive during pollen development, blossoming and pollination, resulting in declined yield production of seed [11]. The consequences of water deficit vary due to varieties, stress duration and intensity, weather, and the stages of *Brassica* growth and development [12]. According to [13] water stress after flowering reduces dry matter and seed production in both mustard and rapeseed, demonstrating that the reproductive phase is the most vulnerable to stress. Water reduced a significant loss in no. of pod plant⁻¹, seeds pod⁻¹, index of seed, yield and oil percentage of 5 rapeseed

germplasm [14]. The concept of combining ability is the basic tools to be used for the improvement of field crops. Between several mating designs, diallel analysis is a methodical technique that has been extensively employed in crop plants to assess genotype performance in hybrid combinations and to describe the kind and extent of gene activity involved in regulating quantitative traits [15]. A plant breeder's preliminary concern when generating potential hybridized varieties with sensible selection of parentage and breeding methods [1]. The effects of general and specific combination are prominent genetic approaches, which show the capacity of genotypes to generate improved crosses combinations in commercial *Brassica* breeding programs. The variances for GCA and SCA contain the additive and non-additive component of the overall distinction, which is mostly caused due to dominant and epistatic variations [16]. The investigation for the selection of breeding techniques and germplasm for hybridization can be beneficial due to combining ability [17]. The variation in results suggests the inheritance designs of plant characteristics giving yield variation with hereditary factual and environmental conditions, implying that genetic information about the breeding material would be explored before selection of the genotypes [18]. This research article clearly hypothesized, because the six drought tolerant mustard genotypes and their respective F₁ hybrids have potential genetic variation against water stress.

Materials and Methods

The existing investigation was conducted to evaluate the drought resistance in mustard cultivars on a genetic basis. Therefore, the six higher yields and stress tolerant mustard cultivars were crossed in 6 × 6 half diallel fashion [19]. Then these six potentially drought tolerant parents (AARI-Canola, Khanpur Raya, Dhoom-1, Super Raya, Galaxy and Coral-432) and their respective 15

F₁ hybrids of mustard were grown and the agronomical and physiological traits measured for genetic analysis in water deficit environments. The study designed in randomized complete block design at the examine field of Sindh Agriculture University, Tandojam, Sindh, Pakistan, with 3 replications and 2 treatments, during 2019-2020. The T₁ Normal: (watered at stem growth, flowering, silique formation and maturity stages) and T₂ water deficit at silique stage: (watered at stem growth and flowering stages). The parameters as plant height, branches plant⁻¹, silique plant⁻¹, seeds silique⁻¹, seed yield plant⁻¹, seed index, relative water content, SPAD chlorophyll, oil content and protein content were studied. All the cultural practices were timely done, and soil analysis was also carried out.

Morphological and physiological parameters

For data collection, ten plants from each genotype were chosen from each replication. The scale of centimeters was used to measure the plant height, and that is done from base to tip of longest inflorescence, when it reached at maturity. The total branches were counted from each selected plant after harvesting and so was also done for number of silique and seeds silique⁻¹. Similarly, the length of silique was noted from base to tip in centimeters. In grams, the seed yield from each silique was weighed from its obtained seeds. However, the total seeds of every particular plant weighed for seed yield plant⁻¹. The seed index was measured after 1000 seeds weighed in grams. Using the [20] technique, relative water content was calculated. By these techniques the sample leaf was taken and weighed as freshly weighed (FW), after that it was wilted in condensed water and turgid weight (TW) was taken, then these leaves were taken and dried for 24 hours at 72 °C to obtain dry weight (DW). In the end, measured the data by given formula.

$$RWC\% = [(FW-DW)/(TW-DW)] \times 100$$

Whereas, by using the SPAD meter SPAD chlorophyll was observed (Model: SPAD-502 Plus).

Oil and protein content

The Soxhlet extract method used for determining the oil content. By this technique, 1st made the individual sachet of dried grains sample of seeds two to three grams, then these sachets were kept in to Soxhlet device. After that the petroleum ether was put into the Soxhlet device with the help of chemical flask. Remove the samples from Soxhlet device after extract become clear and kept in the fresh air then in oven for drying. Finally, note the reading using the formula below as.

$$\text{First reading} - \text{Last reading} / \text{First reading} \times 100$$

The protein content was determined by using the reapproved technique of American Oil Chemists' Society (AOCS) 1997. In the existence of oxygen grains samples were wrinkled and heated at the temperature of (900 °C). As the resultant combustion released the carbon dioxide, water, and nitrogen. Then observed nitrogen content by using the factor 6.25 changed into crude protein content. The exhibited results were displayed in percentage by using the formula $N \times 6.25$.

Soil analysis

Soil texture

The soil texture (distribution of partial sizes) was determined by the technique of Bouyoucos hydrometer [21], by this method sodium hexameta phosphate used ten percent to separate the soil grains. The thickness of soil is determined to silt+clay and clay alone by using a hydrometer. The standard triangle was used to determine the soil texture, and the silt was categorized as clay loam (Table 1).

Soil Electrical Conductivity (EC)

The EC meter was used to determine the electric conductivity through soil water extract at the distilled water extract 1:2 (soil: distilled water) stated by [22] (Table 1). The reading is recorded in dSm^{-1} .

Soil reaction (pH)

The pH meter was used to determine the soil reaction pH through soil water extract at the distilled water extract 1:2 (soil: distilled water) stated by [22] (Table 1).

Organic matter

The organic material of soil was determined by determining soil carbon using the chromic acid titration or Walkley and Black method as reported by [23] (Table 1).

Soil water content

The water content in soil was determined by gravimetric technique reported as [24]. The samples were obtained from the soil of experimental field and promptly moved to crucibles. These were pre-weighed with a tight-fitting cover for the observations. Immediately, the weight of the soil moisture with crucibles was recorded. After removing the cover, the crucibles stayed in oven till 48h at 105°C until they reached on constant weight. After that, immediately remove crucibles from the oven and enclose with the cover lid and chill it in the desiccator. Afterward cooling, the crucibles holding the oven dry soil weight with cover was weighed. The data was recorded in (Table 1) by following formula.

Cane weight with lid = Wc (g)

Moist soil weight with cane and lid = Wcws (g)

Dry soil weight with cane and lid = Wcds (g)

Water loss weight during drying = Wcws - Wcds = Ww (g)

Dry soil weight = Wcds - Wc = Wods (g)

Weight of Moisture = (Ww/Wods) × 100 = Θ_w (%)

Water holding capacity

The capacity of soil for holding the water was obtained by percolation method. A bucket auger was used to collect soil samples from the experimental field, after that the soil was air-dried and crushed using a grinder then passed in a sieve of 2.0 mm for the observations. Filter sheets were used in three funnels, which were labelled X, Y, and Z. Then, the dried samples of soil in 50 grams were placed in X, Y, and Z labelled funnels. After that, take 50ml of water and pour slowly in each funnel. When the water dipping halted from the funnel, then noted the volume in measuring cylinder of filtered water. Finally, the results were calculated (Table 1) with formula as given below.

Soil weight = (X)

Poured volume of water = (Y)

Filtered water volume in measuring cylinder = (Z)

Retained water volume of soil = (Y-Z)

Capacity for Water holding of soil in percentage = $(Y-Z) / X \times 100$

Table 1. The soil data of experimental field

S. #	Deepness (Inc)	Texturing of Soil	Electrical conductivity dSm ⁻¹	Soil pH	Organic matter	Water content %	Water holding capacity%
1	0-12	Silt Clay Loamy	0.60	7.8	0.52	21.24	48.8
2	0-12	Silt Clay Loamy	0.79	7.9	0.64	21.66	50.6
3	0-12	Silt Clay Loamy	0.65	7.8	0.48	21.40	49.6

Meteorological traits

The data for rain and temperature was observed during the growing season, no rain

occurred, While the maximum and minimum mean temperature remained 6.5 (Min) and 36.2 (Max), respectively.

Statistical investigation

The [25] method used for measuring the analysis of variance and determined the superior treatment means with least significant difference (LSD) test. The numerical method of [19] was used for performing the diallel analysis also refer by [26]. The analysis of combining ability, mean squares and estimations, was obtained using method-2, Model-I of [19], which covers parentage and offsprings, by given equation below.

$$Y_{ij} = u + g_i + g_j + s_{ij} + r_{ij} + 1/bc \sum \sum e_{ijkl}$$

The general and specific combining ability were noted for mean squares given as under:

Sum of square for general combining ability = $1/n+2 [\sum (Y_{i.} + Y_{.i})^2 - 4/n \sum Y^2 \dots]$

Sum of square for general combining ability = $\sum \sum Y_{ij}^2 - 1/n+2 \sum (Y_{i.} + Y_{.i})^2 + 2/(n+1) (n+2) Y^2 \dots$

Sum of square for error = SS Error/r

Results and Discussion

Combining ability estimates

In every breeding effort that is aiming at creating improved hybrid genotypes, the evaluation for yield of seeds and its contributing traits of breeding materials for combining ability is required. It is very desirable that productive plants, which may contain genes for increased seed and oil

content, those genes certainly increase production and stability of the genotypes. The creation of such lines depends on the population's understanding of combining ability and genetic architecture [27]. Griffing's approach of diallel analysis proved beneficial in identifying parents for hybrid combinations [28]. In previous years, various breeders of different field crops have evaluated the genetically potential crop varieties using diallel fashion [29-34]. Analysis of variance exhibited significantly differed for all studied traits and general and specific combiner in well-watered and water deficit, demonstrating the role of additive gene effects as well as non-additive gene effects. Nevertheless, the SCA means square highest as compared to the GCA for greatest number of characters under both growing environments (Table 2 & 3). In mustard crops for developing hybrids and open fertilized varieties, GCA and SCA are employed as primary markers, by which identification and selection made possible for suitable inbred lines [35]. The significant general and specific combining effects of mean squares were observed for morphological and yield characters, across diverse populations of mustard by [1, 27, 31, 36].

Table 2. Analysis of variance of mustard genotypes

SOV	Replication	Genotypes	Treatments	G × T	Parents	F ₁ Crosses	Parents vs. Crosses	Error
Degree of freedom	3	20	1	20	5	14	1	123
Parameters								
Plant height	12.7	587.8**	15431.9**	190.0**	420.73**	487.7**	2824.96**	9.7
Branches plant ⁻¹	0.0642	11.8188 **	96.6228 **	0.5632**	1.0793**	6.5463**	139.331**	0.0623
Silique plant ⁻¹	835	246355 **	734450**	2387**	25785**	62523**	3922849**	779
Seeds silique ⁻¹	0.035	24.577 **	217.021**	0.567 ^{ns}	3.2317**	3.713**	32.9304**	0.366
Seed yield plant ⁻¹	3.237	129.152 **	970.293**	1.060 ^{ns}	83.176**	91.290 **	889.101 **	2.240
Seed index	0.0059	0.8745 **	18.0518**	0.1960**	0.29103*	0.6924**	6.3406**	0.0455
SPAD Chlorophyll	4.75	277.34**	3681.94**	3.75 ^{ns}	48.12**	21.97**	4998.63**	5.39
Relative water content	2.46	33.93 **	3137.53**	4.56*	17.367**	6.95**	494.56**	2.29
Oil content	0.186	8.814**	103.212**	1.449**	6.5503**	1.2963**	125.383**	0.289
Protein content	0.076	5.394**	954.334 **	5.952**	8.1152**	3.65 **	16.184**	0.487

** shows the significant level at 1%; * shows the significant level at 5%; ns shows non-significant

Table 3. Analysis of variances for GCA and SCA effects

SOV	Control			Water stress at silique formation		
	GCA	SCA	Error	GCA	SCA	Error
	(5)	(14)	(60)	(5)	(14)	(60)
Plant height	115.90**	98.85**	2.73	278.04**	39.54**	1.96
Branches plant ⁻¹	1.71**	1.89**	0.02	1.75**	1.33**	0.01
Silique plant ⁻¹	26021.37**	34895.58**	150.21	13811.19**	40837.32**	223.46
Seeds silique ⁻¹	1.47**	5.16**	0.16	0.58**	3.27**	0.02
Seeds yield plant ⁻¹	24.21**	15.17**	0.84	20.52**	15.54**	0.28
Seed index	0.04**	0.06**	0.02	0.21**	0.21**	0.01
SPAD Chlorophyll	7.70**	45.55**	1.96	11.85**	45.63**	0.77
Relative water content	0.77**	5.46**	0.54	3.92**	7.61**	0.60
Oil content	0.16**	0.85**	0.02	0.71**	2.52**	0.12
Protein content	0.46**	2.23**	0.15	0.92**	1.28**	0.10

** shows the significant level at 1%; * shows the significant level at 5%; ns shows non-significant

Agronomical and yield traits

Plant height is an essential feature of *Brassica* that is directly related to the time of blooming. The plant becomes taller before flowering due to the main stem growth and development [37]. Two parents AARI-Canola recorded the greater and positive effects of GCA in control (5.49) and water stress conditions (9.20), whereas the genotype Khanpur Raya was next in ranking for GCA effects (Table 4). Six crosses such as Khanpur Raya × Super Raya (16.50), Galaxy × Coral-432 (15.07) and AARI-Canola × Super Raya (9.87) were on top for encouraging the effect of GCA in control condition. However, in water deficit conditions, AARI-Canola × Dhoom-1 (7.14), AARI-Canola × Coral-432 (6.35) and AARI-Canola × Galaxy (6.31) disclosed positively highest SCA effects of plant height (Table 5). This demonstrates their genetic potential to enhancing rapeseed's valuable characteristics. The current plant height findings were consistent with previous studies [27, 31, 36], which showed that there were extremely substantial genotypic variations in plant height in parents and their crosses. Yield related components are important in plant breeding because such traits help to enhance crop yield indirectly. Under two growing conditions, the parent

AARI-Canola displayed positively and greatest effects of GCA like 0.84 & 0.79 in normal and water deficit environments (Table 4). Although crosses discovered highest positive SCA effects of AARI-Canola × Coral-432, AARI-Canola × Dhoom-1, AARI-Canola × Galaxy and Khanpur Raya × Galaxy (1.46, 1.36, 0.90 and 0.81) and AARI-Canola × Dhoom-1, Dhoom-1 × Coral-432, AARI-Canola × Galaxy and AARI-Canola × Super Raya (1.33, 1.19, 0.91 and 0.36) for branches plant⁻¹ under both growing conditions (Table 5). Similarly, three parents including AARI-Canola, Dhoom-1 and Coral-432, while whole set of crosses (15) reported positive GCA effect for silique plant⁻¹ in all growing treatments (Table 4 & 5). The top rank general combiner was AARI-Canola with effects of (stress = 0.77 and non-stress = 0.45), however, top rank specific combiners were Galaxy × Coral-432 (1.90), Super Raya × Coral-432 (1.84), AARI-Canola × Khanpur Raya (1.72) and Khanpur Raya × Dhoom-1 (1.55) in well-watered while, in water stress AARI-Canola × Khanpur Raya, Khanpur Raya × Coral-432 and AARI-Canola × Super Raya effects were (1.76, 1.50 and 1.39) (Table 4 & 5). For seed index, parents AARI-Canola and Galaxy (Control = 0.09 and stress = 0.28,) and (Control= 0.02 and stress =

0.06), while crosses Super Raya × Coral-432 (0.39), followed by Khanpur Raya × Dhoom-1 (0.26) and Dhoom-1 × Super Raya (0.24) under control and Super Raya × Coral-432 (0.86), followed by Galaxy × Coral-432 (0.68) and AARI-Canola × Galaxy (0.38) under water stress exhibited positive effects (Table 6 & 7). The genotypes indicated above tended to exhibit a desired combining ability in subsequent generations with acceptable performance, and so would be favored in mustard breeding efforts. The dominance or dominance × dominance gene action was a possible reason for excellent SCA effects in hybrids of low × low general. However, those crosses could be used for heterosis breeding. Positive and considerable combining ability was also observed for yields contributing traits in mustard genotypes by [1, 27, 31, 36, 38]. The breeder's essential aims and effort is to raise seed production. Combining ability effects in plant breeding is very important to identify possible parentage consents in cross combinations [39]. In this research, some top parents for seed yield

identified significance and favorable GCA effects. In both growing conditions (control and water deficit), the parent AARI-Canola (3.30 and 0.09) under both conditions and crosses AARI-Canola × Coral-432, Dhoom-1 × Coral-432, Khanpur Raya × Dhoom-1, AARI-Canola × Khanpur Raya and AARI-Canola × Galaxy (5.10, 5.01, 3.33, 2.36, 2.66 and 2.31) under control and Super Raya × Coral-432 (4.97), AARI-Canola × Coral-432 (4.41) and Dhoom-1 × Coral-432 (3.76) in water deficit condition (Table 4 & 5) were identified with significant and desirable combining ability effects, suggesting that these genotypes will improve grain yield in hybrids, especially when water is scarce. As a result, these genotypes would be regarded as the finest general and specific combinations. The low × low general combiner gives the good specific combiner in *Brassica* germplasm, Reported like [37, 40]. The positive seed yield effects due to GCA and SCA in *brassica juncea* were also observed by [1, 27, 36, 38, 41-43].

Table 4. Effects of GCA for effects for various agronomical traits

Parents	Plant height		Branches plant ⁻¹		Silique plant ⁻¹		Seeds silique ⁻¹		Seed yield plant ⁻¹	
	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit
AARI-Canola	5.49	9.20	0.84	0.79	3.30	3.14	0.77	0.45	0.11	0.13
Khanpur Raya	0.04	4.82	-0.22	-0.25	-1.26	-0.76	0.06	0.08	-0.07	0.05
Dhoom-1	-3.09	-1.70	0.14	-0.15	-0.84	-0.70	-0.23	-0.09	-0.07	-0.05
Super Raya	-4.83	-5.56	-0.45	-0.29	-1.30	-1.37	-0.51	-0.35	0.00	-0.13
Galaxy	-0.60	-5.54	-0.31	-0.42	0.42	-0.13	-0.09	-0.15	0.001	0.06
Coral-432	3.00	-1.22	0.01	0.33	0.31	-0.18	-0.002	0.05	0.03	-0.07
SE (gi)	0.53	0.45	0.05	0.03	0.30	0.17	0.13	0.05	0.03	0.03

Table 5. Effects of SCA on different agronomical traits

F ₁ hybrids	Plant height		Branches plant ⁻¹		Silique plant ⁻¹		Seeds silique ⁻¹		Seed yield plant ⁻¹	
	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit
AARI-Canola × Khanpur Raya	0.94	4.00	0.77	0.80	2.36	2.36	1.72	1.76	0.73	0.57
AARI-Canola × Dhoom-1	1.80	7.14	1.36	1.33	0.41	0.41	1.23	1.00	0.32	-0.09
AARI-Canola × Super Raya	9.87	3.33	0.64	0.86	2.08	2.08	1.25	1.39	0.65	0.59
AARI-Canola × Galaxy	1.85	6.31	0.90	0.91	2.31	2.31	0.38	0.36	0.66	0.44
AARI-Canola × Coral-432	5.97	6.35	1.46	0.75	5.01	5.01	1.12	0.62	0.49	-0.41
Khanpur Raya × Dhoom-1	-4.95	4.75	0.48	0.10	2.66	2.66	1.55	1.02	0.85	0.68
Khanpur Raya × Super Raya	16.50	3.87	0.75	0.07	-0.42	-0.42	1.12	0.50	0.04	-0.13
Khanpur Raya × Galaxy	-3.90	2.32	0.81	0.48	-0.83	-0.83	0.59	0.70	0.11	-0.34
Khanpur Raya × Coral-432	0.43	2.88	0.50	0.62	1.87	1.87	1.43	1.50	0.64	0.19
Dhoom-1 × Super Raya	-12.41	1.16	0.45	-0.23	-2.37	-2.37	0.72	0.39	0.22	-0.08
Dhoom-1 × Galaxy	7.96	-1.39	0.46	-0.13	0.61	0.61	0.67	0.90	0.29	-0.05
Dhoom-1 × Coral-432	3.76	-0.22	0.79	1.19	3.33	3.33	1.26	0.81	0.73	0.53
Super Raya × Galaxy	4.14	-0.32	0.30	-0.06	-0.46	-0.46	-0.01	0.12	0.54	0.14
Super Raya × Coral-432	-5.17	0.67	0.16	0.38	5.10	5.10	1.84	1.15	0.95	1.13
Galaxy × Coral-432	15.07	-1.74	0.14	0.44	0.41	0.41	1.90	1.15	0.83	1.00
SE (si)	1.47	1.24	0.13	0.09	0.81	0.81	0.36	0.14	0.09	0.08

Physiological traits

The highest effects for chlorophyll of GCA in well-watered were reported in Dhoom-1 (1.03), while under water stress expressed by Coral-432 (1.85) (Table 6). Although, the highest positive effects of SCA exhibited in Khanpur Raya \times Coral-432, Super Raya \times Galaxy and AARI-Canola \times Dhoom-1 with values of 5.70, 5.33 and 5.24, in control conditions respectively, whereas Khanpur Raya \times Dhoom-1 (4.59), Super Raya \times Galaxy (4.55) and AARI-Canola \times Dhoom-1 (4.34) in water stress condition (Table 7). The parent Dhoom-1 was found on top for relative water content with GCA effects highest and positive of 0.46 & 0.86 in both environments (Table 6). Nevertheless, the SCA effects greatest of 3.40, 2.42 and 2.03 were depicted by Khanpur Raya \times Super Raya, Dhoom-1 \times Coral-432 and AARI-Canola \times Dhoom-1, under well-watered condition, whereas under water stress AARI-Canola \times Super Raya (4.98) and Super Raya \times Galaxy (2.89) showed maximum, respectively (Table 7). These parentages and hybrids might be used as possible genotypes for a mustard hybridization program aimed at improving and enhancing physiological characteristics related to water stress resistance. By increasing the frequency of beneficial genes, crosses with at least one parent having good GCA effects will improve crop production. The positive effects of the combined ability for these physiological parameters in control and drought conditions were also observed by [44] in wheat; [45] in sunflower; [46] in potato; [33] in cotton and [47] in rice.

Oil yield traits

Seed oil and protein content are a highly essential trait in determining the economic worth of mustard. The parentage and crosses positive effects due to GCA and SCA being greatly useful in mustard breeding. Coral-432 (0.16) and Galaxy (0.04) had noteworthy GCA effects for oil content in both the growth environments, indicating that these genotypes might contribute favorable alleles for oil improvements (Table 6). [36] reported also positive GCA effects in six parents from nine parents for oil content. The SCA effects discovered maximum and positive under both conditions by Khanpur Raya \times Dhoom-1 (0.94), AARI-Canola \times Khanpur Raya (0.84), Dhoom-1 \times Super Raya (0.66) and Khanpur Raya \times Dhoom-1 (1.91), Khanpur Raya \times Super Raya (1.48), Dhoom-1 \times Galaxy (1.33), respectively, (Table 7). The GCA effects witnessed the largest and positive by parentage Dhoom-1 for protein content with (0.29) and (0.55) in both environments (Table 7). The F₁ such as AARI-Canola \times Coral-432 (2.17), Khanpur Raya \times Galaxy (1.52) and AARI-Canola \times Super Raya (0.66), Khanpur Raya \times Galaxy (0.56) reported the SCA effect in all conditions greatest and positive (Table 7). The selection over the next generations would likely have a significant impact on the oil and protein content by using these crosses. [42] investigated only SCA effects in two successive generations of mustard genotypes, the consequences of the research displayed that eight crosses were in the direction of positive SCA effects in all environments. Similarly, GCA and SCA positive and significant effects also noted by [38] in mustard for oil and protein.

Table 6. Effects of GCA for various yields, physiological and oil traits

Parents	Seed index		SPAD chlorophyll		Relative water content		Oil content		Protein content	
	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit
AARI-Canola	0.09	0.28	-1.32	-1.41	-0.24	-0.68	0.02	0.30	0.21	0.20
Khanpur Raya	0.06	-0.04	-0.18	-0.44	-0.19	0.34	-0.24	-0.50	0.12	0.04
Dhoom-1	-0.11	-0.20	1.30	0.95	0.46	0.86	-0.06	-0.06	0.29	0.55
Super Raya	-0.02	-0.08	0.63	0.00	0.00	-0.58	0.02	-0.07	-0.29	-0.16
Galaxy	0.02	0.06	-0.88	-0.95	0.28	-0.59	0.10	0.04	-0.12	-0.41
Coral-432	-0.03	-0.01	0.45	1.85	-0.31	0.66	0.16	0.30	-0.21	-0.21
SE (gi)	0.04	0.03	0.45	0.28	0.24	0.25	0.04	0.11	0.12	0.10

Table 7. Effects of SCA for different yields, physiological and oil traits

F ₁ hybrids	Seed index		SPAD chlorophyll		Relative water content		Oil content%		Protein content%	
	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit	Normal	Water Deficit
AARI-Canola × Khanpur Raya	0.08	0.29	1.14	2.11	-0.26	1.02	0.84	1.15	0.86	0.06
AARI-Canola × Dhoom-1	0.05	0.32	5.24	4.34	2.03	0.26	0.56	-0.60	-0.26	-0.08
AARI-Canola × Super Raya	0.06	0.26	4.90	3.80	1.95	4.98	0.39	0.52	2.17	1.68
AARI-Canola × Galaxy	0.15	0.38	3.59	3.96	1.50	0.20	0.06	0.56	-0.47	-1.47
AARI-Canola × Coral-432	0.17	-0.01	3.09	3.35	-0.21	-0.73	0.30	0.71	0.49	-0.90
Khanpur Raya × Dhoom-1	0.26	0.15	4.30	4.59	0.61	1.33	0.94	1.91	0.02	-1.48
Khanpur Raya × Super Raya	-0.06	-0.11	3.73	4.16	3.40	1.45	0.58	1.48	0.79	-1.06
Khanpur Raya × Galaxy	0.04	-0.15	1.91	2.90	0.98	2.37	0.56	0.10	1.52	0.56
Khanpur Raya × Coral-432	0.16	0.02	5.70	3.09	0.24	1.65	0.59	0.94	0.86	0.27
Dhoom-1 × Super Raya	0.24	0.08	3.06	2.95	-0.70	-0.76	0.66	1.03	0.77	0.66
Dhoom-1 × Galaxy	0.04	-0.15	3.26	3.77	0.54	0.28	0.26	1.33	0.83	-0.94
Dhoom-1 × Coral-432	-0.04	-0.29	2.44	1.94	2.42	1.02	0.24	1.10	0.50	-0.73
Super Raya × Galaxy	-0.12	-0.57	5.33	4.55	1.65	2.89	0.46	-0.93	0.64	-0.35
Super Raya × Coral-432	0.39	0.86	2.16	2.86	0.80	-0.14	0.47	0.37	0.10	-0.46
Galaxy × Coral-432	0.12	0.68	0.91	3.43	0.80	1.15	0.11	0.55	1.25	0.25
SE (si)	0.11	0.07	1.24	0.78	0.65	0.69	0.12	0.31	0.34	0.28

Conclusion

The significant differences between parents and crosses due to GCA and SCA effects, respectively for studied characters recommend that combining ability is an extensive expression and genetical variation for the studied characters. Two mustard parents, such as AARI-Canola and Dhoom-1, reported positive and maximum desirable GCA effects in control and water scarcity environments for majority of studied characters, showing that improved mustard cultivars would be obtained by utilizing these parents in hybridization program followed by selection with respect to water stress. Five top specific combiners were AARI-Canola × Khanpur Raya, AARI-Canola × Super Raya, AARI-Canola × Galaxy, Khanpur Raya × Coral-432 and Galaxy × Coral-432. These hybrids disclosed good diagnoses for the selection of proper crosses to develop suitable crosses.

Authors' contributions

Conceived and designed the experiments: S Bano & SH Rao, Performed the experiments: S Bano & R Akram, Analyzed the data: S Bano, SH Rao & M Arsal, contributed reagents / materials / analysis tools: H Imran, SA Channa & Asadullah, Wrote the paper: S Bano, SH Rao & AW Baloch.

References

1. Singh I, Kumar R, Kaur S, Singh H & Kaur R (2019). Combining ability studies using diallel mating design in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. *Indian J Agric Res* 53(3): 366-369.
2. Tomat A & Singh M (2017). Genetic components analysis for seed yield and its contributing traits in Indian mustard (*Brassica juncea* (L.) Czern & Coss). *J Pharm. Innov* 6(8): 386-389.
3. Government of Pakistan. Pakistan Economic Survey, Ministry of Finance, Economic Advisor's Wing, Islamabad. 2018-19.
4. Government of Pakistan. Pakistan Economic Survey, Ministry of Finance, Economic Advisor's Wing, Islamabad. 2019-2020.
5. Mahmood T, Mustafa HSB, Aftab M, Ali Q & Malik A (2019). Super Canola: Newly developed high yielding, lodging and drought tolerant double zero cultivar of rapeseed (*Brassica napus* L.). *Gene and Mol Res* 18(2): 16039951.
6. Singh RK, Singh SK & Singh A (2020). Heterosis and Combining Ability Analysis in Indian Mustard (*Brassica Juncea* L.). *IJRSR* 8(12): 1618-1623.
7. Meena HS, Kumar A, Singh VV, Meena PD, Ram B & Kulshrestha S (2017). Genetic variability and inter-relation of seed yield with contributing traits in Indian mustard (*Brassica juncea* L.). *J Oilseeds Res* 8(2): 131-137.
8. Arifullah M, Munir M, Mahmood A, Ajmal SK & Hassan FU (2013). Genetic analysis of some yield attributes in Indian mustard (*Brassica juncea* L.). *Afr J Plant Sci* 7(6): 219-226.
9. Mehmood T & Ashraf M (2009). Does exogenous application of glycinebetaine as a pre sowing seed treatment improve growth and regulate some key adverse effects of drought stress on wheat (*Triticum aestivum* L.) *J Food Qual* 84(2): 192-199.
10. Tuberosa R (2012). Phenotyping for drought tolerance of crops in the genomics era. *Front Psychol* 3(347): 1-26.
11. Faraji A, Latifi N, Soltani A & Shirani-Rad AH (2009). Seed yield and water use efficiency of canola (*Brassica napus* L.) as affected by high temperature stress and supplemental irrigation. *Agric Water Manag* 96(1): 132-40.
12. Reynolds M & Tuberosa R (2008). Translational research impacting on crop productivity in drought-prone environments. *Curr Opin Plant Biol* 11(2): 171-179.
13. Gunasekera CP, Martin LD, French RJ, Siddique KH & Walton GH (2003). Effects of water stress on water relations and yield of Indian mustard (*Brassica juncea* L.) and canola (*Brassica napus* L.). In Proceedings of the 22th Australian Agronomy conference, Geelong.

14. Nasri M, Zahedi H, Moghadam HT, Ghooshci F & Paknejad F (2008). Investigation of water stress on macroelements in rapeseed genotypes leaf (*Brassica napus*). *Am J Agric Res* 3(4): 669-672.
15. Patel AM, Arha MD & Khule AA (2013). Combining ability analysis for seed yield and its attributes in Indian mustard (*Brassica juncea* (L.) Czern and Coss). *Asian J Biol Sci* 8(1): 11-14.
16. Variath MT, Wu JG, Li YX, Chen GL & Shi CH (2009). Genetic analysis for oil and protein contents of rapeseed (*Brassica napus* L.) at different developmental times. *Euphytica* 166: 145-153.
17. Rameeh V (2011). Line \times tester analysis for seed yield and yield components in spring and winter type varieties of oilseed rape. *J Cereal Sci* 2(5): 66-70.
18. Rahman M, Khatun A, Liu L & Barkla BJ (2018). *Brassicaceae* Mustards: Traditional and agronomic uses in Australia and New Zealand. *Mol* 23: 231-236.
19. Griffing B (1956). Concept of general and specific combining ability in relation to diallel crossing system. *Aust J Biol Sci* 9(4): 463-493.
20. Bonnet M, Camares O & Veisseire P (2000). Effect of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. cv Apollo). *J Exp Bot* 51(346): 945-953.
21. Bouyoucos GJ (1962). Hydrometer method improved for making particle size analysis of soils. *J Agron* 54(5): 464-465.
22. Jackson ML (1958). Soil Chemical Analysis. Prentice-Hall Inc., Englewood Cliffs, NJ. 498 p.
23. Rowell DL (1994). The preparations of saturation extracts and the analysis of soil salinity and sodicity. Soil science methods and applications. Rowell, D.L. (Ed.). Longman Group, UK.
24. Ilyas N, Bano A, Iqbal S & Raja N (2012). Physiological, biochemical and molecular characterization of *Azospirillum spp.* isolated from maize under water stress. *Pak J Bot* 44:71-80.
25. Gomez KA & Gomez AA (1984). Statistical procedures for agricultural research. John Wiley & Sons Inc. 2nd (ed.). New York U.S.A.
26. Singh RK & Chaudhary BD (1985). Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi.
27. Kaur S, Kumar R, Kaur S & Singh V (2021). Combining ability for yield and its contributing characters in Indian mustard. *J of Oilseed Brassica* 12(1): 32-37.
28. Sabaghnia N, Dehghani H, Alizadeh B & Mohghaddam M (2010). Diallel analysis of oil content and some agronomic traits in rapeseed (*Brassica napus* L.) based on the additive-dominance genetic model. *Aust J Crop Sci* 4(8): 609-616.
29. Chikuta S, Odong T, Kabi F & Rubaihayo P (2017). Combining ability and heterosis of selected grain and forage dual purpose sorghum genotypes. *J Agric Sci* 9(2): 122-130.
30. Jeeterwal RC, Sharma LD & Anju N (2017). Combining ability studies through diallel analysis in pearl millet (*Pennisetum glaucum* L.) under varying environmental conditions. *J Pharmacogn Phytochem* 6(4): 1083-1088.
31. Chaurasiya JP, Singh M, Yadav RK & Singh L (2018). Heterosis and combining ability analysis in Indian mustard (*Brassica juncea* (L.) Czern and Coss.). *J Pharmacogn Phytochem* 7(2): 604-609.
32. Zakiullah MF, Khan, Mohibullah M, Iqbal M, Irfanullah, Faheemullah, Urooj M & Arif U (2019). Combining ability analysis for morphological traits in 6 \times 6 diallel crosses of maize (*Zea mays* L.). *Sarhad J Agric* 35(1): 182-186.
33. Noor E & Qayyum A (2020). Genetics of physiological, fiber and yield contributing traits in cotton grown under normal and water stress conditions. *Int J Agric Syst* 23(6): 1158-1164.
34. Younas A, Sadaqat HA, Kashif M, Ahmed N & Farooq M (2020). Combining ability and heterosis for grain iron biofortification

- in bread wheat. *J. Sci. Food Agric* 100(4): 1570-1576.
35. Akbar M, Tahira BM & Hussain M (2008). Combining ability studies in *Brassica napus* L. *Int J Agric Biol* (10): 205-208.
 36. Choudhary P, Sharma H, Sanadya SK, Dodiya NS & Bishnoi V (2020). Combining ability for agronomic and quality traits in Indian mustard. *Int J Chem Stud* 8(5): 720-724.
 37. Gul S, Uddin R, Khan NU, Khan SU, Ali S, Ali N, Khan MS, Ibrahim M, Goher R, Saeed M & Hussain D (2019). Heterotic response and combining ability analysis in F₁ diallel populations of (*Brassica napus* L.). *Pak J Bot* 51(6): 2129-2141.
 38. Yadav VN, Singh M, Yadav RK, Singh HC, Maurya AK, Singh AK & Singh SG (2020). Genetics of seed yield in Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] under late sown environment. *J Pharmacogn Phytochem* 9(4): 249-254.
 39. Channa SA, Tian H, Mohammed MI, Zhang R, Faisal S, Guo Y, Klima M, Stamm M & Hu S (2018). Heterosis and combining ability analysis in Chinese semi-winter 3 exotic accessions of rapeseed (*Brassica napus* L.). *Euphytica* 214(134): 1-19.
 40. Kanwal S, Tahir MHN, Sadaqat HA & Sadia B (2019). Development of high yielding types of (*Brassica napus* L.) under salinity stress. *Pak J Bot* 51(4): 1185-1190.
 41. Patel R, Solanki SD, Gami RA, Prajapati KP, Patel PT & Bhadauria HS (2015). Genetic study for seed yield and seed quality traits in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *Electron J Plant Breed* 6(3): 672- 679.
 42. Lal D, Kumar R, Kumar A, Singh SK, Kumar D & Kumar S (2018). Estimation of specific combining ability in Indian mustard (*Brassica juncea* L.). *J Pharmacogn Phytochem* 7(5): 901-905.
 43. Shrimali TM, Chauhan RM, Prajapati KP, Desai SA, Patel JR, Patel PT, Patel PJ & Chaudhary BK (2018). Analysis of yield and its components based on heterosis and combining ability in Indian mustard (*Brassica juncea* L. Czern & Coss.). *Int J Pure Appl Biosci* 6(1): 219-224.
 44. Rad SAH, Abbasian A & Aminpanah H (2013). Evaluation of rapeseed (*Brassica napus* L.) cultivars for resistance against water deficit stress. *Bulg J Agric Sci* (19): 266-273.
 45. Tyagi V, Dhillon SK, Kaushik P & Kaur G (2018). Characterization for drought tolerance and physiological efficiency in novel cytoplasmic male sterile sources of sunflower (*Helianthus annuus* L.). *Agronomy* 8(232): 1-20.
 46. Hirut B, Shimelis H, Fentahun M, Bonierbale M, Gastelo M & Asfaw A (2017). Combining ability of highland tropic adapted potato for tuber yield and yield components under drought. *PLoS ONE* 12(7): e0181541.
 47. Ghidan WF & Khedar RA (2021). Assessment of some agro physiological traits and genetic markers in rice (*Oryza sativa* L.) under normal and water stress conditions. *J Plant Prod* 12(1): 73-86.