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An Analysis of the Total Protein and Genetic Components for Yield and Its Constituents in some Hybrid Rice (*Oryza sativa* L.)

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RICE is the most widely cultivated cereal crop in tropical and subtropical regions around the globe. Rice is also the most commonly ingested staple food by a large portion of the global population. A line × tester experiment was conducted during 2018 and 2019 seasons to evaluate the combining ability, gene action, and heterosis for its yield characters. During 2018 season, four lines were crossed with seven testers in a line-testing method, yielding twenty-eight F1 hybrids. On May 1 of 2019, a randomized complete block design with three replications was used to generate the twenty-eight hybrids and their parents. Number of panicles per plant, panicle length (cm), panicle weight (g), seed set (%), 1000 grain weight (g), and grain yield per plant (g) were all investigated in the data. Analysis of variance revealed significant differences among the lines, testers, and line × testers for the yield and its components. Within CMS lines, G46A had the highest GCA for most of the studied characters. G178M4, Giza 178 Im, and Giza 179 Im represented good general combiners for most testers' tested characters. G46A × Giza178 and G46A × G178M3, IR69625A × Giza178 Im, IR79575A × Giza 179 Im, IR79575A × Giza 178M4, and IR70368A × Giza 178M1, G46A × Giza 179 Im, and IR79575A × Giza 178M4 showed desirable values for specific combining ability (SCA) for all of the evaluated traits.

Keywords: Genetic analysis, Grain yield, Hybrid rice, Total protein.

Introduction

Rice (*Oryza sativa* L.) is a globally important crop that is a member of the Poaceae family. Due to the significance of rice grain production in breeding programs, numerous studies have been conducted to enhance rice yield under normal and stressful conditions (Elkhoby et al., 2013; Abd El-Lattef et al., 2014; El-Hity et al., 2020; Hafez et al., 2020; Mohamed et al., 2021). More than half of the world's population, especially in low- and middle-income countries, depends on rice for calories and protein. By 2025, approximately 756

million tons of paddy would be required to satisfy rising demand, a 70% increase over current output (Duwayri et al., 1999). Protein is one of the two most prevalent components of rice grains, along with carbohydrates. The rice bran and endosperm are protein-rich grain components. Protein bodies refer to cellular organelles that are enclosed by a membrane and are mostly found in the starchy endosperm of cereal grains. These organelles serve as storage sites for proteins (Agboola et al., 2005). Rice proteins are known for their colorlessness, essential amino acids, bland flavor, and hypoallergenic properties (Chrastil, 1992).

Despite the interest in rice proteins as a functional constituent, there are few studies on this topic due to the low water solubility and low protein content of rice. Therefore, their extraction and incorporation into edible products is difficult (Abdelaal et al., 2021). However, consumer awareness of the health benefits of plant proteins is growing. Consequently, numerous extraction methods for the purification of rice proteins, including alkaline, enzymatic, and physical procedures, are being developed and implemented industrially (Fabian & Ju, 2011). A growing number of health-related products incorporate rice proteins as functional ingredients (Amagliani et al., 2017).

Furthermore, breeding programs are an important strategy for the improvement of the grain yield production of rice plants. Hybrid rice, as a type of rice bred from two very different parent varieties, is known to be an effective and economical way of increasing rice production. It can significantly outyield other rice varieties and give higher yields of 15% to 20% (Virmani, 1997). Rice stigma vigor can last 3–7 days after flowering. According to Wang & Hucy (2008), increasing the percentage of sterile male lines with exerted stigmas could promote pollination after flowering. In comparison to non-exerted stigmas, (Tian et al., 1990) found that rice spikelets with exerted stigmas had a higher seed setting rate (64%) compared with non-exerted stigmas (15.7%). A line × tester analysis is a modified form of a top cross that is used for measuring the general and specific combining ability variances and effects in large germplasm lines at a time. This concept of a line × tester analysis was established by Kempthorne (1957).

From 1976 to 1991, breeding programs provided a 30% advantage over conventional pure rice production in China, producing almost 200 million tons of rice (Yuan, 1998). The parent's performance does not always indicate whether it is a good or lousy combiner. Consequently, it is vital to collect information on the nature of gene effects and their expression in terms of combining ability and the type of gene action that is involved in the inheritance of the given character. As a result, this research aimed to evaluate the combining ability of sterile and restorer lines and to identify the nature and magnitude of the gene action for yield and yield-related components, in order to discover the best combinations of sterile and restorer lines.

Materials and Methods

This investigation was conducted in 2018 and 2019 at an experimental farm of the Rice Research and Training Center in Sakha, Kafr Elsheikh, Egypt, 31°5′54″ N 30°57′0″ E. Figures 1A, B display weather data for 2017 and 2018 (rainfall in millimeters, average temperature in degrees Celsius, relative humidity in percent, and radiation in joules per square meter) it can be observed that the rainfall were low during the searson while the temperature was so high, that were collected from a https://power.larc.nasa.gov.
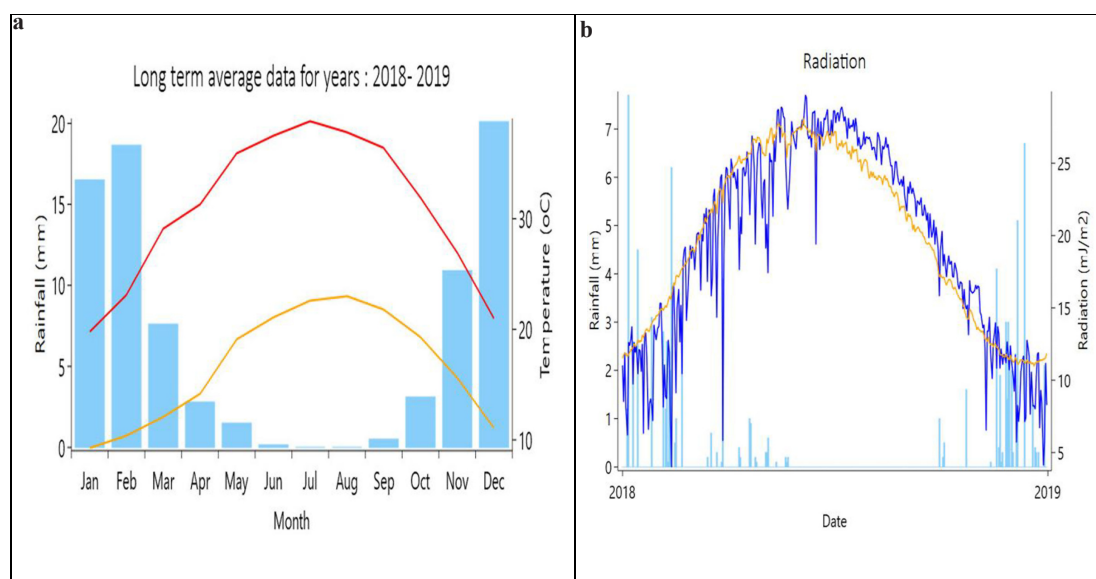

Fig. 1. The weather data (rain in mm/month, the average temperature in °C (a) , and radiation in MJ/m^2 (b))

In total, the following eleven rice varietals were utilized: Four varieties (IR69625A, IR70368, G46A, and IR79575) were used as females (designated as CMS lines), while seven genotypes (Giza 178, Giza 178M1, Giza 178M2, Giza 178M3, Giza 178Im, and Giza 179Im) were utilized as males. The line×tester union design, according to Kempthorne (1957), during the 2018 season, these progenitors were crossed to produce 28 F1 hybrids. The progenitors and their F1 hybrids were fertilized in the nursery on May 1, 2019, and thirty days later, the seedlings were transplanted. The progenitors and their F1 offspring were cultivated using a randomized complete block design (RCBD) with three replications. In each replicate, one row of F1 crosses was positioned between two rows of each parental line; the row was five meters long, 20 centimeters apart, and contained 25 hills. Rice Research and Training Center (RRTC) rice recommendations were applied to all agricultural practices (RRTC, 2018). Ten plants were chosen at random from each replicate to capture data, and the mean values were used for statistical analysis. In accordance with the recommendations of the IRRI Standard Evaluation System (SES) (IRRI, 2008), the number of panicles per plant, panicle weight (g), seed set (%), 1000 grain weight (g), and grain yield per plant (g) were utilized in this study.

Protein extraction buffers

The total proteins were extracted from the leaves using the extraction solution, which contained 2% sodium dodecyl sulfate (SDS), 6M (36.04g) urea and 1.5% 2-mercaptoethanol (2-ME) (Laemmli, 1970). The extraction buffer was freshly prepared for each electrophoretic run. The solution used for the extraction included 0.002% g of tracking dye (bromophenol blue).

Leaf proteins extraction and electrophoresis methods

The leaf proteins that were soluble in water were isolated from 0.5g of the leaf material. Half a gram of the leaves was completely pulverized in a pre-chilled mortar and pestle. The leaf was manually ground into a fine powder under liquid N_2, mixed in a 2mL buffer, and centrifuged at 14,000 rpm 4°C until use (Murray, 1988; Stegemann & Krojerreckenfort, 1995).

The total protein banding patterns were electrophoretically assessed using SDS discontinuous gel slabs (4% and 7.5%, respectively, for the stacking and separating gels), as described by Stegemann & Krojerreckenfort (1995). About 45μL of the isolated proteins were added, and a constant current of 20mA was maintained for about 6h using the Stricter-Hoefer Scientific Instruments, USA. The gel was stained for approximately 2h with Brilliant commission Blue R-250 as a protein thermocouple reagent, and then transferred twice to the distaining solution until the background became clear.

Statistical analysis

The data were analyzed using an analysis of variance using a randomized complete block design, as proposed by Kempthorne (1957), and a line x tester crossing design, as proposed by Panse & Sukhatme (1985). Mather (1949) and Mather & Jinks (1971) defined heterosis as the percentage difference between the middle and top performers of the parents and the average performance of their offspring. Several crucial genetic features were assessed, as detailed by Falconer & Mackey (1996). These included additive variance, non-additive variance, broad-sense heritability (h2b), narrow-sense heritability (h2n), and genetic advancement. We calculated a phenotypic correlation using the method described by Singh & Chaudhary (1977).

Results

Analysis of variance

The analysis of variance for all measured characteristics (Table 1) reveals significant differences and correlations between rice genotypes for all analyzed variables. Moreover, genotypic mean squares were separated into parents, crosses, and parents versus crosses. The parents exhibited significant variation in all the investigated characters. The fact that the variance, owing to the general combining ability (σ^2gca), was lower than the variance due to the specific combining ability (σ^2sca) for all the characters, suggests that dominance genes played a significant role in their inheritance.

Mean performance of genotypes

The average performance of the parents and their F1 crosses in terms of yield attributes were summarized (Table 2). There were significant differences in all the measured traits between the lines. Line G46A recorded the desirable value for a 1000 grain weight, line IR69625A achieved the

highest value for the number of panicles/hills, and line IR70368A achieved the desired values for the seed set % and grain yield (g/plant). On the sister line, most of the yield characters were recorded (B-line). For the testers, IR69625A × Giza 179 produced the desirable values for the number of panicles/plant, 1000 grain weight, and the grain yield/plant, while Giza 178M4 possessed the desirable values for the seed set. IR70368 × Giza 179IM, G46A × Giza 178M4, and G46A × Giza 179Im were the hybrid combinations with the heaviest panicles.

The hybrid combination IR70368A × Giza 178M4 achieved the highest number of panicles per plant and grain yield per plant (Table 2). In contrast, the hybrid combination G46A × Giza 178M4 had the highest value for the 1000 grain weight, and the hybrid G46A × Giza 178M3 achieved the highest value for the % of the seed set. Finally, the hybrids IR69625A × Giza 178M3, IR69625A × Giza 178M4, and G46A × Giza 178M3 demonstrated the highest seed set (%) and grain yield per plant, respectively.

General combining ability

The impacts of the general combining ability were estimated (GCA) for the males and females (Table 3). Among the female (lines) parents, G46A was a good combiner for most of the studied traits, while the CMS line IR70368A recorded the most positive GCA for the number of panicles per plant. Another tester, Giza 178M4, demonstrated

positive GCA effects for the majority of the tested characters. It could be suggested that each parent with the most significant value for its examined characteristics is regarded as a suitable combiner for this trait, and could be utilized in breeding programs to develop new parental lines.

G46A, the paternal line, was revealed to be an excellent general combiner for the grain yield/plant and most of the yield components, and thus might be used in future breeding programs to increase the grain yield. The cross combinations, IR69625A × Giza178 Im and IR79575A × Giza179 Im, possessed highly significant and positive SCA effects for the number of panicles/plant and the panicle weight.

The positive and significant SCA effects for the seed set % were recorded in the cross combination, IR69625A × Giza178, whereas G46A × Giza179 Im exhibited positive and significant SCA effects for the 1000 grain weight, as shown in Table 4. The SCA effects on the yield/plant were positive and highly effective for the cross combination IR79575A × Giza178M$_8$-4. The cross combination of IR79575A × Giza178M8-4 revealed positive and highly significant SCA effects for the yield/plant. Hence, it was well documented that dominance genetic effects could control the inheritance of the studied traits and indicated that the selection for the traits inherited in this manner should be performed in further generations.

TABLE 1. Estimation of the analysis of variance of line x tester analysis for yield and its components during the 2019 season

S.O.V	DF	No. of panicles/plant	Panicle weight (g)	Seed set %	1000 Grain weight (g)	Grain yield/plant (g)
Reps	2	0.06	0.01	0.01	0.08	0.02
Genotypes (G)	38	29.82 **	1.02 **	3.63 **	7.89 **	35.37 **
Parents (P)	10	15.37 **	0.69 **	2.26 **	5.82 **	38.47 **
Crosses (Cr)	27	10.85 **	0.50 **	1.52 **	2.83 **	7.71 **
Pa vs. Cross	1	656.82 **	17.15 **	70.77 **	157.42 **	716.08 **
Lines (L)	3	9.46 **	0.58 **	0.99 **	16.39 **	10.23 **
Testers (T)	6	16.36 **	1.37 **	3.99 **	3.52 **	21.85 **
L × T	18	9.24 **	0.20 **	0.78 **	0.34 **	2.57 **
Error	49	0.03	0.01	0.02	0.03	0.04
GCA	-	0.01	0.0087	0.012	0.04	0.08
SCA	-	3.07	0.05	0.25	0.10	0.84
GCA/SCA	-	0.0001	0.007	0.001	0.0002	0.007

** Significant at 0.05 probability level

TABLE 2. The mean performances of all genotypes for yield and its components during the 2019 season

Genotypes	No. of panicles/plant	Panicle weight (g)	Seed set%	1000 grain weight (g)	Grain yield/plant (g)
Lines					
IR69625B	17.60	3.32	94.07	24.16	37.70
IR70368B	15.38	3.55	94.77	24.52	42.74
G46B	14.46	3.66	94.67	26.27	39.76
IR79575B	16.52	3.49	94.66	23.63	40.22
Mean	15.99	3.50	94.54	24.65	40.10
Tester					
Giza178	18.17	4.11	94.74	22.23	43.87
Giza178M$_8$-1	19.15	4.22	95.19	22.70	44.38
Giza178M$_8$-2	19.56	4.45	95.28	22.76	46.31
Giza178M$_8$-3	20.06	4.58	96.59	22.80	46.87
Giza178M$_8$-4	20.67	4.72	96.87	22.92	47.56
IR69625A/Giza178	19.32	4.26	95.80	24.46	46.73
G46A/Giza179	21.83	4.33	95.78	26.19	48.56
Mean	19.82	4.38	95.75	23.44	46.32
Crosses					
IR69625A × Giza178	22.52	4.63	95.93	24.30	44.92
IR69625A × Giza178M8-1	23.19	4.77	97.11	25.11	47.84
IR69625A × Giza178M8-2	23.49	4.76	97.29	25.51	49.73
IR69625A × Giza178M8-3	23.92	4.81	97.30	25.71	50.09
IR69625A × Giza178M8-4	24.58	5.61	97.23	26.34	50.31
IR69625A × Giza178 Im	23.33	4.24	97.13	25.77	50.94
IR69625A Giza179 Im	23.01	5.54	97.65	26.64	51.83
IR70368A × Giza178	23.11	4.60	96.19	25.54	48.21
IR70368A × Giza178M8-1	23.05	4.78	96.89	25.92	49.55
IR70368A × Giza178M8-2	23.57	4.85	97.78	26.13	50.19
IR70368A × Giza178M8-3	24.04	5.11	97.81	26.51	51.05
IR70368A × Giza178M8-4	24.89	5.29	97.38	26.94	51.23
IR70368A × Giza178 Im	24.17	4.57	96.26	26.56	50.26
IR70368A × Giza179 Im	24.37	5.76	95.22	27.21	50.04
G46A × Giza178	21.65	4.66	95.83	27.13	48.07
G46A × Giza178M8-1	22.41	4.72	96.71	27.42	49.13
G46A × Giza178M8-2	22.56	4.78	97.76	27.85	50.52
G46A × Giza178M8-3	23.15	5.21	97.46	28.09	51.23
G46A × Giza178M8-4	23.57	5.66	97.15	28.31	51.78
G46A × Giza178 Im	23.43	5.65	97.19	27.74	49.66
G46A × Giza179 Im	23.81	5.81	96.66	27.29	50.55
IR79575A × Giza178	22.31	4.33	96.43	25.08	46.55
IR79575A × Giza178M8-1	22.40	4.51	97.84	25.74	48.67
IR79575A × Giza178M8-2	22.94	4.62	97.65	25.78	47.24
IR79575A × Giza178M8-3	23.62	5.18	97.64	26.51	49.04
IR79575A × Giza178M8-4	23.94	5.58	97.70	26.90	49.26
IR79575A × Giza178 Im	23.35	4.75	97.76	26.59	49.12
IR79575A × Giza179 Im	24.06	5.62	96.13	26.11	50.65
Mean	23.69	5.01	97.04	26.46	49.56
G. Mean	22.21	4.75	96.55	25.73	48.01
LSD 0.05	0.17	0.10	0.13	0.15	0.18
LSD 0.01	0.22	0.13	0.18	0.20	0.23

TABLE 3. Estimates of GCA effects (gi) of the CMS lines and restorer lines (testers) for yield and its components during the 2019 season

Genotypes	No. of panicles/plant	Panicle weight (g)	Seed setting %	1000 Grain weight (g)	Grain yield/plant (g)
Lines					
IR69625A	−0.26 **	−0.11 **	−2.81 **	−0.83 **	−0.18 **
IR70368A	0.19 **	−0.02	−3.59 **	−0.05	0.52 **
G46A	−0.75 **	0.20 **	7.81 **	1.24 **	0.57 **
IR79575A	0.82 **	−0.07 **	−1.41 **	−0.35 **	−0.91 **
Tester					
Giza178	−1.30 **	−0.46 **	−4.85 **	−0.94 **	−2.62 **
Giza178M_8-1	−0.93 **	−0.32 **	−3.01 **	−0.41 **	−0.76 **
Giza178M_8-2	−0.55 **	−0.26 **	−0.30	−0.14 **	−0.14 **
Giza178M_8-3	2.24 **	0.06 *	2.62 **	0.25 **	0.79 **
Giza178M_8-4	0.55 **	0.52 **	5.68 **	0.67 **	1.09 **
Giza178 Im	−0.13 **	−0.21 **	3.05 **	0.21 **	0.44 **
Giza179 Im	0.12 **	0.67 **	−3.19 **	0.36 **	1.21 **
L.S.D. for CMS lines 0.05	0.07	0.04	0.73	0.07	0.08
L.S.D. for CMS lines 0.01	0.10	0.06	0.97	0.09	0.01
L.S.D. for testers 0.05	0.10	0.06	0.97	0.09	0.01
L.S.D. for testers 0.01	0.13	0.08	1.29	0.12	0.14

** significant at 0.05 probability level

Genetic parameters and heritability

The estimates of the genetic parameters were summarized (Table 5). For all the characters examined, both the additive and non-additive variances were substantial. However, as evidenced by the degree of the dominance value, the non-additive effects were more significant (d). This parameter was assessed to be more than one in all situations, showing that over-dominance was predominant in controlling the analyzed attributes. The heritability in the broad sense (h^2b) of the examined characters was more remarkable (85%), indicating that the environmental factors had a minor effect on these traits. In all cases, the specific heritability (h^2n) was low (6.78–40.21%). In contrast, the highest specific heritability was calculated for the number of panicles per plant and the grain yield per plant, showing that non-additive influences were significant in controlling these characters.

Phenotypic correlation

The correlation coefficient among the characters was an important factor, especially in economic and complex characters such as yield. Steel & Torrie (1960) stated that correlations are measures of the intensity of association between traits. The

selection for one trait resulted in progress for all the positively correlated characters and retrogress for the negatively correlated traits. The phenotypic correlation result generally indicated that the yield per plant was positively and significantly correlated with the number of panicles/plant and the panicle weight (Table 6).

Total soluble protein analysis

A biochemical analysis was performed on two CMS lines, IR69625A and G46A, two restorer lines, Giza 178-3 and 178-4, and three hybrids, IR69625A × Giza 178-3, G46A × Giza 178-3, and IR69625A × Giza 178-4. The total protein analysis for the parental lines and their hybrids was investigated (Table 7 and Fig. 2). The results indicated that 65 bands with varying molecular weights and densities were detected in the polymorphisms of various rice genotypes. The genotype Giza 178-3R and its hybrids, IR69625A × Giza 178-3R and G46A × Giza 178-3R, exhibited 10 bands with molecular weights that ranged from 7 to 240kDa. Additionally, band 3, with a molecular weight of 100kDa, was found in the three genotypes, indicating that the restorer variety Giza 178-3R had dominant alleles in its progenies. In contrast, the genotypes IR69625A

and G46 A, and the hybrid IR69625A × Giza 178-4R, showed nine bands. Moreover, the restorer line Giza 178-4 showed only eight bands, but its hybrid with IR69625A showed nine bands, indicating that IR69625A had a maternal effect. Furthermore, most of these bands varied in intensity, with bands 4, 7, and 9 being the most intense for the most investigated genotypes.

Correlation analysis

The studied traits were not normally distributed, so the Spearman correlation coefficient was used instead of the Pearson coefficient. Figure 3 revealed that the GYPP was significantly and positively correlated to the GI (0.72), SS (0.65), PW (0.78), and PNPP (0.81). This meant that the PNPP was the most effective trait for the GYPP, followed by the PW, then the GI and SS. The PNPP and PW were significantly and positively correlated (0.81), indicating that the breeding for one led to the other. Additionally, the GI and PW were significantly and positively correlated (0.70), depicting that increasing the GI would cause an increase in the PW.

TABLE 4. Estimated SCA effects (Sij) of hybrid combinations for yield and its components during the 2019 season

Genotypes	No. of panicles/plant	Panicle weight (g)	Seed setting %	1000 Grain weight (g)	Grain yield/plant (g)
IR69625A × Giza178	1.19 **	−0.39 **	2.44 **	−0.26	0.25
IR69625A × Giza178M$_8$-1	−1.96 **	−0.39 **	−0.34 **	0.18 **	0.39 **
IR69625A × Giza178M$_8$-2	−1.90 **	−0.34 **	0.02	0.18 **	0.68 **
IR69625A × Giza178M$_8$-3	−0.69 **	0.58 **	0.21 **	0.11	0.61 **
IR69625A × Giza178M$_8$-4	−0.34 **	0.09	0.02	−0.16 **	−1.75 **
IR69625A × Giza178 Im	2.17 **	1.47 **	−0.31 **	0.18 **	0.59 **
IR69625A × Giza179 Im	1.53 **	−1.03 **	0.12	−0.46 **	0.02
IR70368A × Giza178	−0.68 **	1.12 **	0.30 **	−0.04	−0.54 **
IR70368A × Giza178M$_8$-1	0.50 **	0.30 **	0.59 **	0.07	0.52 **
IR70368A × Giza178M$_8$-2	0.29 **	−0.21 **	0.14	0.11	0.09
IR70368A × Giza178M$_8$-3	0.06	−1.16 **	0.19*	0.12*	0.24 **
IR70368A × Giza178M$_8$-4	−0.25 *	0.31 **	0.13	0.05	−2.08 **
IR70368A × Giza178 Im	−0.10	−0.11	−0.05	−0.23 **	0.46 **
IR70368A × Giza179 Im	0.17	−0.25 **	−0.69 **	−0.21 **	0.41 **
G46A × Giza178	−1.21 **	1.34 **	−0.31 **	0.10	0.37 **
G46A × Giza178M$_8$-1	−0.01	1.53 **	−0.22 **	−0.09	0.01
G46A × Giza178M$_8$-2	1.29 **	1.42 **	−0.38 **	−0.17 **	0.40 **
G46A × Giza178M$_8$-3	0.75 **	0.61 **	−0.02	−0.17 **	0.18
G46A × Giza178M$_8$-4	1.03 **	−0.64 **	−0.06	−0.07	−2.03 **
G46A × Giza178 Im	−1.44 **	−1.54 **	0.50 **	−0.07	0.08
G46A × Giza179 Im	−0.41 **	−2.72 **	0.21 **	0.65 **	0.62 **
IR79575A × Giza178	0.69 **	−2.07 **	−0.02	−0.07	0.75 **
IR79575A × Giza178M$_8$-1	1.47 **	−1.44 **	−0.03	−0.16 **	−0.91 **
IR79575A × Giza178M$_8$-2	0.32 **	−0.88 **	0.22 **	−0.11	−1.18 **
IR79575A × Giza178M$_8$-3	−0.12	−0.03	−0.37 **	−0.06	−1.02 **
IR79575A × Giza178M$_8$-4	−0.44 **	0.23 **	−0.09	0.17 **	5.86 **
IR79575A × Giza178 Im	−0.62 **	0.18 *	−0.13	0.12 *	−1.12 **
IR79575A × Giza179 Im	−1.30 **	4.00 **	0.37 **	0.02	−1.04 **
LSD 0.05	0.22	0.15	0.53	0.28	0.27
LSD 0.01	0.29	0.19	0.70	0.36	0.37

TABLE 5. Estimation of genetic parameters and heritability in broad and narrow senses for growth and yield and its components during the 2019 season

Genetic parameters and heritability	No. of panicles/ plant	Panicle weight	Seed set %	1000-Grain weight (g)	Grain yield/ plant
Additive variance (σ^2A)	0.03	0.017	0.024	0.089	0.17
Dominant variance (σ^2D)	3.07	0.055	0.25	0.10	0.84
Genotypic variance (σ^2G)	3.10	0.072	0.27	0.18	1.01
Environmental variance (σ^2E)	0.097	0.064	0.031	0.10	0.057
Phenotypic variance (σ^2P)	3.19	0.136	0.301	0.28	1.06
Broad sense heritability ($h^2_b\%$)	98.93	86.25	92.93	87.79	96.46
Narrow sense heritability ($h^2_n\%$)	1.06	20.51	8.22	40.21	16.94
Relative importance of gca% *	0.97	23.61	8.89	49.44	16.83
Relative importance of sca% **	99.03	76.39	92.59	55.55	83.16

* Relative importance of gca% = s2A/s2G; and ** relative importance of sca% = s2D/s2G.

TABLE 6. Phenotypic correlation coefficients among botanical yield and its component traits of some rice genotypes during the 2019 season

Traits	NOP	PW	SS	TGW
PW	0.812 **			
SS	0.768 **	0.628 **		
TGW	0.599 **	0.616 **	0.533 **	
GY	0.890 **	0.824 **	0.796 **	0.630 **

** Significant at 0.05 level. N.P.: number of panicles/plant; P.W.: panicle weight; T.G.W.: thousand-grain weight; and G.Y.: grain yield/plant.

TABLE 7. Molecular weight and density bands for different parents and their hybrids

No.	kDa	1	2	3	4	5	6	7
1	240	++	++	++	−	+++	++	+++
2	140	+++	+++	+++	+	+++	+++	+++
3	100	−	−	++	−	++	++	−
4	70	++++	++++	++++	++	++++	++++	++++
5	60	++	++	++	+	++	++	++
6	50	++	++	++	+	++	++	++
7	35	++++	+++	++++	++	+++	+++	++++
8	25	+++	+++	+++	+++	+++	+++	+++
9	15	++++	++++	++++	+++	++++	++++	++++
10	7	++	+++	+++	++	+++	+++	+++
Total		9	9	10	8	10	10	9

1—IR69625 A; 2—G 46 A; 3—Giza 178-3R; 4—Giza 178-4R; 5—IR69625 A/Giza 178-3; 6—G 46 A Giza/178-3; and 7—IR69625 A/ Giza 178-4.
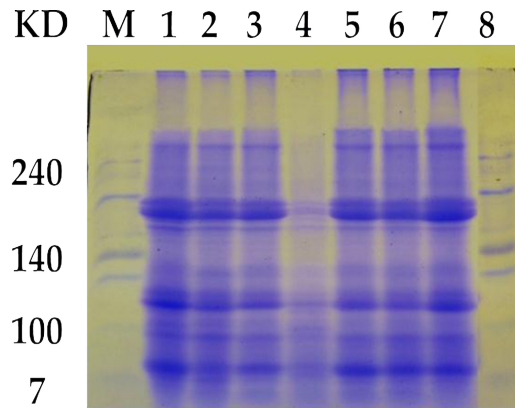
Fig. 2. Total protein analysis for parental lines and their hybrids
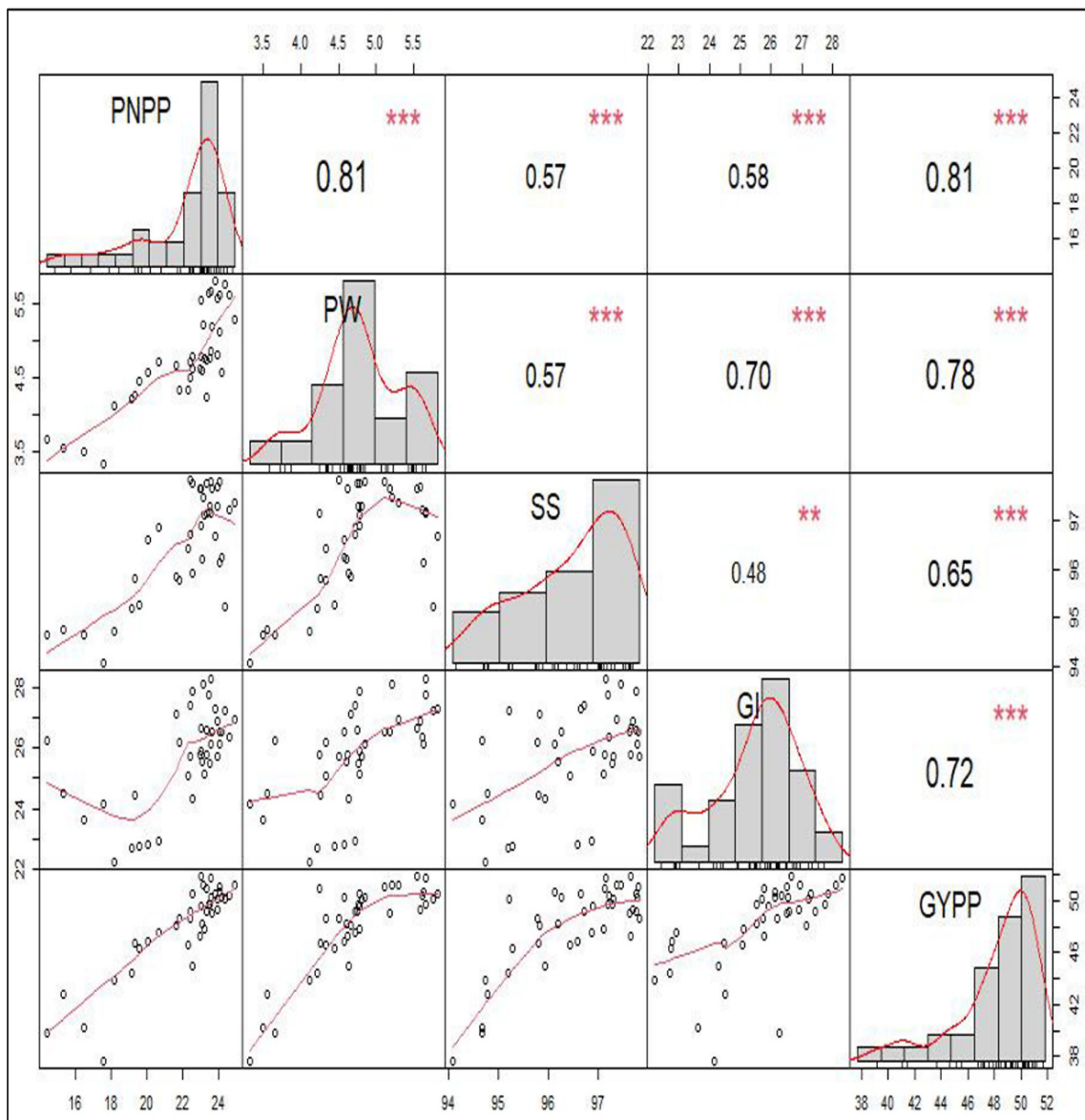

Fig. 3. Spearman correlation matrix among the studied traits [PNPP = panicle number per plant, PW = panicle weight, SS = seed set, GI = grain index, and GYPP = grain yield per plant]

Cluster analysis

As a measure of dissimilarity, the Euclidian coefficient was utilized in a cluster analysis. The clustering method developed by Ward (1963) was utilized to classify the data into clusters and construct the dendrogram illustrating the similarity between the lines, testers, and crosses. Due to the distinct dimensions, the investigated characteristics were normalized prior to computing the distance (Fig. 4). The cubic clustering criterion (Milligan & Cooper, 1985) was utilized to determine whether or not data clusters existed. A total of six methods were evaluated using an agglomerative coefficient to determine the optimal method for data clustering.

Average, generalized average, solitary, and weighted methods were utilized. The respective agglomerative coefficients for each method were 0.807, 0.894, 0.587, 0.831, 0.879, and 0.942. It was evident, based on the agglomerative coefficient, that the Ward method was the correct way to cluster our data. After selecting the most precise method, the appropriate number of clusters was determined through internal validation. The optimal number of data clusters was determined using a total of 30 indices (Charrad et al., 2014). The results of the internal validation indicated that the data consisted of two clusters (Fig. 4), with the averages of the analyzed parameters depicted in Table 8.
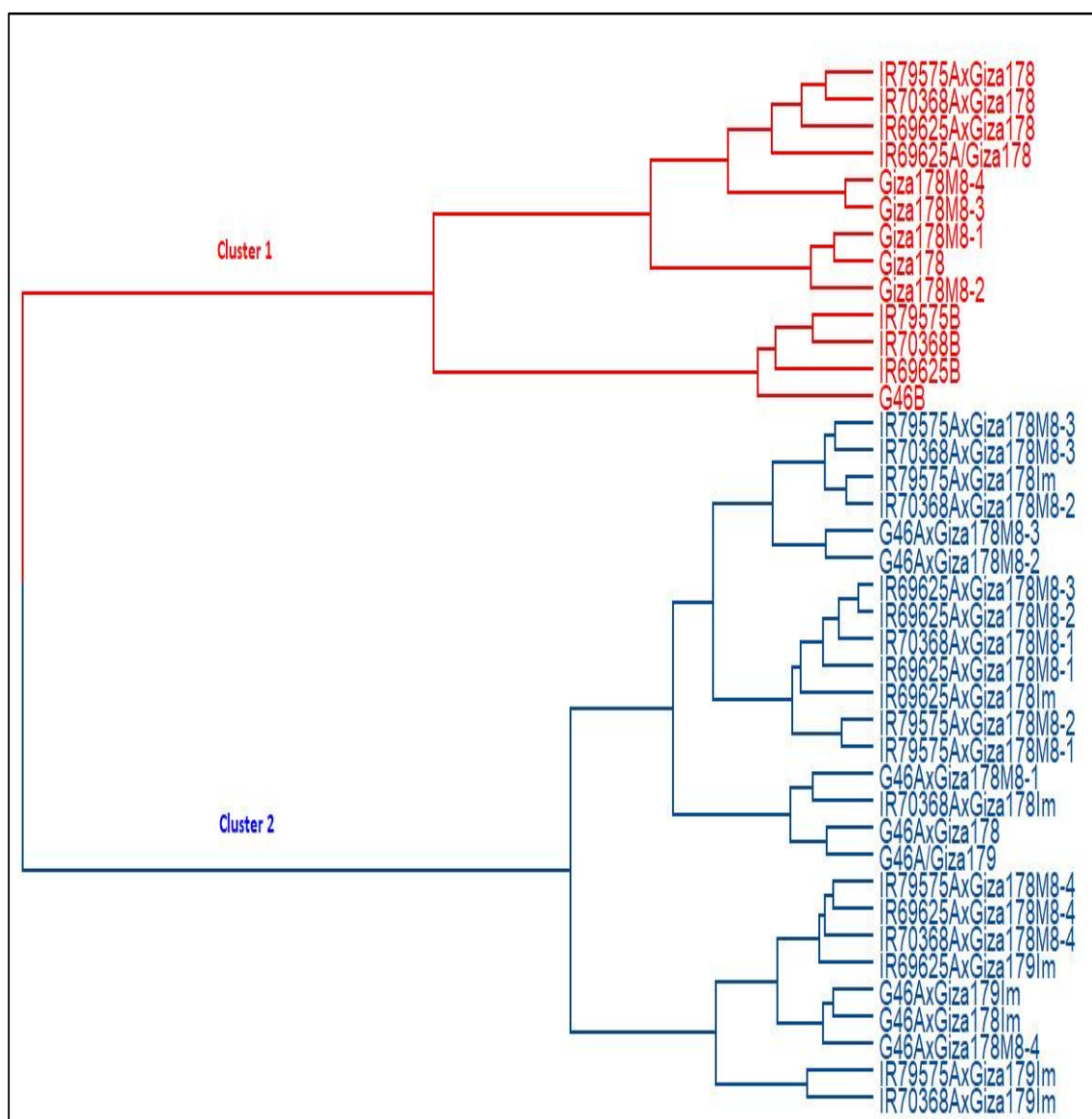

Fig. 4. Dendrogram showing results of cluster analysis based on Euclidian coefficient and Ward method

TABLE 8. Average of the studied parameters for the 2 clusters

Clusters	PNPP	PW	SS	GI	GYPP
Cluster1	19.14	4.15	95.48	23.95	44.29
Cluster2	23.40	5.04	97.09	26.61	49.87

The two clusters of our data that focused on the grain yield per plant (GYPP) and the panicle number per plant (PNPP) were depicted (Fig. 5), and were based on both color scale and size scale. The color scale was used to differentiate between the different values of the GYPP, whereas the size scale was used to distinguish between the different values of the PNPP. This analysis revealed that the crosses IR70368A × Giza178M8-4, IR69625A × Giza179Im, and G46A × Giza178M8-4 had a higher grain yield per plant. However, they were different in their panicle number per plant. The cross IR70368A × Giza178M8-4 was increased in both its grain yield per plant and panicle number per plant, whereas the crosses IR69625A × Giza179Im and G46A × Giza178M8-4 were high in grain yield per plant and lower than the cross IR70368A × Giza178M8-4 in panicle number per plant. This reflected that not only did the panicle number per plant highly contribute to the grain yield per plant for all the crosses, but also to the grain index, with the high yield crosses high in grain index.

Fig. 5. Tree and leaf diagram showing GYPP and PNPP of all the lines, testers, and crosses

Figure 5 displays the dissimilarity among all the lines, testers, and crosses based on the Euclidean metric of the scaled data, depending on a color scale. The higher dissimilarity values are displayed by a darker red color, whereas the lower dissimilarity values are reported in a blue color. According to our analyses (Fig. 6), all the crosses are similar to each other. The lines G46B, IR69625B, IR70368B, and IR79575B, as well as the tester Giza178, were the most dissimilar to all the crosses. The testers Giza178M$_8$-1, Giza178M$_8$-2, Giza178M$_8$-3, and Giza178M$_8$-4, as well as the two crosses IR69625A/Giza178 and IR69625A × Giza178, had a medium similarity with all the crosses.

The relationship between the studied traits and all the lines, testers, and crosses is illustrated in Fig. 7. Using scaled data, the association is explained using a color scale. The crosses had grain yield per plant values that ranged from mid to high. This was associated with a higher panicle number per plant value than grain index value. All the lines and testers, except for G46A/Giza179, had a low grain yield per plant, which was associated with a low panicle number per plant, panicle weight, seed set, and grain index values.

Discussion

Rice (*Oryza sativa* L.) is a valuable crop that is grown worldwide; therefore, many studies have been conducted to enhance rice yield, quality, and quantity through breeding programs (El-Hity et al., 2020, Mohamed et al., 2021). One of the most abundant components in rice grains is protein, which is stored in the rice bran and endosperm (Agboola et al., 2005). Rice proteins are precious because of their colorlessness, essential amino acid content, bland flavor, and hypoallergenic properties (Chrastil, 1992). Therefore, breeding activities are critical for enhancing rice grain yield output. Hybrid rice has been a successful and cost-effective technique for expanding rice productivity and production.

On the sister line, most of the yield characters were recorded (B- line). For the testers, IR69625A × Giza 179 produced the desirable values for the number of panicles/plant, 1000 grain weight, and grain yield/plant, while Giza 178M4 grew the desirable values for the seed set %. These results were consistent with Swati & Ramesh (2004) and Abd El-Aty et al. (2022). Among the hybrid combinations, IR70368 × Giza 179IM, G46A × Giza 178M4, and G46A × Giza 179Im, showed the heaviest panicles.

The hybrid combination IR70368A × Giza 178M4 achieved the highest number of panicles per plant and grain yield per plant. These results may be attributed to the stability of favorable weather conditions and the homogeneity of the F1 crosses. Therefore, these hybrids could be released as promising hybrids into the hybrid rice program.

Additionally, Thalapati et al. (2015) discovered that the yield and its contributing features of hybrids were estimated, as was the variance for the combining ability. As a result, it can be concluded that the crosses involving these parents will result in the identification of superior segregates with favorable grain yield genes, and the related investigation of these crosses will result in the evaluation and understanding of the effect of non-additive gene action on a trait. Moreover, non-additive gene action for a feature is an indicator for hybrid combination selection. As a result, a highly significant SCA effect is desirable for a hybrid breeding program to be successful.

Thus, dominant genetic effects influence the inherited traits' inheritance, indicating that the selection for the qualities inherited in this manner should be conducted in subsequent generations. The results correspond to those of (Kiani, 2019). Numerous researchers have reported the preponderance of dominant gene action for most yield traits (Satyanarayana et al., 2000; Kumar & Chaudhory, 2004). At the same time, Kumar et al. (2006) revealed the predominance of additive gene action and the necessity of using it in combination with other parameters' interactions. (Thirumeni & Paramasivam, 2000; Pradhan et al., 2006) observed a predominance of non-additive gene action in the expression of yield and yield-related attributes.

Ahmadikhah (2008) observed a low specific heritability for yield-related traits, and Wu et al. (1986) reported a low specific heritability for tiller numbers and grain yield. Thus, hybridization appears to be a viable option for using putative heterosis in particular crosses. Correlation coefficients between characters are vital, especially for economic and complex traits such as yield. Steel & Torrie (1984) stated that correlations are measures of the intensity of the links between attributes. Rasheed et al. (2002) also found a clear correlation between the yield/plant and the seed set % at the genotypic level, which implied that increasing the seed set % may increase the grain yield.
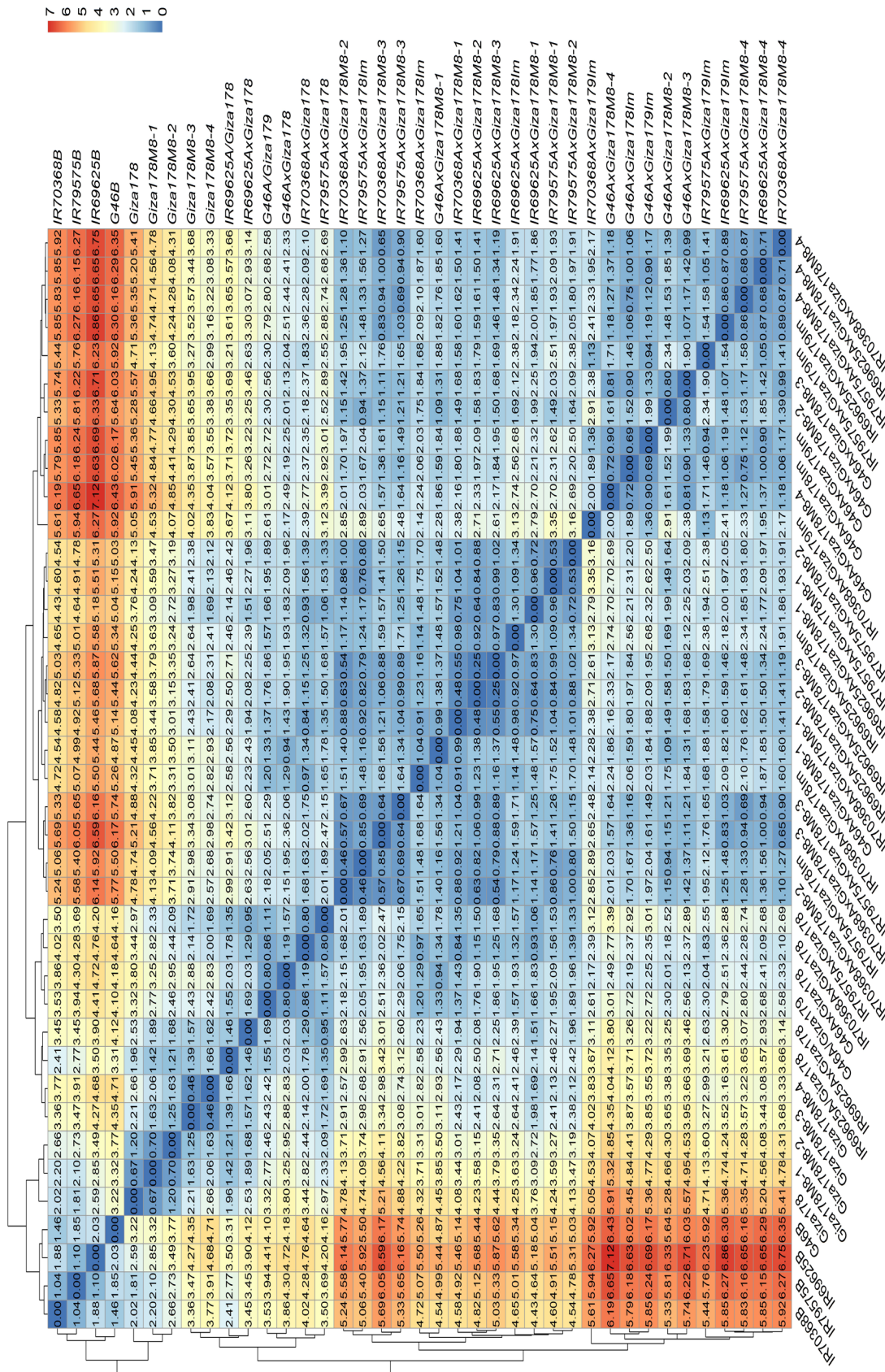
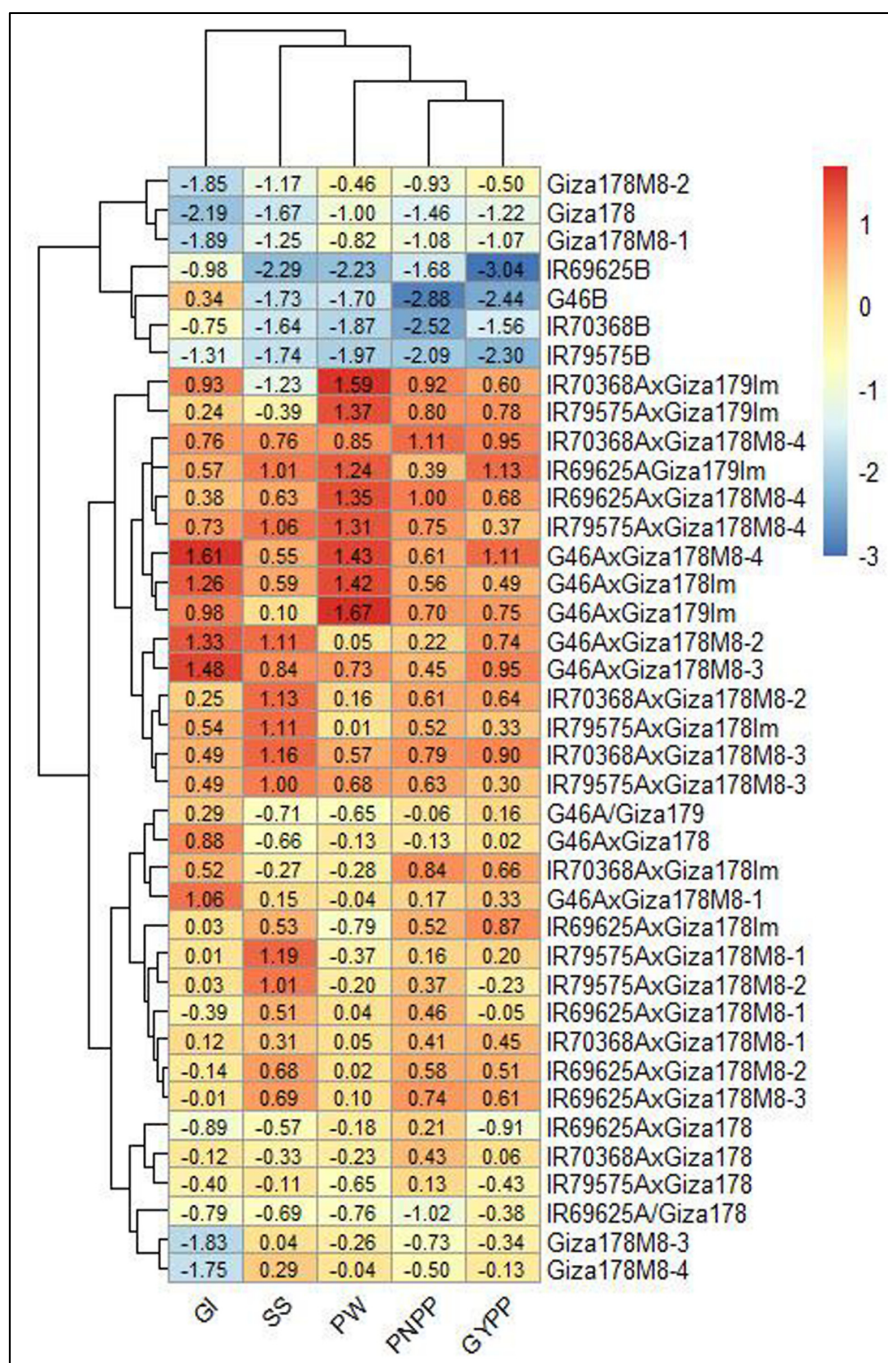**Fig. 6. Heatmap showing the dissimilarity among all the lines, testers, and crosses**

Fig. 7. Heatmap of the relationship among all the lines, testers, crosses, and the studied traits

This revealed a more significant role for non-additive gene action in its expression and a powerful potential for utilizing non-additive genetic diversity for these qualities via hybrid breeding (Annadurai & Nadarajan, 2001; Likittrakulwong et al., 2021). Since strong GCA effects are additive for genetic variation, the parents with the highest positive significant GCA effects are good combinators. The analysis of variance of the general combining ability (GCA) and the specific combining ability (SCA) revealed a predominance of additive gene effects in the grain yield inheritance. These findings are also corroborated by Likittrakulwong et al. (Likittrakulwong et al., 2021). Overall, a total protein analysis may be a proxy for restoring the gene expression in F1 hybrids.

Conclusions

In a randomized complete block design with three replicates, 28 hybrids and their parents were analyzed. Our results indicated that the analysis of variance was highly important for the yield and its components across the lines, testers, and line testers. The variance and ratio of the general and particular combinatorial ability and the degree of dominance indicated a predominance of non-additive gene effects for each attribute. Within CMS lines, G46A had the highest GCA for most of the characters analyzed. Among the testers, G178M4, Giza 178Im, and Giza 179Im were effective general combinators for most of the investigated characters. In addition, broad-sense heritability was high for all the examined traits. The paternal lines G178M4, Giza 178Im, and Giza 179Im could generate novel combinations, and the female line G46A could generate novel combinations, which could be utilized to generate new hybrid combinations for the majority of the examined characters. Thus, a total protein analysis may be a proxy for restoring the gene expression in F1 hybrids, particularly for the seed set %.

Competing interests The authors report no conflicts of interest regarding this work.

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تحليل إجمالي البروتين والمكونات الوراثية للمحصول ومكوناته في بعض أنواع الأرز الهجين

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أجريت تجربة خط × فاحص خلال موسمي 2018 و 2019 لتقييم القدرة على الجمع ، والعمل الجيني ، والتغاير لصفات الإنتاجية. خلال موسم 2018 ، تم عبور أربعة خطوط مع سبعة مختبرين في طريقة اختبار الخط، مما أسفر عن ثمانية وعشرين سيارة هجينة من طراز F1. في 1 مايو 2019 ، تم استخدام تصميم القطاعات الكاملة العشوائية بثلاثة مكررات لإنشاء ثمانية وعشرين هجينًا وآبائهم . كشف تحليل التباين عن وجود فروق ذات دلالة إحصائية بين السلالات والاختبارات والخط × المختبرين للحاصل ومكوناته. كشفت تقديرات التباين ونسبة قدرة التجميع العامة والخاصة ، وكذلك درجة السيادة ، عن رجحان تأثيرات الجينات غير المضافة. ضمن خطوط CMS ، كان G46A لديه أعلى GCA لمعظم الصفات المدروسة. تمثل G178M4 و Giza 178 Im و Giza 179 Im توافقات عامة جيدة لمعظم الشخصيات التي تم اختبارها من قبل المختبرين. كان التوريث واسع النطاق مرتفعًا لجميع الصفات المدروسة ، مما يشير إلى أن العوامل البيئية لها تأثير طفيف. أظهرت النتائج أيضًا أن السلالات الأبوية G178M4 و Giza 178Im و Giza 179IM يمكن أن تخلق توليفات هجينة جديدة ، في حين أن السلالة الأنثوية G46A يمكن أن تجمع معظم الصفات التي تم تقييمها.