HEAT TOLERANCE AND GENETIC DIVERSITY ANALYSES OF RICE ACCESSIONS USING SSR MARKERS

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SUMMARY

The germplasm with heat-tolerant traits is one of the crucial targets effective in rice (*Oryza sativa* L.) breeding for climate change. Hence, the presented research aimed to improve heat-tolerant cultivars through traditional breeding and molecular markers for climate change adaptability. The results showed most of the studied rice genotypes had a wide range of variability for various traits, with this range also reflected among the tested crosses. The best crosses with the highest mean values for all traits were Giza178 × Giza179, Giza178 × IET 1444, Sakha104 × IET 1444, and Giza179 × IET 1444. The general combining ability (GCA) effects revealed cultivars IET 1444, Giza179, Giza178, and Sakha104 with significant positive GCA influences for tillers and panicles plant^{-1}, filled grains panicle^{-1}, and grain yield per plant. The best identified crosses for almost all traits were Giza177 × Giza178, Giza177 × Giza179, Giza177 × Sakha104, Giza178 × IET 1444, and Sakha105 × IET 1444. The principal component analysis (PCA) divided the seven rice genotypes into two groups. The first one included the sensitive rice cultivars, namely, Giza177, Sakha105, and Sakha101, and the second group comprised tolerant genotypes, i.e., Giza178, Giza179, IET144, and Sakha104. Using 18 SSR markers helped assess the genetic diversity in rice genotypes. The studied markers produced 204 alleles, with a mean of 11.33 per locus. A higher number of alleles per locus resulted from primers RM493, RM341, RM3297, and RM3330. The polymorphic information content (PIC), a reflection of allele diversity and frequency, was moderate and ranged between 0.157 for RM504 and 0.872 for RM3330, with an average of 0.756. Based on the SSR cluster analysis, rice genotypes formed two groups; the first group included the sensitive rice genotypes, while the second was the tolerant genotypes.

Key words: Rice (*Oryza sativa* L.), germplasm, breeding, heat tolerance, genetic diversity, GCA and SCA, SSR markers, yield-related traits

Key findings: In the presented study, the four rice (*Oryza sativa* L.) parental genotypes, Giza178, Sakha104, IET 1444, and Giza179, were heat-tolerant, while three genotypes, Giza2177, Sakha 101, and Sakha105, were heat-sensitive. The crosses Giza177 × Giza178, Giza177 × Giza179, Giza177 × Sakha104, Giza178 × IET 1444, and Sakha105 × IET 1444 were notably high-yield crosses. Based on genetic diversity, Giza177, Sakha101, and Sakha105 genotypes were sensitive, and Giza178, Giza179, IET144, and Sakha104 were tolerant. The SSR markers RM493, RM341, RM3297, and RM3330 showed the highest alleles. The promising parental genotypes and their hybrids could be beneficial for developing heat-tolerant rice genotypes.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most pertinent food sources worldwide, and more than half of the world's population depends on it as a staple food, especially in developing countries (Al-Zahrani *et al*., 2018). The global demand for rice, increasing yearly to cope with the population demand, requires a considerable enhancement in rice production (USDA, 2021). However, several constraints in increasing rice yield worldwide are climate change factors, i.e., heat and cold, drought, crop diseases, and high salinity (Kumar *et al*., 2022).

Recently, agriculture production has incurred global warming impacts due to climate change (Dabi and Khanna, 2018; Ezin *et al*., 2022; Saud *et al*., 2022). The effects of global warming and arable land erosion are critical and need thorough attention worldwide for achieving food security (Masutomi *et al*., 2023). High temperatures adversely affect rice plants sensitive to extreme heat, especially at the flowering stage, which leads to sterility and low grain yield (Jagadish *et al*., 2010; Kinose *et al*., 2020). For better rice production, the required optimum temperature is between 25 °C and 35 °C; however, higher temperatures will severely affect the crop, decreasing grain yield by 7%–8% and influencing grain quality traits (Nishad *et al*., 2018; Liu *et al*., 2023). An increase in temperature by 1 °C has led to an increase in sterility by 5%–15% in 62% of rice-growing areas. Also, when the temperature rises by 3 °C degrees, sterility increases by more than 30% (Jagadish *et al*., 2015).

Therefore, studies associated with the inheritance of heat-stress tolerance are crucial because of its polygenic control compared with the inheritance of drought and salt tolerance in rice (Villegas *et al*., 2018; Mohamed *et al*., 2021; Li *et al*., 2023). However, previous studies reported that, at the flowering stage, heat tolerance has recessive gene control in rice (Howarth, 2005; Fu *et al*., 2015). Thus, the hybridization process requires the heat-tolerant parental genotypes' involvement to produce desirable F_1 combinations. But, sometimes, the male parent plays a vital role in heat tolerance compared with female parental lines. In rice, genetic diversity offers an applicable approach to helping the breeders develop new genotypes with a wide range of tolerance to biotic and abiotic stresses by selecting recombinants with desired genes. In this sense, different molecular markers, such as simple sequence repeats (SSR), help choose rice genotypes carrying the desirable genes (Singh *et al*., 2015; Mazal, 2021).

Breeding of heat-tolerant cultivars validates an efficient approach to reducing the impact of global warming on rice and other major crops (Masutomi *et al*., 2023). Conventional rice breeding generally employs two main methods: the modified pedigree and recurrent selection (Villegas *et al*., 2018). However, in the Philippines, the breeding of heat-tolerant rice began in 2010 to develop rice genotypes that could adapt to changing climatic and local farming conditions through a combination of heat-tolerant donor parents (such as, cultivar N22) with high-yielding cultivars (Manigbas *et al*., 2014). The promising study sought to investigate the general and specific combining ability (GCA and SCA) and inheritance of quantitative characters in rice F_1 hybrids to estimate the genetic diversity among rice genotypes and classify them through cluster analysis using SSR markers.

MATERIALS AND METHODS

Seven rice (*Oryza sativa* L.) genotypes comprising four tolerant cultivars (Giza178, Sakha104, Giza179, and IET 1444) and three sensitive cultivars (Giza177, Sakha105, and Sakha101) sustained crossing in a half-diallel design to produce 21 F_1 crosses (Table 1). The 21 F_1 hybrids and their seven parental genotypes' planting occurred during the 2018 and 2019 growing seasons at the research field of the New Valley Agricultural Research Station, Egypt. Transplanting all the rice genotypes transpired in a randomized complete block design (RCBD) with three replications. Each subplot comprised five rows, five meters in length with 20 cm spacing among the rows, to have 25 plants/m^2. The recommended cultural practices for rice cultivation ensured proper employment. The average temperature

(°C) and relative air humidity (%) within each month ensued every 10 days (Table 2). The data recorded on 10 agronomic traits included duration (days), plant height (cm), flag leaf area (cm^2), tillers plant^{-1}, panicles plant^{-1}, panicle weight (g), 1000-grain weight (g), filled grains panicle^{-1}, unfilled grains panicle^{-1}, and grain yield plant^{-1} (g) according to the standard evaluation system (IRRI, 2014).

Genomic DNA isolation

Seven rice genotypes bore screening using 18 simple sequence repeat (SSR) primers related to heat tolerance (RM209, RM493, RM21, RM405, RM7075, RM341, RM518, RM148, RM231, RM3297, RM562, RM206, RM17, RM225, RM153, RM190, RM3843, and RM3330), supplied by the Sangon Company, China. DNA genomic extraction of the rice

Table 1. Rice genotypes with their parentage, origin, type, and tolerance category.

No.	Genotypes	Parentage	Origin	Type	Tolerance category
1	Giza177	Giza171/ Yumji No.1// PiNo.4	Egypt	Japonica	Sensitive
2	Giza178	Giza 171 / Milyang 49	Egypt	Indica Japonica	Tolerant
3	Sakha104	GZ 4096 X GZ 4100	Egypt	Japonica	Tolerant
4	Sakha105	Giza177 / Suwwon349	Egypt	Japonica	Sensitive
5	Giza179	GZ6296/ GZ1368	Egypt	Indica Japonica	Tolerant
6	IET 1444	TN1 x CO 29	India	Indica Japonica	Tolerant
7	Sakha101	Giza 176 / Milyang 79	Egypt	Japonica	Sensitive

Table 2. Maximum and minimum temperature (°C) and relative humidity (%) at New Valley Agricultural Research Station averaged every 10 days during rice seasons, 2019.

Months	Date	New Valley Governorate Average Air Temperature 2019		Relative humidity (%)
		Maximum	Minimum	
May	1-10	39.4	21.2	27
	11-20	40.1	20.7	25
	21-31	40.6	23.4	24
June	1-10	41.7	25.8	23
	11-20	40.0	24.2	24
	21-30	42.4	23.0	27
July	1-10	41.9	23.4	30
	11-20	40.3	23.2	29
	21-31	43.6	25.3	26
August	1-10	45.1	29.8	24
	11-20	44.4	30.7	26
	21-31	40.5	26.2	31
September	1-10	40.3	25.6	31
	11-20	41.2	25.9	31
	21-30	41.1	25.6	29
Range		39.4 – 44.4	20.7 – 30.7	23 – 31

parental genotypes proceeded (Maixner *et al*., 1995) using two biological replicates for DNA extraction and PCR for each variety. After DNA extraction, the extracted DNA storage had a temperature of -20 °C.

Polymerase chain reaction (PCR) assay

The reaction mixture (25 µL) consisted of 12.5 µL of 2× master mix ready-to-use (0.1 U/µL Taq Polymerase, 20 mM Tris-HCl [pH8.3], 100 mM KCl, 3 mM MgCl2, a stabilizer, and enhancer), 10 Pmol of each primer (1.0 µL), 1.0 uL of DNA (50 ng), and 9.5 µL of PCR grade water. Performing amplifications used a thermocycler (Bio-Rad, C-1000, California, USA) with the following steps: (1) initial denaturation at 94 °C for 5 min; (2) 30 s of denaturation at 94 °C; (3) primers' annealing temperatures differ according to their Tm, for 1 min; (4) one-minute extension at 72 °C; (5) repeating steps 2, 3, and 4 40 times; (6) and a final extension of 10 min at 72 °C ensued. The amplified products' analysis continued on a 2% agarose gel electrophoresis containing ethidium bromide, then photographed and analyzed using the BioDoc Analysis software (Biometra, Germany).

Statistical analysis

All the recorded data underwent analysis of variance (ANOVA) according to Steel and Torrie (1980). The GCA and SCA analysis was in accordance with the procedure suggested by Griffing (1956) using method two and model one. Further comparing and separating the means used the least significant difference (LSD) test at $P < 0.05$ (significant) and $P < 0.01$ (highly substantial) (Singh and Chaudhary, 1985). The analysis of genetic relationships relied on the numerical taxonomic approach for assessing the genetic divergence, and dissimilarity coefficients depended on simple matching (Sneath and Sokal, 1973). The total number of amplified bands, the number of polymorphic alleles of the amplified alleles, the polymorphism ratio (P%), and the polymorphism information content (PIC) incurred calculations to determine the diversity among the alleles of the marker locus (Anderson *et al*., 1993). Similarity matrices generation used the NTSYS-pc version 2.1 (Rohlf, 2000), while the principal component analysis (PCA) followed the method of Ariyo and Odulaja (1991). This method reduces the dimensions of multivariate data to a few principal axes, generates an Eigenvector for each axis, and produces component scores for various characters.

RESULTS AND DISCUSSION

Analysis of variance

According to the analysis of variance, results revealed that mean squares due to genotypes were significant ($P \leq 0.01$) for all traits (Table 3). The mean squares due to genotypes sustained further partitioning into parents, crosses, and parents vs. crosses. The differences among parents were also significant ($P \leq 0.01$) for all traits, indicating the presence of wide genetic variability among parents for almost all traits. The mean squares due to crosses for all traits were significant ($P \leq 0.01$). Parents vs. crosses mean squares further revealed meaningful differences in all crosses ($P \leq 0.01$). The data showed that both general combining ability (GCA) and specific combining ability (SCA) variances were significant ($P \leq 0.01$) for the 10 traits in F_1 rice populations (Table 3). It would indicate the importance of both additive and non-additive genetic variances in determining the performance of these traits. In F_1 populations, the GCA/SCA ratio was higher than unity, indicating that additive and additive × additive types of gene action were more valuable in the inheritance of the studied characteristics (Table 3). The results concluded that selection procedures based on the accumulation of additive effects would succeed in improving yield-related traits. The prevailing results were in line with previous findings on the impacts of different temperature conditions on yield and physiological properties of rice (*Oryza Sativa* L.) (Thippeswamy *et al*., 2016; He *et al*., 2018; Perdani *et al*., 2018; Mohamed *et al*., 2021).

Table 3. Analysis of variance among rice genotypes for agronomic traits under heat stress conditions.

S.O.V.	D.f.	DM	PH	FLA	ET	PN	PW	1000 gwt	FGP	SF	GYP
Replications	2	5.28	0.70	1.39	8.42	4.28	0.06	0.25	4.00	23.42	4.69
Genotypes	27	82.28**	93.04**	30.46**	54.20**	40.13**	0.11**	9.36**	473.89**	219.21**	41.45**
Parents	6	139.48**	119.35**	43.56**	44.16**	27.78**	0.20**	11.65**	756.25**	159.33**	41.09**
Crosses	20	64.21**	89.75**	14.32**	31.97**	23.22**	0.08**	9.11**	277.93**	213.74**	19.80**
P. vs. C (H)	1	100.66**	0.95*	274.77**	559.14**	452.52**	0.29**	0.47**	2698.93**	688.02**	476.47**
Error	54	3.88	2.13	1.39	1.27	4.40	0.03	0.36	44.70	23.39	4.84
GCA	6	96.19**	99.97**	16.00**	30.39**	22.15**	0.05**	10.66**	338.30**	123.57**	24.33**
SCA	21	7.78**	11.31**	8.49**	14.55**	10.87**	0.03**	0.96**	106.44**	58.64**	10.81**
Error	54	1.15	0.71	0.46	0.42	1.47	0.01	0.12	14.90	7.80	1.61
GCA/SCA ratio	---	1.59	1.04	0.22	0.24	0.24	0.19	1.39	0.393	0.25	0.27

*,**: significant at 0.05 and 0.01 levels, respectively. DM = Days to maturity, PH = Plant height (cm), FLA = Flag leaf area (cm^2), ET = Effective tillers plant^{-1}, PN = panicles plant^{-1}, PW = Panicle weight (g), 1000 gwt = 1000-grain weight (g), FGP = Filled grains panicle^{-1}, SF = Spikelet fertility (%), and GYP = Grain yield plant^{-1} (g).

Mean performance

In seven rice (*Oryza sativa* L.) parental genotypes and 21 F_1 crosses, most studied traits had a wide range of variability, and this wide range of variations might be due to the diverse genetic background of the studied F_1 hybrids (Table 4). The cross with a high mean value is desirable except for plant height and unfilled grains panicle^{-1}, where the lowest value is preferable (Abdel-Hafez *et al*., 2016). The results showed that the tolerant rice parental genotypes were more adaptive and contributed to the inheritance of such traits under high-temperature conditions. As for the duration attribute, the rice cultivars Giza177 and Sakha105 were early maturing and required 90.59 and 94.29 days to maturity, respectively (Table 4). However, seven F_1 hybrids, namely, Sakha105 × Sakha101, Giza177 × Giza178, Giza178 × Sakha105, Sakha105 × Giza179, Giza178 × Sakha105, Giza177 × Sakha105, and Sakha104 × Sakha105, required a short duration under heat stress conditions (Table 4). These results agreed with past findings on breeding for plant heat tolerance at vegetative and reproductive stages (Driedonks *et al*., 2016; Widyawan *et al*., 2020; Adriansyah *et al*., 2021; Mohamed *et al*., 2021).

For plant height, all rice genotypes only reached less than 100 cm in height due to high temperatures; however, this short stature is desirable to avoid lodging and suitable for mechanical harvesting (Table 4). On flag leaf area, the rice parental genotypes Giza179, IET 1444, Sakha104, and Giza178 showed maximum values, whereas the cultivars Giza177 and Sakha105 had the lowest flag leaf area. The results further revealed that the F_1 hybrids Giza177 × Sakha104, Giza177 × Giza178, Giza178 × Sakha104, Giza179 × IET 1444, Giza177 × Giza179, Giza177 × IET 1444, and Sakha104 × Giza179 proved promising for flag leaf area.

On tillers plant^{-1}, the rice genotypes Giza179, Giza178, IET 1444, and Sakha104 gave more tillers plant^{-1}, i.e., 19.45, 16.74, 13.23, and 12.04 tillers plant^{-1}, respectively (Table 4). Inversely, the genotypes Giza177 and Sakha105 provided the lowest values for the said trait. The results further showed crosses Giza178 × Giza179, Giza178 × Sakha104, Giza179 × IET 1444, Sakha104 × IET 1444, Giza177 × IET 1444, and Giza178 × IET 1444 emerged as promising regarding the high number of tillers plant^{-1}, i.e., 26.03, 23.63, 23.57, 23.23, 22.13, and 21.93, respectively. The parent cultivars and the crosses exhibiting the best performance

Table 4. Mean performances of the rice parental genotypes and F_1 hybrids for agronomic traits under heat stress conditions.

Parental genotypes & F_1 populations	DM	PH	FLA	ET	PN	PW	1000 gwt	FGP	SF	GYP
Giza177	90.59	85.72	20.34	9.30	7.77	2.65	27.20	78.60	27.74	20.42
Giza177 x Giza178	93.63	82.87	32.50	14.57	12.73	3.02	27.87	115.50	40.13	32.80
Giza177 x Sakha104	98.67	95.43	32.57	18.90	15.70	2.91	27.67	117.90	23.30	29.53
Giza177 x Sakha105	95.00	91.10	28.97	16.27	12.87	3.24	27.90	99.73	31.43	26.97
Giza177 x Giza179	94.73	85.49	31.63	16.00	13.23	3.09	27.17	118.63	21.57	27.77
Giza177 x IET 1444	105.53	84.07	31.03	22.13	19.33	3.17	28.00	110.43	23.27	26.70
Giza177 x Sakha101	104.27	88.10	27.23	18.57	15.67	3.16	27.27	111.17	21.27	24.27
Giza178	99.56	86.37	28.06	16.74	14.30	2.63	21.93	105.90	12.75	27.90
Giza178 x Sakha104	100.75	96.83	32.40	23.63	20.50	3.02	25.57	109.73	22.37	28.27
Giza178 x Sakha105	94.57	100.60	28.47	17.80	14.23	2.83	24.43	113.73	21.37	27.47
Giza178 x Giza179	99.10	85.70	30.57	26.03	20.47	2.68	22.17	116.00	19.90	29.40
Giza178 x IET 1444	102.03	82.50	30.30	21.93	18.23	3.03	25.53	123.70	12.27	32.87
Giza178 x Sakha101	99.36	86.77	26.30	19.97	17.43	2.87	24.27	105.50	23.77	27.07
Sakha104	103.42	102.38	28.43	12.04	10.55	3.15	26.39	108.00	11.41	24.66
Sakha104 x Sakha105	95.57	96.60	24.53	16.63	14.57	2.90	24.93	111.50	27.63	26.47
Sakha104 x Giza179	102.03	96.40	31.00	18.20	15.73	2.72	23.87	123.43	14.17	31.83
Sakha104 x IET 1444	102.17	94.10	28.07	23.23	19.40	3.09	24.67	113.33	22.10	28.90
Sakha104 x Sakha101	107.25	91.03	28.17	18.17	15.63	3.16	26.77	113.67	19.33	26.50
Sakha105	94.29	93.40	20.64	9.29	7.23	2.51	26.44	78.71	22.87	17.25
Sakha105 x Giza179	94.60	89.97	30.67	16.50	12.70	2.78	23.30	106.77	21.83	25.40
Sakha105 x IET 1444	97.73	98.97	30.00	16.17	14.20	2.83	25.23	118.53	14.60	29.10
Sakha105 x Sakha101	92.57	90.47	27.47	14.57	11.20	2.89	25.33	90.80	33.63	24.43
Giza179	100.22	88.24	29.59	19.45	14.93	3.09	23.16	118.30	10.47	25.83
Giza179 x IET 1444	101.10	87.43	31.97	23.57	19.43	3.06	24.13	120.53	11.23	31.73
Giza179 x Sakha101	104.90	86.50	28.87	18.60	16.37	2.75	23.00	95.63	37.73	25.47
IET 1444	111.60	83.77	27.03	13.23	10.40	3.02	25.81	107.04	11.94	24.57
IET 1444 x Sakha101	107.43	86.27	28.83	16.70	14.50	3.00	26.17	91.07	40.07	26.60
Sakha101	110.69	90.80	23.86	10.95	8.67	2.72	26.28	87.58	24.21	20.38
LSD$_{0.05}$	2.5	2.0	1.6	1.5	2.9	0.3	0.8	9.1	6.6	3.0
LSD$_{0.01}$	3.6	2.9	2.3	2.2	4.1	0.4	1.2	13.1	9.5	4.3

DM = Days to maturity, PH = Plant height (cm), FLA = Flag leaf area (cm^2), ET = Effective tillers plant^{-1}, PN = panicles plant^{-1}, PW = Panicle weight (g), 1000 gwt = 1000-grain weight (g), FGP = Filled grains panicle^{-1}, SF = Spikelet fertility (%), and GYP = Grain yield plant^{-1} (g).

for tillers plant^{-1} also performed better for the number of panicles plants^{-1}. Therefore, these cross-combinations could be helpful in future breeding programs to develop genotypes with heat tolerance. The results support previous studies on the heat stress effects on yield parameters of selected rice cultivars at reproductive growth stages (Aghamolki *et al.*, 2014).

Concerning panicle weight, results indicated rice parental genotypes Sakha104, Giza179, and IET 1444 gave panicle weights of 3.15, 3.09, and 3.02 g, respectively (Table 4). However, the 11 F_1 hybrids showed higher values for panicle weight, and the promising crosses were Giza177 × Sakha105, Giza177 × IET 1444, Giza177 × Sakha101, and Sakha104

× Sakha101. For 1000-grain weight, the six F_1 hybrids enunciated the higher 1000-grain weight, and the leading parent cultivar was Giza177 (27.20 g) compared with the remaining rice parental cultivars (Table 4). The filled grains panicle^{-1} had 14 crosses recorded with the highest values, and the best performing F_1 hybrids for the said trait were Sakha104 × Giza179 (123.43), Giza178 × IET 1444 (123.7), and Giza179 × IET 1444 (120.53 filled grains panicle^{-1}).

Four rice parent cultivars, namely, Giza179, Sakha104, IET 1444, and Giza178, exhibited low values for unfilled grains panicle^{-1} (Table 4). Likewise, for the said trait, the best F_1 hybrids were Giza179 × IET 1444, Sakha105 × IET 1444, Sakha104 × Giza179,

Giza178 × IET 1444, and Sakha104 × Giza179. On grain yield plant[-1], rice cultivars, viz., Giza178, Giza179, Sakha104, and IET 1444, showed the highest grain yield plant[-1] (27.90, 25.83, 24.66, and 24.57 g, respectively) (Table 4). However, the crosses showed more grain yield than the leading parental cultivars, and the best crosses were Giza178 × IET 1444 (32.87 g), Giza177 × Giza178 (32.80 g), Sakha104 × Giza179 (31.83 g), and Giza179 × IET 1444 (31.73 g). The superior cross combinations with higher mean values for various yield-related traits were Giza178 × Giza179, Giza178 × IET 1444, Sakha104 × IET 1444, and Giza179 × IET 1444, which can benefit from improving these features through future breeding programs under high-temperature conditions. Similar results came from previous works on the effect of heat stress at vegetative and reproductive stages on spikelet fertility in rice (Cheabu *et al*., 2018; Ali *et al.*, 2019; Mazal, 2021).

General combining ability

An estimate of the general combining ability effect is an essential indicator of the potential of parental genotypes for generating superior breeding populations through hybridization (Table 5). The GCA and SCA variances were also highly significant for all 10 rice traits studied in F_1 populations. It would indicate the importance of the additive and non-additive genetic variances in determining the performance of these 10 characteristics. Also, it signifies that the additive and additive × additive types of gene action are more valuable in the inheritance of these qualities.

The results concluded that selection procedures based on the accumulation of additive effects would succeed in improving these rice traits. Using the GCA revealed the nature and activity of genes involved in the trait inheritance; it is vital in cross-pollinated crops because it helps identify potential parents for outcrossing (Abdel-Hafez *et al.*, 2016; Masutomi *et al*., 2023)**.** Estimates of GCA effects showed the impossibility of selecting a better general combiner because not a single parent had a highly significant GCA effect for all 10 agronomic traits. A negligible or negative combining ability effect indicates a poor ability to transfer its genetic superiority to hybrids. However, the largest significant positive values have the most impacts on all traits except plant height and unfilled grains panicle[-1]. In the investigation, three rice cultivars, Giza177, Sakha105, and Giza178, showed significant negative values for maturity days and plant height. The highly notable GCA effects for early maturity (short duration) and plant height are desirable features in genotypes when breeding for heat tolerance (Fu *et al*., 2015; Gaballah and Abu-El-Ezz, 2019). However, the rice cultivars IET 1444, Giza179, and Giza178 signified the most

Table 5. General combining ability (GCA) effects of rice parental genotypes for various traits.

Parental genotypes	DM	PH	FLA	ET	PN	PW	1000 gwt	FGP	SF	GYP
Giza177	-2.15**	-2.64**	-0.42*	-1.64**	-1.28**	0.05	1.86**	-3.32**	4.23**	-0.52
Giza178	-1.54**	-1.58**	0.91**	1.96**	1.74**	-0.08*	-1.10**	3.95**	-1.45	2.23**
Sakha104	1.21**	5.88**	0.57**	0.34	0.67	0.07*	0.30**	5.02**	-2.96**	0.80*
Sakha105	-4.86**	3.58**	-1.89**	-2.58**	-2.48**	-0.10**	0.05	-6.88**	1.98*	-2.14**
Giza179	-0.61	-1.59**	1.72**	2.01**	1.25**	-0.02	-1.51**	6.35**	-3.45**	1.08**
IET 1444	4.09**	-2.37**	0.65**	1.16**	1.04**	0.09**	0.20	3.47**	-3.44**	0.61
Sakha101	3.86**	-1.28**	-1.54**	-1.25**	-0.94*	-0.02	0.20	-8.60**	5.09**	-2.05**
LSD$_{0.05}$	0.66	0.52	0.42	0.40	0.75	0.06	0.21	2.38	1.72	0.78
LSD$_{0.01}$	0.88	0.69	0.56	0.53	0.99	0.09	0.28	3.18	2.30	1.04

*, ** significant at 0.05 and 0.01 levels, respectively. DM = Days to maturity, PH = Plant height (cm), FLA = Flag leaf area (cm²), ET = Effective tillers plant[-1], PN = panicles plant[-1], PW = Panicle weight (g), 1000 gwt = 1000-grain weight (g), FGP = Filled grains panicle[-1], SF = Spikelet fertility (%), and GYP = Grain yield plant[-1] (g).

Table 6. Specific combining ability (SCA) effects of F_1 populations for various traits.

F_1 Populations	DM	PH	FLA	ET	PN	PW	1000 gwt	FGP	SF	GYP
Giza177 x Giza178	-2.98**	-3.19**	3.46**	-3.23**	-2.30*	0.11	1.66**	7.31*	15.06**	4.39**
Giza177 x Sakha104	-0.69	1.91**	3.86**	2.72**	1.73	-0.15	0.07	8.65**	-0.26	2.55*
Giza177 x Sakha105	1.72*	-0.12	2.73**	3.02**	2.05*	0.37**	0.55*	2.38	2.92	2.93**
Giza177 x Giza179	-2.80**	-0.56	1.79**	-1.84**	-1.31	0.13	1.38**	8.04**	-1.51	0.52
Giza177 x IET 1444	3.30**	-1.20	2.26**	5.14**	5.00**	0.10	0.50	2.72	0.18	-0.08
Giza177 x Sakha101	2.26**	1.74**	0.64	3.99**	3.31**	0.20*	-0.24	15.53**	-10.35**	0.15
Giza178 x Sakha104	0.78	2.26**	2.36**	3.86**	3.52**	0.09	0.92**	-6.79*	4.49*	-1.47
Giza178 x Sakha105	0.67	8.32**	0.89	0.95	0.41	0.09	0.04	9.11**	-1.46	0.68
Giza178 x Giza179	0.95	-1.41*	-0.62	4.60**	2.91**	-0.15	-0.67*	-1.86	2.50	-0.61
Giza178 x IET 1444	-0.82	-3.82**	0.19	1.34**	0.89	0.10	0.98**	8.72**	-5.14*	3.33**
Giza178 x Sakha101	-3.26**	-0.65	-1.63**	1.79**	2.06*	0.03	-0.28	2.60	-2.17	0.19
Sakha104 x Sakha105	-1.08	-3.14**	-2.70**	1.40**	1.80	0.00	-0.86**	5.81	6.32**	1.10
Sakha104 x Giza179	1.14	1.83**	0.16	-1.62**	-0.76	-0.27**	-0.37	4.51	-1.72	3.26**
Sakha104 x IET 1444	-3.43**	0.31	-1.71**	4.26**	3.12**	0.00	-1.28**	-2.71	6.21**	0.79
Sakha104 x Sakha101	1.88*	-3.85**	0.58	1.61**	1.33	0.18*	0.82**	9.70**	-5.09*	1.05
Sakha105 x Giza179	-0.23	-2.31**	2.29**	-0.39	-0.64	-0.03	-0.68*	-0.26	1.00	-0.23
Sakha105 x IET 1444	-1.79*	7.48**	2.69**	0.12	1.07	-0.08	-0.46	14.39**	-6.24**	3.94**
Sakha105 x Sakha101	-6.73**	-2.12**	2.34**	0.93	0.05	0.08	-0.36	-1.27	4.26	1.93
Giza179 x IET 1444	-2.68**	1.11	1.05*	2.93**	2.58**	0.06	0.00	3.15	-4.18	3.35**
Giza179 x Sakha101	1.35	-0.92	0.13	0.38	1.48	-0.14	-1.13**	-9.67**	13.79**	-0.25
IET 1444 x Sakha101	-0.82	-0.36	1.17*	-0.68	-0.17	0.00	0.32	-11.36**	16.12**	1.35
LSD$_{0.05}$	1.64	1.28	1.04	0.99	1.85	0.16	0.53	5.91	4.27	1.94
LSD$_{0.01}$	2.18	1.71	1.39	1.32	2.47	0.22	0.71	7.87	5.69	2.59

*, ** significant at 0.05 and 0.01 levels, respectively. DM = Days to maturity, PH = Plant height (cm), FLA = Flag leaf area (cm^2), ET = Effective tillers plant^{-1}, PN = panicles plant^{-1}, PW = Panicle weight (g), 1000 gwt = 1000-grain weight (g), FGP = Filled grains panicle^{-1}, SF = Spikelet fertility (%), and GYP = Grain yield plant^{-1} (g).

meaningful positive GCA effects for tillers plant^{-1}, panicles plant^{-1}, and filled grains panicle^{-1}. The parental cultivars Giza178, Giza179, and Sakha104 displayed noteworthy positive GCA effects for grain yield plant^{-1}. These results indicated that these cultivars could serve in future hybridization programs to improve heat-tolerant traits through transgressive breeding.

Specific combining ability

The results of the specific combining ability effects showed that three F_1 hybrids, namely, Giza177 × Giza178, Giza177 × Giza179, and Sakha105 × Sakha101, gave significant ($P ≤ 0.01$) SCA influences for the traits, i.e., duration and maturity, plant height,

and flag leaf area (Table 6). These crosses would produce high yield, and early duration segregates in later generations. Similar findings have also resulted in the genetic behavior of some rice genotypes under normal and high-temperature stress conditions (Gaballah and Abu-El-Ezz, 2019).

For tillers plant^{-1} and panicles plant^{-1}, eight crosses, namely, Giza177 × Sakha105, Giza177 × IET 1444, Giza177 × Sakha101, Giza178 × Sakha104, Giza178 × Giza179, Giza178 × Sakha101, Sakha104 × IET 1444, and Giza179 × IET 1444, occurred with highly significant positive SCA effects (Table 6). Therefore, these crosses were also optional for heterosis breeding because the usefulness of a particular cross in the exploitation of heterosis depended on specific combining ability effects. Concerning the traits filled grains panicle^{-1} and grain yield plant^{-1}, the four crosses Giza177 × Giza178, Giza177 × Sakha104, Giza178 × IET 1444, and Sakha105 × IET 1444 showed highly significant positive SCA effects. It was apparent that the parental genotypes of all F$_1$ hybrids manifested with high × low GCA combiners, indicating the presence of dominance gene action. In any case, the new cross combinations produced from two good general combiners should produce some useful transgressive segregates; however, this is not normally the case and sometimes due to attributes of overdominance and epistasis (Gaballah and Abu-El-Ezz, 2019). The superiority of average × average combinations might be due to the concentration and interaction between favorable genes contributed by the parental genotypes. Also, the interaction between the positive alleles for good general combiners and negative alleles for poor general combiners also suggested the exploitation of heterosis in F$_1$ populations as their potential would be unfixable in succeeding generations in rice (Sitaresmi *et al.*, 2020).

Principal component analysis

The principal component analysis (PCA) helped explain the genetic diversity among the seven rice genotypes through morphological traits under high-temperature conditions. The cumulative variance of 98.90% for the first five axes with an Eigenvalue of >1.0 indicates that the identified traits within the axes exhibited better influence on the phenotype of the seven rice cultivars (Table 7). The results showed the control on PC1 and PC2 was for the distribution of the genotypes, with all the genotypes widely scattered across different quarters (Figure 1). These rice genotypes incurred divisions into two groups, with the first including three sensitive rice genotypes (Giza177, Sakha105, and Sakha101) and the second comprising four tolerant rice cultivars, i.e., Giza178, Giza179, IET144, and Sakha104. Similar results appeared with the principal component analysis in rice germplasm under high-temperature stress conditions (Mahendran *et al.*, 2015; Soe *et al.*, 2019; Ponsiva *et al.*, 2019; Al-Daej *et al.*, 2023). The PCA of quantitative traits implied that the first principal component accounted for 61.30% of the total variability, recording the traits for filled grains panicle^{-1}, flag leaf area, panicles plant^{-1}, unfilled grains panicle^{-1}, grain yield plant^{-1}, and tillers plant^{-1} with the values, i.e., 0.36, 0.36, 0.34, 0.34, 0.33, and 0.33, respectively (Figure 2).

The second principal component accounted for 20.60% of the total variability. The variables contributing most positively were plant height (0.45), 1000-grain weight (0.35), and days to heading (0.32). The third component accounted for 10.10% of the total variance, in which days to heading (0.74) and plant height (0.61) contributed positively. Thus, the prominent characteristics come together in different principal components and contribute to explaining the variability and have the tendency to remain together. One may also consider this when employing these attributes in breeding programs. However, the quantitative traits and pedigree information can help estimate the genetic distance among the cultivars. Plant breeders can use this information to identify the parental genotypes that are genetically diverse and have the potential to generate wider genetic variability for future crop improvement. Similar results indicated the comparative assessment of genetic diversity and marker-trait association using a marker system in *Oryza sativa* L. (Al-Daej *et al.*, 2023).

Table 7. Eigen value, factor scores, and contribution of the first six principal component axes to variation in rice genotypes.

Parameters	PC1	PC2	PC3
Day to maturity (days)	0.084	0.317	0.747
Plant height (cm)	0.017	0.453	0.618
Flag leaf area (cm^2)	0.362	0.112	0.041
Tillers/plant	0.333	0.224	0.024
Panicles/plant	0.347	0.179	0.045
Panicle weight (g)	0.292	0.298	0.079
1000-grain weight (g)	0.273	0.357	0.063
Filled grains/panicle	0.364	0.047	0.057
Unfilled grains/panicle	0.341	0.056	0.035
Grain yield/plant (g)	0.337	0.014	0.139
Eigenvalue	7.358	2.467	1.206
Variability (%)	61.30	20.60	10.10
Cumulative (%)	61.30	81.90	91.90

Variables (axes First and second component: 81.90%)
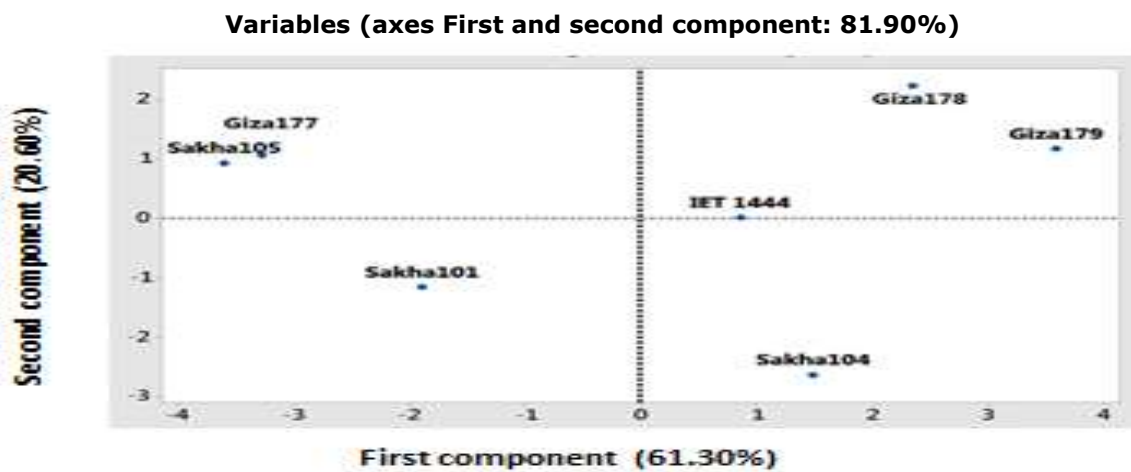
Figure 1. Distribution of seven rice genotypes across two components.

Variables (axes First and second component: 81.90%)
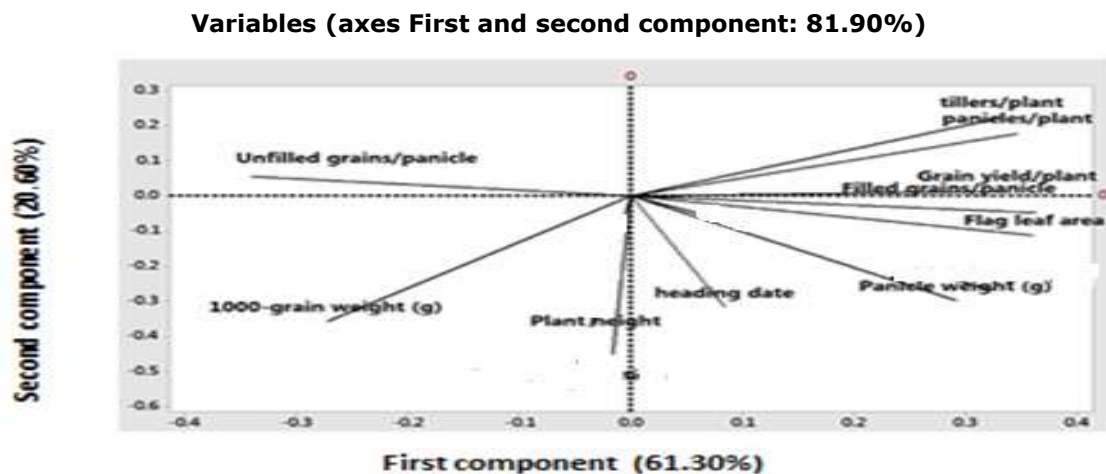
Figure 2. Loading plot of 10 phenotypic traits for seven rice cultivars under heat-stress conditions.

Table 8. The SSR markers, number of alleles, allele size, and polymorphism information content (PIC).

No.	SSR Markers	No. of alleles	Allele Size (bp)		PIC[a]
			Min	Max	
1	RM209	6	183	210	0.847
2	RM493	16	178	250	0.851
3	RM21	12	140	190	0.809
4	RM405	14	186	1300	0.157
5	RM7075	11	376	1200	0.803
6	RM341	16	200	320	0.848
7	RM518	9	193	260	0.820
8	RM148	7	166	430	0.798
9	RM231	11	170	165	0.857
10	RM3297	16	298	440	0.789
11	RM562	10	126	230	0.787
12	RM206	14	152	200	0.851
13	RM17	7	300	1340	0.823
14	RM225	10	220	1250	0.765
15	RM153	9	180	640	0.385
16	RM190	7	250	580	0.748
17	RM3843	14	200	780	0.801
18	RM3330	15	320	1100	0.872
Total		204			
Average		11.33			0.756

*, Polymorphic information content.

SSR Polymorphism and alleles scoring

In the presented study, 18 SSR markers produced 204 alleles, with a mean of 11.33 per locus, detected in the seven rice genotypes, showing polymorphism among them (Table 8). Numerous alleles were evident in primers, i.e., RM493, RM341, RM3297, and RM3330 (Singh *et al*., 2015; Rashmi *et al*., 2017; Jumaili *et al*., 2018; Mazal, 2021). Details of the PCR-amplified products for some SSR markers with seven rice genotypes are available in Figure 3, with multiple alleles per sample observed for all loci under study. However, the polymorphic information content (PIC) employed for each locus assessed the information about each marker, including discriminatory ability, a reflection of allele diversity, and frequency among the rice cultivars. In the pertinent study, polymorphism level assessment among the different rice genotypes calculated the allele number and PIC values for each of the 18 SSR markers (Table 8). The PIC values were high for all SSR markers, with an average of 0.756, and ranged from low (0.157) for RM504 to high (0.872) for RM3330. Reports on similar findings stated a comparative evaluation of rice genotypes for genetic diversity and relationship using SSR markers (Melaku *et al*., 2018; Li *et al*., 2023). Generally, rice is a naturally inbred crop; hence, multiple alleles in a cultivar indicate mixed pure lines or seed mixtures (heterogeneity) rather than genetic heterozygosity (Mazal, 2021). Heterogeneity was evident for all cultivars, which suggested that these SSR markers have a high mutation rate in rice. All the rice cultivars' genotyping relied on a bulk DNA sample; the multiple alleles detected in this data were likely to accurately reflect the degree of heterogeneity encountered in the seed stocks of these rice cultivars (Salgotra *et al*., 2015; Liu *et al*., 2023).

Clustering analysis of rice cultivars based on SSR markers

The dendrogram of the seven rice genotypes appears in Figure 4. The rice genotypes grouping agreed with the parentage presented in Table 1. Cluster analysis divided rice
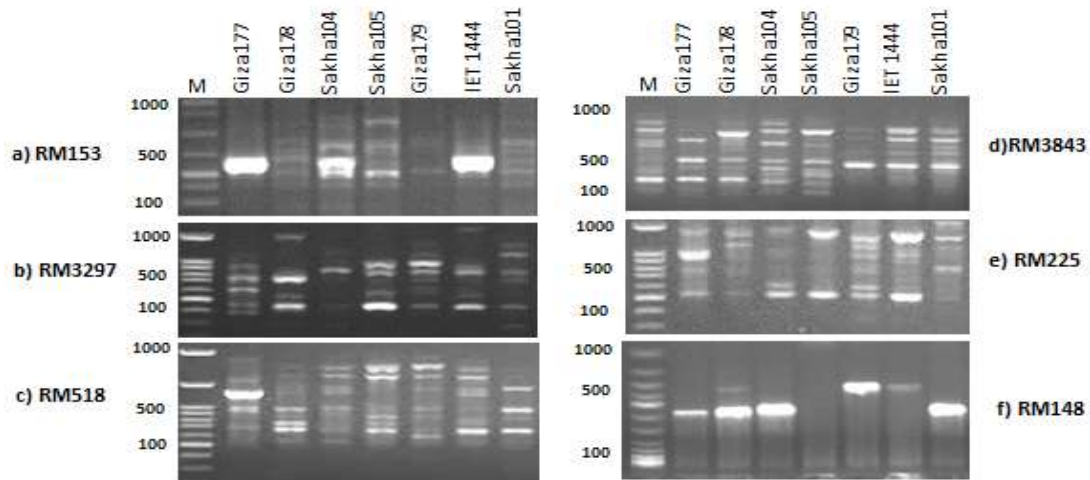
Figure 3. Banding pattern of six SSR obtained from seven rice genotypes with primers a) RM153, b) RM3297, c) RM518, d) RM3843, e) RM225, f) RM148, and M = 100 bp ladder.
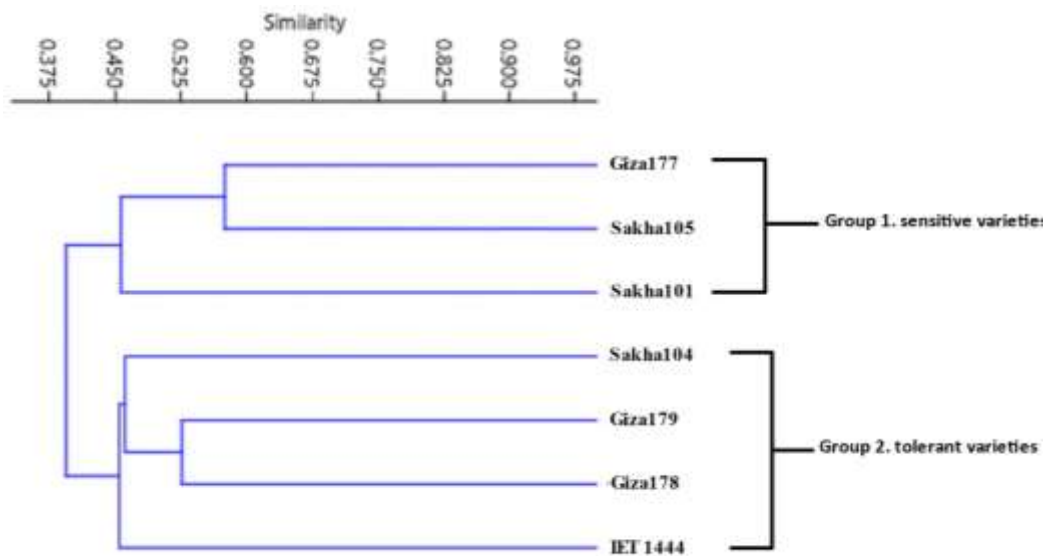

Figure 4. Dendrogram explaining the genetic relationships among tested rice genotypes using SSR markers employing the UPGMA method.

genotypes into two groups. The first group included the Sakha101, Sakha105, and Giza177 (Figure 4). These rice cultivars belonged to a Japonica type, were found sensitive to high temperatures, and their genetic background came from closely related parents, with parent Giza177 being the most common in the production of cultivar Sakha105. The second group included the parental cultivars Giza178, Giza179, IET 1444, and Sakha104. These genotypes belonged to Indica-Japonica except for Sakha104 from a Japonica type. These four genotypes were also tolerant of heat-stress conditions. The results proved a good and stable approach for testing the genetic diversity among the rice genotypes (Rashmi *et al.*, 2017; Mazal, 2021; Al-Daej *et al.*, 2023).

CONCLUSIONS

The variance due to general and specific combining ability was significant ($P \leq 0.01$) for all the traits, indicating that both additive and non-additive genetic variances were essential in controlling the inheritance of these rice traits (*Oryza sativa* L.). The parental genotypes, IET 1444, Gisa179, Gisa178, and Sakha104, emerged as best donors for most traits based on average performance and combining ability effects. However, the rice F_1 populations Giza177 × Giza178, Giza177 × Giza179, Giza177 × Sakha104, Giza178 × IET 1444, and Sakha105 × IET 1444 were notably the best crosses for yield-related traits. Thus, these cross combinations will contribute toward developing promising rice genotypes tolerant to heat stress under Egyptian conditions. The rice genotypes' division resulted in two groups according to the genetic diversity. The first group included the sensitive genotypes, and the second contained the tolerant genotypes. The 18 SSR markers produced 204 alleles, with a mean of 11.33 per locus. The highest number of alleles occurred with four primers, namely, RM493, RM341, RM3297, and RM3330. However, based on 18 SSR markers and the PIC values, a reflection of allele diversity and frequency among the rice cultivars was high for all SSR markers with an average of 0.756 and ranged from a low (0.157) in RM504 to a maximum (0.872) in primer RM3330.

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