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Impact of Climate Changes to Adaptability of Some Rice Germplasm (*Oryza sativa* L.) Under High Temperature through Quantitative Traits and Simple Sequence Repeats (SSR) Marker

Adel A. Rezk[1*], Mohamed M. El-Malky[2], Heba I. Mohamed[3*], Ahmed H. Ismail[4], Othman Al-Dossary[1]

[1]Agricultural Biotechnology Department, College of Agricultural and Food Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia

[2]Rice Research and Training Center, Field Crop Research Institute, Agriculture Research Center, 33717, Egypt

[3]Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, Cairo, 11341, Egypt

[4]Department of Agribusiness and Consumer Sciences, College of Agricultural and Food Sciences, King Faisal

[*]Corresponding authors. E-mail: arazk@kfu.edu.sa; hebaibrahim79@gmail.com

ABSTRACT

The main objective is to study the effect of heat stress on some quantitative traits of 26 different rice genotypes. The results showed that heat stress caused significantly decreased ($p \leq 0.01$) in days to maturity, plant height, flag leaf area, tillers plant^{-1}, panicles plant^{-1}, 1000-grain weight, filled grains panicle^{-1}, sterility %, and grain yield plant^{-1} traits. Among the 26 cultivars studied, 14 exhibited a high grain yield plant^{-1}. The top-performing genotypes were IR72, IR28, Dular, IR82, and Giza178, with yields of 23.27 g, 22.80 g, 21.60 g, 21.20 g, and 21.17 g, respectively. The reduction in yield compared to the normal growing conditions ranged from 26.87% for Hasswi-2 to 47.89% for Giza178. All traits under study exhibited the highest heritability broadly, with the coefficient of variation (PCV) values surpassing the genotypic coefficients of variability (GCV) in all characteristics. The cluster analysis was divided into two main groups, based on the different types of rice. Using 18 SSR markers was essential in assessing the genetic diversity of rice genotypes. The analyzed markers produced 137 alleles, averaging 7.61 alleles per locus. A greater number of alleles per locus was observed with the primers RM547, RM209, RM219, RM205, and RM234. All SSR markers had high polymorphism information content (PIC) values, averaging 0.3891. PIC values represent allele diversity and frequency among the types. Rice genotypes were split into two groups using cluster analysis. IR31775-30-3-2-2, IR2037-93-1-3-1-1, IR29, IR65829-28-H-P, Dular, PUSA Basmati 1, and WAB 880-1-32-1-2-P1-HB were the seven rice genotypes in the first group. The second group included four rice genotypes (Hasswi-1, Hasswi-2, Giza178, and IET 1444) that belonged to the Indica-Japonica type and were produced from different origins. In any case, the Hasswi-1 and Hasswi-2 came from closely related parents.

Keywords: genetic diversity, genetic parameters, heat stress, PIC, rice genotypes, SSR.

INTRODUCTION

The increase in global temperatures resulting from global warming poses a serious threat to global food security, which leads to a significant decline in all agricultural production, especially rice (Zhao et al., 2017; Kinose et al., 2020). For optimal growth and crop maintenance, rice seedlings should be kept between 25 and 28°C (Nishad et al., 2018; Xu et al., 2021). High temperature led to a shortening of the growth period as a result of early flowering, and a decrease in plant height (Mohamed and Abdel-Hamid, 2013; Mohamed et al., 2019; Mohamed et al., 2021; El-Malky, 2024). Also, a clear effect of increasing the sterility grain percentage due to the decrease of pollen grains (Rang et al., 2011) and the lack of filled grains and consequently a decrease in grain yield. Liu et al. (2023) explained that high temperatures decreased yield by 7 to 8% and also affected the quality of grain. Therefore, researchers worked to produce rice varieties that tolerate high temperatures, while maintaining yield and quality characteristics (Masutomi et al., 2023). The growing interest in rice production stems from the need to address the gap between population growth and increased rice consumption. Rice is a major crop globally,

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largely because it is more affordable than many other crops. This makes it particularly suitable for impoverished communities, especially in Asia and Africa (Ndour et al., 2016; El-Beltagi et al., 2022). Therefore, evaluating new varieties in hot areas to obtain new heat-tolerant varieties is useful to confirm suitable varieties that will grow in this region, and it is one of the ways to develop varieties that tolerate high temperatures (Asante et al., 2019; Sitaresmi et al., 2020).

There are other methods to develop new tolerant varieties for high temperatures, such as crossing between donor parents, which carry tolerant genes with local varieties to select new varieties that can withstand high temperatures (El-Malky, 2024; Li et al., 2024). Two primary techniques are typically used in conventional rice breeding: recurrent selection and modified pedigree (Villegas et al., 2018). Nonetheless, heat-tolerant rice breeding started in 2010 to create genotypes of rice that could adjust to local agricultural conditions and change climates by combining high-yielding cultivars with heat-tolerant donor parents (like cultivar N22) (Manigbas et al., 2014). This method requires understanding the genetic background of the parents used, which includes knowing their morphological characteristics and identifying the genes responsible for heat tolerance.

Hence, by selecting recombinants of desired genes, breeders can create new genotypes of rice with a broad range of tolerance to biotic and abiotic stresses. Various molecular markers, such as simple sequence repeats (SSR), aid in the selection of rice genotypes that carry the desired genes (Sadat et al., 2013; Mazal, 2021). The current study aims to 1) evaluate 26 rice genotypes under high-temperature conditions, 2) evaluate superior genotypes under normal and temperature conditions to estimate the reduction in yield, and 3) study the genetic diversity through quantitative traits and the SSR marker.

MATERIAL AND METHODS

Rice genotypes

26 rice genotypes were used in this study, including eleven genotypes belonging to Indica type, ten genotypes from Japonica type, and five belonging to Indica-Japonica type (Table 1). All genotypes were received from Gen Bank at the International Rice Research Institute (IRRI).

Table 1. 26 rice genotypes, parentage, origin, and plant type

No.	Varieties	Pedigree	Origin	Type
1	IR65829-28-H-P	GZ 2175 / GYEHWA 7	IRRI	Indica
2	IR29	IR833-6-1-1/IR1561-149-1//IR1737	IRRI	Indica
3	IR 31775-30-3-2-2	IR 10154-23-3-3 / IR 9129-209-2-2	IRRI	Indica
4	IR 2037-93-1-3-1-1	IR 1697-47-2-2 / IR 1818-2	IRRI	Indica
5	Dular	Dumai / Larkoch	IRRI	Indica
6	IR72	TN 1 x Chianung 242	IRRI	Indica
7	Hasswi-1	Exotic	Kingdom of Saudi Arabia	Indica-Japonica
8	Hasswi-2	RI112/Hasswi-1	Kingdom of Saudi Arabia	Indica-Japonica
9	Novator	PRIKUBANSKY/ITALICA 10	Russia	Japonica
10	VNIRB 572	VNIR 7718/VNIR 1418	Russia	Japonica
11	Suweon 339	SR 9373-71-3 / Pungsan Byeo	Korea	Japonica
12	WAB 880-1-32-1-2-P1-HB	(WAB 56-50/ G14//WAB56-50)	Africa Rice Centre (WARDA)	Indica
13	TKY 1014	J692153X / Fukunishi // Taichung	China	Japonica
14	Koshihikiari	Nourin No.1 × 'Nourin No.22	Japan	Japonica
15	IRAT 170	(IRAT 13/ Palawan)	Ivory Coast	Indica
16	Giza177	Giza171/ Yumji No.1// PiNo.4	Egypt	Japonica
17	Giza178	Giza 171 / Milyang 49	Egypt	Indica-Japonica
18	Sakha104	GZ 4096 X GZ 4100	Egypt	Japonica
19	Sakha105	Giza177 / Suwwon349	Egypt	Japonica
20	Giza175	IR28/IR1541//Giza180/Giza14	Egypt	Indica-Japonica
21	Giza 171	Nahda / Calady 40	Egypt	Japonica
22	Sakha101	Giza 176/Milyang 79	Egypt	Japonica
23	IET 1444	TN1 / Co29	India	Indica-Japonica
24	PUSA Basmati 1	Pusa150 x Karnal local	India	Indica
25	IR82	IR833-6-2-1-1/IR1561-149-1/IR24	IRRI	India
26	IR28	IR833-6-2-1-1/IR1561-149-1/IR24	IRRI	India

Field evaluation under high temperature

In 2020, 2021, and 2022, the 26 rice genotypes were assessed, and each genotype was transplanted using a randomized complete block design (R.C.B.D.) with three replications. Each plot has five rows that are five meters long, with a space of 20 cm between each row and plant, allowing for a total of 25 plants per square meter. The recommended cultural practices for rice growing were employed. The average air temperature in high-temperature conditions ranges between 38.2 to 47.2°C, and the relative air humidity (%) ranges between 21 to 30% in this season. These genotypes were evaluated under these high temperatures in the New Valley Governorate, which had a hot zone under Egyptian conditions (*www.worldweatheronline.com*). While, under normal conditions, it ranged between 28 to 35°C, which is considered the optimal temperature for rice plant growth at the Sakha Agricultural Research Station.

Quantitative Traits Assessment

Ten agronomic traits namely; duration (days), plant height (cm), flag leaf area (cm^2), tillers $plant^{-1}$, panicle weight (g), panicles $plant^{-1}$, 1000-grain weight (g), filled grains $panicle^{-1}$, unfilled grains $panicle^{-1}$ and grain yield $plant^{-1}$ (g) were calculated at the experimental farm. The data for all traits were recorded based on the Standard Evaluation System (SES) for rice (IRRI, 2014). Genetic associations between the 26 rice genotypes under study were constructed using the averages of these features over two years.

Evaluation of selected genotypes

Fourteen rice genotypes, which gave high-yielding and superior agro-morphological traits were evaluated under high temperatures at New Valley Research Station, El-Kharga, New Valley Governorate, Agriculture Research Center (ARC), Egypt, and normal conditions at Sakha Research Station, Rice Research and Training Center (RRTC), Kafr EL-Sheikh Governorate. The reduction of the yield due to the effect of high temperature was estimated. Also, the SSR markers were used to study the genetic diversity among the fourteen rice genotypes.

Genomic DNA isolation

A total of fourteen rice genotypes were screened using 18 simple sequence repeat primers (SSR) purchased from Sangon Company, China (Table 2). DNA was isolated from the 14 rice accessions according to Maixner et al. (1995). Selected leaves with good vitality were washed with clear water. 4 to 10 midribs were cut with a disposable razor blade and 1.0 g of midribs was dispensed in an ELISA sachet and 3.0 mL CTAB extraction buffer. Then the midribs were squashed under cooling at 4°C. 1.5-2.0 mL of midrib juice was transferred to 2 mL tube and kept in a water bath at 65°C for 15 min. This was followed by centrifugation at 3000 for 5 min. One mL of supernatant was collected and transferred to an Eppendorf tube. 1 mL Chloroform-Isoamylic alcohol was then added, and mixed by inverting the tubes several times to obtain an emulsion. The emulsion was then kept in the Centrifuge for 5 min at 14000 ×g. The aqueous phase was collected and transferred to new tubes, and 540 µL Isopropanol was supplied, left at 20°C for 30 min, then centrifuged for 20 min at 14000 ×g. After centrifugation, ethanol was removed without disturbing the small nucleic acid pellet. The pellet was washed with 1 mL ethanol 70%, followed by centrifuge for 10 min at 14000 ×g. Ethanol was removed, and the pellet was dried at speed-VAC*5 min. The dried pellet was re-suspended with 60-100 µL TE 1X (Tris 10 mM EDTA 1 mM PH8) and nucleic acid stored at -20°C.

Table 2. Information on the selected linked molecular markers used for high-temperature stress tolerance

No.	Marker	Chr. No.	Primer sequences used for gene detection (5'-3')	Reference
1	RM219	9	(F): CGTCGGATGATGTAAAGCCT (R): CATATCGGCATTCGCCTG	Wei et al. (2013)
2	RM205	9	(F): CCTAAGAGGAGCCATCTAACAACTGG (R): CTTGGATATACTGGCCCTTCACG	Jafar et al. (2008)
3	RM234	7	(F): TTCAGCCAAGAACAGAACAGTGG (R): CTTCTCTTCATCCTCCTCCTTGG	Jafar et al. (2008)
4	RM547	8	(F): TTGTCAAGATCATCCTCGTAGC (R): GTCATTCTGCAACCTGAGATCC	Ye et al. (2015)
5	RM209	11	(F): ATATGAGTTGCTGTCGTGCG (R): CAACTTGCATCCTCCCCTCC	Bui et al. (2014)
6	RM430	5	(F): AAACAACGACGTCCCTGATC (R): GTGCCTCCGTGGTTATGAAC	Cheng et al. (2012)
7	RM471	4	(F): ACGCACAAGCAGATGATGAG (R): GGGAGAAGACGAATGTTTGC	Xiao et al. (2011)
8	RM405	5	(F): TCACACACTGACAGTCTGA (R): AATGTGGCACGTGAGGTAAG	Zhang et al. (2008)
9	RM235	12	(F): AAGCTAGGGCTAACGAACGAACG (R): TCTCCATCTCCATCTCCATCTCC	Ye et al. (2015)
10	RM1209	1	(F): AATGGAGCTCCTGACTCTAAAGC (R): TGCATCTCCTACAGAAACAAGG	Liao et al. (2011)
11	RM228	10	(F): TCTAACTCTGGCCATTAGTCCTTGG (R): AAGTAGACGAGGACGACGACAGG	Bui et al. (2014)
12	RM336	7	(F): GTATCTTACAGAGAAACGGCATCG (R): GGTTTGTTTCAGGTTCGTCTATCC	Argayoso et al. (2011)
13	RM247	12	(F): AAGGCGAACTGTCCTAGTGAAGC (R): CAGGATGTTCTTGCCAAGTTGC	Antonio et al. (2005)
14	RM249	5	(F): CAACTCCACTCCAGACTCAACTCC (R): GGTATGATGCCATGAAGGTCAGC	Bui et al. (2014)
15	RM314	6	(F): CTAGCAGGAACTCCTTTCAGG (R): AACATTCCACACACACACGC	Bui et al. (2014)
16	RM570	3	(F): AGAAATGGTGAAAGATGGTGCTACCG (R): CTGAATGTTCTTCAACTCCCAGTGC	Ye et al. (2015)
17	RM7364	9	(F): TTTCGTGGATGGAGGGAGTACG (R): TGGCGACTTATGAGCGTTTGTAGG	Wei et al. (2013)
18	RM225	6	(F): TATGTGGTTGGCTTGCCTAGTGG (R): TGCCCATATGGTCTGGATGTGC	Xiao et al. (2011)

Polymerase Chain Reaction Assay

The reaction mixture (25 µL) consisted of: 12.5 µL of 2x master mix ready to use [0.1U/µL Taq Polymerase, 500 µM dNTP, 20 mM Tris-HCl (pH8.3), 100 mM KCl, 3 mM $MgCl_2$ and Stabilizer and enhancer] + 10 Pmol of each primer (1.0 µL) + 1.0 µL of DNA (50 ng) + 9.5 µL PCR grade water. Amplification was performed in a Thermocycler (Bio-Rad, C - 1000) as follows: (1) Initial denaturation at 94°C for 5 min.; (2) Denaturation at 94°C for 30 sec.; (3) Primer annealing temperature differing according to Tm of each primer for 1 min.; (4) Extension at 72°C for 1 min.; (5) Steps 2, 3, and 4 are repeated 40 cycles.; (6) A final extension at 72°C was given for 10 min. Following PCR, the amplified products were examined using a 1.5% agarose gel that contained ethidium bromide at a final concentration of 0.5 µg mL^{-1}. After loading 10 µl of the amplified product into the well, it was run for 45 minutes at 5 volts/cm^2 in a 1x TAE electrophoresis buffer with a 1 Kb plus DNA ladder (Intron Biotechnology Company, Korea). The gels were moved to a UV cabinet when the electrophoresis was completed. Following that, the gels were photographed and examined using BioDoc Analysis software (Biometra, Germany).

Statistical Analysis

For plants, Analysis of Variance (ANOVA) data were gathered by Steel and

Torrie (1980) methodology. However, using Singh and Chaudhary (1985) formula, the means were separated using the least significant difference (LSD) at $p<0.05$ (significant) and $p<0.001$ (highly significant). The program NTSYS-pc version 2.1 was used to create similarity matrices (Rohlf, 2000). The variety of alleles of marker locus was evaluated by calculating the polymorphism information content (PIC), number of polymorphic alleles, number of amplified alleles polymorphism ratio (P%), and total number of amplified bands (Anderson et al., 1993). A dendrogram was created utilizing the Unweighted Pair Group Method with Arithmetic Average (UPGMA) sequential agglomerative hierarchal nested (SHAN) cluster and genetic similarity coefficients.

RESULTS AND DISCUSSION

Analysis of variance for agronomic traits

The performance of the 26 rice genotypes in this study varied greatly across all agronomic characteristics (Table 3). The averages from the two years were used because the results of the ANOVA performed for each year were not significant. The data, which showed very significant variances for all parameters, evidence enormous genetic variability among the genotypes.

Table 3. Analysis of variance for agronomic traits in 2020 and 2021

Traits	Replications (df=2)	Genotypes (df=25)	Error (df=50)	CV (%)
Days to maturity (days)	0.94	682.80**	0.93	15.20
Plant height (cm)	3.39	618.20**	1.22	16.89
Flag leaf area (cm^2)	1.22	53.81**	0.57	14.54
No. of tillers $plant^{-1}$	0.88	17.39**	0.16	15.45
No. of panicles $plant^{-1}$	0.38	14.77**	0.21	15.35
Panicle weight (cm)	0.06	0.14**	0.01	13.34
1000-grain weight (g)	0.20	35.58**	0.15	16.70
No. of filled grains $panicles^{-1}$	1.76	498.06**	3.30	19.97
Sterility percentage	2.58	260.63**	2.04	24.13
Grain yield $plant^{-1}$ (g)	2.43	37.63**	0.33	19.56

** highly significant at 0.01 level.

Mean performance

The mean performance for 26 rice genotypes under high temperatures of ten traits is presented in Table 4. The results indicated a significant variability among the studied traits, likely due to the diverse genetic backgrounds of the genotypes. As for duration trait, 13 genotypes were less than 100 days, while six genotypes recorded long duration (Novator, Giza 171, VNIRB 572, IR2037-93-1-3-1-1, IR29, and IR65829-28-H-P) gave 122.27, 122.07, 120.30, 114.33, 114.23 and 111.43 days, respectively (Table 4).

In plant height, all the genotypes were dwarf and less than 100 cm except the genotypes (Novator, VNIRB 572, and IET1444) which were 113.80, 114.97, and 109.90 cm. Flag leaf area (cm^2), the results showed a significant difference between the genotypes in this trait, the values ranged from 22.7 to 34.4 cm, and heights values were with the genotypes Giza 178, IR82, Hasawi-2, Sakha 101, Hasawi-1, IR65829-28-H-P and IR28 were (34.40, 34.37, 33.85, 33.70, 33.53, 33.40 and 33.23 (cm^2), respectively, while 12 genotypes gave the lowest value of flag leaf. Fourteen rice genotypes exhibited high values for both tillers and panicles $plant^{-1}$. These genotypes belonged to Indica and Indica-Japonica types except the varieties Novator and VNIRB 572 belonged to Japonica type. The values ranged from 12.43 to 19.77 with the varieties Sakha104 and IR72.

Concerning 1000-grain weight, filled grains panicle^{-1}, and grain yield plant^{-1}, the results showed that 14 rice genotypes namely; IR72, IR28, Dular, IR82, Giza178, IR2037-93-1-3-1-1, Hasswi-1, IET 1444, PUSA Basmati 1, IR31775-30-3-2-2, IR29, Hasswi-2, IR65829-28-H-P, WAB 880-1-32-1-2-P1-HB gave 23.27, 22.80, 21.60, 21.20, 21.17, 20.30, 20.00, 19.57, 18.77, 18.30, 17.87, 17.77, 17.00, 16.53 gave high value in these traits compared with the rest genotypes.

Table 4. Mean performance of 26 rice genotypes for teen agronomic traits under high-temperature conditions in New Valley region

No.	Genotypes	Duration (days)	Plant height (cm)	Flag leaf area (cm^2)	Tillers plant^{-1}	Panicle weight (g)	Panicles plant^{-1}	1000-grain weight (g)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Grain yield plant^{-1} (g)
1	IR65829-28-H-P	111.43	83.23	33.40	16.57	1.70	14.50	23.03	63.47	32.37	17.00
2	IR29	114.23	86.70	31.57	13.53	1.80	11.70	22.90	54.40	32.10	17.87
3	IR31775-30-3-2-2	109.00	94.40	29.63	14.53	1.60	13.40	23.37	59.23	37.00	18.30
4	IR2037-93-1-3-1-1	110.90	98.33	32.30	16.50	1.47	15.03	22.67	51.47	35.10	20.30
5	Dular	114.33	96.07	30.50	17.33	1.90	16.03	23.03	76.70	33.63	21.60
6	IR72	109.57	90.10	28.70	19.77	1.70	14.97	23.73	83.23	22.20	23.27
7	Hasswi-1	106.57	88.00	33.53	18.90	1.60	16.20	3.10	69.00	37.50	20.00
8	Hasswi-2	107.27	86.90	33.85	18.07	1.87	15.10	24.37	72.30	29.50	17.77
9	Novator	122.27	113.80	31.40	17.23	1.80	15.33	22.67	74.70	38.80	14.87
10	VNIRB 572	120.30	114.97	30.63	18.03	1.73	14.07	23.37	73.30	35.60	14.47
11	Suweon 339	93.30	64.20	22.70	12.77	1.30	10.97	18.10	53.83	55.60	13.83
12	WAB 880-1-32-1-2-P1-HB	95.20	72.77	24.27	14.43	1.50	12.00	19.47	51.50	49.37	16.53
13	TKY 1014	88.00	68.63	24.33	13.00	1.20	12.13	16.67	57.13	44.27	13.37
14	Koshihikiari	101.27	79.17	30.73	16.07	1.90	14.57	23.10	82.30	35.43	13.73
15	IRAT 170	94.30	65.20	26.73	13.77	1.30	11.87	14.93	50.70	61.80	14.63
16	Giza177	98.23	72.67	24.47	13.03	1.63	11.07	16.20	49.60	49.20	13.87
17	Giza178	101.37	88.10	34.40	18.00	2.00	15.47	22.83	80.97	38.20	21.17
18	Sakha104	75.17	71.23	24.63	12.43	1.60	9.67	18.73	54.33	45.77	13.73
19	Sakha105	78.37	77.10	32.53	14.00	1.60	12.33	23.17	88.67	30.47	14.33
20	Giza175	74.90	84.03	23.30	16.17	1.50	13.77	17.70	51.60	50.27	14.53
21	Giza 171	122.07	69.27	22.83	13.10	1.43	9.63	14.40	55.07	44.27	14.10
22	Sakha101	74.67	72.50	33.70	13.50	2.00	17.43	23.07	88.70	24.13	12.07
23	IET 1444	76.37	109.90	23.90	19.00	1.30	12.00	15.57	53.03	39.37	19.57
24	PUSA Basmati 1	87.07	74.17	25.27	13.97	1.43	13.07	14.20	55.07	38.80	18.77
25	IR82	92.67	94.77	34.37	16.07	1.77	12.10	22.37	63.87	33.20	21.20
26	IR28	98.87	93.23	33.23	16.37	1.67	14.23	23.27	63.67	30.43	22.80
	LSD 0.05%	1.32	1.51	1.03	0.54	0.62	0.13	0.53	2.48	1.95	0.78
	0.01%	1.89	2.16	1.48	0.77	0.89	0.19	0.76	3.56	2.80	1.12

Genetic variability

The mean value, genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in the broad sense (h^2b), genetic advance (GA), and genetic advance in percentage are shown in Table 5.

The 26 rice genotypes showed a wide range of genetic deviations for DM, indicating a significant diversity for this trait (Table 4). Table 5 shows that DM ranged from 74.6 to 122.2 days. DM had a significantly higher phenotypic variance (PV) (228.22) than the GV (227.29). More genetic than environmental influences influenced the

trait's expressivity. Given that PCV (15.24) and GCV (15.21) were near each other, there may not be much of an environmental influence on how the trait manifests. For this variable, the estimate of h^2b was high (99.59), but the GA (30.99) and GA% (31.26) were moderate, suggesting that genotypes were responsible for the apparent variation. With a mean of 84.98 cm and a range of 64.2 to 114.9 cm for the plant height (PH) trait (Table 5), the PV (207.05) was only marginally higher than the GV (205.83), suggesting that the environment had little effect on the expressivity of the genes controlling the trait. According to Table 5, the PCV (16.93) and GCV (16.88) deviations were the lowest for PH. The fact that PH had a high h^2b (99.41) together with high GA (29.47) and GA% (34.68) suggested that genotype was responsible for the apparent difference.

A mean of 20.62 g was found for the 1000-grain weight (1000-GW), which varied from 14.2 to 24.3 g. GV (11.81), PCV (16.78), GCV (16.67), and PV (11.96) show the least amount of environmental effect on the expression of the character. While the h^2b of 1000-GW was very high (98.74), with a significant GA (7.03). Concerning FLA, NET, and NEP traits, a wild range of variations were found in both traits, and PV and PCV, were higher than GV and GCV) showing less environmental influence on FLL (Table 5). The inheritance of FLA and NET were high (96.87 and 97.34), with a high GA (29.32 and 30.73). As for the NFG trait, a wide range was found between these genotypes and the mean was 64.53, while PV (168.22) was higher than GV (164.92), also PCV was a little bit higher than GCV (Table 5). Therefore, the characteristic had low contextual influence, whereas NEG expressivity was strongly influenced by genetics. While the GA was low (26.19), the h^2b of NEG and GA% were also high (98.04 and 40.59). Among the genotypes investigated, significant differences in filled grains panicle^{-1} (NFG) were discovered (Table 5). Furthermore, the SS % genetic data show that the chosen lines have a high level of genetic control. There are considerable differences among the 26 rice genotypes in terms of grain yield plant^{-1} (GYP), with a mean of 18.11 g per plant and a range of 12.1 to 23.2 (g) (Table 5). Using a selection of 26 rice genotypes, GCV (19.47), PCV (19.73), and h^2b (97.73) demonstrated that the environment has less of an impact on GYP.

Table 5. Estimation of genetic parameters for agro-morphological characters in 26 rice genotypes

S.O.V. Traits	Mean	Range	GV	PV	GCV	PCV	h^2b	GA	GA%
DM	99.14	74.6-122.2	227.29	228.22	15.21	15.24	99.59	30.99	31.26
PH	84.98	64.2-114.9	205.83	207.0	16.88	16.93	99.41	29.47	34.68
FLA	29.13	22.7-34.4	17.75	18.32	14.46	14.69	96.87	8.54	29.32
NET	15.85	12.4-19.7	5.74	5.90	15.12	15.33	97.34	4.87	30.73
PW	1.62	1.2-2.0	0.04	0.05	12.86	14.22	81.73	0.39	23.95
NEP	13.68	9.6-17.4	4.85	5.06	16.11	16.45	95.85	4.44	32.48
1000-GW	20.62	14.2-24.3	11.81	11.96	16.67	16.78	98.74	7.03	34.12
NFG	64.53	49.6-88.7	164.92	168.22	19.90	20.10	98.04	26.19	40.59
SS%	38.63	22.2-61.8	86.20	88.23	24.03	24.32	97.69	18.90	48.94
GYP	18.11	12.1-23.2	12.43	12.76	19.47	19.73	97.43	7.17	39.60

GV=genotypic variance; PV=phenotypic variance; GCV=genotypic co-efficient of variation; PCV=phenotypic coefficient of variation; h^2b=heritability in broad sense; GA=genetic advance; GA%=genetic advance in percent of mean; DM=days to maturity; PH=plant height (cm); FLA=flag leaf area; NET= tillers plant^{-1}; NEP=panicles plant^{-1}; PW=panicle weight (g); 1000-GW=1000-grain weight (g); NFG=filled grains panicle^{-1}; SS=sterility %; GYP=grain yield plant^{-1} (g).

Cluster analysis based on quantitative characteristics

This analysis was conducted on 26 genotypes that were grown and evaluated for 10 quantitative traits under high-temperature conditions in the New Valley region.

The cluster analysis divided all the rice genotypes into two major groups, the first one was divided into two sub-groups, the first sub-group included all the indica types except VNIRB572 variety, which belonged to Indica-Japonica type (Table 1). These genotypes could be genetically close to each other for seven quantitative traits namely; duration, plant height, tiller plant^{-1}, panicles plant^{-1}, flag leaf area, 1000-grain weight, and grain yield plant^{-1} (Figure 1). While, the second sub-group included three rice genotypes (Hasswi-2, Hasswi-1, and Giza178) these genotypes belonged to Indica-Japonica types. In any case, these genotypes were similar in duration, plant height, flag leaf area, tiller plant^{-1}, panicles plant^{-1}, 1000-grain weight, and grain yield plant^{-1}. The second major group was divided into two sub-groups, the first one included Japonica type except for IET1444 and Giza175 varieties, which belonged to Indica-Japonica types. All these genotypes were short-duration under heat stress, and lower in grain yield plants which means that these genotypes were sensitive to high temperatures except IET1444 gave high yielding compared with other genotypes (Figure 1).

The rice cultivars Sakha 105 and Giza 177 formed the second sub-group; they had a very similar genetic background because they had a common parent (Giza 177). The plant height, number of tillers per plant, number of panicles per plant, and grain yield per plant were all equal for these two rice varieties. Clustering based on quantitative traits was grouped into many sub-groups, Based on the types of genotypes (Figure 1). All Indica types were in one group except VNIRB572 from Japonica type, this classified due to coming from one origin (IRRI), and there is a close in some quantitative characteristics. On the other hand, the two Saudi varieties came together in the same group and belonged to the Indica-Japonica type. The second group included all Japonica types except for the two (IET1444 and Giza175) that belonged to the Indica-Japonica type.

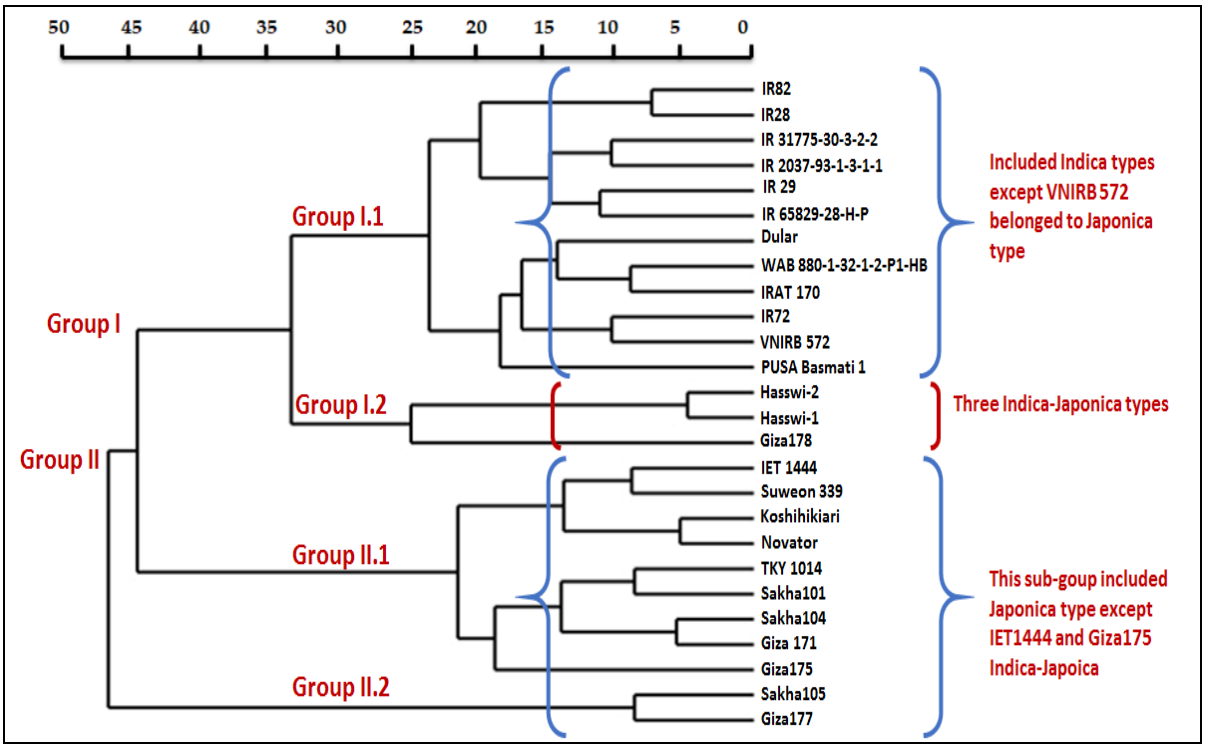

Figure 1. Cluster diagram for 26 varieties classified by teen morphological quantitative characters

Evaluation of tolerant rice genotypes under normal and heat conditions

In this study, fourteen rice genotypes exhibited a high grain yield plant^{-1} (g) under heat stress, which is the primary trait targeted for developing new varieties. Additionally, these genotypes were assessed under normal conditions to estimate the percentage reduction in grain yield plant^{-1} (g). The mean of grain yield plant^{-1} (g) under normal conditions ranged from 24.30 to 38.90 g/plant (Table 6). Giza178 and IET1444 gave high values of about 38.90 and 37.40 g/plant for grain yield under normal conditions, while the lowest value was observed with Hasswi-1 and Hasswi-2 of about 24.80 and 24.30 g/plant. According to the yield redaction %, the results showed that the genotypes Giza178, IET1444, IR65829-28-H-P, WAB 880-1-32-1-2-P1-HB, IR29, and IR 31775-30-3-2-2 gave high percentage 47.89, 47.67, 46.03, 44.83, 41.98 and 41.53%, respectively (Table 6). On the other hand, the lowest redaction was found with Hasswi-1 and Hasswi-2 (19.35 and 26.87%), these two genotypes were growing in Saudi Arabia and indicated that the two genotypes were more adapted under high temperatures.

According to grain yield plant^{-1}, 14 rice genotypes within the 26 genotypes under study gave high yield and ranged between 16.33 to 23.27 (g) compared with this trait under normal conditions, which gave a range between 24.30 to 38.80 g. The reduction in the yield ranged between 19.35 to 47.89% (Table 6).

Table 6. Mean performance of 14 rice genotypes for grain yield plant^{-1} (g) and redaction percentage

No.	Genotypes	Grain yield plant^{-1} (g)			Redaction %
		Normal condition	Heat condition	Yield redaction/plant^{-1}	
1	IR72	34.80	23.27	11.53	33.13
2	IR28	33.40	22.80	10.60	31.73
3	Dular	32.90	21.60	11.30	34.34
4	IR82	34.60	21.20	13.40	38.72
5	IR 2037-93-1-3-1-1	31.70	20.30	11.40	35.96
6	Giza178	38.98	21.17	17.81	47.80
7	Hasswi-1	24.80	20.00	4.80	19.35
8	IET 1444	37.40	19.57	17.83	47.67
9	PUSA Basmati 1	29.90	18.77	11.13	37.22
10	IR 31775-30-3-2-2	31.30	18.30	13.00	41.53
11	IR 29	30.80	17.87	12.93	41.98
12	Hasswi-2	24.30	17.77	6.53	26.87
13	IR 65829-28-H-P	31.50	17.00	14.50	46.03
14	WAB 880-1-32-1-2-P1-HB	29.60	16.33	13.27	44.83

Molecular markers

SSR Polymorphism and Allele scoring under study

In this investigation, 18 SSR markers yielded 137 alleles, with an average of 7.61 alleles per locus, which were found in the 14 rice genotypes. The findings indicated that the genotypes were polymorphic (Table 6). RM547, RM209, RM219, RM205, and RM234 were the primers that indicated a greater number of alleles). Figure 2 highlighted the PCR-amplified results for some SSR markers with 14 rice genotypes. For every locus under investigation, several alleles were seen per sample. Numerous alleles in a variety indicate mixed pure lines or seed mixture (heterogeneity) rather than genetic heterozygozity because rice is a naturally inbred crop. Heterogeneity was observed for all varieties, which suggested that these SSR markers have a high mutation rate in rice. All varieties were genotyped based on a bulk DNA sample; the multiple alleles detected in these data are likely to accurately reflect the degree of heterogeneity encountered in seed stocks of these varieties.

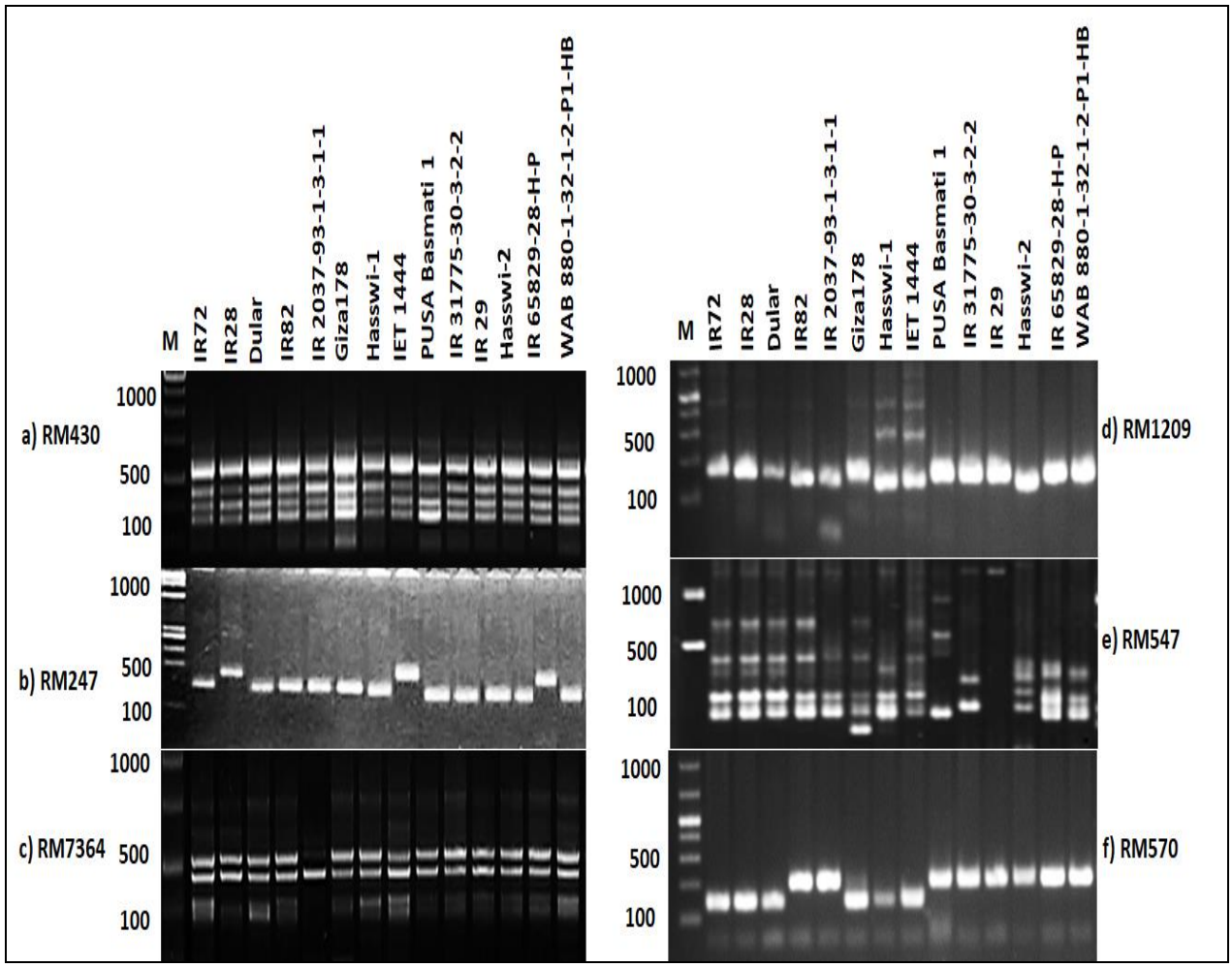

Figure 2. The banding pattern of 18 SSR obtained from 14 rice genotypes with primers
a) RM430, b) RM247, c) RM7364, d) RM1209, e) RM547, f) RM570, and M = 100 bp ladder

Polymorphic information content (PIC)

For each locus, the polymorphic information content (PIC) was used to evaluate the information of each marker, discriminatory capacity, a representation of allele diversity, and frequency among varieties. The allele number and PIC values for each of the 18 SSR markers were computed in this study to determine the degree of polymorphism among the various genotypes (Table 7). Regarding allele diversity and frequency among the types, the PIC values were high for all SSR markers, ranging from a low of 0.2755 for RM249 to a high of 0.4579 for RM228. The average PIC value was 0.3891.

Table 7. SSR loci were used for genotyping a set of 14 rice genotypes and their genetic diversity parameters

SSR Marker	Positive bands	Negative bands	Positive frequency	Negative frequency	No of alleles	PIC value
RM219	4.6	9.5	0.3285	0.6714	10	0.3102
RM205	5.5	8.5	0.3928	0.6071	10	0.4051
RM234	5.0	9.0	0.3571	0.6428	10	0.4224
RM547	7.0	7.0	0.5000	0.5000	11	0.3609
RM209	6.7	7.2	0.4805	0.5194	11	0.4304
RM430	5.0	9.0	0.3571	0.6428	8	0.3750
RM471	7.0	7.0	0.5000	0.5000	8	0.4286
RM405	5.7	8.2	0.4107	0.5892	8	0.3954
RM235	5.3	8.6	0.3809	0.6190	6	0.4456
RM1209	5.6	8.3	0.4047	0.5952	6	0.3741
RM228	7.1	6.8	0.5089	0.4910	8	0.4579
RM336	4.8	9.1	0.3482	0.6517	8	0.3992
RM247	5.0	9.0	0.3571	0.6428	2	0.3980
RM249	4.6	9.3	0.3333	0.6666	6	0.2755
RM314	7.1	6.8	0.5119	0.4880	6	0.3944
RM570	5.4	8.6	0.3857	0.6142	5	0.3214
RM7364	5.8	6.2	0.4142	0.5857	5	0.3849
RM225	5.2	8.7	0.3775	0.6224	7	0.4254
Total					137	
Mean	5.7	8.1	0.4082	0.5916	7.61	0.3891

Clustering analysis of rice cultivars based on SSR markers

The dendrogram illustrating the 14 rice genotypes is shown in Figure 3. The cluster analysis categorized these rice genotypes into two groups. The first group consisted of seven rice genotypes: Dular, PUSA Basmati 1, IR 31775-30-3-2-2, IR 2037-93-1-3-1-1, IR 29, IR 65829-28-H-P, and WAB 880-1-32-1-2-P1-HB. All of these cultivars belong to the Indica type and are tolerant to high temperatures. They were primarily produced at the International Rice Research Institute (IRRI), except for PUSA Basmati 1, which is from India (Table 1).

Four rice genotypes (Hasswi-1, Hasswi-2, Giza178, and IET1444) that were of the Indica-Japonica type and cultivated from various origins were included in the second group. The parents of the Hasswi-1 and Hasswi-2 were, in any event, closely connected (Table 1). The four genotypes in this group - IR28, IR82, and IR72 - belonged to the Indica types and were also resistant to heat stress. Furthermore, Table 1 shows that the IR28 and IR82 were descended from the same parents.

Molecular marker technology was employed to improve breeding programs' effectiveness, identify genes, speed up the transfer of desirable genes between genotypes, and investigate genetic diversity among rice species. Due to their co-dominant inheritance, high abundance, vast amount of allelic variety, and simplicity of determining SSR size variation by PCR using pairs of flanking primers, 18 SSR markers were employed for 14 rice genotypes. There were 137 alleles in all, with an average of 7.61 alleles per locus, found in the 14 rice genotypes. There was variation among the 14 rice genotypes in all 18 primers (Table 7).

Figure 2 provides clarification of the PCR-amplified products for some SSR markers with 14 rice genotypes. Additionally, for every locus under investigation, several alleles were detected per sample. All SSR markers had high PIC values (Table 7), ranging from a low of 0.2755 for RM249 to a high of 0.4579 for RM228. The average PIC value was 0. 3891.

Figure 3 shows a clustering study of rice cultivars into two groups based on SSR markers. Except for PUSA Basmati 1 from Indian rice, all seven of the tolerant rice genotypes in the first group were of the

Indica type and were created at the same International Rice Research Institute (IRRI) (Table 1). The second group included four rice genotypes (Hasswi-1, Hasswi-2, Giza178, and IET 1444) belonging to Indica-Japonica. In any case, the Hasswi-1 and Hasswi-2 came from closely related parents (Table 1). Also, this group included IR28, IR82, and IR72 belonged to Indica types, these four genotypes were also tolerant of heat-stress conditions. In addition, the IR28 and IR82 came from the same parents (Table 1).

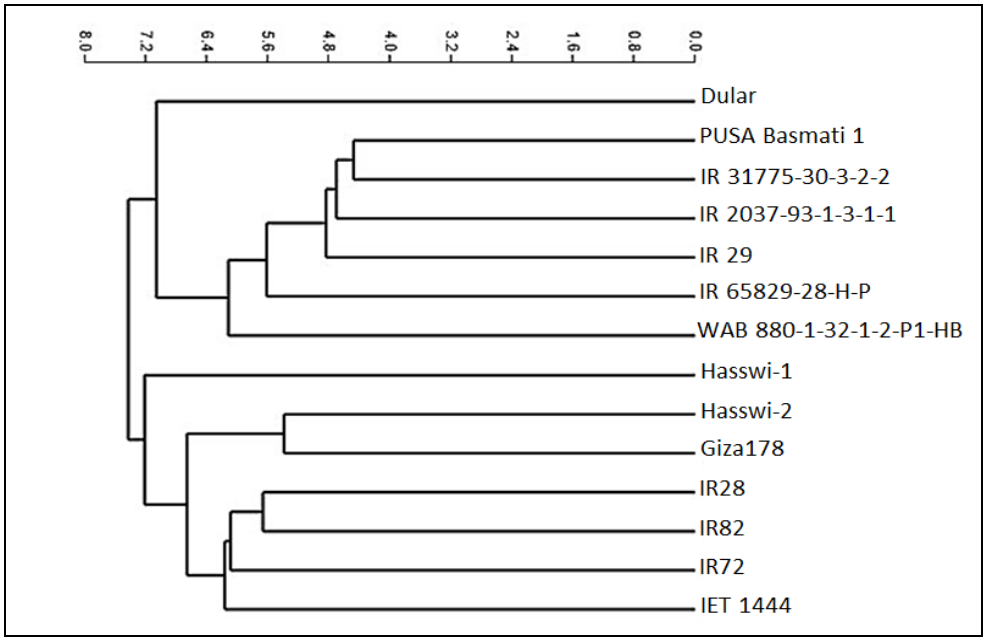

Figure 3. The dendrogram explains the genetic relationships among tested rice genotypes using SSR markers employing the UPGMA method

High temperatures affect the vegetative and reproductive stages of rice, and heat tolerance is defined as the ability of the plant to grow and produce an economical crop at high temperatures (Mohammad and Tarpley, 2010; Permana et al., 2017). In this study, 26 rice genotypes belonging to different types (Indica, Indica-Japonica, and Japonica) were evaluated under high temperatures for some quantitative traits. The results showed that heat stress led to a decrease in the duration, and plant height and also affected the production of pollen grains received by the stigma (Jumiatun et al., 2016; Cheabu et al., 2018; Duan et al., 2020), which led to an increase in the sterility percentage, decrease in the filled grains, 1000 grain weight and thus a decrease in grain yield (Table 4). According to grain yield plant^{-1}, 14 rice genotypes within the 26 genotypes under study gave high yield and ranged between 16.33 to 23.27 (g) compared with this trait under normal conditions, which gave a range between 24.30 to 38.80 g. The reduction in the yield ranged between 19.35 to 47.89% (Table 6). These results indicated that tolerant temperature depends on the genetic background, which is different among rice varieties (Takimoto et al., 2019; Duan et al., 2020). In addition, there is great genetic diversity and differences among the 26 rice genotypes in the quantitative traits under study. These results are similar to El-Malky and Al-Daej (2023) and Rezk et al. (2024). The phenotypic coefficient of variation (PCV%) was greater than the genetic coefficient of variation (GCV%) which indicates this environmental factors and cultural practices constituted the largest part of PCV%. These results are consistent with the results of Faysal et al. (2022) and Rezk et al. (2024). High heritability estimates were found with all traits studied, indicating the presence of both additive and non-additional genetic variances in the inheritance of most traits (Asante et al., 2019; El-Malky and Al-Daej, 2023). Thus, it is possible to draw the conclusion that the selection processes it uses

are effective in enhancing the attributes being evaluated. Some results were previously obtained by Faysal et al. (2022) and Rezk et al. (2024). The quantitative and molecular characterization of heat tolerance in rice has previously been investigated (Pradhan et al., 2016; Mazal 2021; El-Malky 2024). Hence, genetic diversity for this trait is the pre-requisite step for developing high-yielding temperature stress-tolerant rice varieties suitable and suitable for cultivation in hot regions (Ali et al., 2019; Ezin et al., 2022). Clustering based on quantitative traits was grouped into many sub-groups, Based on the types of genotypes (Figure 1). All Indica types were in one group except VNIRB572 from the Japonica type, this classified due to coming from one origin (IRRI), and there is a close in some quantitative characteristics. On the other hand, the two Saudi varieties came together in the same group and belonged to the Indica-Japonica type. The second group included all Japonica types except for the two (IET1444 and Giza175) that belonged to Indica-Japonica type. Similar results were also obtained (Pradhan et al., 2016; El-Malky, 2024; Rezk et al., 2024). However, conventional breeding is generally a good facility for producing new tolerant genotypes (Driedonks et al., 2016; He et al., 2018). Accurate evaluation of the selection of rice genotypes or breeding lines, and successful transfer of heat tolerant traits into specific cultivars with good agronomic performance are of great importance to conventional breeding (Kilasi et al., 2018). Molecular marker technology was used as a tool in breeding programs to increase its efficiency, speed the transfer of desirable genes among genotypes, Identify genes, and study genetic diversity among rice species. 18 SSR markers were used for 14 rice genotypes, which had highly popular genetic markers due to their co-dominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing SSR size variation by PCR with pairs of flanking primers (Rezk et al., 2024). The total number of alleles was 137 with a mean of 7.61 alleles per locus, which were detected in the 14 rice genotypes. All 18 primers showed polymorphism between the 14 rice genotypes (Table 7). The PCR amplified products for some SSR markers with 14 rice genotypes were clarified in Figure 2 also, multiple alleles per sample were observed for all loci under study (Ahmad et al., 2015; El-Malky, 2024; Rezk et al., 2024). PIC values were high (Table 7), for all SSR markers with an average of 0.3891 and ranged from a low of 0.2755 for RM249 to a high of 0.4579 for RM228. Similar results with Melaku et al. (2018) and Mazal (2021). According to this finding, SSR markers were useful instruments for assessing genetic diversity (Aljumaili et al., 2018; El-Malky 2024; Rezk et al., 2024). Clustering analysis of rice cultivars based on SSR markers in (Figure 3) divided into two groups. The first group included seven tolerant rice genotypes, belonging to Indica type, and their produced in the same origin International Rice Research Institute (IRRI) except PUSA Basmati 1 from Indian rice (Table1). The second group included four rice genotypes (Hasswi-1, Hasswi-2, Giza178 and IET 1444) belonging to Indica-Japonica. In any case, the Hasswi-1 and Hasswi-2 came from closely related parents (Table 1). Also, this group included IR28, IR82, and IR72 belonged to Indica types, these four genotypes were also tolerant of heat-stress conditions. In addition, the IR28 and IR82 came from the same parents (Table 1). 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; Almasoud et al., 2024). Finally, the efficiency of SSR analysis was raised in this work to a level that was equivalent to multi-locus fingerprinting methods and measuring the polymorphism between genotypes.

CONCLUSIONS

In conclusion, the 14 genotypes were highly productive under heat stress conditions, and the highest genotypes for grain yield plant^{-1} were IR72, IR28, Dular, IR82, and Giza178. These genotypes can help

breeders use them in breeding programs for heat tolerance. This study also revealed that the reduction in the yield caused heat stress ranging from 16.33 to 23.27 (g). Heritability in the broad sense was high for all traits, and phenotypic variance was higher than the genetic variance for all traits studied. Most of the tolerant genotypes were in groups together in the cluster, while the sensitive genotypes were in another group. SSR marker was a good tool for studying the polymorphism between the genotypes according to genetic background. The study also indicated that these varieties have high genetic diversity. Therefore, it is instrumental in breeding programs to identify and select suitable parents for hybridization to produce varieties that tolerate high temperatures.

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REFERENCES

Ahmad, F., Hanaf, M.M., Hakim, M.A., Rafi, M.Y., Arolu, I.W., Abdullah, S.N.A., 2015. *Genetic divergence and heritability of 42 coloured upland rice genotypes (Oryza sativa) as revealed by microsatellites marker and agro-morphological traits*. PLoS One, 10(9): e0138246. https://doi.org/10.1371/journal. pone.0138246

Al-Daej, M.I., Rezk, A.A., Ismail, M., El-Malky, M.M., Shalaby, T.A., 2023. *Comparative genetic diversity assessment and marker-trait association using two DNA marker systems in rice (Oryza sativa L.)*. Agronomy, 13(2): 329. https://doi.org/10.3390/agronomy13020329

Ali, F., Waters, D.L.E., Ovenden, B., Bundock, P., Raymond, C.A., Rose, T.J., 2019. *Australian rice varieties vary in grain yield response to heat stress during reproductive and grain filling stages*. J. Agron. Crop. Sci., 205: 179-187. DOI:10.1111/jac.12312

Aljumaili, S.J., Rafii, M.Y., Latif, M.A., Sakimin, S.Z., Arolu, I.W., Miah, G., 2018. *Genetic diversity of aromatic rice germplasm revealed by SSR markers*. BioMed Research International 2018: 7658032. https://doi.org/10.1155/2018/7658032

Almasoud, W.A., Abdel-Sattar, M., Sedeek, S., Elgammaal, A.A., El-Refaee, N., Ramadan, I.A., Abdulmajid, D., Rihan, H.Z., 2024. *The Path Towards Novel Varieties: Investigating Phenotypic Genetic Diversity in New Promising Egyptian Rice Lines*. Agronomy, 14: 2775. https://doi.org/10.3390/agronomy14122775

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., Sorrells, M.E., 1993. *Optimizing parental selection for genetic linkage maps*. Genome, 36: 181-186.

Asante, M.D., Adjah, K.L., Annan-Afful, E., 2019. *Assessment of genetic diversity for grain yield and yield component traits in some genotypes of rice (Oryza sativa L.)*. J. Crop Sci. Biotech., 22: 123-130. https://doi.org/10.1007/s12892-019-0008-0

Cheabu, S., Moung-Ngam, P., Arikit, S., Vanavichit, A., Malumpong, C., 2018. *Effects of heat stress at vegetative and reproductive stages on spikelet fertility*. Rice Sci., 25: 218-226. https://doi.org/10.1016/j.rsci.2018.06.005

Driedonks, N., Rieu, I., Vriezen, W.H., 2016. *Breeding for plant heat tolerance at vegetative and reproductive stages*. Plant Reprod., 29: 67-79. DOI:10.1007/s00497-016-0275-9

Duan, H., Tong, H., Zhu, A., Zhang, H., Liu, L., 2020. *Effects of heat, drought and their combined effects on morphological structure and physicochemical properties of rice (Oryza sativa L.) starch*. J. Cereal Sci., 95: 103059. https://doi.org/10.1016/j.jcs.2020.103059

El-Beltagi, H.S., Mohamed, H.I., Aldaej, M.I., Al-Khayri, J.M., Rezk, A.A., Al-Mssallem, M.Q., Sattar, M.N., Ramadan, K.M.A., 2022. *Production and antioxidant activity of secondary metabolites in Hassawi rice (Oryza sativa L.) cell suspension under salicylic acid, yeast extract, and pectin elicitation*. In Vitro Cell Dev. Biol. Plant., 58: 615-629. https://doi.org/10.1007/s11627-022-10264-x

El-Malky, M.M., 2024. *Heat tolerance and genetic diversity analyses of rice accessions using SSR markers*. SABRAO J. Breeding Gene, 56: 519-533. http://doi.org/10.54910/sabrao2024.56.2.6

El-Malky, M.M., and Al-Daej, M.I., 2023. *Assessment of inbreeding depression genetic variability and genetic diversity in rice (Oryza sativa l.) based on agro-morphological traits*. Fres. Environ. Bull., 32(04): 2067-2077.

Ezin, V., Ahanchede, W.W., Ayenan, M.A.T., Ahanchede, A., 2022. *Physiological and agronomical evaluation of elite rice varieties for adaptation to heat stress*. BMC Plant Biol., 22: 236. https://doi.org/10.1186/s12870-022-03604-x

Faysal, A.S.M., Ali, L., Azam, M.G., Sarker, U., Ercisli, S., Golokhvast, K.S., Marc, R.A., 2022. *Genetic Variability, Character Association, and Path Coefficient Analysis in Transplant Aman Rice Genotypes*. Plants, 11(21): 2952. https://doi.org/10.3390/plants11212952

He, L.X., Chen, Y.L., Zhang, T.T., Zheng, A.X., Cheng, Y., Du, P., Lai, R.F., Lu, R.H., Luo, H.W., Liu, Y.F., Tang, X.R., 2018. *Effects of different temperature conditions on yield and physiological properties of rice (Oryza sativa L.)*. Appl. Ecol. Environ. Res., 17: 199-211. https://doi.org/10.15666/aeer/1701_19921

IRRI, 2014. *Standard evaluation system for rice. International Rice Research Institute*. Los Baños, Laguna, Philippines. ISBN: 97897122030469712203042

Jumiatun, A.J., Lubis, I., Chozin, M.A., Miyazaki, A., 2016. *Morphological, physiological and yield responses of some rice varieties (Oryza sativa L.) as exposed under high temperature in Indonesia*. Am. J. Plant. Physiol., 11: 33-41. DOI:10.3923/ajpp.2016.33.41

Kilasi, N.L., Singh, J., Vallejos, C.E., Ye, C., Jagadish, S.V.K., Kusolwa, P., Rathinasabapathi, B., 2018. *Heat stress tolerance in rice (Oryza sativa L.): identification of quantitative trait loci and candidate genes for seedling growth under heat stress*. Front. Plant Sci., 9: 1578. https://doi.org/10.3389/fpls.2018.01578

Kinose, Y., Masutomi, Y., Shiotsu, F., Hayashi, K., Ogawada, D., Gomez-Garcia, M., Matsumura, A., Takahashi, K., Fukushi, K., 2020. *Impact assessment of climate change on the major rice cultivar Ciherang in Indonesia*. J. Agric. Meteorol., 76: 19-28. DOI:10.2480/agrmet.D-19-00045

Li, S., Wu, F., Zhou, Q., Zhang, Y., 2024. *Adopting agronomic strategies to enhance the adaptation of global rice production to future climate change: A meta-analysis*. Agron. Sustain., 44: 12-23. DOI:10.1007/s13593-024-00963-7

Liu, H., Zeng, B., Zhao, J., Yan, S., Wan, J., Cao, Z., 2023. *Genetic research progress: Heat tolerance in rice*. Int. J. Mol. Sci., 24(8): 7140. https://doi.org/10.3390/ijms24087140

Maixner, M., Ahrens, U., Seemüller, E., 1995. *Detection of the German grapevine yellows (Vergilbung skrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure*. Eur. J. Plant. Pathol., 101: 241-250. DOI:10.1007/BF01874780

Manigbas, N.L., Lambio, L.A.F., Madrid, L.B., Cardenas, C., 2014. *Germplasm innovation of heat tolerance in rice for irrigated lowland conditions in the Philippines*. Rice Sci., 21: 162-169. https://doi.org/10.1016/ S1672-6308(13)60180-8

Masutomi, Y., Takimoto, T., Manabe, T., Imai, Y., Tamura, M., Kobayashi, K., 2023. *Breeding targets for heat-tolerant rice varieties in Japan in a warming climate*. Mitig. Adapt. Strateg. Glob. Change, 28: 1-19. https://doi.org/10.1007/s11027-022-10027-4

Mazal, T.M., 2021. *Field evaluation and genetic diversity for heat tolerance using stress indices and SSR markers in rice*. Menoufia J. Plant Prod. 6(2021): 267-287.

Melaku, G., Zhang, S., Haileselassie, T., 2018. *Comparative Evaluation of Rice SSR Markers on Different Oryza Species*. J. Rice Res. Dev., 1(1): 38-48. DOI:10.36959/973/418

Mohamed, A.A.E., Fiaz, S., Wang, X., Ali, M., Parveen, N., Gaballah, M.M., Ali, S., Abdallah, A.A., Ahmed, S.I., 2021. *Performance of some rice (Oryza sativa L.) cultivars under water shortage and high temperature stress*. Sains Malaysiana, 50: 617-628. http://dx.doi. org/10.17576/jsm-2021-5003-05

Mohammed, A.R., and Tarpley, L., 2010. *Effects of high night temperature and spikelet position on yield-related parameters of rice (Oryza sativa L.) plants*. Eur. J. Agron., 33: 117-123. https://doi.org/10.1016/j.eja.2009.11.006

Mohamed, H.I., and Abdel-Hamid, A.M., 2013. *Molecular and biochemical studies for heat tolerance on four cotton genotypes*. Romanian Biotechnol. Lett., 18(6): 8823-8831.

Mohamed, H.I., Ashry, N.A., Ghonaim, M.M., 2019. *Physiological and Biochemical Effects of Heat Shock Stress and Determination of Molecular Markers Related to Heat Tolerance in Maize Hybrids*. Gesunde Pflanz., 71: 213-222. https://doi.org/10.1007/s10343-019-00467-5

Ndour, D., Diouf, D., Bimpon, I.K., Sow, A., Kanfany, G., Manneh, B., 2016. *Agro-Morphological Evaluation of Rice (Oryza sativa L.) for Seasonal Adaptation in the Sahelian Environment*. Agronomy, 6: 8. doi:10.3390/agronomy6010008

Nishad, A., Mishra, A.N., Chaudhari, R., Aryan, R.K., Katiyar, P., 2018. *Effect of temperature on growth and yield of rice (Oryza sativa L.) cultivars*. Inter. J. Chem. Stud., 6(5): 1381-1383.

Permana, H., Murata, K., Kashiwagi, M., Yamada, T., Kanekatsu, M., 2017. *Screening of Japanese rice cultivars for seeds with heat stress tolerance under hot water disinfection method*. Asian J. Plant. Sci., 16: 211-220. https://doi.org/10.3923/ajps.2017.211.220

Pradhan, S.K., Barik, S.R., Sahoo, A., Mohapatra, S., Nayak, D.K., Mahender, A., Meher, J., Anandan, A., Pandit, E., 2016. *Population Structure, Genetic Diversity and Molecular Marker-Trait Association Analysis for High Temperature Stress Tolerance in Rice*. PLoS One, 11(8): e0160027:1-23. doi:10.1371/journal.pone.0160027

Rang, Z.W., Jagadish, S.V.K., Zhou, Q.M., Craufurd, P.Q., Heuer, S., 2011. *Effect of high temperature and water stress on pollen germination and spikelet fertility in rice*. Environ. Exp. Bot., 70: 58-65. doi:10.1016/j.envexpbot.2010.08.009

Rashmi, D., Prashant, B., Saha Shoumik, S., Bapsila, L., Sakshi, S., Pallavi, Singh, P.K., 2017. *Genetic*

Diversity Analysis in Rice (Oryza sativa L.) Accessions using SSR Markers. International Journal of Agriculture Environment and Biotechnology, 10: 457-467. DOI: 10.5958/2230-732X.2017.00057.2

Rezk, A.A., Mohamed, H.I., El-Beltagi, H.S., 2024. *Genetic variability and diversity analysis in Oryza sativa L. genotypes using quantitative traits and SSR markers*. Saudi J. Biol. Sci., 31(3): 103944. https://doi.org/10.1016/j.sjbs.2024.103944

Rohlf, F.J., 2000. *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.1*. Exeter Publishing Setauket, New York.

Sadat, S., Saeid, K.A., Bihamta, M.R., Torabi, S., Salekdeh, S.G.H., Ayeneh, G.A.L., 2013. *Marker Assisted Selection for Heat Tolerance in Bread Wheat*. World Appl. Sci. J., 21: 1181-1189. DOI:10.5829/idosi.wasj.2013.21.8.2866

Singh, R.K., and Chaudhary, B.D., 1985. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publication, New Delhi, India.

Sitaresmi, T., Susanto, U., Pramudyawardani, E.F., Nafisah, E., Nugraha, Y., Sasmita, P., 2020. *Genotype × environment interaction of rice genotype*. IOP Conf. Ser. Earth Environ. Sci., 484: 1-7. https://doi.org/10.1088/1755-1315/484/1/012028

Steel, R.G.D., and Torrie, J.H., 1980. *Principles and Procedures of Statistics, A Biometrical Approach*. 2nd Edition, McGraw-Hill Book Company, New York.

Takimoto, T., Masutomi, Y., Tamura, M., Nitta, Y., Tanaka, K., 2019. *The effect of air temperature and solar radiation on the occurrence of chalky rice grains in rice cultivars 'Koshihikari' and 'Akitakomachi'*. J. Agric. Met., 75: 203-210. https://doi.org/10.2480/agrmet.D-18-00039

Villegas, J.R., Heinemann, A.B., de-Castro, P.A., Breseghello, F., Racines, C.N., Li, T., 2018. *Breeding implications of drought stress under future climate for upland rice in Brazil*. Glob Change Biol., 24: 2035-2050. https://doi.org/10.1111/gcb.14071

Xu, Y., Chu, C., Yao, S., 2021. *The impact of high-temperature stress on rice: Challenges and solutions*. The Crop J., 9: 963-976. https://doi.org/10.1016/j.cj.2021.02.011

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D.B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S., Peñuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D., Liu, Z., Zhu, Y., Zhu, Z., Asseng, S., 2017. *Temperature increase reduces global yields of major crops in four independent estimates*. Proc. Nat. Acad. of Sci. of the USA, 35: 9326-9331. doi:10.1073/pnas.1701762114