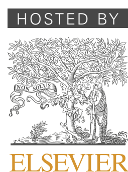

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Genetic variability and diversity analysis in *Oryza sativa* L. genotypes using quantitative traits and SSR markers

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ARTICLE INFO

Keywords:
Cluster analysis
Genetic parameters
Quantitative traits
Rice
SSR markers

ABSTRACT

The present study was aimed at evaluating the genetic variation and population structure in a collection of 22 rice genotypes. Twenty-two rice genotypes were assessed using quantitative traits and SSR molecular markers for genetic variability and genetic diversity. As for genetic diversity, the genotypes were clarified based on twelve quantitative traits. Clustering produced two large groups: the IR70423-169-2-2 variety was in a branch alone due to its long duration, while, the second group included all rest of genotypes and was split up into two sub-groups. The first sub-group included IR67418-131-2-3-3-3, IR67420-206-3-1-3-3, Giza181, Giza182, Sakha104, and P1044-86-5-3-3-2M. However, pedigree played in divided clustering with Giza181 and Giza182, which were belonging to the Indica type and produced from the same parents. SSR markers produced 87 alleles, with a mean of 4.3 alleles per locus, which were detected in 22 rice genotypes. A higher number of alleles were found with primers RM262, RM244, RM3843, RM212, and RM3330. With an overall mean of 0.837, the polymorphic information content values were high for all SSR markers, ranging from a low of 0.397 for M254 to a high of 0.837 for RM244. The dendogram was divided into six groups according to the types of genotypes, with the pedigree playing a major role for the genetic distance. In order to help breeders choose parents and create suitable hybrids to achieve genetic improvement in crops, particularly rice, SSR is a useful technique for analysing genotype diversity and aiding in the genetic fingerprinting of each variety.

1. Introduction

Over half of the world's population consumes rice (*Oryza sativa* L.) as their primary food; for over 3.5 billion people, rice accounts for 20 % of their daily caloric intake (Naomi and Ziska, 2019; Rezk et al., 2023). Regardless, 90 % of the world's production comes from over 100 countries in Asia (Bandumula, 2018). Rice consumption in the world ranges from 100 to 140 kg per person annually in some African and Asian countries (Shallan et al., 2010a, b; Fahad et al., 2019). Also, it is considered an important crop in the world, as it provides humans with more than 21 % of their calorie needs and up to 76 % of calories, especially in developing countries such as Southeast Asia (Zhao et al., 2020; Elkatry et al., 2023). As a result, increasing rice productivity is required, and this can be done by increasing the area planted to rice or the yield per acre.. But there are many constraints for increasing the rice-cultivated area, such as a shortage of water, increased salinity, and climatic changes (Guo et al., 2014; Fahad et al., 2019). However, improving and developing new genotypes is the best way to increase production per unit area (Abdourasmane et al., 2016; Esther et al., 2021; El-Malky and Al-Daej, 2023).

The primary goal and essential step is breeding for high yields and resilience to biotic and environmental stress to prevent these significant obstacles to global rice production (Mohamed et al., 2019; Ashry et al., 2018; Gaballah et al., 2022). This will need to study the genetic diversity and genetic background for each variety, which will be useful to improve the agronomic traits (Sabri et al., 2020; Zhong et al., 2021). Studies on genetic diversity are crucial for breeding and improvement projects because they guarantee the appropriate use of germplasm resources and a productive breeding system for the promotion of closely related crop species (Al-Daej et al., 2022). Until recently, morphological characterization had proven effective in identifying genetic material. However, due to the increased homogeneity of commercial genotypes and the scarcity of available morphological traits, this method is no longer effective in differentiating between commercial genotypes (Shamim and Sharma, 2014).

Breeding programs that are designed using traditional breeding

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https://doi.org/10.1016/j.sjbs.2024.103944
Received 7 January 2024; Received in revised form 21 January 2024; Accepted 26 January 2024
Available online 28 January 2024
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methodologies, which are based on observed variations in traditional varieties or on controlled crosses, primarily depend on the differences and similarities among genotypes. Nevertheless, the length of time needed to achieve the desired gain may make these methods less practical. By utilizing DNA markers in breeding programs, molecular breeding, on the other hand, may expedite the genotype screening procedure and reduce the time needed to get the anticipated gain (Al-daej et al., 2023). Molecular markers are used to characterize the genotypes genetically, serving as a supplement to their morphological identification (Rahman et al., 2009; El-Beltagi, and Mohamed, 2010; Ghonaim et al., 2023). For the purpose of describing genotypes, both approaches are complementary (Muhamad et al., 2017; Mehmood et al., 2021). Breeding programs have employed many DNA markers for various purposes, such as identifying genes related to biotic and abiotic stress (e.g., disease resistance, salinity, drought, heat, and cold tolerance) (Mohamed et al., 2009; Mohamed and Abdel-Hamid, 2013; Mohamed et al., 2018; Ghonaim et al., 2021). Furthermore, estimates of genetic diversity among types heavily rely on DNA markers. Numerous DNA markers exist with varying purposes; however, selection of a DNA marker is largely influenced by criteria such as economic constraints and accuracy. Two of the most often used DNA markers in assessments of genetic diversity are simple sequence repeats (SSRs) and inter-simple sequence repeats (ISSRs) (Al-daej et al., 2023).

Genetic variation assessment is important for crop improvement as well as effective germplasm source conservation and management (Sabri et al., 2020). Our knowledge of rice genetics and the structure and function of genomes has increased because of technological advancements in molecular markers for genetic variation (Krupa et al., 2017). Highly polymorphic Simple Sequence Repeats (SSR) markers are helpful for phylogenetic analysis, genome mapping, and gene labeling (Jumaili et al., 2018). The genetic diversity of a crop is essential for breeding programs to succeed in enhancing the crop and generating genotypes with high yields. Additionally, plant breeders will benefit from the use of genetic diversity when choosing suitable materials for cultivar enhancement and efficient management of rice genetic resources. In order to classify and assess the germplasm, morphological characterization is the initial stage (Muhamad et al., 2017).

The aim of this study was to ascertain the genetic diversity of 22 exotic rice genotypes, detect potential associations among the agronomic traits analyzed, and expand the research to ascertain the genetic diversity among 22 rice genotypes, categorise them via cluster analysis, and assess the genetic diversity among these genotypes through the use of twenty SSR markers.

2. Materials and methods

2.1. Field experiments

During the 2018 and 2019 seasons, 22 rice genotypes were chosen from several types; these included 9 Japonica, 6 Indica-Japonica, and 7 Indica genotypes (Table 1). The genotypes were supplied by International Rice Research Institute (Los Banos, Philippines). The seedlings were moved onto the permanent field after 30 days. Over the course of the two years (2018 and 2019), an RCBD (Randomized Complete Block Design) with three replications was used to transplant seedlings of each genotype. Every plot had three rows, with twenty-five plants per row spaced at 20 × 20 cm.

2.2. Quantitative traits assessment

The average of two years for twelve agronomic traits, namely: days to heading, plant height (cm), flag leaf area (cm^2), tillers plant^{-1}, panicles plant^{-1}t, panicle weight (g), panicle length, 1000-grain weight (g), filled grains panicle^{-1}, spikelet fertility (%), grain yield plant^{-1} (g), and milling rice percentage. The Standard Evaluation System (SES) for rice was used to record the data for every attribute (IRRI, 2014). The two-year averages of these features were employed to establish genetic relationships between the genotypes of rice that were being examined.

Table 1
Parentage, Origen, types and grain shape of the studied 22 exotic rice genotypes.

No.	Genotypes	Parentage	Origen	Type	Grain shape
1	IR70423-169-2-2	IR 19660-73-4/IR 54//IR 9828-36-3	IRRI*	Indica	Long
2	IR67418-131-2-3-3-3	IR 9645-146-2-6-2/IR 62873-278-4-3	IRRI	Indica	Long
3	IR67420-206-3-1-3-3	IR59645-146-2-6-2/IR62873-417-4-1	IRRI	Indica	Long
4	Sakha103	Giza177 / Suwwon349	Egypt	Japonica	Short
5	Giza177	Giza 171/ Yomji No.1//Pi No 4	Egypt	Japonica	Short
6	Giza178	Giza175 / Milyange49	Egypt	Indica-Japonica	Short
7	Giza175	IR28/IR1541//Giza180/Giza14	Egypt	Indica-Japonica	Short
8	Sakha106	Giza177 / Hexi30	Egypt	Japonica	Short
9	IET360	GEB 24/TN1	India	Indica-Japonica	Short
10	ITA257	IRAT13/DP689//TOX490-1	II TA**	Indica	Long
11	P1035-5-6-1-1-1M	P738-137-3-1/P881-191410//P738-137-3-1/P881-19-24-5	IRRI	Indica-Japonica	Short
12	P1035-5-6-1-1-2M	P738-13-7-3-1/P881-19-14-10//P738-137-3-1 / P 881-19-24-5	IRRI	Indica-Japonica	Short
13	Giza171	Nahda/Calady40	Egypt	Japonica	Short
14	Sakha104	Gz4096-8-1/Gz4100-9-1	Egypt	Japonica	Short
15	IAC165	Dourado Precoce/lAC 1246	Brazil	Japonica	Short
16	P1044-86-5-3-1-2M	P738-137-3-1//P738-137-3-1/P881-19-24-4	IRRI	Indica-Japonica	Short
17	Giza182	Giza181/IR39422-163-1-3/Giza181	Egypt	Indica	Long
18	Giza181	IR28/IR22	Egypt	Indica	Long
19	lAC164	Dourado Precoce/lAC 1246	Brazil	Japonica	Short
20	ITA301	IRAT13/Dourado Precoce 689//Padipapayak	II TA	Indica	Long
21	Giza172	Nahda/Kinmaza	Egypt	Japonica	Short
22	P1044-86-5-3-3-2M	P738-137-3-1//P738-137-3-1/P881-19-24-4	IRRI	Japonica	Short

* = International Rice Research Institute.
** = International Institute of Tropical Agriculture.

2.3. DNA extraction

The procedure outlined by Redona and Mackill (1998) was used for extracting DNA from the leaves of the studied genotypes. Before usage, the DNA samples were diluted to 10 ng/μl using a fluorometer (Hoefer Scientific TKO 100, Hoefer Scientific Instruments, San Francisco, CA, USA). The 22 rice genotypes genomic DNA was amplified using 20 microsatellite markers to detect simple sequence repeat polymorphism (SSR) (Table 2). Also, polymerase chain reaction analysis was performed following the procedure recommended by manufacturer (ABI Prism 377 GeneScan Chemistry Guide, PE Biosystems, Foster City, CA) with minor modifications. The PCR reaction was supplemented with fluorescent dCTP (330 nM) labelled with rhodamine dye (R110 and R6G) to facilitate fragment detection in the Perkin-Elmer ABI377 automated sequencing gel system. Green nucleotides were R6G and blue R110 nucleotides.

Table 2
Primer sequences and chromosome number of microsatellite used.

No.	Chromosome Number	Marker	Sequences RM247	Repeat Motif
1	12	RM247	F: TAGTGCCGATCGATGTAACG R: CATATGGTTTTGACAAAGCG	(CT)16
2	6	RM217	F:ATCGCAGCAATGCCTCGT R:GGGTGTGAACAAAGACAC	$(CT)_{20}$
3	11	RM21	F:ACAGTATTCCGTAGGCACGGR: GCTCCATGAGGGTGGTAGAG	GA)18
4	1	RM1216	F: TTCCCCAATGGAACAGTGACR: AGGGTCTACCACCCGATCTC	(AG)14
5	4	RM241	F:GAGCCAAATAAGATCGCTGA R: TGCAAGCAGCAGATTTAGTG	$(CT)_{31}$
6	8	RM44	F: ACGGGCAATCCGAACAACC R:TCGGGAAAACCTACCCTACC	(GA)16
7	11	RM254	F:AGCCCCAATAATCCACCT R: CTGGAGGAGCATTTGGTAGC	$(TC)_6ATT(CT)_{11}$
8	6	RM3330	F: ATTATTCCCCTCTTCCGCTCR: AAGAAACCCTCGGATTCCTG	(CT)15
9	6	RM238B	F:GATGGAAAGCACGTGCACTA R: ACAGGCAATCCGTAGACTCG	$(CT)_{15}$
10	1	RM212	F: CCACTTTCAGCTACTACCAG R: CACCCATTTGTCTCTCATTATG	(GA)24
11	11	RM229	F: CACTCACACGAACGACTGAC R: CGCAGGTTCTTGTGAAATGT	$(TC)_{11}(CT)_5C_3(CT)_5$
12	2	RM262	F: CATTCCGTCTCGGCTCAACT R: CAGAGCAAGGTGGCTTGC	(CT)16
13	10	RM244	F: CCGACTGTTCGTCCTTATCAR: CTGCTCTCGGGTGAACGT	(CT)4(CG)3C(CT)6
14	6	RM225	F:TGCCCATATGGTCTGGATG R: GAAAGTGGATCAGGAAGGC	$(CT)_{18}$
15	9	RM219	F: CGTCGGATGATGTAAAGCCT R:CATATCGGCATTCGCCTG	(GA)17
16	3	RM3	F: ACACTGTAGCGGCCACTG R: CCTCCACTGCTCCACATCTT	$(GA)_2GG(GA)_{25}$
17	11	RM209	F:ATATGAGTTGCTGTCGTGCGR: CAACTTGCATCCTCCCCTCC	$(CT)_{18}$
18	11	RM224	F:AGCCCCAATAAATCCACCT R: CTGGAGGAGCATTTGGTAGC	$(AAG)_8(AG)_{13}$
19	11	M206	F:CCCATGCTTTAACTATTCTR: CGTTCCATCGATCCGTATGG	$(CT)_{21}$
20	4	RM3843	F: ACCCTACTCCCAACAGTCCCR: GGGGTCGTACGCTCATGTC	(GA)23

2.4. Amplification PCR

The PCR reaction was carried out in a Perkin Elmer Thermal Cycler with the following steps: (i) a 3 min 94 °C initial denaturation step; (ii) 35 cycles of 1 min at 94 °C, 2 min at 55 °C, and 1.5 min at 72 °C; or (iii) 5 min at 72 °C. After amplification, 2 units of CIP (Calf Intestinal Alkaline Phosphatase) were added to each sample. CIP is used to remove 5-phosphate from DNA fragments to prevent self-ligation. Gel electrophoresis was conducted using the ABI PRISM 377 DNA Sequencer apparatus (PerkinElmer) using the 1X TBE buffer. The sequencing gel was pre-run at 1000 W until it reached 50 °C. Each sample was heated to 90 °C for 7 min for DNA denaturation and placed directly on ice. 1.5 µl of a mixture consisting of 1 µl Rox 500 size standards (red color) (PE Biosystems, Warrington, UK), 1 µl loading buffer, and 2 µl Formamide were added to 1.5 µl of each sample. Finally, 2 µl from each sample were loaded on the gel. The gel was run at 3000 V until 500 bp size standard passed read window. Fragment length was estimated utilizing the internal size standards by GeneScan Analysis Software.

2.5. Statistical analysis

2.5.1. Analysis for quantitative traits

For every season, an analysis of variance was performed on the assumption that the genotypes being studied are random. Le Clerg et al. (1962) reported that because the experiment's error variances were statistically homogeneous, the two trials were statistically integrated over the duration of the two seasons. The studies were then put through an analysis of variance, which was used to determine the size of each factor and divide the overall phenotypic variability into components resulting from genetic (hereditary) and non-genetic (environmental) factors.

The phenotypic variance, which is explained by genotypic variations between the phenotypes, includes genotypic variance. Comparably, phenotypic variance is defined as the total variation in phenotypes under various relevant contexts (Dudley and Moll, 1969). The variance components, genotypic (Vg), phenotypic (Vp), or error (Ve) variances were therefore calculated utilizing the following formula, which was developed by Wricke and Weber (1986).

The genotypic variances (Vg) = [The mean squares of genotypes – Mean squares of error / Number of replications]

The phenotypic variances (Vph) = [The mean squares of genotypes / Number of replications]

The error variances (Ve) = [Mean squares of error / Number of replications]

According to Allard (1999), genotypic mean basis was used to determine broad-sense heritability (h_2B), which is stated as the percentage of the ratio of genotypic variance (Vg) to the phenotypic variance (Vph).

Dissimilarity coefficients were calculated using the numerical taxonomic approach (Sneath and Sokal, 1973) for evaluating genetic divergence based on simple matching. Similarity matrices were generated using software NTSYS-PC version 2.1 (Rohlf, 2000).

2.5.2. Statistically analysis for DNA data

Generally, the fragments that were discovered were given non-integer base-pair size values by the Applied Biosystems ABI GeneScan programme. Each fragment was given an allele associated with the proper microsatellite locus. During the allele binning procedure, several steps were involved: The fragments were sorted by size, the fragments that were separated by less than two base pairs were binned together, and the allele's molecular weight was determined by computing the mean and rounding it to the nearest full base pair integer. To determine a marker's polymorphism information content (PIC), a condensed version of Anderson et al. (1993) was utilized.

$PIC = 1 - n_{j=1} P_{ij}^2$

where P_{ij} is the frequency of the jth allele for the ith marker, and summed over n alleles. Also, average number of alleles, total heterosygozity and average PIC value were calculated.

For every microsatellite marker, the alleles present and absent were noted for every genotype and turned into a genetic matrix. The genetic separation between two genotypes (Nei and Li, 1979) was calculated as follows:

GD = 1- [2 the number of shared bands /2 the total number of bands for genotypes i + the total number of bands for genotypes j]

Version 2.1 of the Numerical Taxonomy and Multivariate Analysis system (NTSYSpc; Rohlf, 2000) was used to construct the dendogram analysis, genetic distances, and similarity matrix.

3. Results

3.1. Growth and yield attributes of rice

The results of the analysis of variance (Table 3) showed that genotypes expressed a substantial range of variation and had highly significant differences for all the attributes studied.

The mean performance of 22 rice genotypes for studied traits is presented in Table 4. The results show that a short duration was observed in Sakha103 (95 days), Giza177 (95 days), Giza182 (100 days), Giza178 (105 days), IAC164 (108 days) and ICA165 (109 days). While Giza171 was considered to have a long duration, one gave 135 days to heading date, and the other genotypes had a moderate duration. Conspicuously, Table 4 shows that the maximum flag areas were in LAC156, Giza178, Giza175 and IET360. As for plant height, all the genotypes were less than 100 cm except Giza171, Giza172, IR70423-169-2-2, Sakha104, IAC164, IR67418-131-2-3-3-3, IR67420-206-3-1-3-3, and Sakha103. For the number of grains per panicle, the genotypes IR70423-169-2-2, Sakha106, Giza182, IR70423-169-2-2 and IR67420-206-3-1-3-3 gave the highest values (167.00, 157.05, 152.33, 147.67 and 147.67), respectively. Concerning panicle weight, Table 4 showed that the highest genotypes were Giza182 (5.47), P1035-5-6-1-1-2M (5.10), Giza181 (4.90), Giza171 (4.70), Sakha104 (4.23), LAC156 (4.20), and IET360 (4.0 g). While, in grain yield per plant, the genotypes IR67418-131-2-3-3-3, Giza182, Giza181, Sakha106 gave 44.57, 44.20, 40.70, 44.20 and 39.40 (g), respectively.

3.2. Estimates of genetic parameters

The mean performance of the 22 rice genotypes under investigation varied widely. Table 5 displays the computed results of heritability and genetic advancement under selection. All characters studied in various years showed high estimates of heritability, indicating the presence of both additive and non-additional genetic variance in inheritance of most traits, with the exception of panicle weight, which varied between 64.0 % and 74 %. These characteristics, however, are more resilient to shifting environments and cultural norms. This study found that estimations of the heritability of panicle length (cm), plant height (cm), and flag leaf area (cm2) varied in size, indicating that these features were more influenced by environmental influences and cultural norms. Additionally, the best method for enhancing these attributes was the selection process.

3.3. Phenotypic correlation coefficient

Phenotypic correlation coefficients among twelve quantitative traits across twenty-two genotypes were calculated, and the results are illustrated as a heat map (Fig. 1). The heat map showed how well each genotype performed in relation to the attributes for which the data was collected. Darker red strips show strong negative correlations, whereas the positive, darker scale indicates extremely significant correlations for genotypes, according to the blue color scale. The genotypes also exhibit moderate performance in both positive and negative ranges when color intensity decreases. Lucidly, grain yield was positively and strongly correlated with each of the number of grains per panicle and 1000-grain weight. As such, the intended increase in grain yield will result from any selection made in accordance with these characteristics. Also, the number of tillers per plant was positively correlated with the number of panicles per plant, the number of grains per panicle, the 1000-grain weight, and the milling percentage. Duration was positively correlated to the number of panicles per plant, while the number of grains per panicle showed an insignificant positive correlation with 1000-grain weight and milling (%).

3.4. Cluster analysis for studied genotypes based on quantitative characteristics

By clustering genotypes according to how comparable their quantitative traits were, two sizable groups were created (Fig. 2). First, there was the long-duration genotype in the branch only (IR70423-169-2-2); second, there were all the other genotypes and two sub-groups; the first sub-group contained four genotypes of rice: IR67418-131-2-3-3-3, IR67420-206-3-1-3-3, Giza181, Giza182, Sakha104, and P1044-86-5-3-3-2M. However, the genotypes IR67418-131-2-3-3-3 and IR67420-206-3-1-3-3 with the same duration belonged to IRRI genotypes, Indica types, and were produced from the same parents, while the genotypes Giza181 and Giza182 are indica types with long grains and the same pedigree. On the other hand, Sakha104 and P1044-86-5-3-3-2M belonged to the true Japonica type (Fig. 2). The second sub-group was divided into two sub-groups. The first one included rice genotypes ITA257 and ITA301, which were produced from the same parents and similar in number of panicles per plant, number of grains per panicle, number of unfilled grains per panicle, grain yield per plant, and milling percentage. While the second one included rice genotypes, P1044-86-5-3-1-2M, P1044-86-5-3-3-2M, and P1035-5-6-1-1-2M were closest to those coming from offspring; also, these genotypes were smellier in duration, plant height, number of grains per panicle, number of unfilled grains per panicle, grain yield per plant, and milling percentage. On the

Table 3
Mean square estimates for agronomic traits under study.

S.O.V.	D.f.	DH	PH	FLA	NPP	NPT	PW
Replications	2	0.72	0.69	0.22	0.16	0.18	0.002
Genotypes	21	88.50**	32.47**	28.75**	23.07**	22.73**	1.275**
Error	42	4.15	4.16	1.07	1.02	1.01	0.015
S.O.V.	D.f.	PL	X100GW	NPG	NU.GP	GYP	MI
Replication	2	0.19	0.003	0.73	0.17	0.20	0.34
Genotypes	21	7.35**	0.276**	467.52**	30.51**	79.43**	82.89**
Error	42	1.04	0.014	4.16	1.03	1.02	1.21

*, ** significant at 0.05 and 0.01 levels, respectively.DH = Days to heading, PH = Plant height (cm), FLA = Flag leaf area (cm^2), NPP = tillers $plant^{-1}$, NPT = panicles $plant^{-1}$t, PW = Panicle weight (g), PL = panicle length, X100GW = 1000-grain weight (g), NPG = Filled grains $panicle^{-1}$, NU.GP = Spikelet fertility (%), GYP = Grain yield $plant^{-1}$ (g) and MI = Milling%.

Table 4
Mean performance for 22 rice genotypes (average of two years 2018 and 2019).

Genotypes	DH	PH	FLA	NPP	NPT	PW	PL	X100GW	NPG	NU.GP	GYP	MI
IR70423-169-2-2	135	107	28.63	27	25	3.70	24	3.50	167.00	16.0	37.16	67.37
IR67418-131-2-3-3-3	118	102	24.89	26	23	3.90	26	3.70	147.67	14.0	44.57	68.10
IR67420-206-3-1-3-3	115	104	32.98	25	24	3.17	20	3.40	147.67	12.6	37.93	70.17
Sakha103	95	101	25.79	23	22	3.63	21	3.40	141.40	17.6	34.09	72.07
Giza177	95	95	26.20	22	20	3.93	20	3.70	138.67	16.6	37.14	74.07
Giza178	105	99	31.90	29	27	3.13	20	3.00	133.50	12.0	34.44	69.23
Giza175	113	100	31.87	24	22	3.47	24	3.10	122.60	11.3	33.50	65.80
Sakha106	102	98	27.90	28	26	3.70	25	4.22	157.05	10.3	39.40	69.77
IET360	110	100	30.09	23	20	4.00	25	3.20	127.40	8.0	35.80	64.20
ITA257	122	94	29.17	22	19	3.63	23	3.10	119.00	18.6	30.50	65.80
P1035-5-6-1-1-1M	118	96	29.13	20	18	3.90	24	3.00	127.90	15.0	32.70	69.67
P1044-86-5-3-1-2M	120	95	29.56	24	22	5.10	25	3.20	126.30	14.6	31.60	66.10
Giza171	130	117	28.08	23	20	4.70	23	3.00	133.20	15.3	34.00	65.80
Sakha104	112	105	27.03	27	24	4.23	22	3.60	148.50	19.3	36.57	68.77
IAC165	107	100	33.89	24	23	4.20	22	3.10	128.30	15.0	33.30	65.17
P1044-86-5-3-3-2M	113	99	26.39	28	26	3.17	22	3.40	144.10	15.0	30.00	67.97
Giza182	100	98	26.63	29	25	5.47	25	3.50	152.33	11.0	44.20	70.60
Giza181	109	96	28.03	27	26	4.90	21	3.30	139.80	10.3	40.70	66.80
lAC164	108	105	29.17	23	22	3.90	23	3.10	127.10	12.3	33.00	69.37
ITA301	120	99	25.87	24	20	3.50	21	3.00	122.00	19.0	32.90	65.40
Giza172	128	112	27.71	20	18	3.37	21	3.20	134.60	9.3	35.10	64.67
P1044-86-5-3-3-2M	123	95	28.88	21	20	3.60	24	2.90	130.40	14.0	30.20	66.13
LSD at 0.05at 0.01	2.814.08	2.583.74	1.442.08	1.412.04	1.381.99	0.160.24	1.341.94	0.160.23	2.814.08	1.632.36	1.842.66	1.031.88

DH = Days to heading, PH = Plant height (cm), FLA = Flag leaf area (cm^2), NPP = tillers plant^{-1}, NPT = panicles plant^{-1}t, PW = Panicle weight (g), PL = panicle length, X100GW = 1000-grain weight (g), NPG = Filled grains panicle^{-1}, NU.GP = Spikelet fertility (%), GYP = Grain yield plant^{-1} (g) and MI = Milling%.

Table 5
Grand mean, variance, genotypic (GCV) and phenotypic (PCV) coefficients of variability, broad sense heritability (h2B), genetic advance and variability parameters for agronomic traits under study.

Characters	Mean	Vph	VG	VE	PCV	GCV	h^2B	SD	SE (m)	CV (%)
DH	129.5	29.50	28.11	1.39	4.19	4.09	91.03	5.43	1.16	4.19
PH	98.82	10.82	9.44	1.39	3.33	3.11	77.30	3.29	0.70	3.33
FLA	28.62	8.94	8.10	0.44	12.33	12.02	89.42	2.4	0.51	8.38
NPP	24.50	7.69	7.34	0.35	11.32	11.06	91.38	2.77	0.59	11.32
NPT	22.36	7.58	7.23	0.35	12.31	12.02	91.26	2.75	0.59	12.30
PW	3.92	0.42	0.42	0.00	16.64	16.55	97.85	0.63	0.13	15.92
PL	21.32	2.45	2.10	0.35	7.34	6.80	75.22	1.88	0.40	8.23
X100GW	3.29	0.09	0.09	0.00	9.20	8.97	90.44	0.31	0.07	9.09
NPG	137.11	155.84	154.45	1.39	9.10	9.06	98.24	12.48	2.66	9.10
NU.GP	13.96	10.17	9.82	0.35	22.85	22.45	93.41	3.19	0.68	22.85
GYP	36.76	26.48	26.13	0.35	14.00	13.90	97.42	5.14	1.09	14.00
MI	69.23	11.40	4.70	0.30	12.45	9.57	88.56	1.96	0.42	2.81

DH = Days to heading, PH = Plant height (cm), FLA = Flag leaf area (cm^2), NPP = tillers plant^{-1}, NPT = panicles plant^{-1}t, PW = Panicle weight (g), PL = panicle length, X100GW = 1000-grain weight (g), NPG = Filled grains panicle^{-1}, NU.GP = Spikelet fertility (%), GYP = Grain yield plant^{-1} (g) and MI = Milling%.

other hand, the varieties IAC164 and IAC165 were closely related due to the same pedigree. The last sub-subgroups included six rice genotypes: Sakha103 and Giza177, which were in the same branches. These genotypes belonged to Japonica types in terms of short duration, flag leaf area, number of grains per panicle, number of unfilled grains per panicle, panicle length, panicle weight, 1000-grain weight, and grain yield per plant. Also, Giza177 was used as a parent to Sakha103. On the other hand, the genotypes Giza178 and Giza175 were in the branched category due to the fact that these genotypes belonged to Indica-Japonica in terms of duration, flag leaf area, plant height, 1000-grain weight, grain yield per plant, and milling percentage (Fig. 2). Finally, the varieties Giza171 and Giza172 were produced from the common parent (Nahda variety), while IR70423-169 was in branch alone because this variety belonged to the Indica type and long duration one.

3.5. Molecular markers

3.5.1. SSR polymorphism and allele scoring under study

Twenty SSR markers were utilized in this study and produced 87 alleles, with a mean of 4.3 alleles per locus, which were detected in the 22 rice genotypes. All twenty primers showed polymorphism between the 22 rice genotypes (Table 6). A higher number of alleles were found with the primers RM262, RM244, RM3843, RM212, and RM3330. PCR-amplified products for 20 SSR with 22 rice genotypes are shown in Fig. 3. The size standard is elucidated in these figures, and it proved to be useful in determining the molecular weights of all amplified PCR products across all genotypes. Every SSR had a characteristic fragment pattern that was easily distinguishable, and the molecular weight was determined using the most intense fragment. When an amplification product for a certain genotype-X marker pair could not be discovered in repeated trials, a variety was assigned a null allele for that SSR locus. All of the loci evaluated showed null alleles; five out of the 20 loci evaluated showed null alleles for single variety; four loci showed null alleles for two genotypes; and the 20 loci showed null alleles for seven genotypes. Additionally, all of the loci under investigation showed multiple alleles per sample. Observations of heterogeneity for every genotype indicated that the mutation rate of these microsatellites in rice is high. The numerous alleles found in this data probably properly reflect the level of heterogeneity present in seed stocks of these genotypes, as all genotypes were determined using a bulk DNA sample for processing.

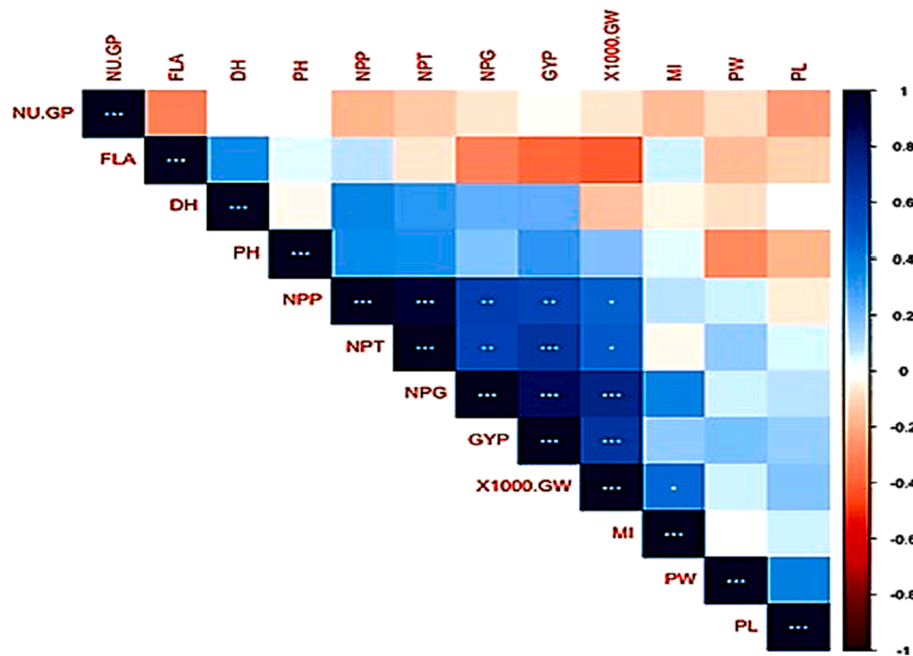

Fig. 1. Pearson's Correlation Coefficients among twelve quantitative traits across twenty-two genotypes. Asterisks indicate significant correlations using a two-tailed *t*-test (**, P < 0.05; ***, P < 0.01).

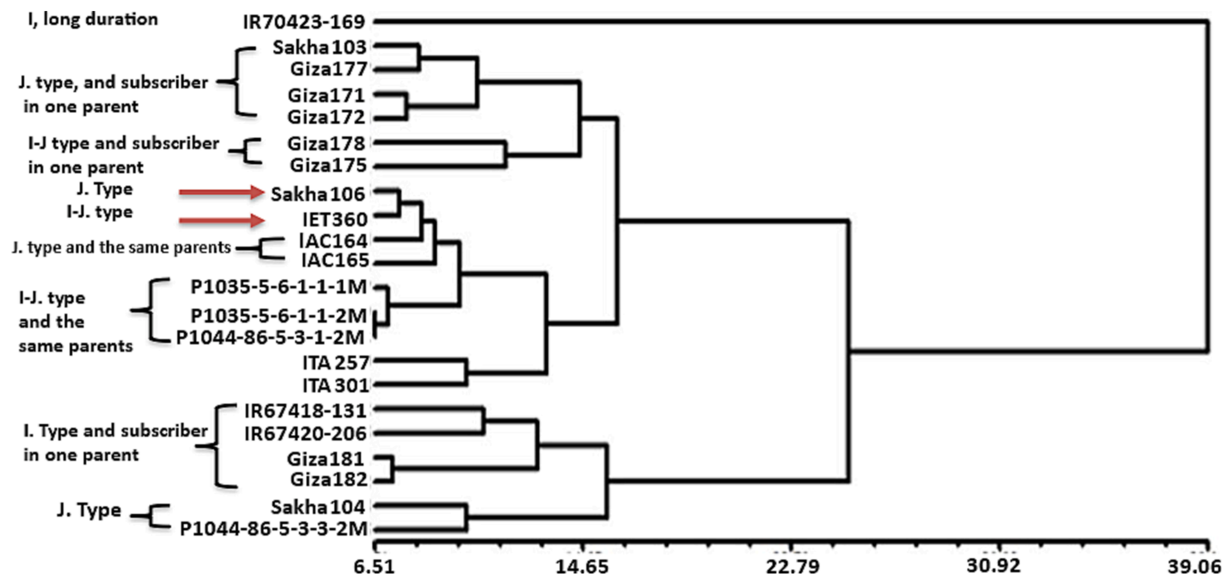

Fig. 2. Dendrogram based on average taxonomic distance for twelve quantitative traits among 22 rice varieties.

3.5.2. *Polymorphic information content (PIC)*

Because it represents allele variety and frequency among genotypes, the polymorphic information content, or PIC, was employed for each locus to assess the information of each marker and its potential for discrimination. In this study, allele counts and PIC values for each of the 20 SSR markers were calculated in order to examine the polymorphism levels among the various genotypes (Table 6).

3.6. *Genetic structure analysis*

The cluster dendogram was analyzed with the software NTSYS, version 2.2. (Fig. 4). The rice genotypes under study were grouped in agreement with the parentage presented in Tables 1 and 7. The results showed that the dendogram divided these genotypes into six groups. The first group included ITA257, Sakha103, Giza177, Giza171, and Giza172. These rice genotypes belonged to the Japonica type, except ITA257, which belonged to the Indica-Japonica. This is due to the fact that the genetic background of these genotypes came from close parents, and there are some parents (Nahda variety) who were common in the production of Giza171 and Giza172. In any case, Giza171 was used as a parent to produce Giza177, which was used to produce Sakha103 (Fig. 4). The second group included Giza178 and Giza175; these genotypes are from Indica-Japonica, and the common parent was the Milyange49 variety, which was crossing and producing Giza178 and Giza175. Owing to their dual parentage (Dourado Precoce/lAC 1246), ICA164 and ICA165, belonging to the japonica type, were included in the third group. On the other hand, the fourth group included Sakha104 and P1044-86-5-3-3-2M, which were true Japonica types. The fifth group included the remaining promising lines, namely: P1035-5-6-1-1M, P1035-5-6-1-1-2M, Giza171, LAC164, ITA301, Giza172, P1044-

Table 6
SSR markers, number of alleles, allele size, expected heterozygosity (HE) and polymorphism information content (PIC).

No.	SSR locus	No. of alleles	Allele size (bp)		HE (%)	Polymorphic information content PIC
			Min	Max		
1	RM247	5	95	1322	0.480	0.652
2	RM217	3	121	152	0.204	0.554
3	RM21	5	135	191	0.423	0.679
4	RM1216	4	85	387	0.330	0.695
5	RM241	3	130	142	0.295	0.554
6	RM44	4	114	1004	0.370	0.715
7	M254	2	288	331	0.182	0.397
8	RM3330	6	148	1398	0.582	0.794
9	RM238B	4	167	250	0.310	0.426
10	RM212	7	244	1370	0.363	0.826
11	M229	3	116	228	0.198	0.657
12	RM262	8	416	860	0.524	0.769
13	RM244	8	98	1054	0.408	0.837
14	M225	3	265	286	0.222	0.505
15	RM219	3	178	260	0.218	0.508
16	RM3	2	135	155	0.243	0.423
17	RM209	3	135	151	0.250	0.663
18	M224	3	253	309	0.260	0.602
19	M206	3	147	206	0.228	0.790
20	RM3843	8	143	1155	0.348	0.745
Mean		**4.3**	**170.6**	**560.5**	**0.322**	**0.640**

86-5-3-1-2M, and LAC156 (Table 7). However, all these genotypes were Japonica types except P1035-5-6-1-1-1M, which is from Indica-Japonica. The six-group included Giza182 and Giza181; these genotypes belonged to the Indica type, and the variety Giza 181 was registered before and used as a parent to produce the Giza182 variety (Fig. 4).

4. Discussion

One of the most crucial elements of any successful breeding strategy, particularly for self-pollinating rice, is the study of genetic variation in rice breeding. Additionally, by responding to different situations and analyzing genetic diversity, breeders can enhance and generate new, high-yielding genotypes by choosing optimal materials for further genotype improvement and effective management of rice genetic resources. This indicates the presence of wide genetic variability among genotypes for all traits, and that could be helpful in searching for desirable traits for breeding programs. These results were similar to those of El-Malky et al. (2013) and Faysal et al. (2022).

In the present study, 22 rice genotypes have shown significant diversity in both quantitative and qualitative traits. Comparable outcomes were discovered by El-Malky et al., (2013), Gaballah et al. (2022), and AL-Khayri and EL-Malky (2023). Each genotype means a square for every characteristic, which is extremely significant, meaning that selecting every trait from these genotypes will effectively improve every genotype attribute. Comparable outcomes were discovered by Abdourasmane et al. (2016) and Esther et al. (2021). In all genotypes for all attributes, the phenotypic coefficient of variability (PCV%) was greater than the genotypic coefficient of variability (GCV%), suggesting that environmental factors and cultural practices accounted for the bulk of PCV%. These results are in agreement with those of Faysal et al. (2022) and Gaballah et al. (2022).

All characters studied in various years showed high estimates of heritability, indicating the presence of both additive and non-additional genetic variance in inheritance of most traits, with the exception of panicle weight, which varied between 64.0 % and 74 %. These characteristics, however, are more resilient to shifting environments and cultural norms. Thus, it is possible to draw the conclusion that the selection processes it uses are effective in enhancing the majority of the

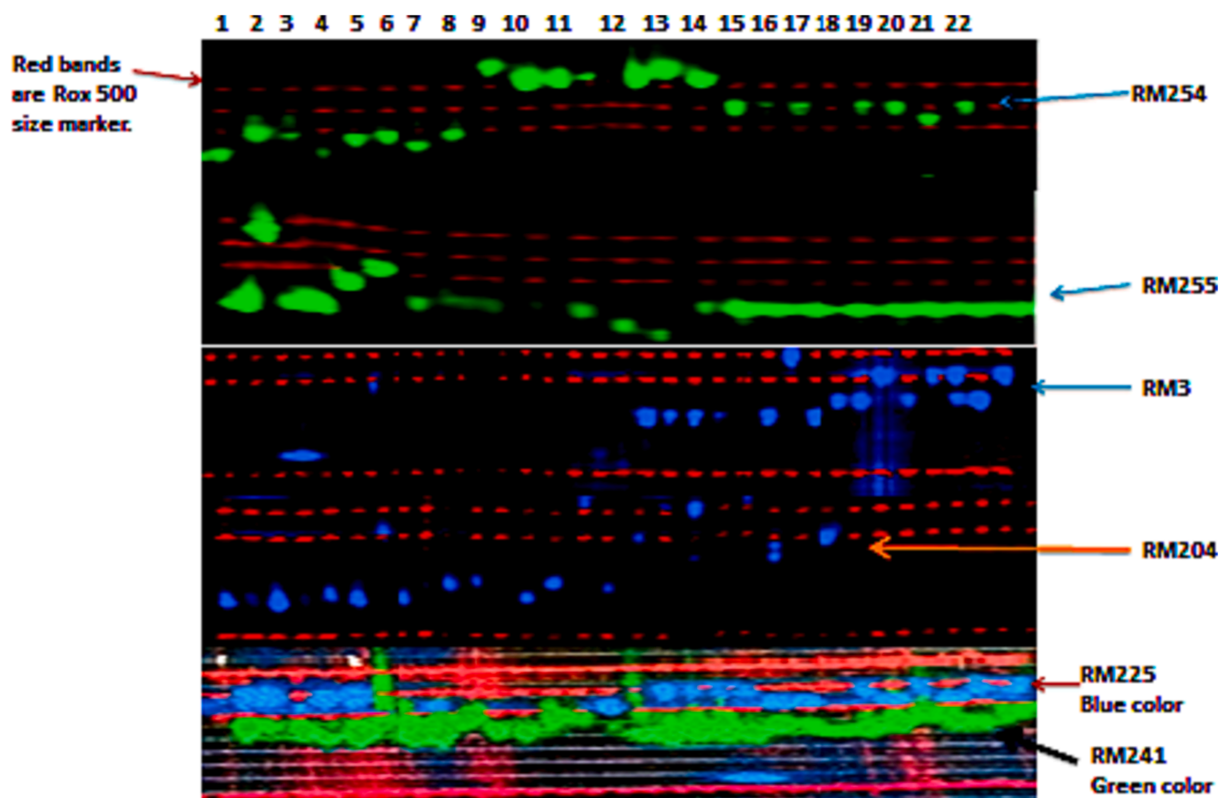

Fig. 3. Microsatellite gel image depicting the reaction product from PCR amplification of genomic DNA from the 22 rice varieties with eight SSR primers (blue); RM3, RM217, RM21, RM50, RM204, RM206, RM209 and RM225. Red bands are Rox 500 size marker. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

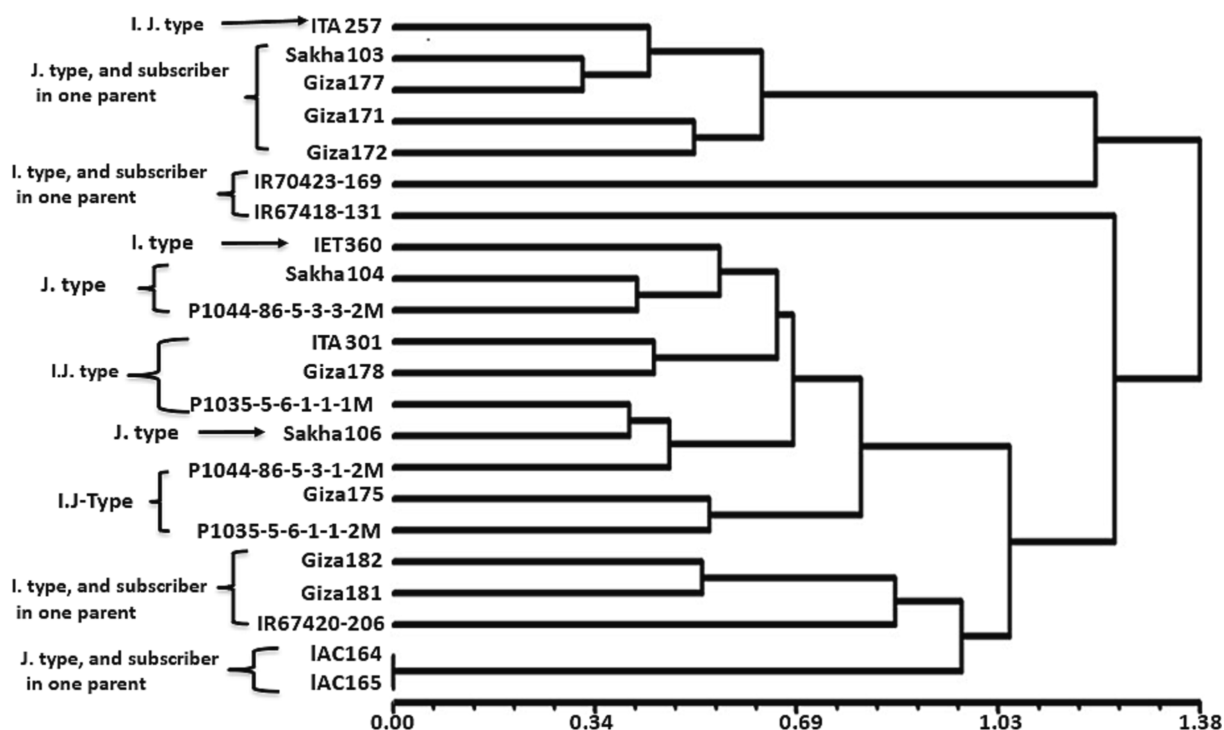

Fig. 4. Cluster analysis of 22 accessions of rice genotypes based on SSR markers polymorphism (Nei and Li, 1979) similarity coefficient.

Table 7
Composition of clusters based on average taxonomic distance for 20 SSR markers in 22 rice genotypes.

Cluster	Number of genotypes	Composition of cluster	Type
I	6	IAT257, Sakha103, Giza177, Giza171, Giza172 and IR67420-206-3-1-3-3, which belonged to Japonic types except IR67420-206-3-1-3-3 belonged to Indica, while IAT257 from Indica-Japonica type.	Japonica, Indica-Japonica and Indica
II	4	IAT301, Giza178, ITA257 and P1044-86-5-3-3-2M	Indica-Japonica
III	2	ICA164 and ICA165	Japonica
IV	5	IR70423-169-2-2, IR67420-206-3-1-3-3, Giza182, Giza181 and IR67420-206-3-1-3-3,	Indica
V	3	P1035-5-6-1-1-1M, Giza175 and P1044-86-5-3-1-2M	
VII	2	Sakha106 and IET360	Japonica and Indica
Total	22		

attributes being evaluated. Some results were previously obtained by Esther et al. (2021) and Faysal et al. (2022). Estimates of heritability showed inconsistency in magnitude under this study; panicle length (cm), plant height, and flag leaf area (cm^2) indicated that they were more sensitive to environmental conditions and cultural practices. Also, the selection procedure was most effective for improving these traits. Thus, it is possible to draw the conclusion that the selection process, which has been shown to be successful in changing gene frequency in situations involving both additive and non-additional genetic variation, would also be successful in enhancing the traits that are being studied. Comparable outcomes were attained by Sabri et al. (2020) and Gaballah et al. (2022). In addition, panicle length was highly significant and positively associated with panicle weight (PW). These results were similar to those of El-Malky and Al-Daej (2023). In contrast, plant height was highly significant and negatively associated with 1000-grain weight and milling (%) (Fig. 1). The characteristics used for this analysis were the same agronomic and quantitative characteristics. Every trait's normality was examined, and the results showed that every trait had a good approximation to a normal distribution (El-Malky et al., 2013). In addition, the Milyang49 variety was used as a parent to produce Giza178 and Giza175. These results agreed well with those of earlier researchers (Salah et al., 2020).

In order to develop genotypes that are tolerant of adverse conditions and high in yield, it is necessary to select parental breeds that have high yielding traits and have acceptable morphological traits (Jumaili et al., 2018; Salah et al., 2020). Hence, the present research has attempted to assess the genetic diversity of different rice genotypes on the basis of different quantitative traits. Molecular marker technology was used in breeding programmes to increase its efficiency, speed the transfer of desirable genes among genotypes, and introgress novel genes from related wild species. Molecular markers would make it simple to tag polygenic markers—which were previously challenging to analyze using conventional breeding procedures. The foundation for tagging the required resistance gene, fine mapping the gene in the rice chromosome, and ensuing marker-assisted selection (MAS) programmes is the screening of markers for parental variation among the rice cultivars.

Twenty SSR markers were used in this study and produced 87 alleles, with mean of 4.3 alleles per locus, which were detected in the 22 rice genotypes. All twenty primers showed polymorphism between the 22 rice genotypes (Table 6). A higher number of alleles were found with the primers RM262, RM244, RM3843, RM212, and RM3330 (Singh et al., 2015; Rashmi et al., 2017; Jumaili et al., 2018). PCR-amplified products for 20 SSR with 22 rice genotypes are shown in Fig. 3. The sizes of 20 SSR primers were predicted from sequencing the clone ustilized to isolate SSR (Vu et al., 2016; Melaku et al., 2018). Since rice is an inbred crop by nature, multiple alleles in a variety indicate heterogeneity—mixed pure lines or seed mixtures—rather than genetic heterozygozity (Jumaili et al., 2018). An excellent test of the microsatellites analysis was the germplasm collection examined in this study. Even when compared to earlier estimates for rice microsatellite (Melaku et al., 2018), PIC values were high, indicating the genotypes that were chosen had a varied range. The allele size ranges described here are probably

high enough for Egyptian rice to be cultivated, given the cross-section of germplasm these genotypes represent (Muhamad et al., 2017; Jumaili et al., 2018). All SSR markers had high PIC values, which show genotype-to-genotype allele diversity and frequency. Low (0.397) for M254 and high (0.837) for RM244 were among the lowest values.. Similar results were found with Hossain et al., (2012), Vu et al., (2016), and Melaku et al. (2018).

Finally, the efficiency of microsatellite analysis was raised in this work to a level that was equivalent to multi-locus fingerprinting methods by the use of automated fluorescence detection. According to this finding, fluorescently tagged microsatellite markers were useful instruments for assessing genetic diversity (Jumaili et al., 2018). This method should significantly improve genetic diversity assessment, variety fingerprinting and identification, marker-assisted selection during breeding, and genetic physical mapping of genes and quantitative trait loci (Hossain et al., 2012; Vu et al., 2016; Melaku et al., 2018).

5. Conclusion

To investigate genetic diversity, 22 genotypes typical of different rice genotypes were used in the study, along with quantitative attributes and microsatellite markers. The results indicated that a wide range was observed between the genotypes under study. The 1000-grain weight and the number of grains per panicle showed a favorable correlation with the grain production per plant. Clustering through quantitative traits produced two large groups, and the pedigree playing in divided the clustering with Giza181 and Giza182, which were belonging to the Indica type and produced from the same parents, while clustering through SSR was more accurate and divided into six groups. Group I included six rice genotypes that belonged to Japonica, while group II included two rice genotypes from Indica-Japonica, and group III included two from promising lines. Group IV included two sister lines and the Japonica type. In any case, the polymorphism information content (PIC) values were high for RM244 (0.837) and low for M254 (0.397). Therefore, SSR is a good technique for studying the diversity between the genotypes and helping to do the genetic fingerprinting for each variety, which will help the breeder in the process of selecting parents and creating appropriate hybrids to achieve genetic improvement in crops, especially rice.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors extend their appreciation the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through project (INST080).

Authors' contributions

Adel A. Rezk performed the overall experiment and data analysis. Heba I. Mohamed and Hossam S. El-Beltagi: performed experiments to confirm the results and wrote the manuscript. Adel A. Rezk, Heba I. Mohamed and Hossam S. El-Beltagi: designed and managed whole experiments and finalized the manuscript. Adel A. Rezk and Hossam S. El-Beltagi got the fund.

Funding

This research funded by Deputyship for Research & Innovation of Education in Saudi Arabia, grant number (INST080).

References

Abdourasmane, K.K., Zongo, A., Kam, H., Sanni, A., Audebert, A., 2016. Genetic variability and correlation analysis of rice (Oryza sativa L.) inbred lines based on agro-morphological traits. African J. Agric. Res. 11 (35), 3340–3346.

Al-Daej, M.I., El-Malky, M.M., Sattar, M.N., Rezk, A.A., Naqqash, M.N., Al-Khayri, J.M., 2022. Rice (Oryza sativa L.) breeding among Hassawi landrace and Egyptian genotypes for stem borer (Chilo agamemnon Bles.) resistance and related quantitative traits. Phyton-Int. J. Exp. Bot. 91 (9), 1905–1922. https://doi.org/10.32604/phyton.2022.020629.

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

Al-Khayri, J.M., El-Malky, M.M., 2023. Genetic parameters and principal component analysis for quantitative traits in rice (Oryza sativa L.). SABRAO J. Breed. Genet. 55 (5), 1547–1560. http://doi.org/10.54910/sabrao2023.55.5.9.

Allard, R.W., 1999. Principles of Plant Breeding, second ed., New York, John Wiley and Sons, U.S.A.

Anderson, J.A., Churchill, J.A., Autrique, J.E., Tanksley, S.D., Sorrells, M.E., 1993. Optimizing parental selection for genetic linkage maps. Genome 36, 181–186. https://doi.org/10.1139/g93-024.

Ashry, N.A., Ghonaim, M.M., Mohamed, H.I., Mogazy, A.M., 2018. Physiological and molecular genetic studies on two elicitors for improving the tolerance of six Egyptian soybean cultivars to cotton leaf worm. Plant Physiol. Biochem. 130, 224–234. https://doi.org/10.1016/j.plaphy.2018.07.010.

Bandumula, N., 2018. Rice Production in Asia: Key to Global Food Security. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 88, 1323–1328. https://doi.org/10.1007/s40011-017-0867-7.

Dudley, J.W., Moll, R.H., 1969. Interpretation and use of estimates of heritability and genetic advance in plant breeding. Crop Sci. 9, 257–262. https://doi.org/10.2135/cropsci1969.0011183X000900030001x.

El-Beltagi, H.S., Mohamed, A.A., 2010. Changes in non-protein thiols, some antioxidant enzymes activity and ultrastructural alteration in radish plant (Raphanus sativus L.) grown under lead toxicity. Not. Bot. Horti Agrobotanici Cluj-Napoca 38, 76–85. https://doi.org/10.15835/nbha3834631.

Elkatry, H.O., El-Beltagi, H.S., Ahmed, A.R., Mohamed, H.I., Al-Otaibi, H.H., Ramadan, K.M.A., Mahmoud, M.A.A., 2023. The potential use of Indian rice flour or husk in fortification of pan bread: assessing bread's quality using sensory, physicochemical, and chemometric methods. Front. Nutr. 10, 1240527. https://doi.org/10.3389/fnut.2023.1240527.

El-Malky, M.M., 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.

El-Malky, M.M., El-Habashy, M.M., Hammoud, S.A.A., Sreif, M.R., 2013. Genetic studies of some rice genotypes for rice stem borer (Chilo agamemnon Bles.) and agronomic characters under Egyptian condition. Egypt. J. Plant Breed. 17 (2), 196–212.

Esther, F.D., Adjei, R.R., Boadu, S.E., Oduro-Owusu, A.S., Durowaa, A., 2021. Genetic variability, heritability and path analyses of yield and yield related traits of newly developed rice (Oryza sativa L.) genotypes in lowland ecology in Ghana. J. Innovat. Agric. 8 (1), 16–20. https://doi.org/10.37446/jinagri/rsa/8.1.2021.16-20.

Fahad, S., Adnan, M., Noor, M., Arif, M., Alam, M., Khan, I.A., Ullah, H., Wahid, F., Mian, I.A., Jamal, Y., Basir, A., 2019. Major constraints for global rice production. Adv. Rice Res. Abiotic Stress Tolerance. https://doi.org/10.1016/B978-0-12-814332-2.00001-0.

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.

Gaballah, M.M., Attia, K.A., Ghoneim, A.M., Khan, N., El-Ezz, A.F., Yang, B., Xiao, L., Ibrahim, E.I., Al-Doss, A.A., 2022. Assessment of genetic parameters and gene action associated with heterosis for enhancing yield characters in novel hybrid rice parental lines. Plants 11 (3), 1–18. https://doi.org/10.3390/plants11030266.

Ghonaim, M.M., Mohamed, H.I., Omran, A.A.A., 2021. Evaluation of wheat salt stress tolerance using physiological parameters and retrotransposon-based markers. Genet. Resour. Crop Evol. 68, 227–242. https://doi.org/10.1007/s10722-020-00981-w.

Ghonaim, M.M., Attya, A.M., Aly, H.G., Mohamed, H.I., Omran, A.A.A., 2023. Agro-morphological, biochemical, and molecular markers of barley genotypes grown under salinity stress conditions. BMC Plant Biol. 23, 526. https://doi.org/10.1186/s12870-023-04550-y.

Guo, L., Gao, Z., Qian, Q., 2014. Application of resequencing to rice genomics, functional genomics and evolutionary analysis. Rice 7 (1), 1–10. https://doi.org/10.1186/s12284-014-0004-7.

Hossain, M.M., Islam, M.M., Hossain, H., Ali, M.S., Teixeira da Silva, J.A., Komamine, A., Prodhan, S.H., 2012. Genetic diversity analysis of aromatic landraces of rice (Oryza sativa L.) by microsatellite markers. Genes Genomes. Genomics 6 (11), 42–47.

IRRI, 2014. Standard evaluation system for rice. International Rice Research Institute, Los Banos, Manila, Philippines, p. 56.

Jumaili, 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. Res. Inter. 2018 https://doi.org/10.1155/2018/7658032.

Krupa, K.N., Shashidhar, H.E., Dalawai, N., Reddy, M., Vijaykumara Swamy, H.V., 2017. Molecular Marker Based Genetic Diversity Analysis in Rice Genotypes (Oryza sativa L.) using SSR Markers. Int. J. Pure App. Biosci. 5 (2), 668–674.

Le Clerg, E.L., Leonard, W.H., Clark, A.G., 1962. Field Plot Technique. Burgress Pub. Co., Minnesota, U.S.A.

Mehmood, S., Ud Din, I., Ullah, I., Mohamed, H.I., Basit, A., Khan, M.N., Shah, S.S.H., Rahman, A., 2021. Agro-morphological and genetic diversity studies in Rice (Oryza sativa L.) germplasm using microsatellite markers. Mol. Biol. Rep. 48, 7179–7192. https://doi.org/10.1007/s11033-021-06710-5.

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.

Mohamed, H.I., Abdel-Hamid, A.M.E., 2013. Molecular and biochemical studies for heat tolerance on four cotton genotypes (Gossypium hirsutum L.). Romanian. Biotechnol. Lett. 18 (6), 7223–7231.

Mohamed, H.I., Akladious, S.A., Ashry, N.A., 2018. Evaluation of water stress tolerance of soybean using physiological parameters and retrotransposon-based markers. Gesunde Pflanz. 70, 205–215. https://doi.org/10.1007/s10343-018-0432-1.

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.

Mohamed, A.A., El-Beltagi, H.S., Rashed, M.M., 2009. Cadmium stress induced change in some hydrolytic enzymes, free radical formation and ultrastructural disorders in radish plant. Electron. J. Environ. Agric. Food Chem. 8, 969–983.

Muhamad, K., Ebana, K., Fukuoka, S., Okuno, K., 2017. Genetic relationships among improved genotypes of rice (Oryza sativa L.) in Indonesia over the last 60 years as revealed by morphological traits and DNA markers. Genet. Resour. Crop Evol. 64, 701–715. https://doi.org/10.1007/s10722-016-0392-1.

Naomi, K.F., Ziska, L.H., 2019. Rice: Importance for Global Nutrition. J. Nutri. Sci. Vitaminol. 65, S2–S3.

Nei, M., Li, W.H., 1979. Mathematical model for studing genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76, 5269–5273. https://doi.org/10.1073/pnas.76.10.5269.

Rahman, M.S., Molla, R.M., Alam, S.M., Rahman, L., 2009. DNA fingerprinting of rice (Oryza sativa L.) genotypes using microsatellite markers. Australian J. Crop Sci. 3, 122–128.

Rashmi, D., Bisen, P., Saha, S.K., Loitongbam, B., Singh, S., 2017. Genetic Diversity Analysis in Rice (Oryza sativa L.) Accessions using SSR Markers. Inter. J. Agric. Environ. Biotechnol. 10 (4), 1–11. https://doi.org/10.5958/2230-732X.2017.00057.2.

Redona, E.D., Mackill, D.J., 1998. Quantitative trait locus analysis for rice and grain characteristics. Theor. Appl. Genet. 96, 957–963.

Rezk, A.A., El-Malky, M.M., El-Beltagi, H.S., Al-daej, M., Attia, K.A., 2023. Conventional breeding and molecular markers for blast disease resistance inrice (Oryza sativa L.). Phyton-Int. J. Exp. Bot. 92 (3), 725–746. https://doi.org/10.32604/phyton.2023.024645.

Rohlf , F.J., 2000. NTSYS-pc: numerical taxonomy and multi- variate analysis system, version 2.2. Exeter Software, Setauket.

Sabri, R.S., Rafii, M.Y., Ismail, M.R., Yusuff, O., Chukwu, S.C., Hasan, N.A., 2020. Assessment of agro-morphologic performance, genetic parameters and clustering pattern of newly developed blast resistant rice lines tested in four environments. Agronomy 10 (8), 109. https://doi.org/10.3390/agronomy10081098.

Salah, M.G., Ehab, M.M., Mohamed, M.E.M., 2020. Combining ability and heterosis studies for some economic traits in rice (Oryza sativa L.). Res. J. Biotechnol. 15 (1), 101–111.

Shallan, M.A., El-Beltagi, H.S., Mona, A.M., Amera, T.M., 2010a. Chemical evaluation of pre-germinated brown rice and whole grain rice bread. Electron J. Environ. Agric. Food Chem. 9 (5), 958–971.

Shallan, M.A., El-Beltagi, H.S., Mona, A.M., Amera, T.M., Sohir, N.A., 2010b. Effect of amylose content and pre-germinated brown rice on serum blood glucose and lipids in experimental animal. Aust. J. Basic Appl. Sci. 4, 114–121.

Shamim, M.Z., Sharma, V.K., 2014. Study of genetic diversity among different rice genotypes using quantitative characters. Oryza 51 (1), 86–89.

Singh, A., Saini, R., Singh, J., Arya, M., Ram, M., Singh, P.K., 2015. Genetic diversity studies in rice (Oryza Sativa L.) using microsatellite markers. Inter. J. Agric. Environ. Biotechnol. 8, 143–152. https://doi.org/10.5958/2230-732X.2015.00019.

Sneath, P.H.A., Sokal, R.R., 1973. The principle and Practices of Numerical Classification In: Numerical Taxonomy. Freeman San Francisco: 573.

Vu, H.T.T., Nguyen, H.T.T., Tran, K.D., Khuat, T.H., Nakamura, C., 2016. Genetic diversity of Vietnamese lowland rice germplasms as revealed by SSR markers in relation to seedling vigour under submergence. Biotech. Biotech. Equip. 30, 17–25.

Wricke, H., Weber, W.E., 1986. Quantitative genetics and selection in plant breeding. Walter de Gruyter and Co, Berlin.

Zhao, M., Lin, Y., Chen, H., 2020. Improving nutritional quality of rice for human health. Theor. Appl. Genet. 133, 1397–1413. https://doi.org/10.1007/s00122-019-03530-x.

Zhong, H., Liu, S., Zhao, G., Zhang, C., Peng, Z., Wang, Z., Yang, J., Li, Y., 2021. Genetic diversity relationship between grain quality and appearance in rice. Front. Plant Sci. 12, 708996 https://doi.org/10.3389/fpls.2021.708996.