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Full Length Article

Genetic Diversity and Grouping of New Rice Accessions in Morocco Based on Yield and Seed Quality

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Abstract

This study aimed to determine the genetic diversity among 33 rice accessions introduced into Morocco to develop new, improved varieties and enrich the Moroccan rice gene pool. The plant material was collected from the leaves 13 days after seed germination. The development of a genomic DNA extraction protocol and SSR characterization methods preceded the molecular analysis of the accessions. The microsatellite markers used are co-dominant, reproducible, and highly polymorphic. Our study highlighted significant genotypic diversity among the 33 rice accessions. Using five SSR loci enabled us to define different SSR profiles, and most of the genotypes determined are each present in a single zone. The five primers used to amplify DNA from each accession proved polymorphic. Seventy-five reproducible polymorphic bands were identified, which could be proposed as primers specific to the *Oryza sativa* species. Statistical analysis revealed broad genetic diversity. However, the PCA constructed from the statistical analysis of these markers showed a high degree of genetic diversity and a significant rearrangement of accessions. Upon analysis, the rice accessions were categorized into four distinct groups. The first group, comprised of seven accessions (KF20017, KF190018, KF20019, KF190061, KF18045, KF190063 and KF190052), demonstrated unique genetic characteristics. The second group, encompassing ten accessions (KaWS 9294292, KF18044, CB MS11, KF190051, KF190065, KF190064, KF190027, KF20006, KF190026 and KF190066), stood out due to the presence of two genetically close accessions, KF190066 and KF190026. Remarkably, this genetic closeness occurred despite geographical distance, possibly attributed to dissemination or genetic drift. The third group, consisting of nine accessions (KF190022, KF18046, KF20034, KF190112, KF20059, KF20058, KF20045, KF20035 and KF20018), showcased distinct genetic profiles. Finally, the fourth group, composed of seven accessions (KF190136, KF190006, KF20036, KF190114, KF20046, KF20005 and KF20013), exhibited unique genetic characteristics contributing to overall diversity. Thus, the inter-provenance polymorphism revealed in these analyses testifies to the discriminating power of SSR markers.

Keywords: Rice; Genetic diversity; PCR; SSR markers; Polymorphism; Morocco

Introduction

Rice (*Oryza sativa* L.), a crucial cereal crop renowned for its nutritional and agronomic benefits, falls within the Graminae family and Oryzoidea subfamily (Fazal *et al.* 2023). It stands out as one of the most essential food crops globally, catering to over half of the world's population (Qiao *et al.* 2021; Huang *et al.* 2022). However, with the global population anticipated to reach 9.7 billion by 2050 (United Nations Department Economic and Social Affairs 2022), particularly in Asia and Africa, the demand for rice is expected to rise significantly.

The rice sector holds significant socio-economic importance in Morocco, having demonstrated notable growth in recent years through initiatives under the Green Morocco Plan. These measures have effectively organized the sector, providing a stable income for 2,500 farmers and generating approximately 1.5 million working days annually, with 87% upstream and 13% downstream. Technologically, rice cultivation has experienced a dynamic surge, enabling domestic production to fulfill over 72% of the country's consumption needs (MAPM 2020). While concentrated in the Gharb and Loukkos regions, particularly larache, successful rice production in Morocco heavily

relies on efficient irrigation practices. Projections for the upcoming 2023–24 season indicate a stable harvested area of around 8,250 hectares, with an expected production of 45,000 metric tons, reflecting a notable increase of 7.1% from the previous year. This growth is primarily attributed to favorable weather conditions, as the United States Department of Agriculture reported in 2023.

The varietal profile used in the rice sector in Morocco is a little diversified and concerns both round rice varieties and medium and long rice varieties. All Moroccan rice farmers use selected seeds imported from abroad, despite the existence of 17 varieties of Moroccan origin selected at National Institute of Agronomic Research (INRA) between 1987 and 2001. The return to these Moroccan varieties and diversification through developing new national varieties is essential. It will help develop a diverse national genetic heritage to meet farmers' needs and make production costs profitable by reducing foreign seed imports and exchange outflows. However, several constraints weigh on the development of the sector and the objectives of the government's agricultural development strategy. The main constraints to the development of the sector are related to the high cost of inputs, dependence on selected seeds imported from abroad by rice farmers and certain seed marketing companies, and late planting by the majority of rice farmers, sometimes causing considerable damage to rice production due to autumn rains. In this case, the search for short-cycle varieties with high yields and good quality seeds and their blast resistance would be of paramount interest. Implementing a program to improve the quality and diversification of rice products from domestic production (development of national varieties) could help improve rice productivity in Morocco. This need for research and development is perfectly in line with the vision of the Green Generation strategy for the rice sector (2020-2030).

The primary components of the rice program developed within the new "Green Generation" strategy focused on establishing a production program and multiplying nationally certified rice seeds. Emphasis is placed on promoting the use of high-yield varieties, encouraging widespread early sowing, and implementing weed and disease control measures. Within this framework, the development of new national rice varieties aligning with these objectives is conducted by the National Institute of Agronomic Research (INRA) at the Sidi Allal Tazi Experimental Unit, affiliated with the Regional Centre for Agricultural Research in Kenitra. This initiative operates under the KAFACI project (Korea-Africa Food and Agriculture Cooperation Initiative) since 2017. In Morocco, like other regions, continuous efforts are directed toward improving rice production by introducing novel accessions featuring enhanced traits. The significance of genetic diversity within rice populations cannot be overstated, given its pivotal role in influencing crop adaptation, productivity, and overall agricultural sustainability. Recognizing the critical significance of assessing the genetic composition of recently acquired rice accessions, this study aimed to fulfill three primary objectives. The first objective involved an exploration of the genetic variability among these newly acquired rice accessions in Morocco. To achieve this, molecular markers and genetic profiling techniques were employed, providing insights into the extent of genetic diversity within the rice population. The second objective sought to contribute valuable insights to ongoing efforts in rice crop improvement programs. By comprehending the genetic makeup and performance of the new accessions, the study aimed to play a role in enhancing both the production and quality of rice in the region. Lastly, the research aimed to identify and characterize superior rice varieties, showcasing desirable traits such as high yield and optimal seed quality. These identified varieties could then be recommended for cultivation or integrated into further breeding programs in Morocco. In essence, the study addressed a multifaceted approach, combining genetic exploration, program improvement insights, and the identification of superior varieties to contribute comprehensively to the advancement of rice agriculture in Morocco.

Materials and Methods

Plant material

The plant material covered 33 accessions selected for the experimental field of Sidi Allal Tazi, located about 57 km from Kenitra, extending along the Atlantic coast between latitudes 34° and 34° 45' N. It covers a vast hydrogeographic region spanning 7,500 square kilometers. In this area, rice is grown on gray alluvial soils. These soils are mainly clayey, generally poor in humus phosphoric acid, and often poor in nitrogen. They have a slightly alkaline pH. The region's climate is Mediterranean, with significant water resources from the Sebou River, its tributaries, the Merjas, and groundwater. KAFACI (Korea-Africa Food and Agriculture Cooperation Initiative) lines were selected from 100 lines introduced by the Rice Center for Africa in Saint-Louis, Senegal, and tested in November 2021 with three INRA Morocco varieties. Selection is based on yield, components, early maturity, dwarf plant and overall performance. The plant material used in this experiment was seedlings from seed germination at the laboratory level (Table 1).

Plant material preparation

The first step is to prepare the seeds for germination. Each variety of rice is soaked in a water tube for a few hours and then dried with absorbent paper.

Germination of rice seeds

In order to accelerate the germination process of rice seeds from different adhesions, the seeds were soaked in a tube filled with water for a few h and then dried with absorbent paper.

Once the seeds are dried, they are deposited in sterilized petri dishes containing absorbent paper soaked with water (Fig. 1a). This operation was carried out under a laminar flow hose. The petries are thus stored in a culture chamber at 27°C. After three days, all the seeds have germinated (Fig. 1b) and are placed in pots filled with a mixture of soil and compost (Fig. 1c). Then, they are placed inside the greenhouse at a temperature of 25°C. After two weeks, we harvested the stems, cut the leaves with a scissor (Fig. 1d), and stored them in aluminum paper at -20°C (Fig. 1e).

DNA extraction

DNA extraction was performed using the Doyle method (Doyle and Doyle 1987). It is a conventional method commonly used in plants and is based on the precipitation of polysaccharides by CTAB and the elimination of proteins through extraction by chloroform isoamyl alcohol, which leads to obtaining relatively pure DNA. Note that this protocol is well adapted to the conditions of less equipped laboratories since the extraction of DNA was successful without liquid nitrogen, protein, or Arnase. Thus, 0.1 g of leaves are ground in a mortar with 1 mL of CTAB extraction buffer 2% (1 *M* Tris-HCI pH 8.0; 1.36 *M* NaCI; 0.5 *M* EDTA pH 8.0; 0.5 *M* PVP; and CTAB 2 g). The resulting shred is transferred to 2 mL microtubes and incubated in a 65°C water bath for 30 min, stirring every 10 min. The sample is washed with 700 μL of chloroform isoamyl alcohol (24:1) to remove cellulose and protein debris. After 10 min of centrifugation at 10,000 rpm, the overflowing agent is transferred back into a 1.5-mL microtube to resume the same washing operation. The overflowing agent is transferred into a 1.5-mL microtube to precipitate the DNA to which the same volume of chlorophorm and isopropanol is added. The solution is then centrifugated at 10,000 rpm for 10 min. The overflowing agent is transferred again by adding 400 μL of chlorophyll isoamyl alcohol. The solution is then incubated at -20°C for 12 h. After this step, centrifugation is carried out at 14,000 rpm for 15 min, after which the hose is recovered and washed by adding 500 mL of a solution composed of ammonium acetate and 76% ethanol. The microtubes are then centrifuged at 13,000 rpm for 2 min. After removing the overagent, the hose is removed with 700 μL of ethanol, followed by centrifugation at 13,000 rpm for 2 min. Finally, the recovered jacket is dried out in the open air for 40 min. The DNA solution for each rice variety was thus obtained by adding 100 μL of pure, sterile water and storing it at -20°C.

Assessment of DNA quality

The quality of the extracted DNA is assessed by horizontal electrophoresis. This technique separates nucleic acids by molecular weight using an electric field. Agarose gel electrophoresis separates DNA fragments from 100 base pairs (bp) up to 60,000 bp. negatively charged DNA migrates from the cathode to the anode, depending on its molecular weight.

Revelation is achieved by maintaining fluorescence under ultraviolet (UV) light (Mahuzier and Hamon 1989). The extracted DNA is in a super-resolution agarose gel at 0.8%. The gel was prepared using 0.8 g of agarose gel in buffer (TBE) for a 100-mL solution. A volume of 15 μL of each amplification product previously mixed with loading buffer (0.040% bromophenol, 7% glycerol and 6 m*M* EDTA) was deposited in the gel wells to enable migration at 120 V for 40 min. After migration at 120 V in TBE x1 buffer, the agarose gel was placed in a BET solution for 20 min.

SSR markers used in PCR

Thirteen (13) pairs of SSR primers were tested on the 33 accessions for reproducibility. The results showed that five of the thirteen primers had precise readings, demonstrating the genetic diversity between the 33 accessions in Table 2.

Microsatellite marker (SSR) amplification protocol

DNA amplification using the PCR technique in the presence of SSR markers was carried out according to the method of Caruso *et al.* (2010). For each DNA sample, PCR was performed in a total volume of 20 μL of reaction mixture (Table 3). Thus, a total of 165 PCR reactions (5 primer pairs x 33 accessions) were performed.

The polymerization program used includes a 6-min initial denaturation phase at 94°C, followed by 40 cycles with a denaturation of 94°C for 30 s, hybridization according to the temperature maintained for each priming torque for 45 s and elongation at 72°C for 1 min and 30 sec finally, a final stretch at 72°C for 10 min. The analysis of PCR amplification products was performed on an agarose gel at 1.8% for 2 h at 150 volts.

Statistical analysis

For each SSR, the observed bands were deciphered in dominant read mode, which assumes a band as a site for a given locus. Thus, the raw database corresponds to a contingency matrix that matches each accession with a code (similar to a barcode) as a combination of the presence and absence of bands (DNA fingerprints) shared between all the SSRs tested. The analysis of the genetic fingerprints was conducted by various software programs dealing with the polymorphism of molecular markers. A multivariate analysis in the form of PCA was performed by GENALEX v. 6.5, respectively (Yeh and Boyle 1997).

This analysis aims to identify the clusters of similarities between the different accessions and to see to what extent the spatial structuring obtained based on genetic data is related to the geographical origin of the names of the accessions tested.

The following parameters were calculated for each locus using FSTAT (version 2.9.3.2) (Goudet 2001) and

Genepop (version 4) (Rousset 2008):

•Allelic frequency distribution (*AFD*):

$$AFD_i = \frac{\text{Number of alleles of type } i}{\text{Total number of alles at the locus}}$$

Where, *AFDi* represents the allelic frequency of the *i*-th allele at a specific locus.

•Observed heterozygosity (Ho):

$$\text{H0} = \frac{\text{Number of heterozygous individuals at the locus}}{\text{Total number of individuals at the locus}}$$

•Expected heterozygosity in the population (Hs):

$$\text{Hs} = 1 - \text{n}_{i=1} P_i^2$$

Where *pi* is the frequency of the *i*-th allele at the locus.

•Expected total heterozygosity (Ht):

$$\text{Ht} = \frac{1}{\text{L}} \; L_{j=1} \; Hs_j$$

Where, L is the total number of loci and *Hsj* is the expected heterozygosity at the *j*-th locus.

•CuLtivar Differentiation Proportion (GST):

$$\text{GST} = \frac{\text{Ht} - \text{Hs}}{\text{Ht}}$$

GST is the cultivar Differentiation Proportion.

Ht is the expected total heterozygosity, measuring total genetic diversity in the population.

Hs is the expected heterozygosity at each locus, estimating the expected heterozygosity in the population according to allelic frequencies.

In addition, Genepop was used to test the pair-binding equilibria at all loci between two groups, thus allowing the calculation of the FST pair genetic differentiation statistic (Raymond and Rousset 1995), which measures genetic differentiation between populations or groups.

Philip 3.5c (Felsenstein 1995) was used to Determine the standard genetic distance of Nei (Ds): The standard genetic distance of Nei (Ds) (Nei 1975) was Calculated. This distance measurement quantifies genetic differences between populations or individuals based on their allelic frequency data.

Finally, a factorial correspondence analysis (FCA) was performed to illustrate the dissimilarity between cultivars as a function of their allelic variability. FCA is a multivariate technique used to visualize and analyze patterns of dissimilarity or similarity between entities based on their genetic data.

Results

Structure of SSR molecular polymorphism within the 33 accessions Genetic variation analysis is a significant factor in the development of rice lines, which can be obtained through DNA profiling techniques that display high loci quantity for high variability. Samples or accessions of collected rice cultivars were analyzed using SSR markers, essentially PCRs. Microsatellites are viral genetic markers because of their co-dominant heredity, high abundance, enormous extent of allelic diversity, and ease of evaluating PCR SSR size variation with flank primer pairs. The reproducibility of microsatellites is such that different research laboratories can effectively use them to produce consistent data. Examining the structure of SSR molecular polymorphism within the cohort of 33 accessions, we employed five pairs of SSR initials as genetic footprints, providing a nuanced understanding of the population's genetic makeup. This exploration is pivotal for simplifying research on marker-trait associations and streamlining the incorporation of diverse genotypes into breeding plans to produce superior varieties. The results yielded diverse profiles, each distinct from one SSR pair to another. The size of the detected fragments exhibited a range spanning from 156 to 247 pairs of bases (PB), as visually depicted in Fig. 2. Notably, the bands selected for polymorphism analysis fell within the pb interval previously identified in Helsen *et al.* (2007) and Finti *et al.* (2013) SSR marker works. The figures revealed a high efficiency in identifying intraspecific polymorphisms among the rice varieties and lines included in the study. Notably, two SSR markers emerged as particularly informative, showcasing their efficacy in discerning genetic variations within the studied set of microsatellite markers. Within the scope of our study, the 75 reproducible polymorphic bands represent a rich tapestry of genetic variations among the examined rice varieties and lines. These polymorphisms encompass diverse alterations in the DNA sequences of the microsatellite markers used, highlighting the inherent genetic diversity within the population. Elaborating on the specific traits or characteristics associated with these polymorphic bands and their relevance in breeding programs could provide valuable insights for readers seeking a more comprehensive understanding of the study's implications.

Analysis of polymorphism within and between 33 accessions

A comprehensive examination of all tested markers revealed ten discernible bands in the meticulous analysis of polymorphism within and between the 33 accessions under scrutiny. Intriguingly, the highest number of bands (10) was conspicuously observed in accessions A1 to A19, A21 to A24, A26 to A28 and A30 to A33. Notably, accessions A20 and A30 exhibited a slightly reduced band count of (8), while the lowest band count of (4) was identified in accession A25. Notably, the combinations delineating the distribution of the presence and absence of bands, as discerned through the analysis of five SSR markers, exhibited a distinctive pattern for accessions A20, A30 and A25 when compared to all other accessions (Table 4). The dissimilarity in-band distribution underscores unique genetic profiles for these specific accessions, emphasizing the

Table 1: List of 33 rice accessions used

N° accession	Serial	Entry n°	Pedigree
1	KF190136	2019WS /9230343	HR32057F1-4-4-1-1-5-1
2	KF190006	2019WS / 9210008	KR55-2-4
3	KF20005	2020WS/ 20210008	HR32058F1-1-10-1-4-1-1
4	KF190112	2019WS / 9230259	HR32054F1-2-17-1-1-3-3
5	KF20017	2020WS/ 20220007	KR55-2-1-1-2-1
6	KF20018	2020WS /20220008	KR55-2-3-4-1-1
7	KF190114	2019WS /9230265	HR32054F1-2-17-1-1-5-1-2
8	KF20036	2020WS/ 20270275	Ohselfed-1-4-2-3
9	KF20058	2020WS/ 20120139	HR32058F1-4-18-1-2-3-3
10	KF20045	2020WS/ 20120083	HR32051F1-3-11-1-5-4-2
11	KF20046	2020WS/ 20120007	HR32054F1-2-17-1-1-5-1-2
12	KF20006	2020WS/ 20210013	HR32058F1-4-18-1-3-1-1
13	KF20013	2020WS/ 20220029	HR32056F1-4-14-1-4-2-2
14	KF20034	2020WS/ 20270262	ARS1960-5-1-1-2
15	KF20059	2020WS/ 20120106	HR32058F1-4-18-1-5-1-2
16	KF190018	2019WS /9210033	SR35266-2-11-4-1-1
17	KF20019	2020WS/20220009	KR55-2-3-4-1-2
18	KF190061	2019WS /9220061	SR35266-HB3580-110
19	KF20035	20270273	Ohselfed-1-4-2-1
20	KF18045	2018WS / 8220057	SR35357F1-1-3-4-1
21	KF18046	2018WS / 8220058	SR35357F1-1-3-4-1
22	KaWS 9294292	2019ws	
23	KF18044	2018WS / 8220056	SR35357F1-1-3-4-1
24	CB MS11	2019ws	
25	KF190063	2019WS /9220065	SR35285-HB3573-48
26	KF190026	2019WS /9210046	ARS1974-5-2
27	KF190051	2019WS /9220038	SR34574-HB3565-285
28	KF190027	2019WS /9210047	ARS1974-5-3
29	KF190052	2019WS /9220039	SR34574-HB3565-290
30	KF190064	2019WS /9220066	SR35285-HB3573-72
31	KF190065	2019WS /9220067	SR35285-HB3573-75
32	KF190022	2019WS /9210037	SR34045-HB3487-11-1-1
33	KF190066	2019WS /9220069	SR34567-HB3573-113

Table 2: Characteristics of SSR breakfast pairs used

Code/SSR	SSR primer pair sequences (5'- 3'): forward and reverse	TH (°C)
SSR 1	F: ATC CAT GTC CGC CTT TAT GAGA GA R: CGC TAC CTC CTT CAC TTA CTA GT	59,5
SSR 2	F: GCG GGA GAG GGA TCT CCT R: GGC TAG GAG TTA ACC TCG CG	58,2
SSR 3	F: CTT ACA GAG AAC GGC ATC G R: GCT GGT TTG TTT CAG GTT CG	54,6
SSR 4	F: GGT AAA TGG ACA ATC CTA TGG R: GAC AAA TAT AAG GGC AGT GTG C	54,4
SSR 5	F: CAA GAA ACC TCA ATC CGA GC R: CTC CTC CCG ATC CCA ATC	54,5
SSR 6	F: CAA GAA ACC TCA ATC CGA GC R: CTC CTC CCG ATC CCA ATC	57,3
SSR 7	F: ACG CGA ACA AAT TAA CAG CC R: CTT TGC TAC CAG TAG ATC CAG	56,9
SSR 8	F: GTT GCT CTG CCT CAC TCT TG R: AAC GAG CCA ACG AAG CAG	56,8
SSR 9	F: AAA CGA GAA CCA ACC GAC AC R: GGA GGG AGG AAT GGG TAC AC	54
SSR 10	F: GCT CCA CAG AAA AGC AAA GC R: TGC AAC AGT AGC TGT AGC CG	58,5
SSR 11	F: GCA GAT CAA GTA TGC CTG CC R: TCG CTA GAT AGG GGA TGT GG	56,4
SSR 12	F: CCC TTC TTT TCA ACT GAA TA R: TTG TAA CAA TGA ACT CGT TC	48,5
SSR 13	F: AGC GAC GGA TGC ATG ATC R: TTG AGC CGG TAG TCT TG	56,5

significance of the detected polymorphism. This nuanced level of analysis delves into the intricacies of molecular markers, highlighting their potential to unravel the genetic intricacies of the plant material under investigation.

Table 3: Composition of the PCR reaction mixture

Composition	Vi/réaction	Concentration finale
H_2O Milli-Q sterile	8,92 μL	
Tampon 5x	4 μL	1x
dNTP 25 m*M*	0,2 μL	1 U
$MgCl_2$ 25 m*M*	0,4 μL	0,2 m*M*
Amorce Forward 100 μM	1,6 μL	2 m*M*
Amorce Reverse 100 μM	0,06 μL	0,3 μM
ADN 10 ng/μL	0,06 μL	0,3 μM
Taq polymerase (Promega) 5 U/μL	5 μL	2,5 ng/μL
Total	20 μL	

Table 4: Estimated genetic parameters for the 5 SSRs studied in 33 rice additions

SSR	number of alleles	Gst**	Fis**
SSR1	19	0.888	0.257
SSR2	31	0.959	0.328
SSR3	5	0.537	0.167
SSR4	6	0.588	0.285
SSR5	14	0.917	0.304
Moyenne	15	0,778	0,268

Fig. 1: The process of germination of rice seeds from different accessions: **a)**; after three days the seeds germinated: **b)**; growing rice plants in pots: **c)**; after 15 days, the stems are harvested and the leaves cut off with a chisel: **d)**; conservation of rice in aluminium paper: **e)**

However, it is imperative to acknowledge that, at this descriptive level, the power of molecular markers is considered neutral concerning environmental influences. This neutrality ensures that the observed polymorphism is attributed to inherent genetic factors rather than external environmental variables, providing a more robust understanding of the nature of the plant material studied.

Analysis of genetic parameters

For all markers tested, a total of 75 alleles were detected (Table 4). The genetic diversity parameters calculated for each locus (5 SSR primers) and their mean for all 33 accessions studied show an overall excess of heterozygosity, knowing that the SSR marker contributes the most to the structures of the most heterozygous genotypes, which is consistent with the high number of alleles detected (75). However, with 75 alleles, the SSR2 marker is less rich in heterozygous structures. Hence, it contributes most to genetic differentiation, with SSR2/GST = 0.95. In addition, the 5 SSR markers used contribute an average of 50% (average Gst x 100) to genetic divergence, which gives them a strong power of identification among the 33 accessions studied. This shows the low exchange of gene flows between

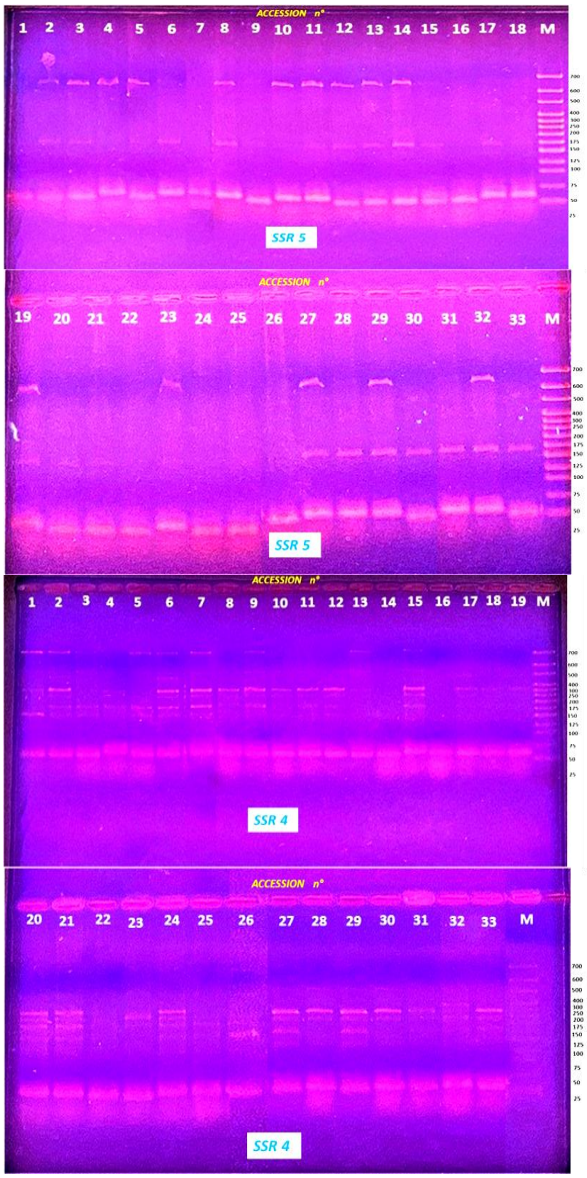

Fig. 2: SSR Molecular Profiles Developed on Agarose Gel (2% SFR). Each profile corresponds to a specific accession number, and the associated information includes the accession number and its corresponding identifier. N°1 (KF190136); N°2 (KF190006); N°3 (KF20005); N°4 (KF190112); N°5 (KF20017); N°6 (KF20018); N°7 (KF190114); N°8 (KF20036); N°9 (KF20058); N°10 (KF20045); N°11 (KF20046); N°12 (KF20006); N°13 (KF20013); N°14 (KF20034); N°15 (KF20059); N°16 (KF190018); N°17 (KF20019); N°18 (KF190061); N°19 (KF20035); N°20 (KF18045); N°21 (KF18046); N°22 (KaWS 9294292); N°23 (KF18044); N°24 (CB MS11); N°25 (KF190063); N°26 (KF190026); N°27 (KF190051); N°28 (KF190027); N°29 (KF190052); N°30 (KF190064); N°31 (KF190065); N°32 (KF190022); N°33 (KF190066)

accessions and reinforces the idea of isolation under anthropogenic effects. Indeed, accessions generally correspond to plantations multiplied by the same genotype or clone. The calculated differentiation coefficients (Fis) between accessions clearly illustrate the high level of genetic differentiation, varying between 0.1 and 0.3 (Table 4).

**Wright (1965-1988) defined the FST index (standardized variance) as the heterogeneity of allele frequencies between population subdivisions. It represents the correlation between alleles within a subpopulation versus all subpopulations. This parameter is used hierarchically. L et T is a set of S populations, each composed of individuals. Population-to-total differentiation (TSF) is calculated based on FIS (differentiation of individuals within populations) and FIT (differentiation of individuals from total). The relationship binds them: FST = 1 (1 FIT)/ (1 FIS).

According to Wright 1988: 0 < FST < 0.05: low

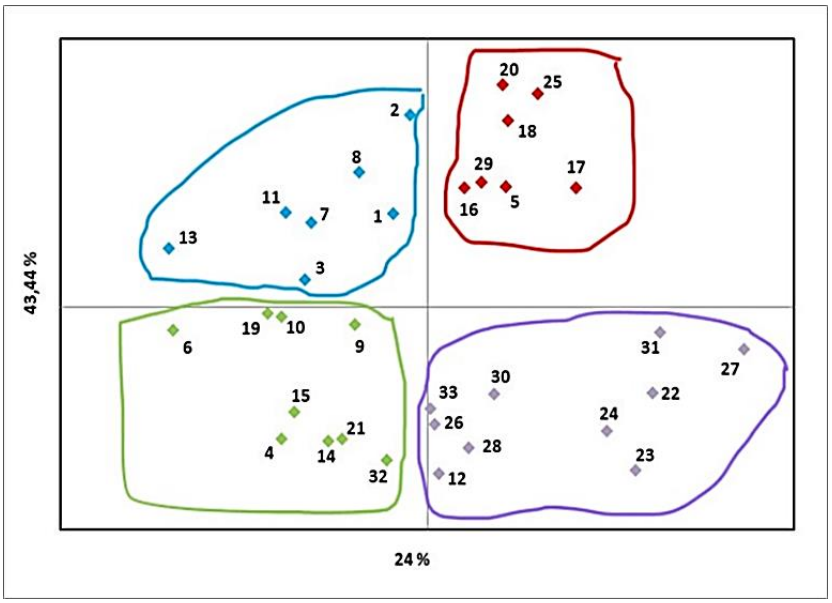

Fig. 3: Cultivars projected in the factor plane based on the dissimilarity of their allelic variability Principal Coordinates N°1 (KF190136); N°2 (KF190006); N°3 (KF20005); N°4 (KF190112); N°5 (KF20017); N°6 (KF20018); N°7 (KF190114); N°8 (KF20036); N°9 (KF20058); N°10 (KF20045); N°11 (KF20046); N°12 (KF20006); N°13 (KF20013); N°14 (KF20034); N°15 (KF20059); N°16 (KF190018); N°17 (KF20019); N°18 (KF190061); N°19 (KF20035); N°20 (KF18045); N°21 (KF18046); N°22 (KaWS 9294292); N°23 (KF18044); N°24 (CB MS11); N°25 (KF190063); N°26 (KF190026); N°27 (KF190051); N°28 (KF190027); N°29 (KF190052); N°30 (KF190064); N°31 (KF190065); N°32 (KF190022); N°33 (KF190066)

differentiation.
0.05 < FST < 0.15: moderate differentiation
0.15 < FST < 0.25: significant differentiation
FST > 0.25: significant differentiation
**GST: The FST parameter is often replaced by an analogous parameter, the GST, defined by the formula:

$$\mathrm{GST} = \frac{Ht - Hs}{Ht} = 1 - \frac{Hs}{Ht}$$

Since HS is the average (across all populations) of intra population and HT genetic diversity, overall genetic diversity is considered a single population (total diversity). Regarding several loci, HS and HT become the averages (overall loci) of previous diversities.

The analysis of the allelic combination between the loci shows a clear separation between the 33 accessions studied (Table 4). This variability depends on the number of mutations detected. Their projection (PCA) (Fig. 3) shows that all accessions are divided into four subgroups without structure related to their geographical origin. The PCA constructed from the statistical analysis of these markers showed a high degree of genetic diversity and a significant rearrangement of accessions.

Discussion

The genetic diversity and grouping of new rice accessions in Morocco, particularly concerning yield and seed quality, are vital aspects that warrant discussion. Understanding the genetic diversity within a collection of rice accessions is crucial for informed breeding strategies and the development of improved varieties. The grouping of accessions based on yield and seed quality parameters provides valuable insights into the potential for enhancing agricultural productivity. Identifying distinct groups within the rice accessions allows for a targeted approach to breeding programs. In this study, the utilization of SSR markers revealed a high polymorphic information content and allelic diversity, signifying the presence of a broad genetic foundation within this collection (table 4). However, we will present a refined analysis of these combinations in the polymorphism analysis within and between 33 accessions introduced in Morocco. The observed gene diversity and heterozygosity were in line with findings from other research, underscoring a significant level of genetic variation in the rice germplasm (Verma *et al*. 2023). By elucidating the genetic factors contributing to variations in yield and seed quality, breeders can focus on specific traits within each group to develop new varieties with enhanced performance.

The implications of this genetic diversity are multifaceted. First and foremost, it provides a foundation for selecting parental lines with desirable traits for future crosses. Moreover, the grouping of accessions allows for the identification of commonalities or unique characteristics within each group, aiding in the formulation of tailored breeding strategies. In the context of yield improvement, knowledge about genetic diversity can guide the selection of accessions with high-yielding traits. Identifying unique and

rare alleles in the SSR profiles of numerous accessions is evident. Compared to the literature, the results show that RAPD markers (Branco *et al.* 2023) also differentiate the same accessions studied without systematically grouping them according to geographical proximity. However, SSR markers (GST = 0.77) appear more discriminating than RAPD markers (GST = 0.29). Nevertheless, these two markers (RAPD and SSR) show the absence of a systematic grouping according to the geographical proximity of the accessions studied. Similarly, genetic information becomes instrumental in pinpointing accessions with the desired characteristics for seed quality enhancement, such as nutritional content or disease resistance. Advanced analytical techniques, like principal component analysis (PCA), can further refine our understanding of the genetic relationships between specific groups. PCA allows for a comprehensive assessment of the factors contributing to variations in yield and seed quality, aiding in identifying vital genetic markers associated with these traits. The PCA (Fig. 3) illustrates the clustering of accessions carried out independently of the region of origin since genotypes grouped into all groups do not diverge significantly, although they come from different localities. Analysis of the allelic combination between loci shows a clear separation between the 33 accessions studied. This variability depends on the number of mutations detected; their projection (PCA) (Fig. 3) shows that four subgroups represent all accessions. Results compared to those in the literature show that SSR markers (Amegan *et al.* 2020) also made it possible to differentiate the same accessions studied without systematic grouping based on geographical proximity.

The strategic utilization of genetic diversity in rice accessions can significantly impact rice husbandry programs in Morocco. It facilitates the development of varieties that are well-adapted to local conditions and possess the desired agronomic traits. The scientists pursued an alternative approach to address this issue by introducing molecular markers. Molecular markers are tools to evaluate the genetic diversity among various rice varieties, enabling the analysis of quantitative and inherited traits (Kshirsagar *et al.* 2012). In this study, five SSR loci were employed to establish distinct SSR profiles, with most genotypes being unique to a specific zone. The five primers utilized for DNA amplification in each accession exhibited polymorphism. Seventy-five reproducible polymorphic bands were identified (Table 4), suggesting their potential as *Oryza sativa*-specific primers. Markers employed in this study unveiled a 20% polymorphism across diverse rice genotypes (Gao and Innan 2021). In a parallel investigation by Gao and Innan (2021), 15 varieties were genetically characterized using 30 distinct SSR primers, revealing discernible polymorphisms among the examined varieties. Additionally, improved seed quality can lead to better market value and increased resilience against environmental challenges.

The genetic diversity and grouping of new rice accessions in Morocco provide a foundation for targeted breeding programs to enhance yield and seed quality. The insights gained from these analyses contribute to developing resilient and high-performing rice varieties, crucial for sustainable agriculture and food security.

Conclusion

The practical applications of the study's findings hold considerable significance for rice breeding and variety development in Morocco. Moreover, the study's contribution to ongoing rice crop improvement programs is pivotal for sustainable agricultural practices. Understanding the genetic makeup and performance of the new accessions equips agricultural practitioners and policymakers with actionable insights. This knowledge can guide decisions on adopting specific varieties, agronomic practices, and resource allocation, ultimately contributing to enhanced rice production and quality. Identifying and characterizing superior rice varieties, particularly those with high yield and optimal seed quality, hold immediate applications for cultivation practices. Farmers can benefit from cultivating these superior varieties, experiencing increased yields and improved seed quality. Additionally, the findings serve as a valuable resource for further breeding programs in Morocco. The recommended varieties can be used as foundational material for developing new cultivars that align with the objectives of the "Green Generation" strategy.

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Author Contributions

All authors actively contributed to this paper's formulation, discussion of findings, and composition, collectively assuming responsibility for its content.

Conflicts of Interest

The authors assert that they have no conflicts of interest to declare.

Data Availability

The data underpinning the conclusions of this study can be obtained from the corresponding author upon a reasonable request.

Ethics Approval

Not applicable

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