ASSESSING GENETIC DIVERSITY OF VENEZUELAN ELITE RICE LINES USING MORPHO-AGRONOMIC AND MOLECULAR MARKERS

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ABSTRACT

The genetic base of irrigated rice in Venezuela, and Latin America, is narrow and the Venezuelan National Project for the Improvement of Genetic Rice (PNMGA) has applied strategies to increase it. The objective of this work was to characterize the genetic diversity of 13 irrigated elite rice lines and four check varieties of economic importance, by means of morpho-agronomical and molecular markers, as well as to determine their association using the Mantel test. A total of 17 genotypes were planted on a trial during the 2014 irrigated cycle in the experimental field of the National Agricultural Research Institute (INIA)-Guárico using a completely randomized block design with three replicates, in a 20 m^2-plot with standard agronomical management. Thirteen quantitative characters were evaluated and high variability among genotypes was found. Principal Components Analysis (PCA) showed that four attributes comprised 71 % of the total phenotypic variance. The UPGMA conglomerates analysis for both types of markers using Euclidean and Dice distance allowed the discrimination of the 17 genotypes in five and four groups, respectively, showing that the molecular analysis was more accurate and informative. The multiple correspondence factor analysis indicated that ISSR 880, 834 and 850 markers discriminated the 17 genotypes, and the Mantel test detected a low correlation between distance matrices. Both analyzes contribute to characterize genetic diversity in irrigated rice germplasm.
Additional keywords: ISSR, *Oryza sativa*, plant breeding, Venezuela

RESUMEN

Diversidad genética de líneas élite de arroz de Venezuela mediante marcadores morfo-agronómicos y moleculares
La base genética del arroz cultivado bajo riego en Venezuela, y en América Latina es limitada por lo que el Proyecto Nacional para Mejoramiento Genético del Arroz (PNMGA) ha aplicado estrategias para aumentarla. El objetivo de este trabajo fue caracterizar la diversidad genética de 13 líneas de elite de arroz de riego del PNMGA y cuatro variedades testigo de importancia económica mediante marcadores morfo-agronómicos y moleculares, así como determinar su asociación mediante la prueba de Mantel. Se plantaron 17 genotipos en un ensayo durante el ciclo de riego 2014 en el campo experimental del INIA-Guárico utilizando un diseño de bloques completos al azar con tres repeticiones, en una parcela de 20 m^2 con un manejo similar al de los productores. Se evaluaron trece caracteres cuantitativos y se encontró una alta variabilidad entre los genotipos. El análisis de componentes principales mostró que cuatro atributos agrupan 71 % de la varianza fenotípica total. El análisis UPGMA para ambos tipos de marcadores usando distancia Euclidiana y Dice permitió discriminar los 17 genotipos en cinco y cuatro grupos, respectivamente, mostrando que el análisis molecular fue más preciso e informativo. El análisis factorial de correspondencias múltiples indicó que los marcadores ISSR 880, 834 y 850 discriminaron los 17 genotipos, y la prueba de Mantel detectó una correlación baja entre las matrices de distancia. Ambos análisis contribuyen para caracterizar la diversidad genética en el germoplasma de arroz irrigado.
Palabras clave adicionales: Fitomejoramiento, ISSR, *Oryza sativa*, Venezuela

INTRODUCTION

Rice crop improvement in Venezuela began in 1943 with the introduction of genetic materials (Torres et al., 2006). The contribution of this genetic improvement in terms of yield has been

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around 33.7 kg·ha^{-1}·year^{-1} in the last 50 years. Regarding to grain quality, there has been genetic progress because of a reduction in the percentage of chalkiness plus white belly and an increment of amylose content; however, there is not a linear relationship with the percentage of whole milled grain (Pieters et al., 2011).

Genetic improvement programs of self-pollinated species make use of available variability from local or introduced varieties. When this variability is low or does not exist, the breeder must create new populations on which to carry out the selection process; such populations must possess medium and high variance values for the desired characteristics. Generally, crossings are made between elite lines that in some way share a large number of genes in order to achieve those variance levels. These crosses between related lines and the repeated use of certain parents, lead to increased inbreeding, which consequently reduces the genetic base. In addition, the lack of outcrossing within the population, due to the predominant breeding system of autogamous species, naturally restricts recombination. In this way, there is an increase in similarity among the cultivars used, limiting the differentiation between them, reducing genetic diversity and increasing vulnerability to biotic stresses. Therefore, it is strongly recommended to increase the genetic base for rice crop genetic improvement in Venezuela, since it would allow obtaining in the short or medium term a greater gain by selection, besides avoiding possible genetic vulnerability.

Studies on the genetic diversity of irrigated and rainfed rice crop in Latin America concluded that there is a narrow genetic base, increasing the kinship among the cultivars (Cuevas et al., 1992, Guimarães et al., 1996). In Brazil, Rangel et al. (1996) noted that genetic gain by selection was low due to the narrow genetic base found among the materials used. In Venezuela, Acevedo et al. (2007) concluded that the rice genetic base is narrower than that found elsewhere in Latin America and similar to that of irrigated rice in Brazil. Pérez et al. (2011b) using the coefficient of kinship and microsatellites markers in commercial rice cultivars, pointed out that molecular analysis is more accurate than kinship coefficient analysis; nevertheless, both analyzes agreed that the materials were highly correlated. However, Berrio

et al. (2016) studied the genetic diversity of rice varieties released between 2003-2014 in 13 member countries of the Latin American Fund for Irrigated Rice (FLAR), stressing that the 51 varieties had a kinship coefficient rxy = 0.19 on average, which is considered intermediate. Comparing this parameter among the member countries, it fluctuated from a minimum of 0.13 in Ecuador and a maximum of 0.31 for Venezuelan varieties, therefore it was concluded that the genetic base has been broadened in the region.

Studies of genetic diversity in rice germplasm are based on morphological and molecular markers. Fonseca et al. (2002) highlighted that rice morphological characteristics are grouped in qualitative or constant characters and quantitative or variable characters. The first are those that define the line or cultivar and are generally controlled by few genes, have high heritability and they are little influenced (or not) by the environment, while the second are controlled by several genes, have low heritability, they are highly affected by the environment and also considered as the result of the interaction of the environment and the genotype.

Diversity studies have been used to evaluate genetic variability and phylogenetic analysis (Virk et al., 2000; Joshi et al., 2000); analysis of SSR frequency in cultivars and identification of cultivars (Blair et al., 1999); gene tagging and marker-assisted selection (Reddy et al., 2002). DNA-based markers have been extremely useful for characterization of genotypes, cultivar discrimination, genetic seed purity and genetic diversity studies (Joshi et al., 2000). Advantages of this type of markers include not being influenced by the environment, plant physiological state or age. In addition, they provide a robust estimate of genetic similarity allowing the distinction among varieties that cannot be obtained using morphological data only. Furthermore, association of molecular markers to morphological characteristics is of interest for breeding programs.

Inter-Repeated-Simple-Sequence (ISSR) markers (Zietkiewicz et al., 1994) amplify through the polymerase chain reaction (PCR) genomic regions between microsatellites, present in all eukaryotic organisms, by use of specific sequences, generating multiloci markers (Reddy et al., 2002). Primers designed for ISSR-PCR are longer (16-25

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bp) than those used for Random Amplified Polymorphic DNA (RAPD) markers (10 bp) and the reaction occurs at a higher hybridization temperature, which favors greater specificity in the process. These markers are of dominant type and do not require knowledge of the organism's genomic sequence.

Application of ISSR is a simple, fast, efficient, relatively inexpensive and reproducible method, combining most of the microsatellite (SSR) and Amplified Fragment Length Polymorphism (AFLP) advantages with the universality of RAPD. ISSR are highly polymorphic and are useful in studies of genetic diversity, phylogeny, gene tagging, genomic mapping and evolutionary biology (Reddy et al., 2002).

In a recent study, the genetic diversity within 76 cultivars of rice was evaluated for the Rice Program in Ecuador (Pérez et al., 2019). In Venezuela, however, there have been few works on rice genetic diversity that provide sustainability

for genetic improvement programs. The present work was proposed to characterize the genetic diversity present in 13 elite lines and four varieties of irrigated rice, based on quantitative morpho-agronomical and ISSR molecular markers, as well as to study the association between both marker types.

MATERIALS AND METHODS

A total of 17 genetic materials were studied (Table 1), distributed in two groups: 13 experimental elite lines belonging to the National Rice Genetic Improvement Project (PNMGA) of Venezuela (developed under the agreement between the National Institute of Agricultural Research-INIA and the National Rice Foundation-FUNDARROZ) and four rice irrigated commercial varieties, used as controls and selected based on their commercial importance.

Table 1. Description of the rice germplasm used in this genetic diversity study

N°	Code	Origin	Developer
1	SD20A	Check line	DANAC Foundation
2	Venezuela 21	Check line	INIA-FUNDARROZ
3	Payara	Check line	APROSCELLO
4	Soberana Fl	Check line	INIA-FUNDARROZ
5	PN01B037	Elite line	PNMGA
6	PN06V007	Elite line	PNMGA
7	PN07V010	Elite line	PNMGA
8	PN08I017	Elite line	PNMGA
9	PN08I18	Elite line	PNMGA
10	PN08I025	Elite line	PNMGA
11	PN09I049	Elite line	PNMGA
12	PN09I050	Elite line	PNMGA
13	PN04I051	Elite line	PNMGA
14	PN09I051A	Elite line	PNMGA
15	PN09I051B	Elite line	PNMGA
16	PN09I051C	Elite line	PNMGA
17	PN09I052	Elite line	PNMGA

INIA: National Agricultural Research Institute; PNMGA: National Project for the Improvement of Genetic Rice

The trial was planted during the 2014 irrigated rice cycle in the experimental field of INIA-Guarico State, a tropical dry forest area located at 8º 44' N, 67º 32' W, 72 meters above sea level, with annual average values of 1,460 mm rainfall, 1,700 mm evaporation, 27.5 ºC temperature, 6.9 h daily sunshine and 70.6 % relative humidity, according to the data obtained at the local

meteorology unit (INIA, 2017).

Planting was done using a completely randomized block experimental design with three replicates and plots of 20 m^2, with pregerminated genetic seed at a dose of 100 kg·ha^{-1}. The agronomical management was similar to that of the regional commercial crop planting. Thirteen morpho-agronomic variables were considered,

according to the Standard Evaluation System for Rice (IRRI, 2002), as follows: (1) lodging incidence, Lg; (2) panicle exsertion, Exs; (3) panicle density, PnD; (4) panicle length, PnL; (5) phenotypic acceptability, PAcp; (6) plant height at harvest, Ht; (7) 50 % flowering, Fl 50 %; (8) sterility percentage, PnS; (9) number of grain per panicle, Granpp; (10) grain yield adjusted to 12 %, Yld; (11) milled grain yield, MRY; (12) percentage of white center grain, PWCG and (13) percentage of chalkiness plus white belly, PCG.

Statistical analysis for morpho-agronomical markers. An analysis of variance was performed to detect differences among morpho-agronomic variables using the SAS 9.1 program (Cary, NC, USA). Next, principal components analysis (PCA) was carried out with the help of the statistical program InfoGen/E (Balzarini et al., 2010), including the 13 morpho-agronomic characters, in order to identify the main variables that explain the total variance observed. Subsequently, the data matrix was subjected to a multivariate analysis of hierarchical conglomerates using the algorithm UPGMA (unweighted pair method method using arithmetic averages). The average Euclidean distance was used as a cut-off value for the description of the groups.

Genomic DNA extraction. Ten seeds of each rice material were grown in 100 mL glass bottles with water-moistened absorbent paper under sterile conditions for approximately one week at the Agricultural Biotechnology Unit (UBA) of the National Center for Agricultural Research of the National Institute of Agricultural Research (INIA-CENIAP). Then, 0.5 g of foliar tissue were taken and the DNA extracted following the methodology described by Pérez et al. (2011a).

ISSR technique. A total of 12 ISSR primers were selected to characterize the rice genotypes. Polymerase chain reactions were performed in a 20 μL volume, in a microtube containing 2.5 ng of genomic DNA, 3 mM MgCl2, 1X Buffer PCR, 0.2 mM dNTPs, 0.25 μM of primer, and 0.1 U of Taq DNA polymerase (Promega). The PCR cycles were done using a thermal cycler (BioRad). The PCR profiles were initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at recommended temperature for 1 min; extensión at 72 °C for 2 min; final extension at 72 ºC for 7 min. The amplification products were separated by horizontal electrophoresis in 1.5 % agarose gels, prepared in TBE 1X buffer, which was also used as running buffer. ISSR molecular analyzes were carried out in the Molecular Biology Laboratory, National Center for Agricultural Biotechnology, Institute for Advanced Studies (IDEA), in Sartenejas, Caracas.

Three μL of DNA 200 bp step ladder Promega were used as a standard of comparison. Determination of the molecular weight of the bands was done using the ImageQuant TL V 2005 software of Amersham Biosciences (GE).

Statistical analysis for molecular markers. The amplification fragments were coded according to the dominant nature of the marker assigning (1) for presence and (0) for absence of bands, generating one column per locus for each primer. The level of polymorphism and the discriminatory capacity of each initiator were assessed through the content of polymorphic information (PIC). Subsequently, the matrix was subjected to a multivariate analysis of hierarchical conglomerates through the algorithm UPGMA from the estimated distance values of Dice. The degree of adjustment of the similarity matrix was measured using the cophenetic correlation. The average genetic distance of Dice was used as a cut-off value for the description of the groups. The analysis was done using NTSYS version 2.1 (Rohlf, 2000).

In order to determine the importance of each ISSR marker used in the determination of the relationships or distances between the genetic materials, a multiple correspondence factor analysis (MCFA) was carried out according to the statistical program InfoGen/E (Balzarini et al., 2010).

Relationship between morpho-agronomical and molecular markers. The correspondence between the distance matrices generated by the Euclidean distances for quantitative characters (morpho-agronomical markers) and Dice distances for qualitative characters (molecular markers) was analyzed using the Mantel Z statistic (Mantel, 1967). The main interest was to learn if there was linear correlation between the matrices of considered distances. The Z statistic of Mantel was estimated by the following formula:

$$Z_{xy} = \frac{SP\,(X,Y)}{\sqrt{SS(X)SS(Y)}}$$

where, the numerator represents the covariance between distance matrices and the denominator is the square root of the product of the variance of each distance matrix. Since the Mantel test measures the correlation between distance matrices, when the correlation is significant, it can be concluded that there is a linear spatial structure.

RESULTS AND DISCUSSION

Analysis of morpho-agronomical characters. In ten (76.9 %) of the assessed morpho-agronomic variables statistical differences were detected ($P \leq 0.05$). The results showed wide genetic variability among rice lines, an important feature taken into account during the development of breeding genotypes. Phenotypic variability assessed through comparison of maximum, average and minimal values of rice varieties performance are shown in Table 2.

Among morpho-agronomic markers that showed significance ($P \leq 0.01$), grain yield presented an average of 6.91 Mg·ha^{-1} indicating the productive potential of the majority of lines. Flowering at 50 % also showed high variability discriminating early flowering genotypes (82 days) from late materials (98 days) and average ones (89 days), similar to control varieties. Milled grain yield was another trait with high variability, with maximum, minimal and average values of 62, 49 and 58, respectively, showing that most of the elite lines surpassed the industry set standards for this character. On the other hand, several characters presented little genetic variability ($P > 0.05$) although they are of considerable agronomical importance such as lodging, phenotypic acceptability, panicle exsertion and density; this might be explained by the fact that the genetic materials under selection become highly homozygous after several years of selection across diverse environments trial.

Table 2. Maximum, average and minimal values of morpho-agronomical characteristics assessed on Venezuelan rice varieties

Characteristic	Lg	Exs	PnD	PnL (cm)	PAcp	Ht (cm)	Fl 50 % (days)	PnS (%)	Granpp	Yld (Mg·ha^{-1})	MRY (%)	PWCG (%)	PGC (%)
Minimum	1	1	1	18	3	92	82	5	138	4.63	49	1	10
Average	2	1	5	21	4	101	89	9	151	6.91	58	1	15
Maximum	7	3	5	25	5	110	98	16	168	9.63	62	2	43

(1) lodging incidence (Lg); (2) panicle exsertion (Exs); (3) panicle density (PnD); (4) panicle length (PnL); (5) phenotypic acceptability (PAcp); (6) plant height at harvest (Ht); (7) 50 % flowering (Fl 50 %); (8) sterility percentage (PnS); (9) number of grain per panicle (Granpp); (10) grain yield adjusted to 12 % (Yld); (11) milled grain yield (MRY); (12) percentage of white center grain (PWCG) and (13) percentage of chalkiness plus white belly (PCG).

The PCA for the 13 morpho-agronomic characters showed that four traits explained 71.3 % of the total phenotypic variation observed, namely yield of whole grain, yield of paddy grain, 50 % flowering and white center, given in order of importance to discriminate the 17 genotypes. The rest of the variables presented a high correlation, which could be explained by two factors: the genetic materials used are fixed lines with high homozygosis (F_7 generation), and secondly, the phenotypic variances obtained are influenced by the interaction genotype-environment.

The multivariate analysis of hierarchical conglomerates through the UPGMA algorithm, using average Euclidean distance, yielded five groups at an average cut-off distance of 0.85.

Group I formed by the genotype PN09I052 and Group II by PN01B037, presented the greatest ultrametric distances with average values of 2.40 and 1.58, respectively (Figure 1). PN09I052 is a line from the International Center of Tropical Agriculture (CIAT) rice pre-breeding program, with grain yield under 4.5 Mg·ha^{-1} and low overall grain quality, while the PN01B037 line presented high yield of 9.0 Mg·ha^{-1} and regular grain quality; both are long cycle materials (more than 90 days to reach 50 % flowering plants). Group III formed by the siblings PN09I051C, PN09I051B, PN09I051A and Soberana FL variety with high grain yield of 8.8 Mg·ha^{-1}, good grain quality and intermediate cycle (86 days for 50 % flowering), presented an average Euclidean distance of 1.28.

The fourth group formed by the lines PN08I018, PN09I049, PN07V010, PN04I051 and the variety SD-20A had an average yield of 6.5 Mg·ha^{-1}, medium grain quality and late cycle (90 days for 50 % flowering), average distance of 1.15. The fifth group, formed by the elite lines PN09I050, PN08I017, PN08I025, PN06V007 and varieties Venezuela 21 and Payara, had lower grain yield among the studied materials nevertheless yielded

5.9 Mg·ha^{-1} which is higher than the national average; grain quality was high (translucent grains) and medium cycle (88 days, 50 % flowering). The cophenetic correlation was 0.87 indicating a good fit between the generated dendrogram and the Euclidean distance matrix. The general analysis of the pedigrees of this group indicates that the materials share common progenitors, hence their genetic similarity.
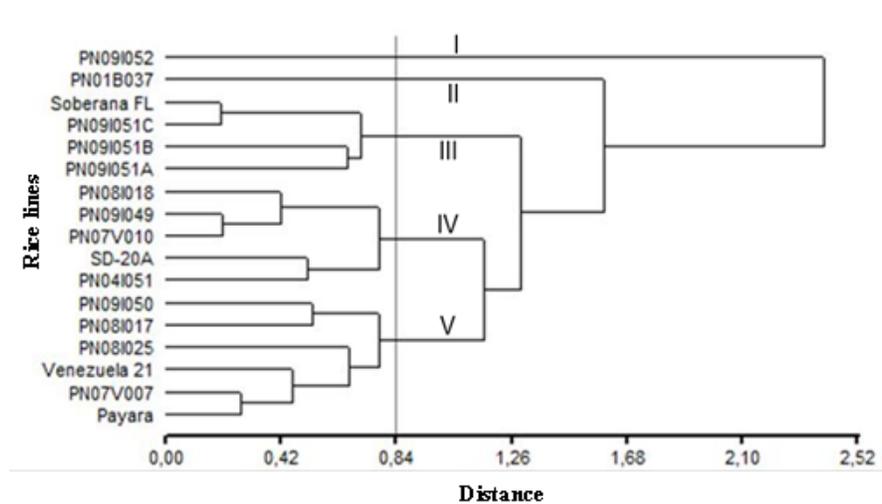

Figure 1. Dendrogram showing the genetic relationships between 17 rice genetic materials generated by cluster analysis using the UPGMA method with Euclidean distance

These results once again demonstrated the excellent grain quality of the variety Venezuela 21, which is currently used as the benchmark in the different milling quality research laboratories of the country. It should be noted that the genotypes subjected to this analysis were not grouped by their genetic origin and there was no clear genetic diversity among elite lines and varieties used as controls. However, the plausible explanation for the phenotypic grouping obtained here could be attributed to the fact that such characterization was carried out based on the morpho-agronomical study of phenotypic variables of polygenic characters, highly affected by the interaction genotype-environment. These results agree with those obtained in Venezuela by Pérez et al. (2011b) and Acevedo et al. (2007).

Molecular analysis using ISSR. A total of twelve ISSR random primers were used (Table 3), and according to the observed polymorphism, eight were selected for molecular analysis (834, 841, 864, 880, 888, 850, 855, and 891), which generated high resolution bands for all samples

(Table 3); the rest of initiators (812, 815, 835 and 890) were not considered to avoid false positives since they did not present good resolution in the gels. The eight initiators produced a total of 112 bands, 105/112 were polymorphic (93.75 %), with a size range between 360-3900 bp. The selected initiators generated information measured in terms of the number of polymorphic fragments and Polymorphic Information Content (PIC); the 850, 888, and 891 primers with PICs greater than 50 % stand out, which makes them selectable for future molecular studies with these rice genotypes (Table 3). The 880 primer generated the lowest number of polymorphism, and the 891 primer the highest, with 21 polymorphism fragments.

Figure 2 shows a dendrogram generated by cluster analysis using UPGMA and the distance Dice. The analysis allowed distinguishing up to four well-differentiated groups with an average ultrametric distance of 0.43. Group I consists of the elite line PN09I052 from the CIAT pre-breeding program, whose genetic origin is not related to CIAT's active rice germplasm or FLAR,

since the wild species *Oryza rufipogon* is found among its progenitors. This elite line, introduced in an additional nursery of the FLAR in Venezuela, presented high grain yield potential

with limitations due to problems of lodging or overturning, and quality of the grain; however, it has good general adaptability and it is a progenitor within the PNMGA of the country.

Table 3. Descriptive statistics of polymorphism observed in 8 ISSR primers used for genotyping 17 rice materials in this study

ISSR Primer	Nucleotide sequence	Size range (bp)	AP	PIC	SE	PMF(95)	PDICMA
834	5´-AGAGAGAGAGAGAGAGAGCTT-3	700-3000	6	40.3	0.04	0.86	2,00E-06
841	5' -GAGAGAGAGAGAGAGAGAYC -3'	620-2050	18	46.4	0.01	1	3,90E-14
850	5´-GTGTGTGTGTGTGTGTGTYC-3	500-1740	7	51.5	0.01	0.88	1,80E-10
855	5´-ACACACACACACACACCTT-3	910-3900	16	43.8	0.01	1	2,10E-16
864	5´-ATGATGATGATGATGATG-3	500-2900	13	38.7	0.03	0.93	1,80E-08
880	5'- GGAGAGGAGAGGAGA- 3'	600-2250	5	45.1	0.01	0.83	4,40E-10
888	5´-HVHTGTGTGTGTGTGTGTG-3´	360-2470	19	52.9	0.02	0.95	7,50E-10
891	5'-GGAGAGGAGAGGAGA -3'	400-2670	21	54.2	0.01	0.91	6,30E-12

Bases abreviation: N = (A, G, C, T); R = (A, G); Y = (C, T); B = (C, G, T); (not A); D = (A, G, T) (not C); H = (A, C, T) (not G); V = (A, C, G) (not T). H= Not G, y V= not T; bp = base pair; AP: number of polymorphic alleles; PIC: polymorphism information content; SE = standard error; PMF(95): polymorphic marker frequency; PDICMA: probability that two individuals share the same allele by chance
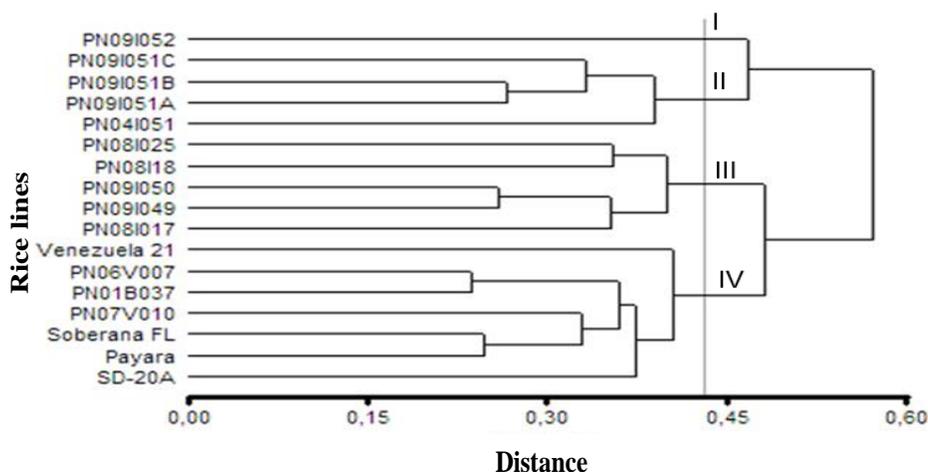

Figure 2. Dendrogram generated by cluster analysis by the UPGMA method showing the genetic relationships among 17 rice

The group II is formed by the elite lines PN09I051A, PN09I051B, PN09I051C and PN04I051; the first three correspond to siblings with good adaptability but differing in their precocity; whereas PN04I051 is a line with high yield potential; however, it has an important heterozygosity that makes it divergent to the rest of the materials evaluated, but not to the group of line PN09I051 siblings. Group III was composed of five elite lines (PN08I025, PN08I018, PN09I050, PN09I049 and PN08I017) with genetic

similarity of 0.41, coming from four different crosses, under which the lines PN08I017 and PN08I018 are complete siblings, with excellent phenotypic acceptance and milling quality of grain.

The fourth group, with of about 37 % similarity, was made up of seven materials, three of which correspond to elite lines PN01B037, PN06V007 and PN07V010 and the rest comprises the group of controls made up of the commercial varieties (Venezuela 21, Soberana

FL, Payara and SD-20A). This result is consistent with other studies that explored genetic diversity using molecular markers in Venezuelan varieties (Arnao et al., 2008; Ghneim et al., 2008; Pérez et al., 2011b), which report low diversity between the materials because of the origin of the germplasm (CIAT/FLAR), and for sharing common parents.

The molecular markers allowed discrimination of the 17 genotypes of rice according to their genetic origin. The results together show that the PNMGA of Venezuela, in recent years, has made modifications incorporating new germplasm that allowed to increase genetic diversity among potential new cultivars. However, there are lines (PN01B037, PN06V007 and PN07V010) that share up to 60 % genes with the control varieties, although there are other important groups of materials that share less than 43 % of the genes.

These results demonstrate the genetic diversity among irrigated rice germplasm in the country, in agreement with the results of Berrio et al. (2016)

who indicated that regarding genetic diversity of rice varieties in Latin America, in general, an expansion of the genetic base of germplasm has been achieved, and mainly in Venezuelan materials.

Multiple correspondence factor analysis. There are few published papers on studies of rice genetic diversity using molecular markers in Venezuela. In this sense, the MCFA allowed the association of the ISSR markers with the irrigated rice germplasm studied. The spatial distribution of the eight polymorphic markers ISSR and the 17 genetic materials of rice are presented in Figure 3. The axis 1 with 53.2 % inertia allowed the identification of the markers 880 and 834 to discriminate mainly the commercial varieties in contrast with the marker 850, more frequent for elite lines with high genetic divergence; the axis 2 with an inertia of 17.98 % discriminated contrasting genotypes with the 855 and 864 markers. The ISSR 864, 888 and 841 are most frequently present in the elite lines.
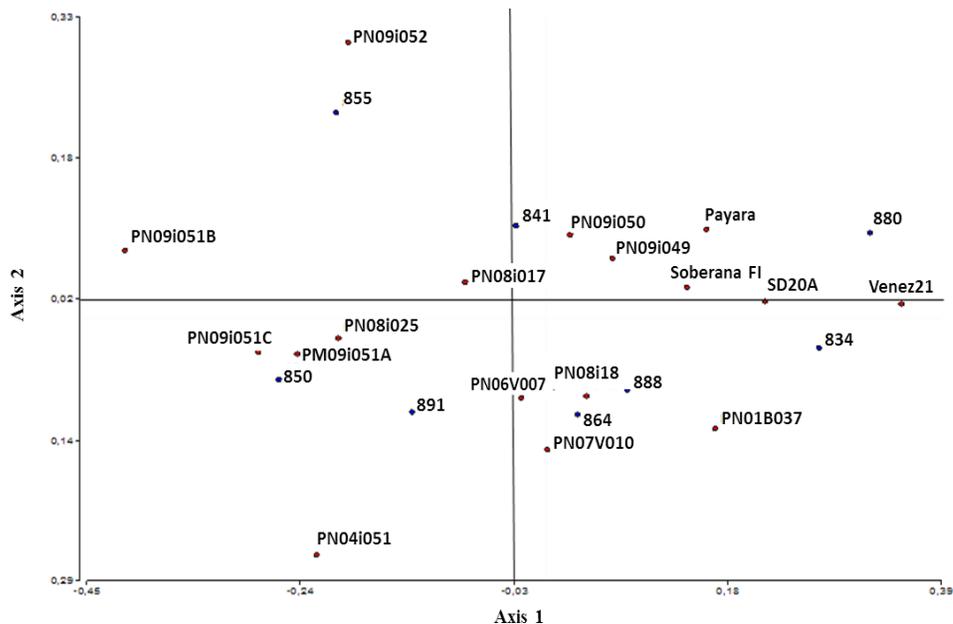

Figure 3. Spatial distribution of eight ISSR primers and 17 irrigated rice genotypes used in the Venezuelan breeding program

In general, this analysis confirms what was found in the previous analyzes. The efforts made within the PNMGA to increase the genetic diversity in the germplasm of irrigated rice grown in Venezuela have been positive, contrary to what had been observed in several diversity studies in

rice genetics in Latin America and the Caribbean (Cuevas et al., 1992); and in studies in Venezuela conducted by Acevedo et al. (2007) and Pérez et al. (2011b).

Relationship between morpho-agronomical and molecular markers. The Mantel test resulted in a

not significant low correlation coefficient (r = 0.14). This result demonstrates the low linear association found between the Euclidean distances for quantitative characters (morpho-agronomical markers) and Dice distances for the qualitative characters (molecular markers). That is, both analyzes grouped most of the genotypes differently, with low similarity. However, the analysis with molecular markers was more precise and informative and yielded such grouping, according to the genetics, differing with the morpho-agronomical markers. The elite lines PN08I017 and PN08I018, using morpho-agronomical markers, were located in different groups, what is due to the fact that both lines come from the same crossing, being sister lines. Similar analysis applies to the varieties Venezuela 21, Payara FL and Soberana FL, since the study of the pedigree detected common ancestry, and the morpho-agronomical markers (Figure 1) located them in different groups, in contrast to the molecular ones (Figure 2). An additional explanation that could be given to the low correlation found in the Mantel test is that both analyzes (morpho-agronomical and molecular) complement each other and are not mutually exclusive, as reported by Vieira et al. (2007) and Vieira et al. (2013).

On the other hand, the low correlation could be attributed to: a) the morpho-agronomical markers associated with phenotypic characters are biased by the uncertainty that the genotype-environment interaction confers on them, b) both morpho-agronomical and molecular markers evaluate different characters, in other words, there is no association between phenotype and genotype; and c) the ISSR markers explore a portion of the rice genome; however, these regions may not code for agronomic traits of interest and may not be selected as is the case with morpho-agronomical markers.

CONCLUSIONS

The studied rice germplasm showed high genetic variability for quantitative traits, yield of whole grains, paddy grain yield, flowering 50 % and white center. The diversity analyzes with morpho-agronomical and molecular markers discriminated the 17 rice genotypes, being more accurate and informative when the ISSR markers were used. The multiple correspondence factor analysis detected that the ISSR type markers that best discriminated the irrigated rice germplasm were 880, 834 and 850 primers. The Mantel test presented a low correlation, suggesting that both ISSR marker and morpho-agronomic analyzes complement each other and they constitute the best strategy for studying germplasm diversity of irrigated rice.

LITERATURE CITED

1. Acevedo, M., E. Torres, O. Paez, R. Álvarez, O. Torres, W. Castrillo et al. 2007. Base genética de los cultivares de arroz de riego liberados en Venezuela. Agronomia Tropical 57(3): 197-204.

2. Arnao, E, Y. Jayaro, P. Hinrichsen, C. Ramis, C. Marín and I. Pérez-Almeida. 2008. Marcadores AFLP en la evaluación de la diversidad genética de variedades y líneas élites de arroz en Venezuela. Interciencia 33(5): 359-364.

3. Balzarini, M., C. Bruno, A. Peña, I. Teich and J.A. Di Rienzo. 2010. Estadística en Biotecnología. Aplicaciones en Info-Gen. Universidad Nacional de Córdoba. Córdoba, Argentina.

4. Berrio-Orozco, L., E. Torres-Toro, J. Barona-Valencia and J. Cuasquer-Sedano. 2016. Diversidad genética de las variedades de arroz FLAR liberadas entre 2003-2014. Agronomia Mesoamericana 27(2): 217-231.

5. Blair, M.W., O. Panaud and S.R. McCouch. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L). Theoretical & Applied Genetics 98(5): 780–792.

6. Cuevas-Pérez, F., Guimaraes, E.P., L.E. Berrio and D. Gonzales. 1992. Genetic base of the irrigated rice in Latin America and the Caribbean. Crop Science 32(4): 1054-1059.

7. Fonseca, J., V. Cutrim and P. Rangel. 2002. Descritores Morpho agronômicos e fenológicos de cultivares comerciais de arroz de várzeas. Embrapa. Brasília, DF. 22 p.

8. Ghneim, T., D. Posso, I. Pérez-Almeida, G. Torrealba, A. Pieters, C.P. Martinez and J. Tohme. 2008. Assessment of genetic diversity

in Venezuelan rice cultivars using simple sequence repeats markers. Electronic Journal of Biotechnology 11(5): 3-4.

9. Gupta, M., Y.S. Chyi, S.J. Romero and J.L. Own. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theoretical and Applied Genetics 89(7-8): 998-1006.

10. Guimarães E.P., J. Borrero and J. Ospina-Rey. 1996. Genetic diversity of upland rice germoplasm distributed in Latin America. Pesquisa Agropecuaria Brasileira 31(3): 187-194.

11. INIA. 2017. Red de Agro-meteorología. Estación Bancos de San Pedro, Calabozo, estado Guárico. http://www.inia.gov.ve/index.php/institucional-inia/red-de-agrometeorologia-del-inia (retrieved March 12, 2017).

12. IRRI (International Rice Research Institute). 2002. Sistema de evaluación estándar de arroz. Manila, Filipinas. 55 p.

13. Joshi S., V. Gupta, R.R. Aggarwal, P. Ranjekar and D. Brar. 2000. Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theoretical & Applied Genetics 100(8): 1311–1320.

14. Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research, Chestnut 27(2): 209-220.

15. Pérez-Almeida, I., L.R. Angulo, G. Osorio, C. Ramis, A. Bedoya, R. Figueroa-Ruiz et al. 2011a. Método modificado de obtención de ADN genómico en orquídeas (*Cattleya* spp.) para amplificación con marcadores moleculares. Bioagro 23(1): 27-34.

16. Pérez-Almeida, I., E. Torres, L. Angulo and M. Acevedo. 2011b. Genetic diversity among Venezuelan rice cultivars based on parentage coefficient estimation and analysis using microsatellite molecular markers (SSR). Interciencia 36(7): 545-551.

17. Pérez-Almeida, I., R. Celi-Herán, F. Sánchez-Mora, L. Paz-Carrasco and B. Ramos-Viteri.

2019. Assessment of molecular genetic diversity of Ecuadorian rice cultivars using simple sequence repeat markers. Bioagro 31(1): 3-12.

18. Pieters, A., E. Graterol, E. Reyes, R. Alvarez and A. Gonzalez. 2011. Cincuenta años de mejoramiento genético del arroz en Venezuela. ¿Qué se ha logrado? Interciencia 36(12): 943-948.

19. Rangel, P., E.P. Guimarães and P. Neves. 1996. Base genética das cultivares de arroz (*Oryza sativa* L.) irrigado do Brasil. Pesquisa Agropecuaria Brasileira 31(5): 349-357.

20. Reddy, M.P., N. Sarla and E.A. Siddiq. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128(1): 9-17.

21. Rohlf, F. 2000. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. New York: Exeter Software. 98 p.

22. Torres, O., M. Salazar, M. Navas, R. Álvarez, E. Reyes, O. Moreno et al. 2006. Mejoramiento genético de arroz en Venezuela. Resumen histórico. INIA Divulga 8: 14-18.

23. Vieira, E., F. Carvalho, I. Bertan, M. Kopp, P. Zimmger, G. Benin et al. 2007. Association between genetic distances in wheat *Triticum aestivum* as estimated by AFLP and morphological markers. Genetics and Molecular Biology 30(2): 392-399.

24. Vieira E., J. Fialho, F. Faleiro, G Bellon, K. Fonseca, M. Silva et al. 2013. Caracterização fenotípica e molecular de acessos de mandioca de indústria com potencial de adaptação às condições do Cerrado do Brasil Central**.** Semina: Ciências Agrárias 34(2): 567-582.

25. Virk, P.S., J. Zhu, H.J. Newbury, G.J. Bryan, M.T. Jackson and B.V. Ford-Lloyd. 2000. Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa*) germplasm. Euphytica 112(3): 275–284.

26. Zietkiewicz, E., A. Rafalski and D. Labuda. 1994. Genome finger-printing by Simple Sequence Repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20(2): 176-183.