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Genetic diversity of African's rice (*Oryza glaberrima* Steud.) accessions cultivated under iron toxicity

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Abstract

Iron toxicity stress is one of the most important constraints to rice production in Togo. Although several methods were explored to control this stress, the best one is still the genetic control through the use of tolerant or resistant varieties. Our hypothesis is that African's rice, *Oryza glaberrima,* accessions contain sources of tolerance or resistance to the iron toxicity stress. Thus, the aim of this study was to determine the level and the structure of the African's rice, *Oryza glaberrima,* genetic diversity and to identify tolerant genotypes. Two hundred and four (204) accessions obtain from Africa Rice genebank and eight control varieties were evaluated under iron toxicity conditions using alpha lattice design with three replications. There was significant variability among accessions for height of mature plants and weight of 1000 seeds. The coefficient of variation values ranged from 10.56% for the 50% flowering to 77.47% for the sterility rate. The principal component analysis (PCA) with all the measured characteristics revealed that the first four axes accounted for 59.93% of the total variability. The coordinates of the variables showed that, six (6) accessions (T30, T60, H60, Tf, yield and Tox60) are associated to the factor F1 with an eigenvalue of 2,81. A high correlation between 50% flowering and plant height, fertile tillers and number of tillers, sterility rate and total number of seeds and yield were also observed. The accessions were divided into three (3) distinct groups. Sixty (60) accessions and four (4) controls (CG14, IR64, Azucena Whyte and NERICA L-20) identified as tolerant genotypes were clustered together in Group 3. These tolerant accessions could be exploited in the rice breeding program for the tolerance to iron toxicity stress.

Keywords: Agromorpological variability, Genotypes, *Oryza glaberrima*, Iron toxicity stress.

Abbreviations: CTOP_ Coordination Togolaise des Organisations Paysannes et de Producteurs Agricoles, DF_50% flowering, DFA_Discriminant Factor Analysis, Exe_panicleexsertion, F_F de Fisher, G/p_ number of seeds per panicle, H^2_ broad sense Heritability**,** H60_Height of plants at 60 days after transplanting, HAC_Hierarchical Ascending Cluster, Hmat_Height of mature plant, Min._Minimum, Max._ Maximum, O. glaberrima_Oryza glaberrima, PCA_Principal Component Analysis, Pr._ Probability at 5%, r_coefficient of Pearson correlation, SD_Standard Deviation, Ster_ sterility rate, Tf_ number of fertile tiller , T30_number of tillers at 30 days after transplanting, T60_number of tillers at 60 days after transplanting, , Tox35_scoring of symptoms of iron toxicity 35th day after transplanting , Tox60_scoring of symptoms of iron toxicity 60th after transplanting , Toxmat_ scoring of symptoms of iron toxicity at maturity, VG_Genotype Variance, VP_Phenotypic Variance, W1000_ weight of 1000 seeds, WAAP_ West African Agriculture Productivity Program, WARDA_ West Africa Rice Development Association.

Introduction

Iron toxicity stress is one of the most important abiotic counstraints to rice in addition to drought and submersion (Brady, 1982). It is recognized as a widespread food disorder affecting mainly lowland rice's growth (Dobermann and Fairhurst, 2000). In West Africa (Ivory Coast, Ghana, Guinea), more than 55% of the low-lying lands are affected by iron toxicity and nearly 10% of the arable land in rice is abandoned due to this constraint (Cherif et al., 2009).
Depending on the severity of the iron toxicity stress and the tolerance of the varieties, rice yield losses in the lowlands in Africa are estimated from ten (10) to 90% (Abifarin, 1988, Audebert and Fofana, 2009, cherif et al., 2009). Studies in Togo's lowlands have shown the yield losses about 30 to 80% (Aboa et al., 2006), jeopardizing the food security in the country. Iron toxicity is influenced by multiple factors, making its control difficult. Several methods such as transplanting on ridges, water management and early submersion, development of shallows, fertilizer supply for better absorption have, however, been carried out in order to fight this constraint (Ponnanperuma, 1977, Sahrawat, 1979, Abu et al., 1989). Despite the success achieved with these methods, their combination with the resistant or

tolerant varieties might be the most effective way to overcome the iron toxicity stress (Abifarin, 1989). Plant genetic studies will provide to farmers, resistant or tolerant and adapted varieties to agro-pedoclimatic conditions and stress inherent in rice cultivation. African's rice, *Oryza glaberrima,* accessions might countain sources of tolerance or resistance to the iron toxicity stress. Indeed, *O. glaberrima* is considered as a rich reservoir of source of tolerance to several biotic and abiotic stresses (WARDA, 1993, Jones et al., 1997, Futakuchi et al., 2012). However, previous studies have concerned only a few Glaberrima accessions (Sikirou et al., 2018). In breeding programs, varieties characterization based on multiple phenotypic traits, can be used as a management tool to validate the identity of accessions. It seems necessary to evaluate the genetic variability of *O. glaberrima* accessions to identify potential high tolerant or resistant genotypes to iron toxicity stress that could be exploited in rice breeding programs (Lineares, 2002; Sarla et al; Sikirou et al., 2015). The present study aims to determine the effect of iron toxicity on the *O. glaberrima* diversity level and structure in order to identify tolerant or resistant genotypes.

Results

Variation in the quantitative characteristics of Oryza glaberrima accessions under iron toxicity stress

Analyse of variance (Table 1) revealed significant differences among the 212 accessions for all the measured traits except number of tillers at 30 days, plant height at maturity, and weight of 1000 seeds. The coefficient of variation values ranged from 10.56 for the 50% flowering to 77.47 for the sterility rate. The broad sense heritability (H^2) was high for all the measured traits (>40%) and varied from 48.1% for the number of tillers at 30 days after transplanting to 72.8% for the 50% flowering.

Relationships between characters

The Pearson correlation matrix (Table 2) showed many significant correlations at 5% and 1% thresholds. Thus, the 50% flowering was negatively correlated with plant height at 60 days (r = -0.56). The plant height at maturity was positively correlated with the sowing to heading cycle (r = 0.17). The number of fertile tillers was also positively correlated with the number of tillers at 30 and 60 days (r = 0.52 and r = 0.7). The number of seeds per panicle was positively correlated with the 50% flowering and the plant height at maturity (r = 0. 29 and r = 0.35). The sterility rate was positively correlated with the number of seeds per panicle (r = 0.65). On the other hand, the weight of 1000 seeds was negatively correlated with the 50% flowering and yield (r = -0.38 and r = -0.27). The yield was positively correlated with the number of tillers at 60 days, and at 60-day plant height. It was also correlated with maturity height, and to number of tillers fertile but negatively correlated with the sowing-heading cycle (r = 0.27, r = 0.36, r = 0. 24, r = 0.35, r = -0.25). Iron toxicity score at 60 days was negatively correlated with yield (r = -0.23), while panicle exsertion correlated positively with plant height at maturity (r = 0.3672).

Variability through principal component analysis (PCA)

The principal component analysis (PCA) with all the measured traits revealed that the first four axes accounted for 59.93% of the total variability (Table 3). The coordinates of the variables showed that out of the 14 variables used for analysis, six (6) (T30, T60, H60, Tf, yield and Tox60) were associated to the factor F1 with an eigenvalue of 2,81 (Figure 1) ; three (03) (DF, G / p and Ster) to the factor F2 with an eigenvalue of 2.35 and two (02) (P1000 and Toxmat) to the factor F4 with an eigenvalue of 1,45. The factor F3 had an eigenvalue of 1.78 but weakly associated with the studied variables.

Accessions variability structure

The hierarchical ascending clustering performed on weighted averages of Euclidean distances (Figure 2) revealed a distribution of the 212 accessions in three (3) groups.The group 1 consisted of 54 accessions including NERICA L-19, Souakoko8 and CK73 and accounted for 25.47% of the total accessions. The group 2 clustered 98 control CK4 together and represented 46.23% of the total accessions, while the group 3 (28.3% of the total accessions) grouped 60 accessions including the controls CG14, IR64, Azucena Whyte and NERICA L- 20 (Supplementary table 2).
The discriminant factor analysis (DFA) characterized the three groups obtained based on six (6) variables (T30, Hmat, yield, P1000, Tox35 and exe). The projection of the groups in the canonical axes system 1 and 2 (Figure 3) indicated that these two first axes had a discriminating power of 100%. Thus, the relationship of the groups with the axes showed that the three groups were more correlated with the axis 1 than the axis 2. The group 1 is composed of individuals of small size, long 50% flowering, low yield with a high sterility rate, while group 3 contained high-performance individuals with average plant height at 97 cm maturity, short 50% flowering of less than 85 days with high fertile tillers, good yield with a low sterility rate and good panicle exsertion. Group 2 was that of the average performers compared to the other two groups. These results reflect the existence of variability between identified groups.

Discussion

The results from the agro-morphological evaluation showed the existence of variability within 204 accessions under iron toxicity stress condition. Indeed, the maximum number of tillers at 60 days after transplanting and fertile tillers of 15 and 13, respectively with coefficients of variance of more than 28 testified the effect of iron toxicity on accession because without iron toxicity, accessions and controls Sativa would have had more tillering. Similarly, sterility rates were quite high with relatively low yields and a low number of seeds per panicle. This would be due to the effect of iron toxicity on these accessions. Accessions that have shown relatively good performance would have a good level of tolerance. Thus, the iron toxicity might reduce the number of tillers as well as the number of fertile tillers. It also acts by increasing the sterility rate of varieties and reduces the number of seeds per panicle and yield (Dobermann and Fairhurst, 2000; Aboa and Dogbe, 2006; cherif et al., 2006).

Table 1. Summary of descriptive analyzes and performance of *O. glaberrima* accessions.

Variable	Min.	Max.	Mean	SD	CV (%)	H^2(%)	F	Pr > F
T30	1,000	10,000	3,361	1,384	41,174	48,1	0,933	0,695
H 60	47,167	122,500	80,564	12,926	16,044	64,6	1,835	< 0,0001
T 60	1,667	15,333	6,934	1,987	28,650	58,7	1,429	0,005
Hmat	70,667	131,833	95,232	10,949	11,497	54,8	1,216	0,076
DF	67,000	111,000	87,733	9,263	10,559	72,8	2,688	< 0,0001
Tf	2,833	13,833	6,526	1,910	29,270	56,4	1,302	0,027
Gr/p	39,398	217,000	89,496	21,462	23,981	65,3	1,891	< 0,0001
Ster	0,901	105,639	20,445	15,840	77,473	69,8	2,319	< 0,0001
yield	125000,000	7100000,000	2202010,618	1002651,029	45,533	58,5	1,417	0,005
W1000	9,950	49,383	29,729	7,081	23,820	51,9	1,083	0,280

Legend : Number of tillers at 30 days after transplanting (T30); Number of tillers at 60 days after transplanting (T60); number of fertile tiller (Tf); Height of plants at 60 days after transplanting (H60) Height of mature plants (Hmat); 50% flowering(DF); sterility rate (Ster), number of seeds per panicle (G / p); weight of 1000 seeds (P1000) yield (yield).

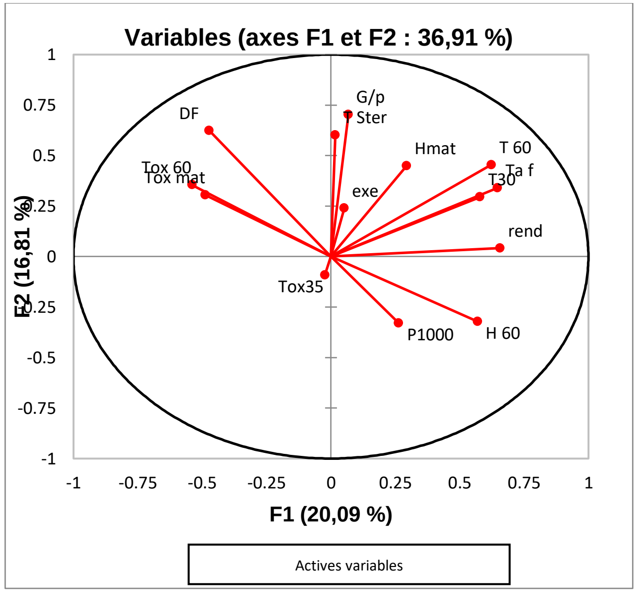

Fig 1. Principal component analysis (PCA). Legend : Number of tillers at 30 days after transplanting (T30); Number of tillers at 60 days after transplanting (T60); number of fertile tiller (Tf); Height of plants at 60 days after transplanting (H60) Height of mature plants (Hmat); 50% flowering(DF); sterility rate (Ster), number of seeds per panicle (G / p); weight of 1000 seeds (P1000) yield (yield).

Table 2. Correlations between the measured characters.

Traits	T30	T 60	H 60	DF	Hmat	Tf	G/p	Ster	yield	W1000	Tox35	Tox60	Toxmat
T30	1,000												
T 60	0,591*	1.000											
H 60	0.033	0.025	1.000										
DF	-0.098	0.034	-0.559	1.000									
Hmat	0.118	0.178	0.293*	0.168	1.000								
Tf	0.520*	0.704*	0.119	-0.070	0.079	1.000							
G/p	0.030	0.128	-0.024	0.2916*	0.3476*	0.065	1						
Ster	0.026	0.116	-0,065	0.164	0.222*	0.032	0.649*	1					
yield	0.269*	0.271*	0.362*	-0.248*	0.24*	0.347*	0.109	-0.021	1				
W1000	0.1506	-0.037	0.196	-0.376	0.0640	-0.034	-0.273*	0.033	0.1707	1			
Tox35	0.0360	-0.002	0.046	-0.046	0.022	-0.039	-0.092	-0.1	-0.059	0.022	1		
Tox60	-0.029	-0.090	-0.313	0.315	-0.017	-0.067	0.011	0.055	-0.225*	-0.044	0.065	1	
Tox mat	-0.025	-0.114	-0.231*	0.214*	0.029	-0.103	-0.039	0.089	-0.143	-0.019	0.037	0.754*	1

Legend : Number of tillers at 30 days after transplanting (T30); Number of tillers at 60 days after transplanting (T60); number of fertile tiller (Tf); Height of plants at 60 days after transplanting (H60) Height of mature plants (Hmat); 50% flowering(DF); sterility rate (Ster), number of seeds per panicle (G / p); weight of 1000 seeds (P1000) yield (yield).

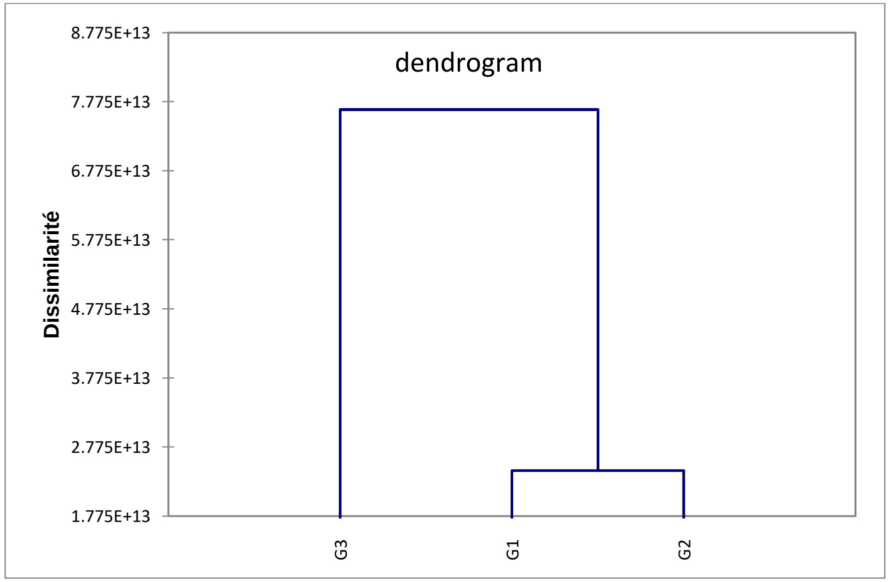

Fig 2. Hierarchical ascending classification of the 212 *O. glaberrima*accessions. Group 1 individuals of group 1; group 2: individuals of group 2; group3 : individuals of group 3

Table 3. Eigen values and percent change expressed for the first five axes from the 14 characters in principal component analysis.

Main Component	F1	F2	F3	F4
Eigen value	2.813	2.354	1.775	1.448
Variability (%)	20.093	16.814	12.675	10.346
% cumulated	20.093	36.907	49.582	59.928
T30	0.579	0.295	0.481	0.069
T 60	0.623	0.454	0.423	-0.156
H 60 (cm)	0.569	-0.322	-0.349	0. 329
DF	-0.473	0.623	0.025	-0.226
Hmat (cm)	0.294	0.449	-0.433	0.419
Tf	0.647	0.340	0.447	-0.121
G/p	0.068	0.703	-0.476	-0.137
Ster	0.017	0.602	-0.371	0.030
yield (g)	0.656	0.041	-0.057	0.195
W1000 (g)	0.263	-0.329	0.035	0.528
Tox35	-0.024	-0.092	0.095	0.273
Tox 60	-0.539	0.355	0.415	0.491
Tox mat	-0.488	0.305	0.367	0.581
Exe	0.052	0.239	-0.410	0.284

Legend : Number of tillers at 30 days after transplanting (T30); Number of tillers at 60 days after transplanting (T60); number of fertile tiller (Tf); Height of plants at 60 days after transplanting (H60) Height of mature plants (Hmat); 50% flowering(DF); sterility rate (Ster), number of seeds per panicle (G / p); weight of 1000 seeds (P1000) yield (yield

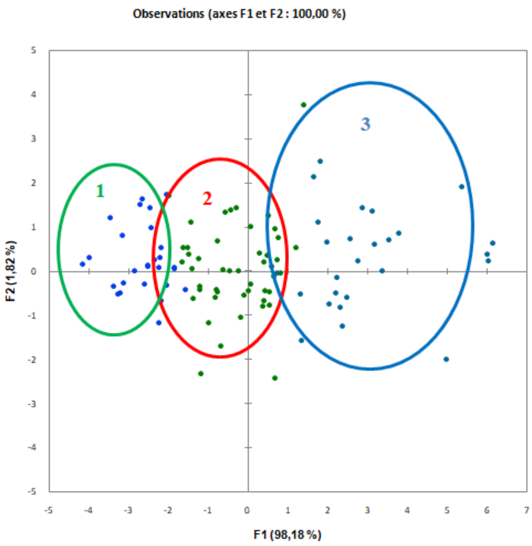

Fig 3. Position of agro-morphological groups of *O. glaberrima* in iron toxicity condition in Dicriminate Factorial Analysis. Legend : 1 : individuals of group 1 ; 2 : individuals of group 2 ; 3 : individuals of group 3.

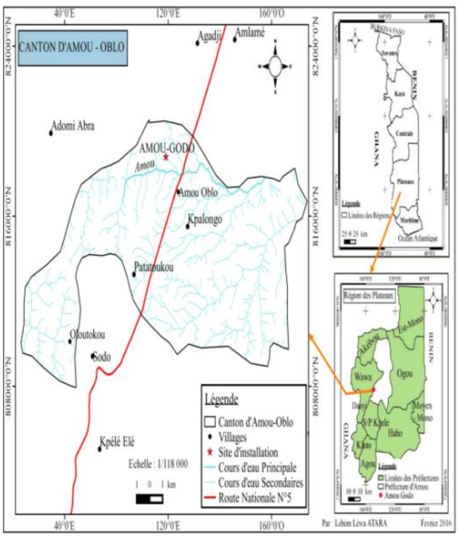

Fig 4. Location of the experimental site.

Several traits discriminate accessions and prove the existence of variability within the *Oryza glaberrima* accessions. The major differences between the minimum and maximum, and high coefficients of variation of the quantitative traits also reflect the existence of large morphological variability within the accessions.
The variability observed might due to the diversity of accessions origins and their behavior with respect to iron toxicity. Indeed, the *O. glaberrima* accessions were collected in different countries of West Africa and represent the diversity of this species in this area. These results differ from those of Ndjiondjop et al. (2010) and Sow et al. (2014) who worked on accessions from Niger and Mali in non-stress conditions. However, the high coefficient of heritability in the broad sense for most of the traits studied showed that the tolerance reaction to iron toxicity stress is more related to the intrinsic characteristics of the accessions. The high heritability values, however, do not explain genetic gain alone. This confirms that *O. glaberrima* is a rich reservoir of genes for the improvement of rice against several stresses (Futakuchi et al., 2012, Agnoun et al., 2012, Gnacadja et al., 2018).
Significant correlations were observed among several traits of interest such as the 50% flowering and plant height, the number of fertile tillers and the total number of tillers, the sterility rate and the total number of seeds and yield. These last different correlations (the sterilite rate with number of seeds and sterilite rate with yield) constitute an indispensable tool for the breeders in the choice of traits to be integrated in the breeding programs. This indicates that accessions with desired morphological traits with regard to tolerance to iron toxicity (number of tillers, low sterility rate, height at maturity) can be selected and used in development of tolerant varieties.
Groups from the ascending hierarchical classification presented individuals with similar characteristics. Thus, group 3 regrouped the best accessions with regards to tolerance to iron toxicity. The performance of some individuals in this group was higher than all the checks used and showed better adaptation to iron toxicity stress. This indicates that group 3 presents accessions whose in-depth analysis could lead to the development of elite genotypes that can be used in breeding for tolerance to iron toxicity. The genetic difference adaptated to soil tolerance under iron toxicity stress has already been the subject of several studies and continues to be exploited for the development of rice cultivars with tolerance to iron toxicity (Sahrawat and Sika, 2002, Sahrawat, 2005). Group 2, on the other hand, regrouped individuals with average performance and could also be subjected to an in-depth analysis to see if certain characteristics of these accessions cannot be exploited to improve tolerance to iron toxicity.

Materials and methods

Plant material

Plant material was consists of two hundred and four (204) *Oyza glaberrima* accessions obtained from the Africa Rice genebank in Africa and eight (08) varieties used as susceptible checks to iron toxicity stress (Supplementary table 1). These accessions represent the diversity of West African *O. glaberrima*.

Experimental site

The experiment was conducted at Amou-oblo, one of the largest villages of the Amou's prefecture located at 40 km southwest of Atakpamé city which distant about 180 km at north of the capital, Lomé. The Amou's prefecture is located in the Plateaux region, one of the five economic regions of Togo which covers an area of 17,000 km^2 or 28.26% of the total area of the country (Tchamie, 2000). It is located between Kpalimé and Atakpamécities at 7º28 'north latitude and 0º54' east longitude (Figure 4). The trial was set up in the village of Amou- oblo, on the edge of the river Amou, 2 km from the national road Nº5, on the western side. The annual average rainfall is 1500 mm with the greatest rainfall in June. As for the temperature, the monthly averages varied between 27 ° C and 36 ° C.

Experimental design

The experiments were conducted during 2014-2015 raining season. The design used is an alpha lattice with three replications. After 21-day nursery,each accession was transplanted into three lines of 2 m long with a spacing of 0.2 m between lines and 0.2 m between hills. The replications were spaced by one meter. Weeds were controlled by a pre emerging herbicide total systemic herbicide with dose of 4l per hectare after planting. Also, when necessary, manual weeding was done to keep the plots free of weeds. Nitrogen, phosphorus and potassium were applied at 50 kg/ha as NPK 15.15.15 at 35 days after tranplanting. Later, nitrogen was top dressed with 25 kg/ha at 60 days after tansplanting.

Agromorphological characters measured

Fourteen (14) traits were evaluated to characterize *Oryza glaberrima* and study the diversity under iron toxicity stress. These traits were number of tillers after transplanting at two development stages (T30 at 30 days, T60 at 60 days), number of fertile tillers (Tf), plant height at two development stages (H60 at 60 days, Hmat at maturity), 50% flowering (DF) which is the days between the sowing and the half of plot heading, seeds numberper panicle (G/p), sterility rate (Ster), weight of 1000 seeds (P1000) and yield (yield). Others traits evaluated are symptoms of iron toxicity at three development stages (Tox35 at 35 days, Tox60at 60 days, Toxmat at maturity). Iron toxicity evaluation was done according to symptoms severity (brozing yellowing) using a standard evaluation system (SES) (Bioversity-International et al., 2007) with scores varying from one (1) to nine (9).

Statistical analysis

Data collected were analyzed with xlstat 2018 software. Analysis of variance was done in order to determine the discriminant traits. The broad-sense heritability (H^2) of each discriminant traits was calculated from the relationship between genotype variance (VG) and phenotypic variance (VP) according to the formula $\mathrm{H}^2(\%) = \frac{VG}{VP} \mathrm{X}\,100$, to determine the share of genotypes in the expression of traits. Relationship between traits was determined through Pearson correlation test while the organization of the variability was determined by the principal component analysis (PCA). The hierarchical ascending clustering was performed according to the aggregation method of Ward based on ten-character means of vegetative growth (T30, T60, Taf, H60, Hmat), yield (yield, P1000), 50% flowering (DF) and tassel (G/p, Tster) and the average of four characters for the assessment of symptoms of toxicity and panicle excretion. Groups obtained were characterized according to the discriminant factor analysis (DFA) based on six (6) variables (T30, Hmat, yield, P1000, Tox35 and exe).

Conclusion

This study carried out under iron toxicity condition showed genetic variability within *O. glabberima* accessions. This variability is mainly due to the intrinsic characteristics of the accessions. Accessions also expressed different tolerance levels to iron toxicity stress. Two groups of accessions (groups 2 and 3) contained potential elite genotypes that could be used in rice breeding programs to control iron toxicity stress. Nevertheless, molecular characterization of these accessions using molecular markers could be carried out to deepen and confirm the variability observed and possibly detects QTLs associated with the tolerance to iron toxicity for use in marker assisted selection programs.

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