

GENETIC DIVERSITY ASSOCIATED WITH AGRONOMIC TRAITS AND BIOTIC STRESSES USING SSR MARKERS IN SOME EGYPTIAN RICE GENOTYPES

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ABSTRACT

Genetic diversity underlies the improvement of crops by plant breeding. In Egypt, pedigree analyses indicate that the rice varieties currently under cultivation are closely related in their genetic background. Effective breeding programs, based on the information about the genetic diversity of cultivars, are needed to broaden the genetic bases of local rice germplasm in the country. The present study has been conducted to evaluate the pattern, type and extent of genetic variability and relatedness among some rice varieties and new promising lines of Egypt based on important agronomic traits and some biotic stresses of some common diseases using simple-sequence-repeat (SSR) markers. In this study, we used a set of twelve SSR markers to assess the genetic diversity of 22 Egyptian rice cultivars and new promising lines, which released by the National Rice Breeding Program between years of 1975 and 2010. The 12 microsatellite markers used in this study produced a total of 73 alleles. The number of alleles per marker ranged from 3 (RM282) to 9 (RM488), with an average of 6.08. The polymorphism information content (PIC) values were high for all microsatellites with average of 0.588 and ranged from a low of 0.345 for RM249 to a high of 0.716 for RM488 and RM144. UPGMA-cluster-analysis based on genetic distance coefficients clearly separated all the genotypes, and showed that the Egyptian rice varieties are closely related. Although the genetic diversity was low, SSRs proved to be an efficient tool in assessing the genetic diversity of rice genotypes. Implications of the low genetic diversity detected and relative relationship among Egyptian varieties are discussed. Varieties like Giza177, Sakha 102 and Sakha 103 are still resistant for most of the biotic stresses and become good sources for biotic stress resistance relating to different disease blast in the national breeding program.

Keywords: Rice, genetic diversity and SSR markers, Rice diseases, biotic stresses

INTRODUCTION

Rice (*Oryza Sativa* L.) is one of the most important staple food crops supporting the world population. Compared with other crop species, the genetic diversity in the world rice germplasm is quite large. Three subspecies, i.e., indica, japonica, and javanica, comprise a large reservoir of rice germplasm including a variety of local landraces and cultivars (Khush, 1997; Garris *et al.*, 2005 and Lu *et al.*, 2005). In addition, there are a number of wild relatives that provide potentially valuable resources for the improvement of cultivated rice (Khush, 1997 and Ren *et al.*, 2003). Despite the richness of genetic resources, only a small proportion of the world rice germplasm collections have been used in breeding programs. As a consequence a high genetic similarity is found within several commercial rice germplasm

worldwide. The limited use made of the rice genetic diversity available worldwide has been a concern in Egypt since late 1980s. Knowledge regarding the amount of genetic variation of germplasm accessions and genetic relationships between genotypes are important issues for designing effective breeding programs. In the past, the characterization of germplasm diversity was carried out by means of morphological and biochemical markers which, in many cases, did not have the resolution power for revealing polymorphisms in genetic analyses and/or for differentiating between closely related genotypes.

Advances in plant genetics and molecular breeding and biology have led to the development of many types of molecular markers which can be used to characterize germplasm beside the morphological characterization. Microsatellites (also known as simple sequence repeats) are simple; tandemly repeated 5-20 fold; often di-to tetra-nucleotide; sequence motifs; each flanked by unique sequences. They are valuable as genetic markers because: they are co-dominant in nature; show high allelic diversity; are easily and economically assayed by PCR; and their use may be automated. Tens of thousands of potential SSRs have been identified in rice, and over 25,000 have been developed as molecular markers (Temnykh *et al.* 2000 and McCouch *et al.* 2007). These markers are currently being used to develop high density genetic maps, genotype rice accessions, determine the genetic structure, optimize the assembly of core collections, and for marker-assisted breeding (Yu *et al.*, 2003, Garris *et al.*, 2005 and McCouch *et al.*, 2007). In rice, SSR markers have been effectively utilized for many purposes including (i) genome mapping (Temnykh *et al.*, 2000, McCouch *et al.*, 2007 and El-Refaee, 2009); (ii) assessment of the genetic diversity and relatedness among various cultivars including both aromatic and non-aromatic rice (Ravi *et al.*, 2003; Jain *et al.*, 2004; Saini *et al.*, 2004; Siwach *et al.*, 2004 and Ghneim Herrera *et al.*, 2008); (iii) identification and purity testing of varieties (Nagaraju *et al.*, 2002; Singh *et al.*, 2004 and Joshi and Behera 2006) and (iv) determination of the genetic relationship between several sub-species (Ni *et al.*, 2002).

Biotic stresses are considered as one of the major constraints for maximum yield production because of its high level of variability and quick spread in case of susceptible cultivars. Blast resistance has consistently been one of the most important objectives of rice breeders in Egypt. Breeding of disease resistance varieties probably is the most cost-effective and reliable method of disease management. Breeding for high yielding and improved resistance are the two most economically and environmentally benign methods of maintaining stable rice production.

In Egypt, the major rice diseases are fungal diseases, rice blast caused by *Pyricularia oryzae* Cav., and the brown spot caused by *Helminthosporium oryzae* Breda De Hann. Brown spot disease is common only in poor soils and when using drainage and low quality water for irrigation. Other minor diseases isolated and identified are false smut caused by *Ustilaginoidea virens*, bakanae caused by *Fusarium moniliforme* Sheldon., Kernel smut disease, *Tilletia barclayana* (Bref.) Sacc. & Syd., Sheath rot caused by *Sarocladium oryzae* (Sawada) and white tip nematode disease

caused by *Aphelenchoides besseyi* Christie. (EL-Shafey, 2002; Sehly *et al.*, 2002; Osman, *et al.*, 2005; Tahooun, 2005; El-Shafey, 2007; Sehly *et al.*, 2008 and Gabr, 2010)

The present study has been conducted to evaluate the pattern and extent of genetic variability and relatedness among some Egyptian rice genotypes based on important agronomic traits and some biotic stress using SSR markers. DNA marker analysis will help in identification and differentiation of genotypes with different genetic make-up. The information will enable maximized selection of diverse parents and assist in broadening the germplasm base of future rice breeding programs.

MATERIALS AND METHODS

Plant materials:

Twenty two Egyptian rice genotypes were used in this study. A detailed description of the materials used in the present investigation is shown in Table 1.

Table 1: Pedigree, type and year of release of the studied twenty two rice genotypes

No.	Entry	Pedigree	Type	Year of Release
1	Rieho	Akiyotaka / Ayanishki	J	1982
2	Giza159	Agmi M1 / Giza 14	J	1964
3	Giza171	Nahda / Calady 40	J	1977
4	Giza172	Nahda / Kinmaze	J	1977
5	Giza175	IR 28/ IR 1541/Giza 180 // Giza14	I/J	1989
6	Giza176	Calarose76/Giza172//GZ242-5	J	1991
7	Giza177	Giza171/Yomji No.1//Pi No.4	J	1995
8	Giza178	Giza175/Milyang49	I/J	1995
9	Sakha101	Giza176/Milyang79	I	1997
10	Sakha102	GZ4096-7-1/GZ4120-2-5-2 (Giza177)	I	1997
11	Sakha103	Giza177/Suweon349	J	1999
12	Sakha104	GZ4096-8-1/GZ4100-9	J	1999
13	Sakha105	GZ5581-46-3/GZ4316-7-1-1	J	2010
14	Sakha106	Giza177/Hexi30	J	Under release
15	Giza181	IR24/IR22	I	1988
16	Giza182	Giza181/IR39422-161-1-3 // Giza181	I	1999
17	Egyptian Yasmin	IR262-43-8-1 / NAHNG SARN	I	1992
18	GZ1368	IR1615-31 / BG90-2	I	Promising Line (Salt)
19	GZ6296	AC1225/Hua Lien Yu202	I/J	Promising Line Under release
20	GZ6903	GZ4596-3-4-2/Suweon372	J	Promising Line Under release
21	GZ7576	GZ5418/Milyang79	J	Promising Line Under release
22	GZ7769	Sakha104/Akiyutaka	J	Promising Line Under release

J: Japonica Type, I: Indica Type and I/J: Indica japonica Type

Microsatellite marker analysis:

Total genomic DNA was extracted from five individuals of each genotype using CTAB (Cetyl-tetramethyl ammonium bromide) method according to Murray and Thompson (1980) at twenty one days age. Purity and concentration of DNA was monitored spectrophotometrically at a wavelength of 260 and 280 nm using Nano-Drop ND-1000 Spectrophotometer (Wilmington, USA). All DNA samples were diluted to a working concentration of 20 ng/μl with TE before use. An equal amount of genomic DNA from 5 individuals of each genotype was mixed to make a bulk sample for microsatellite PCR analysis.

Twelve primer pairs were selected for the genetic diversity analysis on the basis of published rice microsatellite framework map (Table 2). The original source, repeat motifs, primer sequences and chromosomal positions for these markers can be found in the rice genome database (<http://www.gramene.org>).

Table 2: SSR markers used in the current study and some of their basic features.

Marker Name	Chr.	Exp. Pro. size	Motif	F primer seq.	R primer seq.
RM579	1	182	(GA)25	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT
RM488	1	177	(GA)17	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCCAGCGTATGC
RM341	2	172	(CTT)20	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
RM475	2	235	(TATC)8	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
RM282	3	136	(GA)15	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG
RM241	4	138	(CT)31	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
RM249	5	121	(AG)5A2 (AG)14	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC
RM454	6	268	(GCT)8	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
RM 70	7	170	(ATT)33	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC
RM215	9	148	(CT)16	CAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
RM228	10	154	(CA)6 (GA)36	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
RM 21	11	157	(GA)18	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG

SSR analysis was performed following the protocol of Ravi *et al.*, (2003) with minor modifications. PCR amplification reactions were carried out in a total volume of 20μl containing; 10 mM Tris HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl₂; 200 μM each of deoxynucleotide triphosphate (dNTP); 0.2 μM of each forward and reverse primer; 1 unit Taq DNA polymerase (Fermentas Life Sciences); and 20 ng of template DNA. The PCR amplifications were carried out using a MyGene Series Peltier Thermal Cycler (UniEquip GmbH, Munich, Germany). Thermal cycler was programmed to 1 cycle of 5 min at 94°C as an initial hot start and strand separation step. This was followed by 35 cycles of 1 min at 94°C for denaturation, 1 min for annealing temperature depending on the marker used (55°C-65°C) and 2 min at 72°C for primer elongation. Finally, 1 cycle of 7 min at 72°C was used for final extension. Amplified products were stored at -20°C until further use. The reproducibility of the amplification products was checked twice for each primer.

Allele scoring and data analysis:

Ethidium bromide staining of agarose gels generally showed several bands. The size of the most intensively amplified band for each microsatellite marker was determined based on its electrophoretic mobility relative to molecular weight markers (increments of 50 bp). Amplified products from SSR analysis were scored qualitatively for presence and absence of each marker allele-genotype combination. Each SSR band amplified by a given primer was treated as a unit character. Data was entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character. The most informative primers were selected based on the extent of polymorphism. The polymorphic information content (PIC) value of a marker was calculated according to Anderson *et al.*, (1993). Mean allele numbers, PIC values, and genetic similarities were calculated on the basis of different rice genotypes and microsatellite classes. Genetic similarities between all pair of the genotypes were calculated according to Nei and Li (1979). A cluster diagram was constructed based on these distances by the UPGMA (average linkage) method to develop a dendrogram. The similarity matrix, genetic distances and dendrogram analysis were computed using Numerical Taxonomy and Multivariate Analysis system, Version 2.1(Rolhf, 2000 NTSYSpc).

Experimental field:

The present experiment was carried out at Research Farm of Rice Research and Training Center, Sakha, Kafr EL-Sheikh, Egypt, during 2009 and 2010 summer seasons. Twenty two Egyptian rice genotypes were used (as shown from table) to study genetic diversity assessment using morphological and SSR markers. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Thirty day old seedlings of each genotype were individually transplanted in seven rows/plot/replicate with spacing of 20 x 20 cm a part between rows and plants. Plant height (cm), number of panicles/plant, number of filled grains/panicle, 1000-grain weight (g), flag leaf area and grain yield (t/ha) were recorded according to the Standard Evolution System (SES) for rice, IRRI, 1996. Total chlorophyll content in flag leaf area was recorded using chlorophyll meter (5 SPAD-502 minolta Camera Co.ltd., Japan) at heading stage. All recommended agricultural practices were applied for the permanent rice field. Weeds were chemically controlled by 2 liters of Saturn. The collected data were analyzed for analysis of variances according to Gomes and Gomes (1984).

Biotic Stresses Scoring

Blast nursery test:

Twenty two Egyptian rice genotypes were evaluated under field conditions at three locations i.e. Sakha, Gemmiza and Zarzora for blast resistance at seedling stage for major gene resistance with natural infection at blast nursery test (as recommended for disease assessment). Seedbeds were prepared during the first week of July in each season and fertilized with nitrogen in the form of urea (46.5%N) at the rate of 60 Kg nitrogen per feddan and manured (8 m³ farm-yard manure /fed) and prepared for seeding the

varieties. Width of the seedbed was one meter and 10.5 m long, at the beginning and end of each seedbed, five rows of Giza 159 (blast susceptible spreader) were sown, then five of the considered varieties, and again one row of the spreader, with 15 cm apart. Another five varieties were sown, followed by one row of the resistant check (Giza 181). The susceptible and resistant checks were sown alternatively, surrounding five of the considered varieties. The varieties were left exposed for natural blast infection at seedling stage. About forty-days from sowing, the typical blast lesions were scored, according to the standard evaluation system using 0-9 scale (IRRI 1996) as follows:

- 1-2 = resistant (R)
- 3 = moderately resistant (MR)
- 4-6 = susceptible (S)
- 7-9 = highly susceptible (HS)

Artificial inoculation:

All varieties were evaluated for all diseases under natural infection except brown spot and kernel smut. Blast, brown spot and bakanae were tested at seedling stage, while neck blast infection, false smut, kernel smut and sheath rot after complete heading. Only white tip nematode was tested after forty five days from transplanting during appearance of symptoms. Concerning artificial inoculation for brown spot, Twenty two Egyptian varieties were seeded in plastic trays (30 x 20 x15 cm.). Each tray comprised 20 rows representing twenty varieties in four replications. The trays were kept in the greenhouse at 25-30°C, and fertilized with Urea 46.5%N (5 gm/tray). The plants were inoculated for evaluation under greenhouse conditions with virulent isolate of brown spot. Seedlings were ready for inoculation at 3-4-leaf stage, about 3-4 weeks after sowing.

Twenty days old seedlings, in the trays, were inoculated by spraying with water suspension of isolates. Spore suspension(100 ml) was prepared and adjusted to 5×10^4 spores/ml. Gelatin was added to the spore suspension at a concentration of 2.5 g/L (Bastiaans, 1993) to enhance the adhesion of spores on leaf surfaces and sprayed using electrical spray gun. The inoculated seedlings were held in a moist chamber with at least 90% R.H. at 25-28 oC for 24 hr, and then moved to the greenhouse. Ten days after inoculation, severe symptoms were appeared on different rice cultivars. Brown spot infection was scored, according to the Standard Evaluation System of IRRI, 1996.

For kernel smut, all cultivars were inoculated three times during flowering stage at the period of florets opening (11-1 pm) with suspension of 2×10^5 sporidia/ ml (allantoid sporidia). Gelatin was added to the inoculation with concentration of 2.5 g/L to enhance the adhesion of spores on florets surfaces. Samples were taken at the end of the season.

All diseases infection was scored, according to the Standard valuation System of IRRI 1996.

RESULTS AND DISCUSSION

Mean performance:

Mean performance for all studied traits is presented in Table 3. The data revealed highly significant mean squares for all studied traits, suggesting the presence of genetic variations among the rice genotypes for all studied traits. Regarding the chlorophyll content of leave, data in Table 3 indicated that rice varieties varied significantly in their chlorophyll content. Sakha 101, Sakha 104 and Giza 176 gave the highest values 46 and 45 SPAD while, Reho recorded the lowest one 33 SPAD, The differences among the tested rice varieties in chlorophyll content may be attributed to the background of rice variety, which is mainly affected by genetic and partially by the environmental factors such as fertilizer, soil conditions and weather. Similar findings were reported by Abd Alla (1996).

Regarding to flag leaf area, data in Table 3 indicated that rice varieties differ significantly in their flag leaf area. Egyptian Yasmine, GZ1368 and Giza 181 gave the highest flag leaf area 45, 44 and 43 cm², respectively. Meanwhile, Giza 177 and GZ7576 recorded the lowest flag leaf area 26.67cm². The differences among the rice varieties in their flag leaf area mainly due to nature of rice variety, similar trend found by Sedeek *et al.*, (2009).

For growth duration (days) the rice genotypes; Sakha 103, GZ7769 and GZ 6522 were earlier than the old rice varieties Giza 171, Giza 172, Giza 181 and Egyptian Yasmine by 25 days at least, thus these rice varieties consume less irrigation water, than the others.

Regarding to plant height, a significant differences among all the studied varieties were shown. Giza 171 recorded the tallest plant height of 127 cm while GZ6296 recorded the shortest one (86cm).

The rice lines, Giza 178, Sakha 101, Sakha 104, GZ6522 and GZ7955 recorded the highest values of number of panicles/plant.

For panicle length, data in table 3 showed that the rice genotypes; Giza 181, Giza 182, Egyptian Yasmine and Giza 178 gave the highest values of panicle length as compared with the other rice genotypes.

For number of filled grains/panicle data in table 3 showed that the rice genotypes; Giza 181, Giza 182, Egyptian Yasmine and Giza 178 gave the highest values of number of filled grains/panicle as compared with the other rice genotypes, indicating that there are positive correlation between panicle length and number of filled grains/panicle.

As for 1000-grain weight, Sakha 102 and GZ7955 recorded the heaviest 1000-grain weight 28.3 and 28 g while Giza 178, Giza 171 and GZ1368 gave the lowest 1000-grain weight (21, 22.6 and 23 g , respectively). These differences among the genotypes may be due to the differences in their genetic background. These results are in close agreement with those obtained by Hammoud *et al.*, (2006).

The rice lines, Giza 178, Sakha 101, Sakha 104, GZ6522 and GZ7955 recorded the highest values of grain yield (t/ha). The superiority of these varieties in grain yield might be due to their higher number of panicles/plant and their agronomic efficiency.

Table 3: Mean performances of the rice genotypes for some agronomic studied traits.

No.	Variety	chlorophyll content (SPAD) (mg/g)	Flag leaf area (cm ²)	Duration (day)	Plant height (cm)	No. of panicle/ plant	Panicle length (cm)	Filled grains/ panicle	1000-grain weight (g)	Grain yield (t/ha)
1	Reiho	33.0	30.2	144.0	113.0	21.0	21.7	133.3	26.3	9.3
2	Giza 159	38.0	32.2	146.3	122.0	19.3	21.7	125.0	26.0	7.4
3	Giza 171	37.0	28.0	159.0	127.0	20.7	20.7	140.0	22.6	6.8
4	Giza 172	38.0	29.0	150.0	122.0	20.3	23.0	129.0	24.3	7.1
5	Giza 175	37.0	27.0	131.3	94.0	22.3	22.7	134.3	20.7	9.8
6	Giza 176	45.0	30.0	149.0	99.0	21.0	20.7	141.3	25.3	8.8
7	Giza 177	44.0	26.7	124.7	101.0	19.0	22.0	119.0	27.7	9.8
8	Giza 178	42.0	41.0	135.0	100.0	22.3	25.3	158.3	21.0	10.5
9	Giza 181	35.7	43.0	150.0	100.0	21.0	26.7	151.3	25.0	8.3
10	Giza 182	36.0	40.7	127.0	98.0	22.3	22.3	155.7	25.3	9.3
11	Sakha 101	46.0	34.0	141.0	89.0	23.0	24.0	151.3	27.7	10.9
12	Sakha 102	42.7	28.0	125.0	106.7	20.0	21.7	129.3	28.3	9.7
13	Sakha 103	43.0	29.0	122.0	98.0	21.0	22.3	128.0	25.0	10.0
14	Sakha 104	45.0	30.7	135.0	107.7	21.7	26.0	137.7	26.7	10.4
15	E.Yasmin	39.0	45.0	150.0	105.0	21.7	25.0	167.0	26.0	7.5
16	GZ 1368	35.0	44.0	147.0	105.0	24.0	22.0	153.7	23.0	9.8
17	GZ6296	44.0	29.0	126.0	86.0	22.0	23.3	114.0	25.0	9.0
18	GZ6522	41.7	37.0	125.0	98.3	20.0	21.7	131.0	27.0	10.7
19	GZ6903	44.0	32.0	134.3	94.7	21.3	21.7	130.0	27.7	10.0
20	GZ7576	37.3	26.7	127.0	101.3	21.0	21.7	124.7	26.0	9.4
21	GZ7769	39.0	28.7	123.0	104.0	21.7	21.7	142.3	27.0	9.7
22	GZ7955	42.0	34.0	128.0	107.0	22.7	23.0	155.7	28.0	10.9
	LSD 5 %	1.6	1.4	1.7	1.8	1.4	1.7	3.9	1.4	0.2

Biotic Stresses Results:**Blast disease**

For blast disease assessment as shown in Table 4, old rice cultivars such as Giza 159, Giza 176, Giza 171 and Giza 172 were highly susceptible. While, resistance to blast for Sakha 101 and Sakha 104 was broken-down in 2004 with the appearance of the specific variulant races and became susceptible. Still Giza 177, Sakha 102 and Sakha 103 shown resistant and became good sources for blast resistance. Giza 178 and other indica rices appeared to be resistant. All new promising lines also were resistant (GZ 6522, GZ6903 and GZ7955) (RRTC, 2009a)

Brown spot disease

Concerning brown spot under artificial inoculation (Table 4), all tested indica rices exhibited high level of resistance such as Giza 178, Giza181 and GZ 6296. All japonica rice cultivars were susceptible and shown highly susceptibility. Under natural infection all tested cultivars exhibited resistance more than 90 % except Giza 171, Giza 177; Sakha 102 and Sakha 104 were highly susceptible. These results in accordance with RRTC, (1994) and Osman: *et al.*, (2005) which they found that infection with brown spot varied according to different tested varieties. The infection was higher on Giza 177 and Giza 171 rice cultivars as compared with the other cultivars tested while the least infected cultivar is Sakha 104. The cultivar Giza 171 had the highest

percentage of discolored grain, followed by Giza 176, while Giza 178 was the least one.

False smut disease

False smut disease of rice caused by *Ustilagoidea virens* (Cooke) Takahashi, a major disease causing significant losses in yield in certain areas of the world, (Ou, 1985). In Egypt false smut disease was observed for the first time, in 1997 at Kafr El-Sheikh governorate on the rice plants of Cv. Giza 171. Now, it was found in some fields at the 6 rice growing governorates on both early and late maturing cultivars. In general no resistant genotype were shown for all genotypes (Sehly *et al.*, 2000; Tahoon, 2005 and RRTC, 2009 b)

Kernal smut disease

Kernel smut disease was shown in score (Table 4), all the tested varieties exhibited different levels of infection, the old cultivars such as Giza 171, Giza 172, Giza 159, Giza 175, Giza 177 and Giza 178 were highly susceptible. Also Reiho, Sakha 102 and Sakha 103 were susceptible. The new GZ lines and the rest of tested varieties exhibited low level of infection and are moderately resistant. Although Sakha 101 and Sakha 104 occupied more than 70 % of the total cultivated area but exhibited low level of infection.

White tip nematode disease

For White tip nematode score (Table 4), all indica rices were resistant to *Aphelenchoides besseyi*; Giza 175, Giza 181, Giza 182 and Egyptian yasmen. On the other side, all japonica rices were susceptible except Giza 159, Giza 176 and Sakha 104. While, indica japonica cultivars were resistant; Giza 178 and GZ 6296. All GZ lines under study were susceptible but exhibited high level of tolerance and gave acceptable production and yield. These results are in harmony with the data obtained by El-Shafey (2007).

Rice bakanae disease

For Bakanae, under natural infection (Table 4), Sakha 101 and Giza 177 were highly susceptible and showed high level of infection in nursery bed. Also, GZ 6522 was susceptible to *Fusarium moniliforme*. The rest of japonica, indica japonica and indica rices were resistant. These results were in accordance with the data obtained by Gabr (2010) due to the genetic make up of those genotypes.

Sheath rot

Sheath rot, disease of rice caused by *Sarocladium oryzae* (Sawada) it is present and very common in Southeast Asia, India and different rice ecosystems (Ou, 1985). In Egypt, it was considered a minor disease, the fungus infected individual panicles in some rice varieties.

For sheath rot (Table 4), the most of rice varieties were resistant, except some old commercial cultivars; Giza 159, Giza 176 and Reiho exhibited individuals infected panicles. For indica type, only; Giza 181, Giza 182 and Egyptain Yasmin exhibited a minor infection. Also, Sakha 104 recorded the lowest level of infection under natural infection. From the reaction of rice varieties to different diseases, these varieties exhibited a broad spectrum of variability, so it could be identified as resistant sources for transferring the resistance from such varieties.

Table 4: Reaction of the studied twenty two rice genotypes to different diseases during season.

No	Varieties	Type	Blast	Brown spot	False smut	Kernel smut	White tip neamtode	Bakanae	Sheath rot
1	Reiho	J	HS	HS	S	S	HS	R	S
2	Giza 159	J	HS	HS	S	HS	R	R	S
3	Giza 171	J	HS	HS	HS	HS	HS	S	R
4	Giza 172	J	HS	HS	HS	HS	HS	R	R
5	Giza 175	I/J	R	MR	S	HS	R	R	R
6	Giza 176	J	HS	S	S	MR	R	R	S
7	Giza 177	J	R	HS	HS	HS	S	HS	R
8	Giza 178	I/J	R	MR	S	HS	R	R	R
9	Giza 181	I	R	MR	S	MR	R	R	S
10	Giza 182	I	R	MR	S	MR	R	R	S
11	Sakha 101	J	HS	S	S	MR	S	HS	R
12	Sakha 102	J	R	HS	HS	S	S	R	R
13	Sakha 103	J	R	MR	S	S	S	R	R
14	Sakha 104	J	HS	S	S	MR	R	R	S
15	E. yasmine	I	R	MR	S	MR	R	R	S
16	GZ 1368	I	R	MR	S	MR	R	R	R
17	GZ6296	I/J	R	MR	S	MR	R	R	R
18	GZ6522	J	R	S	S	MR	S	S	R
19	GZ6903	J	R	HS	S	MR	S	R	R
20	GZ7576	J	R	S	S	MR	S	R	R
21	GZ7769	J	R	MR	S	MR	HS	R	R
22	GZ7955	J	R	MR	S	MR	S	R	R

R, Resistant; S, susceptible; HS, highly susceptible; MR, Moderately resistant.

Clustering of the genotypes based on biotic stresses reaction:

A dendrogram was constructed based on Numerical Taxonomy System of Multivariate Programs (NTSYS) similarity coefficients, in which the twenty two rice genotypes were grouped into two major groups (Fig. 1). Group I consisted of the most susceptible Egyptian rice varieties for biotic stresses (Giza171 and Giza172). Group II consisted of 20 rice genotypes, which are further subdivided into two clusters, the most cultivated Egyptian rice varieties and they became nowadays susceptible for a lot of different rice diseases Sakha101 and Sakha104 come separately alone in cluster-I. Cluster-II containing 18 genotypes, further divided into three sub clusters. Sub cluster-I containing the most resistant Egyptian rice genotypes (Giza177 and Sakha102) with 0.54 similarities, and they came together because they sharing have the same reaction for resistance score for most of the biotic stresses. Sub cluster-II containing three rice genotypes (Reiho, Giza159 and Giza176); they came together because they sharing for most of the susceptible score reactions. Sub cluster-III containing remains of the 13 rice genotypes, which are further subdivided into five sub-sub clusters. The Egyptian indica/japonica rice variety (Giza178) comes separately alone because it has most of the resistance score reaction for most of the biotic stresses. The Egyptian indica rice varieties (Giza181, Giza182 and GZ1368) come alone because they have the genetic background and also, they have

most of the resistance score reaction for most of the biotic stresses.

The results indicated that most of the Egyptian japonica rice varieties, which are the widest distributing and cultivating, they have the most of susceptible sources for biotic stresses. So, nowadays it is become clearly essential to broaden the genetic base of the rice varieties cultivated in Egypt to reduce its vulnerability to diseases and insect pests, El-Shafey, 2007 and Gabr, 2010.

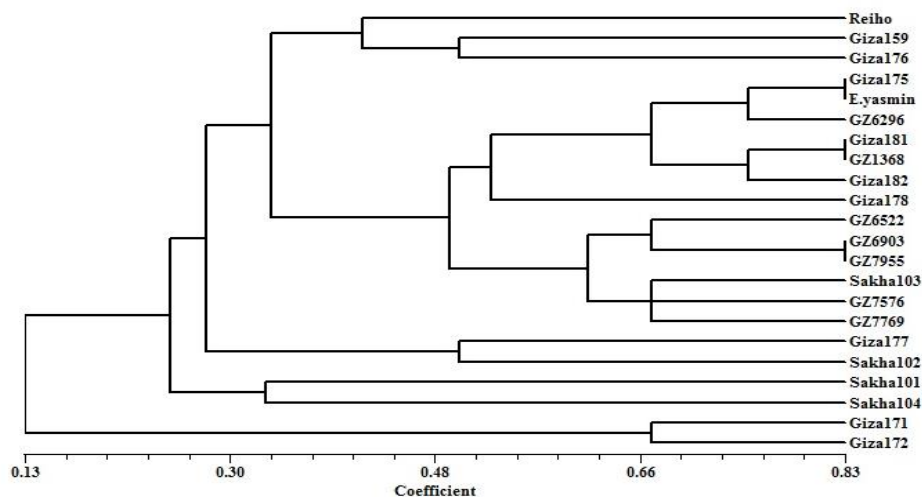


Fig. 1. UPGMA cluster analysis showing the diversity and relationship among 22 Egyptian rice genotypes based on the biotic stresses reaction.

Microsatellite markers diversity

Most of the rice genotypes used in this study can be considered as standard rice genotypes for evaluating allelic diversity in Egyptian rice varieties (Fahmi *et al.*, 2005 and El-Malky *et al.*, 2007). All twelve rice microsatellite markers that used in this study were found to be polymorphic among the twenty two rice genotypes and produced a total of 73 alleles (Table 5). Figure 2 shows the PCR amplified products from the twenty two genotypes for specific SSR markers to RM488 and RM341. Also, these figures for example, show the size standard, which was effective for estimating the molecular weights for all PCR products amplified across all rice genotypes. These sizes were predicted from sequencing the clone used to isolate microsatellites (McCouch *et al.*, 2002). Each individual microsatellite had a typical fragment pattern that could be easily recognized and the most intense fragment was evaluated for molecular weight.

The maximum number of polymorphic alleles (i.e. 10 bands) was obtained with the marker RM488, while the minimum number of polymorphic bands (3 alleles) was amplified with the marker RM282 (Table 5). The average number of polymorphic alleles per locus was 6.08.

Table 5: Characterization of the SSR markers used in the current study.

SSR Locus	Number of alleles	Allele size (bp)		Difference (bp)	PIC*
		Min	Max		
RM 579	6	152	190	38	0.518
RM 488	10	147	240	93	0.716
RM 341	6	155	200	45	0.642
RM 475	5	185	235	50	0.593
RM 282	3	126	180	54	0.667
RM 241	5	138	170	32	0.592
RM 249	7	111	165	54	0.345
RM 454	5	228	310	82	0.617
RM 70	5	150	220	70	0.677
RM 215	7	148	210	62	0.494
RM 228	6	114	170	56	0.592
RM 21	8	115	195	80	0.716
Mean	6.08				0.588

* Polymorphic information content

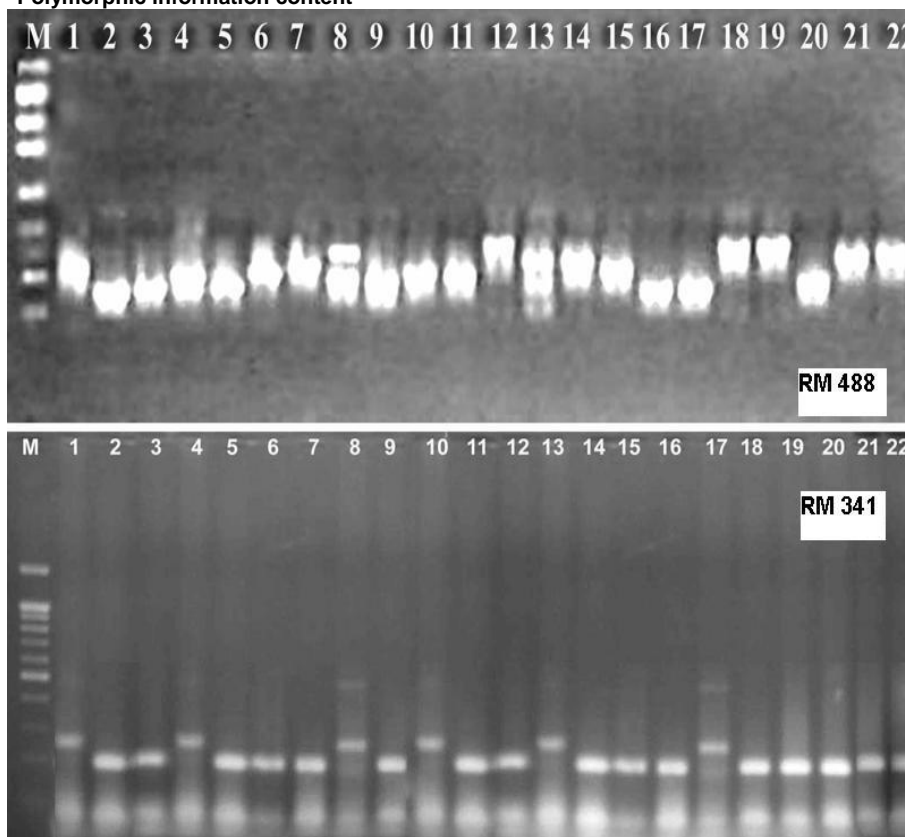


Fig. 2: Genomic amplification using primers specific to RM488 in the upper image showed 8 alleles in all the rice genotypes and RM341 in the down image showed 6 alleles in all the rice genotypes. M is 100bp ladder.

The overall size of the amplified product varied from 111 bp (RM249) to 310 bp (RM454). The molecular size difference between the smallest and the largest allele for a given locus varied from 32 to 93 bp. The level of polymorphism among the genotypes was evaluated by calculating alleles number and PIC values for each of the 12 SSR markers evaluated (Table5). The PIC values, a reflection of allele diversity and frequency among the genotypes, were high for all microsatellites with average of 0.588 and ranged from a low of 0.345 for RM249 to a high of 0.716 for RM488 and RM144.

Genetic relationships among rice genotypes

The UPGMA cluster diagram grouped the 22 Egyptian rice genotypes into two major groups at 100% confidence interval, effectively differentiating the indica rice genotypes from the japonica and indica/japonica ones at similarity coefficient value of 0.69 (Figure 3). The classification of the Egyptian rice genotypes was in agreement with the parentage presented in Table 1 and with the previous studies of the same genotypes (Fahmi *et al.*, 2005 and El-Malky *et al.*, 2007).

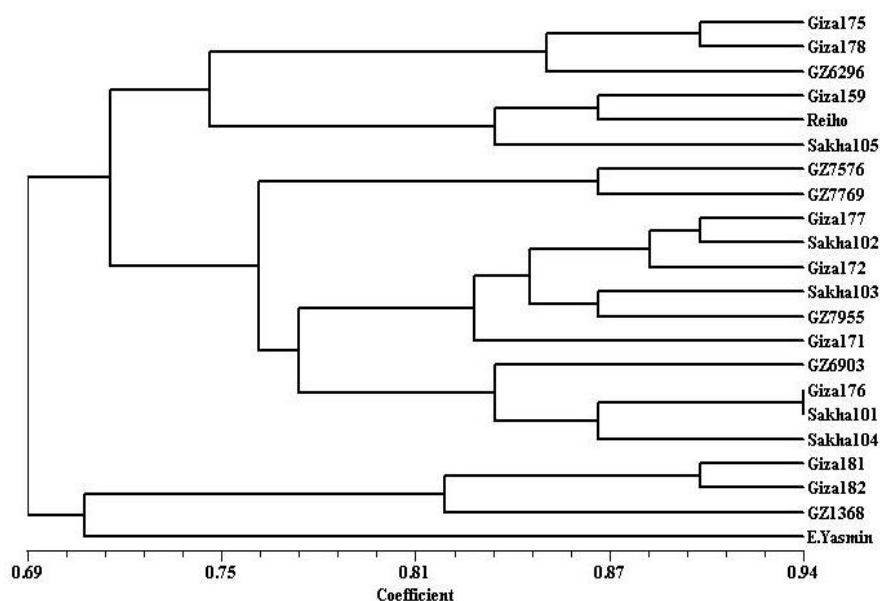


Fig. 3: UPGMA cluster analysis showing the diversity and relationship among 22 Egyptian rice genotypes based on 73 alleles generated by 12 SSR markers.

Group I is comprised of indica varieties and consisted 4 genotypes, which are further subdivided into two clusters, the Egyptian aromatic indica variety (Egyptian Yasmin) comes separately alone in cluster-I. Cluster-II containing three genotypes (Giza181, Giza182 and GZ1368), further divided into two sub clusters with 0.82 genetic similarities.

Group II is comprised japonica and indica/japonica varieties and consisted 18 genotypes, which are further subdivided into two clusters, cluster-I containing six genotypes, further divided into two sub clusters with 0.74 genetic similarities. Sub cluster-I containing the Egyptian indica/Japonica genotypes (Giza175, Giza178 and GZ6296) with 0.84 genetic similarities, and they came together because Giza175 was used for producing Giza178 and became one of the ancestors for the new promising line GZ6296. Sub cluster-II containing three genotypes (Giza159, Reiho and Sakha105) with 0.82 genetic similarities. Cluster-II containing the Egyptian japonica genotypes with 0.74 genetic similarities, which are further subdivided into three sub-clusters. Sub cluster-I containing two genotypes GZ7576 and GZ7769 (new promising lines). Sub cluster-II containing six genotypes (Giza172, Giza177, Giza171, Sakha102, Sakha103 and GZ7955 (Sakha106)) with 0.82 genetic similarities. The Sub cluster-II came together because Nahda variety was used for producing Giza171 and Giza172 where these two varieties played a major role for producing Giza177, Sakha102 and Sakha103 therefore; they became the ancestors of Sakha106. Sub cluster-III containing four genotypes (Giza176, Sakha101, Sakha104 and GZ6903) with 0.83 genetic similarities. The closest genotypes are Giza176 and Sakha101 with 0.94 genetic similarities, because Giza176 was used for producing Sakha101. This result proved that microsatellite markers were a good tool for testing genetic diversity (Nagaraju *et al.*, 2002; Ni *et al.*, 2002 and Jain *et al.*, 2004).

One main cause of eradication of plant genetic sources has been the adaptation of narrowly based advanced varieties for intensive cultivation practices. The low genetic diversity found among the Egyptian rice genotypes evidence the narrow genetic bases used in our breeding programmes. A pedigree analysis included in this work, showed a strong contribution of only few progenitors to main varieties and new promising breeding lines. The results indicated that it is essential to broaden the genetic base of the rice varieties cultivated in Egypt to reduce its vulnerability to diseases and insect pests. Recent studies carried out by the International Rice Research Institute showed there is still a tremendous amount of unexploited genetic diversity in the primary gene pool of rice that can be used for enhancing the diversity in local germplasms and their performance under diverse agroecological conditions (Ali *et al.*, 2006 and Lafitte *et al.*, 2006). Wild species of *Oryza* also represent a potential source of new alleles for improving yield, quality, and stress resistance in rice cultivars (McCouch, 2004; Kovach and McCouch, 2008). Several studies report improvements in performance because the introgression of valuable genes from wild germplasm into elite rice cultivar. Lines derived from crossing the wild species *Oryza rufipogon* with *Oryza Sativa* cultivars showed higher yields than their progenitors and are tolerant to several abiotic stresses (Nguyen *et al.*, 2003; Tian *et al.*, 2006 and McCouch *et al.*, 2007). Yield and grain quality enhancing alleles have also been identified from *O. glaberrima* (Li *et al.*, 2004 and Sarla and Swamy, 2005) and *O. glumaepatula* (Brondani *et al.*, 2002 and Rangel *et al.*, 2005). Utilization of these "novel" gene sources will be of great importance in national rice breeding program for maximizing the yield and providing new sources of the biotic stresses resistance.

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التنوع الوراثي المرتبط ببعض الصفات المحصولية و الأمراض باستخدام المعلمات الجزيئية في بعض أصناف الأرز المصرية

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يعتبر التنوع الوراثي ذو أهمية كبرى في تحسين المحاصيل. وفي مصر استخدام سجلات النسب الأكثر شيوعاً في تربية الأرز تعطي مؤشرات إلى أن الأصناف المزروعة حالياً ذات درجة قرابة وراثية عالية. ولذلك تظهر الحاجة إلى الاعتماد على برامج تربية فعالة تقوم على أساس معرفة التنوع الوراثي للأصناف لتوسيع القاعدة الوراثية في أصناف الأرز المحلية. أجريت هذه الدراسة لتقييم نمط ومدى التنوع الوراثي و الصلة بين بعض أصناف الأرز والسلالات المباشرة في مصر على أساس الصفات المحصولية الهامة و بعض الأمراض الهامة التي تصيب الأرز باستخدام المعلمات الجزيئية. تم استخدام ١٢ معلم جزيئي لتقييم التنوع الوراثي لـ ٢٢ صنف أرز مصري وسلالات جديدة مباشرة تم إنتاجها بواسطة البرنامج القومي لبحوث الأرز ما بين الفترة من ١٩٧٥ و حتى ٢٠١٠. و لقد أنتج الـ ١٢ معلم جزيئي المستخدم في هذه الدراسة ٧٣ البلا. وتراوحت عدد الأليلات لكل معلم جزيئي ما بين ٣ (RM282) إلى ٩ (RM388) بمتوسط قدره ٦,٠٨. و كانت قيم محتوى المعلومات ذو تعدد الأشكال المظهرية (PIC) كانت عالية لكل المعلمات الجزيئية المستخدمة بمتوسط ٠,٥٨٨ وتراوحت ما بين ٠,٣٤٥ (RM249) إلى ٠,٧١٦ (RM488, RM144). لقد أظهر تحليل UBGMA المعتمد على المسافة الرائية للتركيب الوراثية المستخدمة أن أصناف الأرز المصرية ذات درجة قرابة وراثية عالية و على الرغم من أن التنوع الوراثي ما بين التركيب الوراثية المستخدمة كان منخفضاً إلا أن تقنية المعلمات الجزيئية كانت أداة فعالة في تقييم التنوع الوراثي. ولقد تم مناقشة الآثار المترتبة على انخفاض التنوع الوراثي بين التركيب الوراثية المستخدمة. و من جهة أخرى أظهرت أصناف Sakha102, Giza177 و Sakha103 أنها لازالت تحتفظ بأكثر المصادر لمقاومة الأمراض و بالتالي تكون أداة فعالة في برامج التربية لمقاومة الأمراض.

قام بتحكيم البحث

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