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ASSESSMENT AND EVALUATION OF GENETIC DIVERSITY OF SOME LINES DEVELOPED FROM SAKHA101 CV. OF RICE BY USING SSR MARKERS

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ABSTRACT

Two experiments were conducted in this study, the first one was conducted at the Institute of Genetic Engineering and Bio Technology, Menoufia University Sadat branch using SSR markers during 2013. The second one was carried out at Rice Research and Training Center Sakha, Kafr El-Sheikh, Egypt during 2005 to 2013 season. Seven genotypes comprise five derived lines from the cross of Sakha101 X HR4856// Sakha101 were used to study the genetic diversity by using six SSR markers (SSR206; SSR216; SSR302; SSR209; SSR262 and SSR244). SSR markers (SSR206; SSR209 and SSR216) gave two alleles, while the primers of SSR302, SSR244 and SSR262 gave three alleles two one and four alleles for last one, respectively. Cluster analysis divided into two main groups, the first one included only SP70-101-1 line. This line was produced from back cross (Sakha101 X HR4856 //Sakha101), while the second group was divided into two subgroups; the first one included SP70-1،1-3. While, the second subgroup included HR4856 variety in one branch alone. On the other hand, the second subgroup included Sakha101 alone. Similarity distance showed that the two lines; SP70-101-2 and SP70-101-4 were closely related and similarity distance percentage between these lines is about 91%, while the far distant one was SP70-101-1 line and the similarity distant percentage was 60%. Mean square analysis was found to be highly significant for all yield and its component traits under studied at the last two years 2012 and 2013 and their combined data. Also, the interaction of genotypes with years was highly significant for plant height, duration, flag leaf area, number of panicles/ plant, number of grains/panicle, grain yield/plant, hulling %, milling % and head rice %, revealing that these genotypes behaved differently from one year to another year or can be affected by environment. The phenotypic coefficient variability (PCV) showed higher than genotypic coefficient variability (GCV) under two years and their combined data, indicated that effects of environment condition on these traits. Heritability estimates in broad sense was high for all studied traits and ranged between 91.85% for 1000-grain weight to 98.99% for plant duration. Genetic advance percentage (Δg %) value varied for all traits. Grain yield t/ha showed highly significant positive correlation with number of panicles/plant, number of grains/ panicle, flag leaf area and total duration.

INTRODUCTION

SSR markers are highly informative polymerase chain reaction (PCR) based markers that detect length polymorphisms at loci with simple sequence repeats (Powell *et al.,* 1996). Microsatellites or SSR are co-dominant markers and their map positions on the rice genome are well known. Over 500 microsatellite markers have been developed for rice (*Oryza sativa* L.), and their chromosomal location and level of polymorphism have been

determined (Temnykh *et al*., 2001). Also, McCouch *et al.*, (2002) have developed and mapped 2240 SSR markers for rice. They vary in the polymorphism and they detect, which depends on their position in coding or non-coding segments of the genome and the length and sequence of the repeat motif they contain (Cho *et al*., 2000 and Temnykh *et al*., 2000).

Rice microsatellites are ideal markers for characterizing genetic diversity among closely related rice varieties (Yang *et al*., 1994; Xiao *et al*., 1996; Olufowote *et al*., 1997; Chakravarthi and Naravaneni, 2006; El-Malky *et al.,* 2007 and Hammoud *et al.,* 2007).

The breeders in Egypt in 1996 made cross among resistant varieties to develop new verities i.e Sakha101 and Sakha104. Those varieties are the highest yielding and cover most of the cultivated area. Starting from 2004, those varieties have become moderately resistant to blast disease. The variety Sakha101 is Japonica type and released in 1997, from local cross between Giza176 and Milyang79. This variety had high yielding ability about 10t/ha, with 145 – days from total growth duration, resistant to lodging, resistant to blast until 2004 growing season, it had an excellent cooking quality (Rice in Egypt 2006).

Since, 2004 the variety Sakha101 was broken down by blast disease and still cultivated in large area with two fungicide treatments to avoid the yield losses. Fungicide application increases the pollution and raises production cost. However, the economical way is to produce resistant varieties, using Sakha101 as a donor for high yield and desirable characters. The main objective of this study is to use useful SSR markers for studying genetic diversity among the selected lines from the progeny after segregating generation; estimate some genetic parameters as will as to study the relationship and correlation coefficient between some morphological and the yield and its components traits for the selected promising lines and their parents.

MATERIALS AND METHODS

1- DNA experiment:

DNA was extracted from leaves during seedling stage of these varieties was carried out using CTAB method (Murray and Thompson, 1980). Available six SSR markers were used to amplify simple sequence repeat polymorphism (SSR) of genomic DNA from the seven rice genotypes (Table 1). The SSR markers were chosen on the basis of their published molecular weight, reliability of amplification signal and polymorphism information content (Table 2). The PCR was performed in 10μl PCR volume containing 50ng of template DNA, 5pmole of each of forward and reverse primers, 0.1mM dNTP`s, 1x PCR buffer (10mM Tris, pH 8.0, 50mM KCl and 50mM ammonium sulphate), 1.8mM $MgCl_2$, and 0.2units of Taq DNA polymerase. Initial denaturation at $94C^{o}$ for 5 minutes was followed by 35 cycles of amplification with template denaturation at $94C^{o}$ for 1 minute, primer annealing at $55.7C^{o}$ for 1 min and primer extension at $72C^{o}$ for 2min. At the

end of the 35th cycle, a final extension at 72C° for 7min was given followed by storage at 4.0C°. The PCR products were separated using 1.5% agarose gel stained with Et Br solution (1mg/l). The banding pattern was then scored and used to prepare the matrix. Employing the computer package NTSYS .pc (Rohlf, 2000), Jaccord`s similarity coefficients were calculated and used to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering.

Table 1: Parentage and the origin of the seven rice genotypes.

Genotypes	**Parentage**	**Origin**
Sakha 101	Giza 176 X Milyang 79	Egypt
SP 70-101-1	Sakha101 X HR4856 // Sakha101	Egypt
SP 70-101-2	Sakha101 X HR4856 // Sakha101	Egypt
SP 70-101-3	Sakha101 X HR4856 // Sakha101	Egypt
SP 70-101-4	HR4856 X Sakha101 // HR4856	Egypt
SP 70-101-5	HR4856 Xsakha101// HR4856	Egypt
HR4856-1-1-1-1-2	IRI346 X HR3499	South Korea

Table 2: Primer sequences and chromosome number of SSR markers were used.

No.	**Marker**	**Primers**
1	SSR206	Forward CCCATGCGTTTAACTATTCT Reverse CGTTCCATCGATCCGTATGG
2	SSR216	Forward GCATGGCCGATGGTAAAG Reverse TGTATAAAACCACACGGCCA
3	SSR302	Forward TCATGTCATCTACCATCACAC Reverse ATGGAGAAGATGGAATACTTGC
4	SSR209	Forward ATATGAGTTGCTGTCGTGCG Reverse CAACTTGCATCCTCCCCTCC
5	SSR262	Forward CATTCCGTCTCGGCTCAACT Reverse CAGAGCAAGGTGGCTTGC
6	SSR244	Forward CCGACTGTTCGTCCTTATCA Reverse CTGCTCTCGGGTGAACGT

2-1-Field experiment:

The present research was carried out at the farm of Rice Research and Training Center (RRTC), Sakha Kafr El- Sheikh, Egypt. Eight successive seasons starting from 2005 to 2013 (Table 3), two rice varieties; Sakha101 as (Egyptian variety) and HR4856-1-1-1-1-2 as (South Korean variety) were used as a parents. The cross between the two parents have started in 2005 to produce F_1 and backcrossed with Sakha101and HR4856 in 2006 and the experiment was laid out in a Randomized Complete Block design (RCB) with three replications. F_1, F_2, BC_1, BC_2 and parents were grown in five mater long as individuals plants by 20 X 20 cm and applied all the recommended cultural

practices for rice. Breeding scheme to form experimental materials from 2005 up to 2013 seasons clear in (Table 3). Ten agronomic traits were studied i.e. plant height (cm), duration (day), flag leaf area (cm^2), number of panicles/plant, 1000-grain weight (g), number of grains/ panicle, grain yield, hulling%, milling% and head rice%. Analysis of variance was computed by IRRISTAT program. While, heritability percentage was estimated on a plot basis as the ratio of genotypic and phenotypic variance, according to Johanson *et al*., (1955). Phenotypic, genotypic coefficients of variability and the expected genetic advance from selection (Δg %) were calculated according to Burton (1952).

Table 3: Breeding scheme of the materials started from 2005 up to 2013 growing seasons

No.	Years	Activity/process
1	2005	Crosses among two the parental varieties.
2	2006	F1 growing, backcrossing BC1, BC2
3	2007	Growing F2, BC1, BC2 and selection
4	2008	Selection
5	2009	Selection
6	2010	Selection
7	2011	Select 15 lines and blast screening.
8	2012	Growing the five lines in RCBD in deferent locations.
9	2013	Growing the five lines in RCBD in deferent locations.

2-2-Blast reaction under field and greenhouse conditions:

Seeds of each genotype were growing in plastic trays (30 x 20 x 15cm), each tray comprised the two parents i.e. Sakha101 and HR4856 and five derived lines. The trays were incubated in the greenhouse at 25 to 30C^o, and fertilizered with Urea 45.5 %. Seedlings were ready for inoculated at 3 to 4 leaf stage, about 3 - 4 weeks after sowing. All genotypes under this study were inoculated with 14 rice blast isolates i.e. IC-13, IC-15, II-1, ID-13, IC-30, ID-15, II-2, IC-15, II-3, II-4, II-5, II-6, IC-3 and IC-1I at 3-4 leaf stage. Rice seedlings of about 20 days old in the trays were inoculated by spraying with spore suspension (100ml) adjusted to 5 x 10^4 spores/ml., each isolate was sprayed using electrical spray gun. The inoculated seedlings were kept in chamber with 90% RH and 25 to 28C^o for 24 hr, and then moved to the greenhouse seven days of the inoculation. The blast reaction was recorded according to the Standard Evaluation Systems using 0 - 9 scale (IRRI 1996).

RESULTS AND DISCUSSION

3- Genetic diversity under Lab conditions:

Rice is a self-pollinated diploid crop, microsatellite markers (SSR) usually reveal single-copy, homozygous loci and allelic heterogeneity is rare in pure line varieties. These facts simplified the work of microsatellites for the

analysis of genetic diversity of rice cultivars. In this study, the level of polymorphism among genotypes was evaluated by six SSR markers and the data (Fig.1) showed that the primers SSR206, SSR2009 and SSR216 showed two alleles are presented. While, the primers SSR302 and SSR262 gave three and four alleles, respectively. Figure 1 shows that the primer SSR206, produced two alleles with two different molecular weights. The first allele (550Kb) was found in all genotypes except SP70-101-3 line (lane 4). While, the second allele (300Kb) was found in all genotypes except the same line (lane 4).

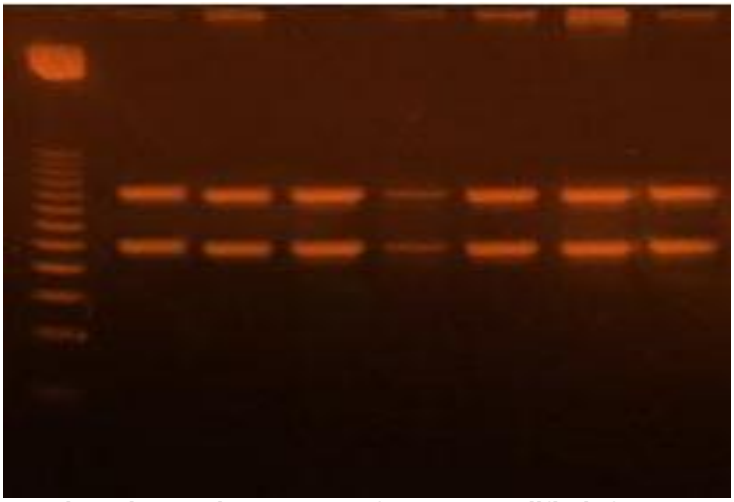

Figure 1: The electrophotogram of DNA amplified fragments using SSR2،6 for selected genotypes. First lane, 100bp DNA ladder; 1, Sakha101; 2, SP70-101-1; 3, SP70-101-2; 4, SP70-101-3; 5, SP70-101-4; 6, SP70-101-5 and 7, HR4856-1-1-1-1-2.

For RM209, Figure 2 shows that two alleles were found; the first one (300Kb) was found in all genotypes except SP70-101-1; 3 (lane2), whereas the second one (170Kb) was found in Sakha101; SP70-101-2; SP70-101-3 and SP70-101-4 (lanes1, 3, 4, and 5). The second allele (170Kb) gave the same trend.

For RM216, (Figure 4) shows that two alleles were present; the first one (850Kb) was found in genotypes; SP70-101-2; SP70-101-3; SP70-101-4; SP70-101-5 (lanes3, 4, 5, and 6). While, the second one (570Kb) was found in all genotypes except SP70-101-4; and SP70-101-5(lanes; 5 and 6). The marker SSR244 gave two alleles; the first one (300Kb) was found in all genotypes, whereas the second one (250Kb) was found in the lines; SP70-101-4 and SP70-101-5 (lanes5 and 6).

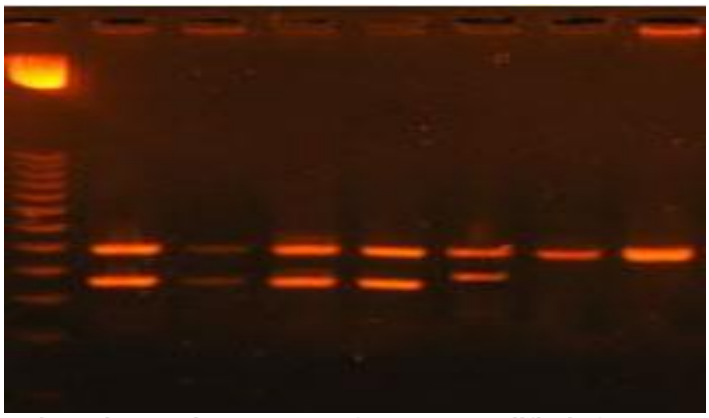

Figure 2:The electrophotogram of DNA amplified fragments using SSR209 for selected genotypes. First lane, 100bp DNA ladder; 1, Sakha101; 2, SP70-101-1; 3, SP70-101-2; 4, SP70-101-3; 5, SP70-101-4; 6, SP70-101-5 and 7, HR4856-1-1-1-1-2.

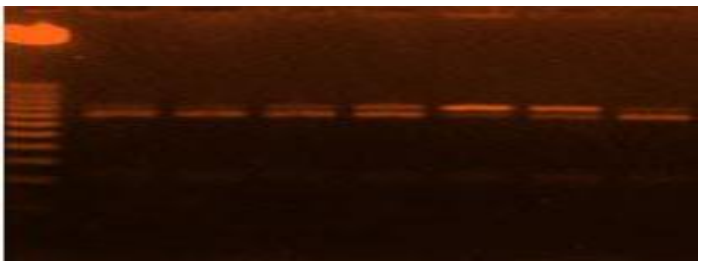

Figure 3: The electrophotogram of DNA amplified fragments using SSR216 for selected genotypes. First lane, 100bp DNA ladder; 1, Sakha101; 2, SP70-101-1; 3, SP70-101-2; 4, SP70-101-3; 5,SP70-101-4; 6,SP70-101-5 and 7, HR4856-1-1-1-1-2.

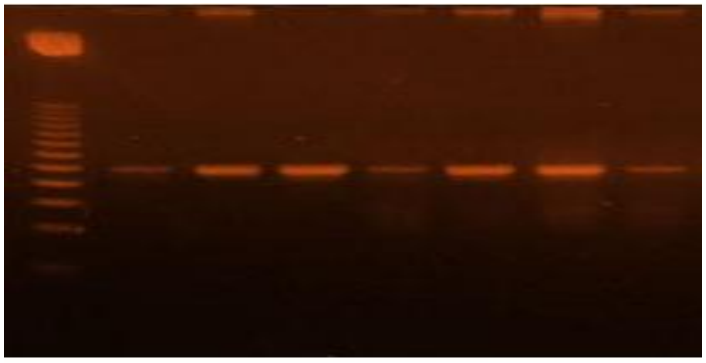

Figure 4: The electrophotogram of DNA amplified fragments using SSR244 for selected genotypes. First lane, 100bp DNA ladder; 1, Sakha101; 2, SP70-101-1; 3, SP70-101-2; 4, SP70-101-3; 5, SP70-101-4; 6, SP70-101-5 and 7, HR4856-1-1-1-1-2.

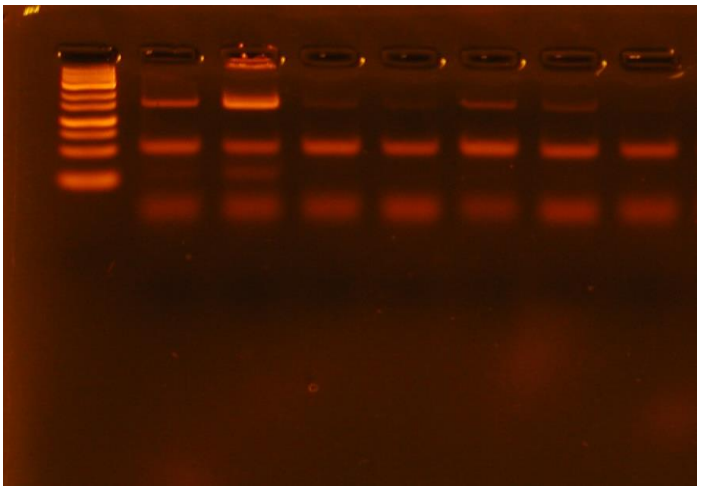

Figure 5: The electrophotogram of DNA amplified fragments using SSR262 for selected genotypes. First lane, 100bp DNA ladder; 1, Sakha101; 2, SP70-101-1; 3, SP70-101-2; 4, SP70-101-3; 5, SP70-101-4; 6, SP70-101-5 and 7, HR4856-1-1-1-1-2.

For SSR302, Figure 6 shows that three alleles were found; the first one (500Kb) was found in genotypes Sakha101, SP70-101-1, SP70-101-4 and SP70-101-5 (lanes1, 2, 5, and 6). While, the second one (200Kb) was found in all genotypes and the third was (100Kb) in Sakha101 and SP70-101-1 (lanes1 and 2).

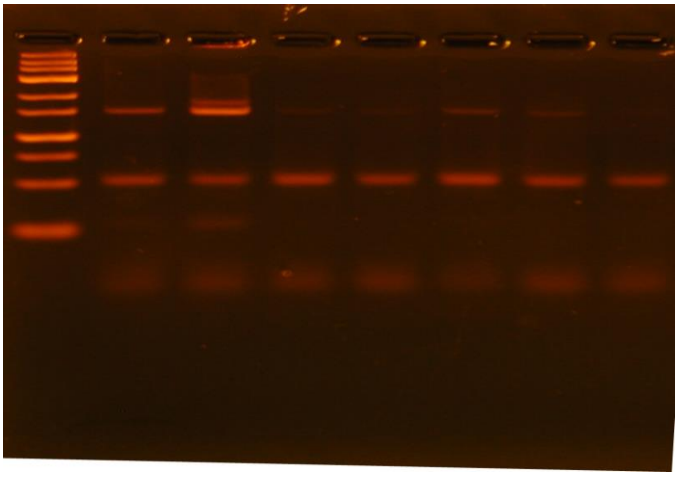

Figure 6: The electrophotogram of DNA amplified fragments using SSR302 for selected genotypes. First lane, 100bp DNA ladder; 1, Sakha101; 2, SP70-101-1; 3, SP70-101-2; 4, SP70-101-3; 5, SP70-101-4; 6, SP70-101-5 and 7, HR4856-1-1-1-1-2.

4- Cluster analysis:

The UPGMA cluster diagram shows the genetic variation pattern, and a coefficient ranged between 0.42 to 0.91; these values indicated that the genotypes used were relative in their genetic background (Figure 7). The classification of the genotypes were divided into two main groups, the first one included only SP70-101-1 line. This line was produced from the parentage (Sakha101 X HR4856) // Sakha101. The second group was divided into two subgroups; the first one included SP70-1-1-3 produced from the same genetic background. The second subgroup included HR4856 variety in branch one alone. On the other hand, the second subgroup included Sakha101 alone. In other words, the former one included SP70-101-5; SP70-101-4 and SP70-101-2. Distance similarity presented in Table 4 and show that the two lines SP70-101-2 and SP70-101-4 were closely and the similarity between these lines is about 91%, while the far one was SP70-101-1 with distance similarity of 60%.

Therefore, this analysis can be expected to greatly increase the efficiency of genetic-diversity assessment, variety fingerprinting and identification, the genetic physical mapping of genes and quantitative trait loci and marker-assisted selection during breeding process. These results proved that microsatellite markers were a proper tool for testing genetic diversity Yu *et al.,* 2003; McCouch *et al.*, 2004; Fahmi *et al*., 2005; Thomson, *et al*., 2005.; Chakravarthi and Naravaneni, 2006; El-Malky, *et al* ., 2007 and Hammoud, *et al*., 2007).

Table 4: Similarity matrix of the seven genotypes based on 6 SSR markers.

Genotypes	Sakha 101	SP70-101-1	SP70-101-2	SP70-101-3	SP70-101-4	SP70-101-5	HR4856-1-1-1-1
Sakha101	1.00						
SP70-101-1	0.600	1.00					
SP70-101-2	0.769	0.571	1.00				
SP70-101-3	0.615	0.428	0.800	1.00			
SP70-101-4	0.846	0.642	0.909	0.727	1.00		
SP70-101-5	0.769	0.692	0.818	0.636	0.909	1.00	
HR4856-1-1-1-1-2	0.616	0.538	0.800	0.666	0.727	0.800	1.00

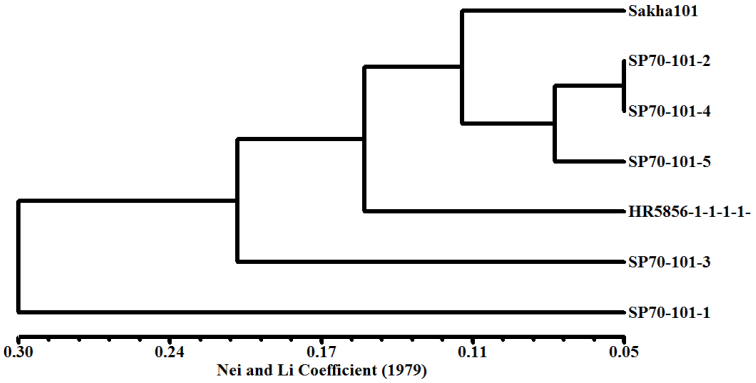

Figure 7: Dendogram derived from UPGMA cluster analysis of the seven rice genotypes based on Jaccord`s coefficient using six SSR markers.

5- Agronomic traits:

5-1-Genetic parameters:

5-1-1- Mean performance:

Agronomic traits for the seven genotypes including two parents; Sakha101 and HR4856 and their derived five promising lines; SP70-101- 1; SP70-101-2; SP70-101- 3; SP70-101-4 and SP70-101-5, were evaluated for two seasons at 2012 and 2013, and their combined date are presented in Table 5. The results showed a wide range of variability for all studied traits. This wide range reflected the variation and among variability tested the genotypes. The two promising lines of SP70-101- and SP70-101-4 gave the highest yield (11.41 to 11.24 t/ha, respectively) compared with their parents; Sakha 101and HR 4856 (11.16 and 6.57 t/ha, respectively) and these lines are superior for all traits.

Table 5: Mean performance of agronomic characters at the two years of 2012-2013 and their combined data.

No.	Genotypes	Plant height (cm)			Duration (day)			Flag leaf area (cm^2)		
		2012	2013	Comb.	2012	2013	Comb.	2012	2013	Comb.
1	Sakha101	87.39	88.18	87.78	145.00	145.33	145.17	29.13	28.31	28.72
2	SP70-101-1	91.78	92.84	92.31	135.67	136.00	135.83	32.44	31.01	31.73
3	SP70-101-2	93.72	93.85	93.79	136.33	135.00	135.67	31.14	29.78	30.46
4	SP70-101-3	94.18	92.91	93.54	134.00	134.67	134.33	31.44	30.78	31.11
5	SP70-101-4	90.80	92.64	91.72	136.67	135.33	136.00	30.15	29.05	29.60
6	SP70-101-5	99.34	95.98	97.66	130.00	129.33	129.67	29.27	31.61	30.44
7	HR4856-1-1-1-1	73.02	76.48	74.75	105.00	107.67	106.33	18.85	20.29	19.57
Range		73.02-99.34	76.48-95.98	74.75-97.66	105.00-145.00	107.67-145.33	106.33-145.17	18.85-32.44	20.29-31.61	19.57-31.73
L.S.D. at 0.05		2.43	2.77	0.96	2.01	2.33	2.06	2.39	1.31	1.82
at 0.01		3.41	3.82	1.31	2.82	3.27	2.80	3.35	1.83	2.47

Table 5:Cont.

No.	Genotypes	No. of panicles/ plant			1000-grain weight (g)			No. of grains/ panicle			Grain yield t/ha		
		2012	2013	Comb.	2012	2013	Comb.	2012	2013	Comb.	2012	2013	Comb.
1	Sakha101	23.15	24.75	23.95	27.97	28.11	28.04	146.00	157.00	151.50	10.99	10.99	11.16
2	SP70-101-1	22.85	23.16	23.00	30.40	30.77	30.58	153.33	147.67	150.50	11.32	11.32	11.41
3	SP70-101-2	22.93	22.89	22.91	29.90	30.33	30.12	156.67	157.05	156.80	10.78	10.78	11.06
4	SP70-101-3	21.76	21.57	21.67	32.20	31.67	31.94	163.67	138.67	151.20	11.01	11.01	10.97
5	SP70-101-4	21.90	22.05	21.98	30.83	30.10	30.47	164.67	147.67	156.20	11.17	11.17	11.24
6	SP70-101-5	18.45	19.02	18.73	30.87	31.86	31.36	145.67	152.33	149.00	10.44	10.44	10.59
7	HR4856-1-1	13.27	9.97	11.62	25.47	24.06	24.76	76.33	80.67	78.50	6.52	6.52	6.57
Range		**13.27-23.15**	**9.97-24.75**	**11.62-23.95**	**25.47-32.20**	**24.06-31.86**	**24.76-31.94**	**76.33-164.67**	**80.67-157.05**	**78.50-156.80**	**6.52-11.32**	**6.52-11.32**	**6.57-11.41**
L.S.D. at 0.05		**2.61**	**1.13**	**1.91**	**1.54**	**0.94**	**1.23**	**10.48**	**13.21**	**21.84**	**0.82**	**0.88**	**0.63**
at 0.01		**3.66**	**1.58**	**2.58**	**2.16**	**1.37**	**1.66**	**14.69**	**18.52**	**29.59**	**0.90**	**0.97**	**0.85**

Table 5:Cont.

No.	Genotypes	Hulling %			Milling %			Head rice %		
		2012	2013	Comb	2012	2013	Comb.	2012	2013	Comb.
1	Sakha 101	81.75	82.39	82.07	71.83	70.57	71.20	64.52	63.45	63.98
2	SP70-101-1	82.10	82.79	82.45	72.03	72.05	72.04	65.64	63.14	64.39
3	SP70-101-2	82.43	82.76	82.60	73.64	72.44	73.04	64.35	63.17	63.76
4	SP70-101-3	84.70	84.91	84.80	75.57	74.24	74.90	66.48	65.37	65.92
5	SP70-101-4	78.68	80.28	79.48	70.54	70.36	70.45	66.19	60.58	63.38
6	SP70-101-5	84.50	86.20	85.35	77.33	76.08	76.45	68.33	66.63	67.48
7	HR4856-1-1-1-1	72.69	70.81	71.75	57.81	57.81	57.81	48.17	45.50	46.83
Range		72.69-84.70	70.81-86.20	71.75-85.35	57.81-77.33	57.81-76.08	57.81-76.45	48.17-68.33	45.50-66.63	46.83-67.48
L.S.D. at 0.05		1.91	2.04	2.03	1.72	1.69	1.72	2.60	2.09	2.19
at 0.01		2.67	2.76	2.84	2.41	2.37	2.41	3.65	2.93	2.87

5-1-2-Variation and interaction with years:

The ordinary analysis of variance of each year along with the combined data for yield and its component attributes are presented in Table 6. Year mean squares of genotypes were found to be highly significant for all yield and its components for all traits in two years. The combined data indicated overall differences among these traits. The interaction of genotypes with years showed highly significant for plant height, duration, flag leaf area, number of panicles/ plant, number of grains/ panicle, grain yield t/ha and head rice %, revealing that these genotypes behaved differently from one year to another or are more affected by environmental conditions. Similar results were obtained with El-Wahsh and Hammoud (2007); Hassan, *et al*., (2012); Abd El-had, *et al*., (2012) and Sedeek, *et al*., (2012).

Table 6: Mean square estimates of the ordinary analysis for grain yield and its components in 2012 (Y_1) and 2013 (Y_2) and their combined data (comb.).

S.O.V	Single	Comb.	Plant height (cm)			Duration (days)			Flag leaf area(cm^2)		
			Y1	Y2	Comb.	Y1	Y2	Comb.	Y1	Y2	Comb.
Years		1			7.57**			6.09**			8.53**
Reps with years		2			1.45			1.33			1.50
Genotypes	6	6	208.83**	129.55**	330.83**	479.87**	410.08**	887.02**	63.41**	45.16**	105.23**
Genotypes X years		6			7.18**			4.93**			5.35**
Error	12	24	1.87	2.35	2.10	1.28	1.72	1.50	1.80	0.54	1.17

Table 6: Cont.

S.O.V	Single	Comb.	No. of panicles/plant			1000-grain weight(g)			No. of grains//panicle		
			Y1	Y2	Comb.	Y1	Y2	Comb.	Y1	Y2	Comb.
Years		1			9.18**			6.49**			37.52**
Reps with years		2			0.76			0.23			68.17
Genotypes	6	6	39.18**	73.69**	109.43**	15.17**	22.21**	36.37**	2823.30**	2184.87**	4748.48**
Genotypes X years		6			6.45**			1.01			259.69**
Error	12	24	2.16	0.40	1.28	0.75	0.30	0.53	34.68	60.54	47.61

Table 6: Cont.

S.O.V	Single	Comb.	Grain yield t/ha			Hulling %			Milling %			Head rice %		
			Y1	Y2	Comb.	Y1	Y2	Comb.	Y1	Y2	Comb.	Y1	Y2	Comb.
Years		1			10.52**			12.33**			12.80**			13.83**
Reps with years		2			0.05			1.61			0.83			1.59
Genotypes	6	6	8.66**	9.12**	17.72**	52.11**	76.64**	126.74**	121.73**	106.11*	227.21*	140.35**	153.17**	289.59**
Genotypes X years		6			4.25**			2.11			0.63			4.92
Error	12	24	0.13	0.15	0.14	1.15	1.46	1.30	0.93	0.93	0.93	1.79	1.38	1.58

***and** significant at 0.05 and 0.01 levels, respectively.**

5-3-Phenotypic, genotypic coefficient of variability and genetic advance:

stimates of phenotypic, genotypic coefficient of variability are presented in Table 7. The rice cultivars showed a wide range of variation for all studied traits in two years and their combined data, where the cultivar mean squares was found to be highly significant for all studied characters Table (7). Thus, the selection for improved traits among these cultivars would be effective in all traits. Similar results were obtained by Hammoud (2004) El-Wahsh and Hammoud (2007) and El Sherif, (2011).

Table 7: Genetic parameters for some agronomic characters over two years.

Traits / Genetic parameters	Plant height (cm)	Duration (day)	Flag leaf area (cm^2)	No. of panicles/plant	1000-grain weight(g)
Genotypes variance	330.83**	887.02**	105.23**	109.43**	36.37**
Mse	2.10	1.50	1.17	1.28	0.53
Mean	90.22	131.86	28.80	20.55	29.61
Ge V	54.79	147.59	17.34	18.03	5.97
PhV	56.89	149.09	18.51	19.31	6.50
GCV%	8.20	9.21	14.46	20.66	8.25
PCV%	8.36	9.26	14.94	21.66	8.61
Heritability %	96.31	98.99	93.68	96.38	91.85
Δg	14.97	24.90	8.30	8.74	4.73
Δg%	16.59	18.88	28.83	42.53	15.97

Table 7:Cont.

Traits / Genetic parameters	No. of grains /panicle	Grain yield (t/ha)	Hulling %	Milling %	Head rice %
Genotypes variance	474.84**	17.72**	126.74**	227.21**	289.59**
Mse	47.61	0.14	1.30	0.92	1.58
Mean	142.00	10.43	81.21	70.88	62.25
Ge V	783.48	2.93	20.91	37.72	48.00
PhV	831.09	3.07	22.21	38.64	49.58
GCV%	19.71	16.41	5.63	8.67	11.13
PCV%	20.30	16.80	5.80	8.77	11.31
Hetitability %	94.27	95.44	94.15	97.62	96.81
Δg	55.98	3.44	9.14	12.50	14.04
Δg%	39.42	32.98	11.26	17.64	22.55

The phenotypic coefficient of variability (PCV) gave higher values than genotypic coefficient variability (GCV) under two years and their combined data, indicated that the environment effects are pronounced on the traits. Some results were obtained by Hassan, *et al*. (2012); Sedeek, *et al*., (2010) Abd El-hadi, *et al*., (2012) and Sedeek, *et al*., (2012).

The genetic coefficient of variability (GCV) for all studied traits ranged between 5.63% for hulling % to 20.66% for number of panicles/ plant over the two seasons, indicating that these traits might be more genotypically predominance and it would be possible to achieve further improvement for them.

Estimates of heritability in broad sense % were in general high for all studied traits and ranged between 91.85% for 1000-grain weight to 98.99% for plant duration (days). The heritability percentage was estimated as a ratio between the genotypic variance (numerator) and the total phenotypic variance (denominator) and the latter was reduced by the small component of the G x E interaction which was significant for most studied traits. Different

estimates were recorded for the same traits by Hassan, *et al*., (2012); Sedeek, *et al*., (2010); Abd El-hadi, *et al*., (2012) and Sedeek, *et al*., (2012).

Genetic advance under selection, presented in Table 7, showed the possible gain from selection as parent increase the ratio of genetic advance from one generation to another when the selected most desirable 5% from plants. The genetic advance percentage (g %) value ranged from 11.26% for hulling (%) to 42.53% for number of panicles/ plant. The same results were obtained by Hassan, *et al*., (2012) and Abd El-hadi, *et al*., (2012).

5-4- Estimates of phenotypic correlation coefficients:

Correlation coefficients of ten studied traits were estimated and the results were presented in Table 8. The grain yield (t/h), showed highly significant positive correlation with number of panicles/ plant, number of grains/ panicle flag leaf area and duration. Plant height was highly significant positive correlations for 1000-grain weight, number of panicles/ plant, hulling (%), milling (%), head rice (%) and flag leaf area. Total duration showed highly significant positive correlation with 1000-grain weight while, it was significant with number of grains/ panicle. On the other hand, flag leaf area gave highly significant positive correlation with 1000-grain weight, number of panicles/ plant, hulling %, milling % and head rice %. Head rice % exhibited highly significant positive correlation values with 1000-grain weight, number of panicles/ plant, hulling (%) and milling (%). As for hulling (%), highly significant positive correlation was found only with 1000-grain weight but milling (%) gave highly significant positive correlation with 1000-grain weight and hulling (%). Number of grains/ panicle showed highly significant positively correlated with number of panicles/ plant and 1000-grain weight. It could be concluded that the grain yield (t/h) affected more with increase in yield component traits. On the other hand, the grain quality affected more by increased 1000-grain weight and total duration was affected by increased of yield component traits. Similar results obtained by Hassan, *et al*., (2012); Sedeek, *et al*., (2010); Abd El-hadi, *et al*., (2012) and Sedeek, *et al*., (2012).

Table 8: Estimate of correlation coefficient for agronomic traits over the two years.

Traits	No. of Panicles/ plant	1000-grain weight (g)	No. of grains/ panicle	Hulling %	Milling %	Head rice %	Flag leaf area (cm^2)	Duration (day)	Plant height (cm)
No. of Panicles/ plant	-								
1000-grain weight (g)	-0.016	-							
No. of grains/ panicle	0.339**	0.285**	-						
Hulling %	0.121	0.551**	0.195	-					
Milling %	-0.015	0.566**	0.188	0.695**	-				
Head rice %	0.007	0.501**	0.298**	0.474**	0.590**	-			
Flag leaf area (cm^2)	0.162	0.504**	0.275*	0.340**	0.405**	0.344**	-		
Duration (day)	0.617**	-0.127	0.270*	-0.058	-0.095	0.038	0.151	-	
Plant height (cm)	-0.039	0.517**	0.238*	0.552**	0.623**	0.406**	0.351**	-0.166	-
Grain yield ton/ha	0.574**	0.150	0.338**	-0.120	-0.029	0.025	0.223*	0.412**	-0.119

***and** significant at 0.05 and 0.01 levels, respectively.**

6-Blast reaction:

6.1- Under field conditions:

Promising lines and their parents were evaluated to blast under field of Sakha conditions at the adult stage. The results showed that all promising derived lines were resistant to blast disease under natural infection compared with their parents.

6.2- Under artificial inoculation:

The results showed that all promising lines; SP70- 101 -1, SP70- 101- 2, SP70- 101- 3, SP70- 101- 4 and SP70- 101 – 5 derived from crossing between Sakha 101 x HR 4856 // Sakha 101 were resistant to all aggressive blast races. On the other hand, the two parents were found to be susceptible for all races except race II-1; IC-3; ID-15; II-2; II-5; IH-6 and IC-1I. On the contrary, the HR4856 was resistant to most races except race II-4 and IC-3. It can be concluded that all promising lines were resistance for all races under artificial inoculation and were field conditions. These new promising lines derived from the cross Sakha 101 x HR 4856 // Sakha 101 could be grown on large scale to attain more yield and blast resistance.

Table 9:Performance of seven rice genotypes under artificial inoculation of 14 races during 2013.

No.	Genotype	Blast races														% of blast resistance
		Ic-13	Ic-15	II-1	ID-13	IC-30	ID-15	II-2	IC-15	II-3	II-4	II-5	IH-6	IC -3	IC-11	
1	Sakha 101	8	7	1	9	1	1	1	9	4	7	1	1	7	1	50
2	SP 70-101-1	2	1	1	2	1	1	1	1	1	1	1	1	1	1	100
3	SP 70-101-2	1	1	1	2	1	1	1	1	1	1	1	1	1	1	100
4	SP 70-101-3	1	1	1	2	1	1	1	1	1	1	1	1	1	1	100
5	SP 70-101-4	1	1	1	2	1	1	1	1	1	1	1	1	1	1	100
6	SP 70-101-5	1	1	1	2	1	1	1	1	3	1	1	1	2	2	100
7	HR4856-1-1	1	1	1	2	1	1	1	1	4	1	1	1	5	3	85.71

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تقدير وتقييم التنوع الوراثي لبعض السلالات الناتجة من الصنف سخا ١٠١ باستخدام المعلمات الجزيئية SSR

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اشتملت هذه الدراسة على تجربتين أجريت التجربة الأولى بغرض تقدير الاختلافات الوراثية بمعلمات تكنيك SSR بمعهد الهندسة الوراثية – جامعة المنوفية – فرع السادات، كما أجريت التجربة الثانية فى هذه الدراسة فى مزرعة مركز البحوث والتدريب فى الأرز خلال المواسم من ٢٠٠٥ وحتى ٢٠١٣ وكانت اهم أهداف الدراسة:

دراسة التنوع الوراثي للسلالات المبشرة الناتجة من الهجين Sakha 101 x HR 4856 دراسة المكونات الوراثية لصفات المحصول ومكوناته. تم دراسة درجات المقاومة لمرض اللفحة مقارنة بالآباء وذلك من خلال العدوى الصناعية بالصوبة بقسم بحوث الأرز بسخا. وكان التصميم الخاص بالتجربة هو القطاعات الكاملة العشوائية فى ثلاثة مكررات فى موسمي ٢٠١٢ و ٢٠١٣ لدراسة التنوع الوراثي باستخدام ستة برايمر تبين وجود اختلافات فى عدد الاليلات حيث أعطى البرايميرات (SSR206 و SSR209وSSR216) أليلين ، بينما البرايمرات (SSR262 , SSR244 ,SSR302) أعطت ٣ و ٤ أليلات . كما أوضحت الشجرة الوراثية اختلافات أدت إلى تفرعها إلى مجموعتين رئيسيتين ، شملت المجموعة الأولى السلالة SP70-101-1 فقط وهذه السلالة ناتجة من التهجين بين (Sakha101 X HR4856)Sakha101 بينما المجموعة الثانية شملت جميع السلالات الباقية والتي تفرعت بدورها إلى تحت مجموعتين. تحت المجموعة الأولى كانت السلالة SP70-101-٣ وهى الناتجة من التركيبة الوراثية ، بينما تحت المجموعة الثانية شملت الصنف HR4856 وهو الصنف من الأصناف الكورية ومبكر النضج وناتج من أباء مختلفة عن باقي السلالات. كما اشتملت تحت المجموعة الثانية علي الصنف Sakha101 فقط وهو من الأصناف المصرية والذي لعب دورا كبيرا فى السلالات المبشرة الناتجة منه. كما أوضحت النتائج أن السلالتين SP70-101-٢ و SP70-101-4 هما أقرب السلالتين تشابها وبلغت نسبة القرابة بينهما ٩١% تقريبا بينما أظهرت السلالة(SP70-101-1) ٦٠% قرابة.

كما تم تقدير التباين المظهري والوراثي والكفاءة الوراثية والتحسين الوراثي المتوقع حيث وجدت اختلافات عالية المعنوية للتراكيب الوراثية المدروسة فى كلا السنتين والتحليل المشترك لها لجميع الصفات المدروسة، كما كان التفاعل بين التراكيب الوراثية والبيئة عالي المعنوية لجميع الصفات المدروسة عدا صفات وزن ال١٠٠٠ حبة، عدد الحبوب/دالية، النسبة المئوية للتبييض و النسبة المئوية للأرز السليم فى كلا السنتين والتحليل المشترك . كان التباين المظهري أعلى من التباين الوراثي لمتوسط السنتين والتحليل المشترك لها فى جميع الصفات المدروسة. وجد أن الاختلافات الوراثية عالية المعنوية لجميع الصفات المدروسة لمتوسط السنتين والتحليل المشترك لها. وكذلك وجد أن التحسين الوراثي المتوقع كان عاليا عند انتخاب أفضل ٥ % من النباتات لصفات عدد الداليات/نبات، عدد الحبوب/دالية والمحصول بالطن/هكتار.

كما أظهرت قراءة اللفحة فى الحقل مقاومة السلالات الناتجة من تهجين الصنف Sakha101 بالصنف HR 4856 مقاومة عالية لفطر اللفحة. كما أظهرت قراءة الصوبة تفوق السلالات الناتجة من الصنف Sakha101 X HR4856 تفوقا كبيرا فى مقاومة مرض اللفحة لعشرة سلالات فطرية ممرضة.

الخلاصة من هذه الدراسة: تم الحصول على مجموعة من السلالات المبشرة عالية المحصول ومقاومة لمرض اللفحة ومبكرة فى النضج وذو جودة حبوب عالية مقارنة بالآباء الناتجة منها. ومن خلال دراسة المعلمات الجزيئية تبين وجود إختلافات وراثية بين السلالات المبشرة وآبائها على المستوى الجزيئى كتراكيب وراثية جديدة.

قام بتحكيم البحث

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