

## EVALUATION OF SOME RICE GENOTYPES FOR STEM BORER RESISTANCE USING GENETIC AND MOLECULAR MARKERS ANALYSES

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(Received: Oct. 19, 2014)

**ABSTRACT:** Rice is the most important staple food crops for more than half of the world's population. The stem borer *Chilo agamemnon* Bles, is one of the most devastating pests of rice, reducing yield worldwide. The use of resistant cultivars remains one of the most reliable methods to pest's management. In this study, some rice genotypes were genetically assessment for the stem borer resistance. Two varieties, Sakha101 (Egyptian rice variety, resistant to stem borer) and Giza178, (Egyptian rice variety, susceptible to stem borer) and their  $F_1$ ,  $F_2$  and  $F_3$  were evaluated. Results showed that resistance to stem borer appears to be under polygenic control. Identifying microsatellite markers association to stem borer resistance was another objective of this investigation. Five markers (RM164, RM166, RM263, RM201 and RM566) showed polymorphism among susceptible and resistant genotypes. However, RM263 and RM201 showed complete association with resistance The SSR markers would be helpful in the breeding program application.

**Key words:** Rice - stem borer - SSR markers and genetic diversity.

### INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops that provide food for about half of the world population. Egypt is one of the few countries, which produce high yielding rice varieties and succeeded to achieve one of the highest productivities per unit area through the last decade. Among the many biotic and abiotic stresses that influence the yield of rice, plant pests and diseases are the most important Heinrichs, (1998). The rice stem borer (*Chilo agamemnon* Bles.) is a major pest if it isn't controlled. Larva of striped stem borer goes into the plant stems and feed on plant nutrients causing severe crop loss Beever *et al.* (1990). Rice plants are most prone to stem borer infestation at the tillering and flowering stages Viajante & Heinrichs (1987). Rice stem borers have been reported to cause yield losses ranging from 30 to 80%. A 100% loss has been recorded in worst affected fields in Nigeria Imolehin and Ukwungwu, (1992). In many parts of Africa it has been reported that borers destroy 30-50% of plant tillers during the wet cropping season thereby reducing significantly the whole harvest Dakouo *et al.* (1991). Therefore, efforts to find resistant varieties to this pest are very important. The mechanism of tolerance depends on many

factors that time and environmental conditions are more important and effective. Resistance to stem borers appears to be under polygenic control (Khush, 1984 and Sarwar, 2013).

The complex nature of the trait and the inherent difficulties in screening have consequently made breeding for stem borer resistance a difficult task. El -Malky *et al.* 2008 and El -Namaky *et al.* (2010) concluded that additive and additive x additive gene action were more important in the inheritance of stem borer resistance. Marker-assisted selection is especially helpful when the characters studied are polygenic, a situation particularly common for resistance traits (Selvi, *et al.*, 2002). This study was aimed to identify molecular markers linked to major loci conferring stem borer resistance in rice using microsatellites (LinXianWen, 2010).

### MATERIAL AND METHODS

This research was carried out at the Experimental Farm of the Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt during the rice seasons of 2010, 2011 and 2012. Two rice varieties, Sakha101 (Egyptian rice variety, resistant to stem borer) and Giza178, (Egyptian rice

variety, susceptible to stem borer), and their F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> were evaluated. Bulk emasculation was conducted using the hot-water technique of Jodon (1938) as modified by Butany (1961). Plant height (cm), days to maturity (days), number of tillers /plant, flag leaf area, total chlorophyll content in the flag-leaf was recorded at heading stage using a chlorophyll meter (5 SPAD-502, Minolta Camera Co. Ltd, Japan), stem diameter(mm), white head %,1000-grain weight (g), panicle length(cm), panicle weight (g), number of panicles/plant , spikelets fertility (%), and grain yield/plant(g) were measured at harvest.

**Stem borer evaluation:** parents, F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> offspring were evaluated for stem borer infestation. The reaction of evaluated genotypes was classified into five categories according to the Standard Evaluation System of the Rice Research and Training Center (RRTC, 2006), Sakha, Egypt:

< 3% whiteheads (WH) resistant (R), 3–6% WH moderately resistant (MR), 6–9% WH moderately susceptible (MS), 9–12% WH susceptible (S) and > 12% WH highly susceptible (HS).

The infestation percentage was calculated using the following formula:

$$\text{Percentage of infestation} = \frac{\text{No. infested hills} \times 100}{\text{Total no. rice hills}}$$

### **Generation mean and variance analysis:-**

The mean and variance were calculated for P<sub>1</sub> (Sakha101), P<sub>2</sub> (Giza178), F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub>. The population means and variances were used to estimate the following parameters :

**1- Scaling test:-** Adequacy of scale must satisfy two conditions namely, additive of gene effects and independence of heritable component from non-heritable ones. The test of first condition provides information regarding absence or presence of gene interactions. The test of adequacy of scales is important because in most of the cases the estimation of additive and dominance components of variances were estimated assuming the absence of gene interactions. Mather (1949) and Hayman and Mather (1955) gave two tests for scale effects D and C. The values of D and C

should equal to zero within the limit of their standard error. The significance of any one of these scales would be used to indicate the presence of non-allelic interactions. The calculated values of "t" were compared with the tabulated value of "t" at 0.05 and 0.01 levels of significance. In each test, the degree of freedom is sum of the degrees of freedom of various generations involved.

**2- Types of gene action:-** Data were analyzed by generations mean analysis method as follows: The means of the five populations in each cross were used to estimate the five parameters of gene effects, according to Gamble (1962). Where, the parameters m, d, h, i and l refer to, mean effects, additive, dominance, additive × additive and dominance × dominance gene effects, respectively. Estimates of gene effects were tested for significance from zero by using t- test.

### **Molecular analysis:-**

Genomic DNA was isolated from 0.5 g of three weeks old leaves of the used rice genotypes according to Murray and Thompson(1980). The quantification of the extracted DNA was determined on 0.8 % agarose gel comparing to known concentrations of λ uncut genomic DNA. The concentration of the genomic DNA was adjusted up to approximately 15 ng / µl for PCR reaction.

From twenty tested SSR primers associated with three morphological traits, (stem diameter, stem borer resistance and silica content) only five primers were showed polymorphism for the studied genotypes. Primer name, sequence, associated trait and reference located in Table 1.

PCR amplification reactions were done in 25 µl reaction mixtures, containing 1 µl of DNA template, 2.0 µl of each forward and reverse primer(10 pmol/ µl), 12.0 µl of PCR master mix (Ferments) and 10 µl dd H<sub>2</sub>O. Thermal cyler was used with the following PCR profile: 95°C for 5 min (initial denaturation step) 35 cycles for of extension, 94°C for 1min, 55°C for 1min (primer annealing), primer elongation at 72°C for 2min and 72°C for 7min and stored the last temperature was 4°C.

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**Table (1): Primer name, sequence, associated trait and reference**

Primer name	Sequence		Associated trait	Reference
	Forward 5' → 3'	Reverse 5' → 3'		
RM566	ACCCAAC TACGATCAGCTCG	CTCCAGGAACACGCTCTTTC	Stem diameter	Mallikarjuna Swamy <i>et al.</i> (2011)
RM164	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	Stem borer resistance	LinXianWen (2010)
RM166	GGTCCTGGGTCAATAATTGGGTACC	TTGCTGCATGATCCTAAACCGG		
RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA		
RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	Silica content	Bryant <i>et al</i> (2011)

Ten microliters of PCR amplified product were loaded into 1.8 % agarose gel supplemented with ethidium bromide. The TAE buffer 1X was used as a running buffer and 50bp DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for 3 h. Gels were then visualized and photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany).

Separated bands were scored for each SSR marker based on the presence and absence of bands, generating a binary data matrix of 1 and 0 for each marker system. The presence/absence matrix for amplified DNA fragments was analyzed using the PAST program, version 1.90 (Hammer *et al.*, 2001).

## RESULTS AND DISCUSSION

### Mean performance of vegetative and yield traits.

The data presented in Table 2 and 3 revealed that differences among the generation means for all agronomic traits (days to maturity, plant height, chlorophyll content, flag leaf area, no. of tillers /plant, stem diameter, white head%, no. of panicles /plant, panicle length, panicle weight, spikelet fertility%, 1000 – grain weight and grain yield per plant) during all the generations of the crosses Sakha101 x Giza178 rice, indicating the presence of genetic diversity for these traits in the above cross. This result in according with Li *et al.*

(2000) who found significant difference among hybrid generation in trait measurements of rice.

### Scaling test and gene action

The scaling test parameter D and C showed non- significant values in all studied traits in Table 3, which indicated the absence of all types of non-allelic interaction. The F<sub>2</sub> mean values (m) were highly significant in all traits except white head %. Additive gene effects (d) were high significant in heading date, stem diameter, white head%, no. of panicles / plant, panicle length, 1000-grain weight, spikelet fertility % and grain yield / plant. They were non significant in chlorophyll content, flag leaf area, no. of tillers / plant and panicle weight. Dominance gene effects (h) were highly significant in all studied traits except white head % was non- significant.

Additive × additive (i) epistatic effects were highly significant in all studied traits except flag leaf area and panicle length were non- significant. On the other hand, dominance × dominance (1) epistatic effects were non- significant in all traits. The additive effect was non significant indicating the presence of complementary additive epistasis for these characters. The presence of digenic epistatic effects of additive × additive gene action for the character indicated that epistasis also played an important role in determining the inheritance of these characters. Similar results for 1000 grain weight were reported by Misra *et al.*

Table (2): Mean performance and standard error for some agronomic traits of parents, F<sub>1</sub> and F<sub>2</sub> for the Sakha101 x Giza178 cross.

Parents / generations	Heading date(day)	Plant height	Chlorophyll content	Flag Leaf area	No. of tillers/plant	Stem diameter	White head%	No. of panicles/plant	Panicle length	Panicle weight	Spikelet fertility	1000-grain yield	Yield per plant
Sakha101	134.65	94.04	43.50	26.24	21.00	2.78	0.46	25.31	21.02	4.20	97.00	27.6	11.36
Giza178	128.85	102.96	39.82	33.27	23.19	4.40	7.99	18.52	18.00	3.53	89.19	21.0	10.30
Parent's Mean	131.75	98.5	41.66	29.755	22.095	3.59	4.225	21.915	19.51	3.865	93.095	24.3	10.83
F <sub>1</sub>	140.78	104.81	47.77	32.92	22.83	5.01	2.66	64.77	23.52	4.45	99.09	27.88	12.08
F <sub>2</sub>	131.79	99.72	41.93	37.99	25.08	4.51	12.12	24.71	23.41	2.65	91.80	19.65	11.64
S.E	14.7	10.22	5.23	12.77	7.78	1.05	3.75	11.03	2.71	0.99	6.35	8.91	0.81

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**Table (3): Gene action and Scaling test of some agronomic traits of the cross Sakha101 xGiza178**

Characters	Scaling test		Gene action				
	C	D	Mean m	Additive d	Dominance h	Additive x additive i	Dominance x dominance l
Heading date (day)	-17.91ns	-29.58ns	131.29**	2.90**	688.82**	-1653.3**	-16.09ns
Plant height (cm)	-7.73ns	35.86ns	99.72**	-4.46**	557.51**	4727.8**	58.12ns
Chlorophyll content	-11.14ns	-0.81ns	41.93**	1.84ns	226.62**	466.89**	13.78ns
Flag leaf area (cm <sup>2</sup> )	26.63ns	27.73ns	27.99**	-3.51ns	206.34ns	18.93ns	1.05ns
No. of tillers / plant	10.45ns	3.71ns	25.08**	-1.10ns	130.74**	-174.6ns	-8.99ns
Stem diameter (mm)	0.84ns	-1.12ns	4.51**	-0.81ns	22.42**	-9.96**	-2.61ns
White head%	34.71ns	4.23ns	12.12ns	-3.77**	48.15ns	-288.9**	-45.98ns
No. of panicles / plant	24.29ns	14.04ns	23.77**	11.65**	119.22**	-256.97**	-14.23ns
Panicle length	7.57ns	8.15ns	23.41**	1.51**	125.17**	11.58ns	0.76ns
panicle weight	-6.01ns	-1.37ns	2.65**	0.33	16.5**	17.18**	6.18ns
1000-grain weight	2.22ns	26.21ns	19.65**	-10.50**	111.44**	441.98**	31.99ns
Spikelet fertility	-17.10ns	-4.28ns	91.80**	3.90**	493.3**	1266.4**	17.09ns
Yield-plant	0.74ns	-0.59ns	11.64**	0.53ns	60.89**	-16.27**	-1.77ns

\* and \*\* Significant at 0.05 and 0.01 levels, respectively

(1994) and Similar results for stem borer resistance reported by El - Malky *et al.* 2008 and El - Namaky *et al.* 2010 These results were not in concurrence with the results of Shekawat *et al.* (2000), who reported that the grain yield / plant was mostly governed by dominance effect (h) and dominance x dominance gene effects (l), with larger

magnitude but was unexploitable due to duplicate type of gene action.

**Molecular markers analysis:-**

Ten extremes F<sub>2</sub> progenies along with the parents were phenotyped for white head reaction and other agronomic traits under stress conditions in the fields. Five stem

borer resistant and five susceptible rice individuals were selected out of 150 F<sub>2</sub> plants derived from Sakha101 and Giza178 and the selection was based on their white head reaction. Phenotypic studied at white head % revealed that the F<sub>1</sub>s were intermediate for resistance to stem borer and in the F<sub>2</sub> population.

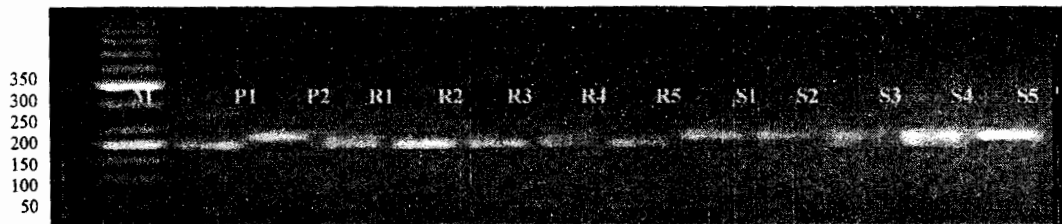
The results in Figure 1 showed that the primer RM566 demonstrated two polymorphic bands (alleles) with sizes of 244 bp for resistant parent Sakha101 and seven lines from F<sub>2</sub> extremes R1, R2, R3, R4, R5, S3, and S4, while the band with the size of 271 bp was presented in the susceptible parent Giza178 and F<sub>2</sub> extremes R1, R4, S1, S2, S3, S4. The individuals R2, R4, S3 and S4 have two alleles from their parents these results showed that the primer RM566 was found to be segregating with resistant and susceptible individuals in a co-dominant fashion

In addition the primer RM164 demonstrated two polymorphic bands (alleles) in Figure 2 with sizes of 317 bp presented in the resistant parent Sakha101 and five F<sub>2</sub> extremes R1, R2, R3, R4 and R5. While, the band with size of 280 bp was

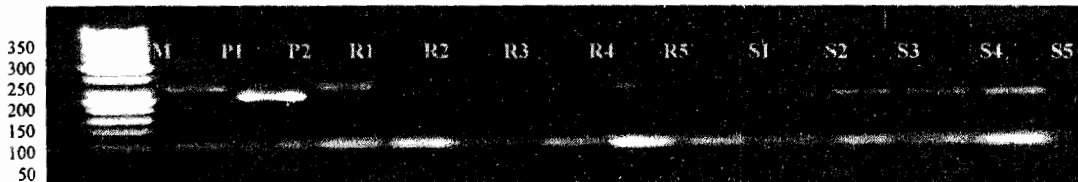
presented in the susceptible parent Giza178 and extremes F<sub>2</sub> R1, R3, S1, S2, S3, S4 and S5. the R1 and R2 have the two alleles with two parents (heterozygous) and resistance to stem borer these results mean resistant allele was dominant.

Results in Figure 3 showed that RM166 demonstrated two polymorphic bands (alleles) with sizes of 382 bp was presented in the resistant parent Sakha101 and all F<sub>2</sub> extremes. While, the band with size of 336 bp was presented in the susceptible parent Giza178 and F<sub>2</sub> extremes R1, R3, R5, S2, S3, S4 and S5.

The results demonstrated primer pairs RM263 gave two polymorphic bands (alleles) as presented in Figure 4. The band with the size of 187 bp was presented in resistant parent Sakha101 and five F<sub>2</sub> extremes R1, R2, R3, R4, and R5. While the band of size of 228 bp was presented in the susceptible parent Giza178 and F<sub>2</sub> extremes S1, S2, S3, S4 and S5. RM263 located in chromosome 2 showed complete association with resistant and susceptible individuals amplifying a 187 bp fragment in resistant and 228 bp in the susceptible individuals.



**Fig. (1).** Alleles showing co-segregation of RM566 among individual F<sub>2</sub> rice plants. M, 50 bp ladder; P<sub>1</sub>, Sakha101; P<sub>2</sub>, Giza178; (R1 - R5), stem borer resistant; (S1 - S5) stem borer susceptible.



**Fig. (2).** Alleles showing co-segregation of RM164 among individual F<sub>2</sub> rice plants. M, 50 bp ladder; P<sub>1</sub>, Sakha101; P<sub>2</sub>, Giza178; (R1 - R5), stem borer resistant; (S1 - S5) stem borer susceptible.

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Fig. (3). Alleles showing co-segregation of RM166 among individual F<sub>2</sub> rice plants. M, 50 bp ladder; P<sub>1</sub>, Sakha101; P<sub>2</sub>, Giza178; (R1 - R5), stem borer resistant; (S1 - S5) stem borer susceptible.

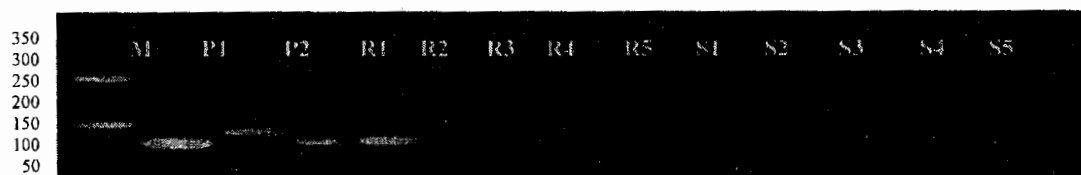


Fig. (4). Alleles showing co-segregation of RM263 among individual F<sub>2</sub> rice plants. M, 50 bp ladder; P<sub>1</sub>, Sakha101; P<sub>2</sub>, Giza178; (R1 - R5), stem borer resistant; (S1 - S5) stem borer susceptible.

The primer RM201 demonstrated two polymorphic bands (alleles) as shown in Figure 5 and Table 4, the band with size of 148bp was presented in the resistant parent Sakha101 and five F<sub>2</sub> extremes R1, R2, R3, R4, and R5. While the band with size of 168bp was presented in the susceptible parent Giza178 and F<sub>2</sub> extremes S1, S2, S3, S4 and S5. These results found to be exactly segregating between the stem borer resistant and stem borer susceptible as that of the parents.

### Phylogenetic analysis as revealed by SSRs technique

The phylogenetic analysis using different markers system showed clear isolation among the F<sub>2</sub> population genotypes belonging to the parent's. The dendrogram in Figure 6 showed the main cluster is divided into two sub-clusters; the first sub-cluster was separated into two groups the first was divided into two branches. The first branch included the resistant parent; Sakha101 and R2, and the other branch included R4. The second group is divided into two braches, the first one contained R1 and the second one contains R3 and R5. The second sub-cluster was divided into two sup-groups. The first one contained the susceptible parent; Giza178, and the other sub-group is divided into two branches, the

first one contained S1, S2 and S5 and the second one contained, S3 and S4.

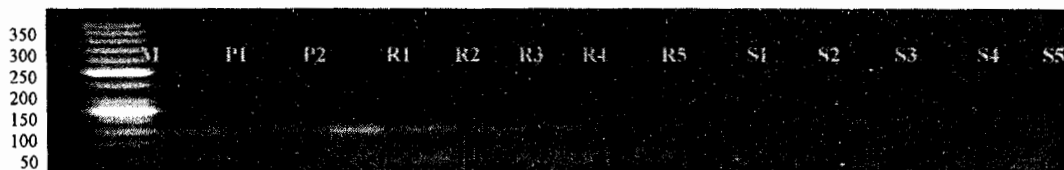
Results indicated polygenic inheritance involving a few genes with major effects. Polygenic nature of trait was also reported by Kalode *et al* (1987) and Selvi *et al.* (2002). Only five SSR primers among twenty markers were found to be segregating with resistant and susceptible extreme in a co-dominant fashion (RM566, RM164, RM166, RM263 and RM201) as gel Figures 1, 2,3,4 and 5 showed and the sizes of bands were showed in Table 4. Among the five microsatellite markers, RM263 and RM201 located on chromosome 2 and 9 respectively showed complete association with resistant individuals. The pooling of DNA from extreme phenotypic classes allowed us to rapidly detect markers associated with respective phenotypes.

The markers RM263 and RM201 were used to screen Egyptian genotypes, thus confirming association with the trait. A survey of microsatellite markers on chromosome 2 and 9 respectively is suggested to establish close association with SSB resistance.

Marker analysis on F<sub>2</sub> lines resulted from a cross between Sakha101 (resistant) x Giza178 (susceptible) using SSR markers

revealed polymorphism between parents and their F<sub>2</sub> plants. The identified markers showed linkage with stem resistance. Five markers, RM566, RM164, RM166, RM263 and RM201 were found to be associated with the trait. Further marker analysis is required to place more markers closer to the genes (s) for stem borer resistance. The SSR markers could help in the application in

breeding program. The study revealed that the ability of SSR markers to detect and to identify the allelic diversity and genetic variation among the studied rice genotypes. In addition, RM201 and RM 263 marker elucidated the possibility to use it in MAS for stem borer resistant in the studied Egyptian rice genotypes. Similar results recorded by (Selvi, *et al.* 2002 and Swamy, 2012).

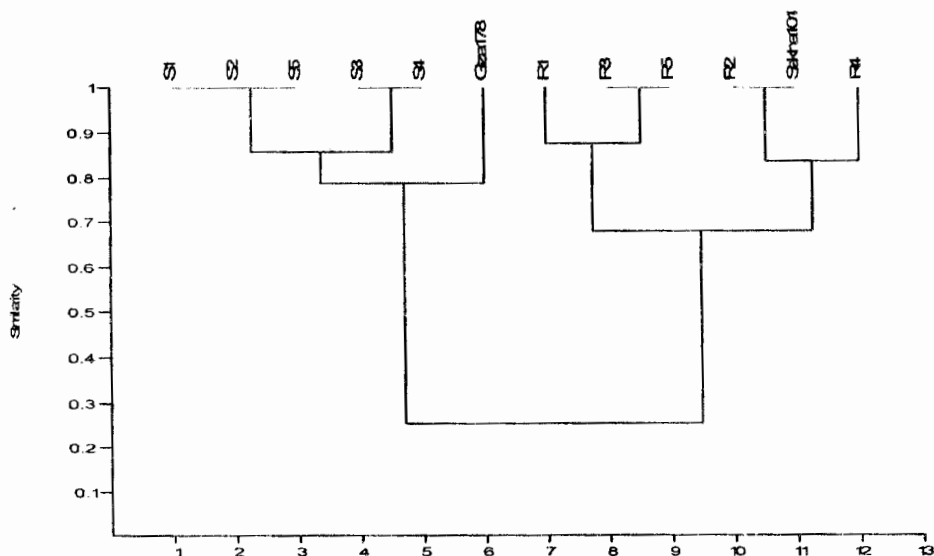


**Fig. (5).** Alleles showing co-segregation of RM201 among individual F<sub>2</sub> rice plants. M, 50 bp ladder; P<sub>1</sub>, Sakha101; P<sub>2</sub>, Giza178; (R<sub>1</sub> - R<sub>5</sub>), stem borer resistant; (S<sub>1</sub> - S<sub>5</sub>) stem borer susceptible.

**Table (4):** Showed presence and absence matrix

Genotype Alleles No.	Primer name	Sakha101	Giza178	R1	R2	R3	R4	R5	S1	S2	S3	S4	S5	(bp)
1	RM566	0	1	1	0	0	1	0	1	1	1	1	1	271
2		1	0	1	1	1	1	1	0	0	1	1	0	244
1	RM164	1	0	1	1	1	1	1	0	0	0	0	0	317
2		0	1	1	0	1	0	0	1	1	1	1	1	280
1	RM166	1	0	1	1	1	1	1	1	1	1	1	0	382
2		0	1	1	0	1	0	1	0	0	0	0	1	336
1	RM263	0	1	0	0	0	0	0	1	1	1	1	1	228
2		1	0	1	1	1	1	1	0	0	0	0	0	187
1	RM201	0	1	0	0	0	0	0	1	1	1	1	1	168
2		1	0	1	1	1	1	1	0	0	0	0	0	148





**Fig. (6). Dendrogram of SSR marker analysis using Average Linkage (Between Groups)**

**Conclusion**

In conclusion, this study indicated that presence of genetic diversity among the generation means for all studied traits. The additive effect was significant in stem borer trait indicated that additive effect played an important role in determining the inheritance of this trait. SSR markers, can be used to identify molecular markers associated to stem borer resistance in rice. Once these markers were identified they can be used in breeding as a selection tool in early generation.

**Acknowledgments**

We are very grateful to Prof. Dr. Mahmoud Ramzy Sherif and Prof. Dr. Ahmed Samir Hendawy, Plant Protection Research Institute for assistance provided in conducting the experiment.

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## التقييم الوراثي لبعض تراكيب الأرز لمقاومة ثاقبة الساق باستخدام المعلمات الجزيئية

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### الملخص العربي

الأرز هو أهم المحاصيل الغذائية الأساسية لأكثر من نصف سكان العالم .وثاقبة الساق ( *Chilo agamemnon*) هي واحدة من الآفات الأكثر تدميرا للأرز والتي تحد من عائداته في جميع أنحاء العالم .إن استخدام الأصناف المقاومة لا تزال أكثر الوسائل الموثوق بها لمكافحة الآفات. في هذه الدراسة تم التقييم الوراثي لمقاومة ثاقبة الساق في بعض أصناف الأرز المصرية وقد أجريت التجربة في المزرعة التجريبية لمركز البحوث والتدريب (RRTC)، سخا، كفر الشيخ، مصر. وتم استخدام صنفين أساسيين في هذه الدراسة Sakha101 وهو مقاوم لثاقبة الساق و Giza178 وهو قابل للإصابة بثاقبة الساق. تم التهجين بين الصنفين السابقين لتتبع سلوك الصفة وتم تقييم ال  $F_1$  و  $F_2$  و  $F_3$  . وأظهرت النتائج أن صفة المقاومة لثاقبة الساق يبدو أنه يتحكم بها العديد من الجينات. وقد تم استخدام خمس دلائل جزيئية (RM164 و RM166 و RM263 و RM201 و RM566) لدراسة ارتباطها بصفة مقاومة ثاقبة الساق وأظهر RM201 and RM263 تعدد الأنماط المظهرية لهذه الصفة. وتعتبر دلائل ال- SSR مفيدة للتطبيق في برنامج التربية حيث تساعد في الانتخاب السريع لمثل صفة مقاومة ثاقبات الساق.