GENETICAL IMPROVEMENT FOR ALLELOPATHIC ACTIVITY IN SOME RICE GENOTYPES

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ABSTRACT

The present investigation was conducted at Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, and Laboratory of Genetics Department, Faculty of Agriculture, Tanta University, Egypt. during the three successive rice growing seasons; 2012, 2013 and 2014. Two rice varieties; SK103 and Rikuto Norin 22 as a parents of the running study and their F_3, F_4 and F_5 were evaluated against the *Echinochloa crus -galli* (barnyard grass)under natural infestation. The results showed that, these varieties showed biologically active suppression of *E.crus-galli* under field conditions were values ranged from 2.33 to 12.6 cm for radial area and from 41.33 to 87% of weed control. The selected genotypes are important and suitable for direct seeded rice, also it could be utilized in the breeding programs to transfer allelopathic activity for commercial varieties to suppress weeds and lowering pollution. The genetic coefficient of variability refers to genotypic effect which played an important role in the inheritance of all studied traits were the heritability in broad sense ranged from 98.06 to 99.98 for allelopathic activity. Two of the ten tested SSR markers (Table 1); RM439 and RM164 showed polymorphic DNA pattern were the primer RM164 exhibited the band with molecular weight 296bp in all genotypes had strong to allelopathic activity and it was absent in poor rice variety. The SSR markers would be helpful and may identify important of DNA sequencing that help in breeding program.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in all over the world. In Egypt, it is one of the major cereal crops. The common annual cultivated area of rice crop is about 1.35 million feddans, produced about 5.3 million tons of paddy rice with an average of 4.15 t/fed, which was considered one of the highest average yield in the world (RRTC, 2016).Rice productivity has remarkably increased year after year according to the percentage replacement of the rice area with the modern varieties to realize a maximum yield average (10tha-1) in the year 2014 against (5.7tha-1) for the period of 1986-1998, because of adopting of the new short duration rice varieties, about 30% of the irrigation water consumption was saved every year (Aidy and Maximos, 2006). However, the weeds grown in rice fields are the main suppressor of rice growth and significantly affecting rice grain yield. Also the chemical treatments or

herbicides for weed control are very dangerous due to the pollution and high production costs. Allelopathy is the result of biochemical interactions between plants and represents an economic way to control weeds in rice fields. It is caused by toxic chemicals released by the plant through volatilization, leaching, and root exudation or produced during decomposition of plant residues in the soil (Chou,1995). Success of breeding programs depends on the magnitude of genetic variability and the extent to which the advantageous characteristics are heritable (Mruthunjaya and Mahade 1993). Therefore, the study of genetic variability in rice is not only essential for selecting valuable genotypes and predicting the effect of selecting best genotypes but it will also aid breeders in simultaneous improvement of characteristics through selection (Patil *et al.,* 1993). The aims of this investigation were to study the inheritance of allelopathic activity, some morphological, yield and its components traits through early generations, of some rice genotypes, Haul Sutrex and Necmi. Beser (2005), indicated that selections for grain weight and number of grains per panicle were effective in early generations. The study revealed that the ability of SSR markers to detect and to identify the allelic diversity and genetic variation among the studies rice genotypes.

MATRIALS AND METHODS

One hundred lines produced from the cross between Two rice varieties (*Oryza sativa.*) SK103 and Rikuto Norin 22 of the proves study to screaning against the *Echinochloa crus -galli* (barnyard grass).The experiments were conducted at the Experimental Farm of the Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh, Egypt, and Laboratory of Genetics Department, Faculty of Agriculture, Tanta University, Egypt. during the three successive rice growing seasons; 2012, 2013, and 2014 throught F3, F4 and F5 generation. All genotypes were evaluated under natural infestation of weeds without using chemical control to determine the allelopathic activity by using the radial area and weed control % around each individual rice plant of the F3, F4 and F5 generation with their parents which sown in the nursery during the last week of May for each season and transplanted after 30 days at a spacing of 20 x 20 cm, with three replications in randomized complete block design, the remain cultural practice were applied as recommended by RRTC (2011), data were recorded on morphological and yield characters as, days to heading (day)**,** seed set (%), grain yield plant-1 and harvest index %.

Molecular analysis ; Ten SSR markers (RM164, RM439, RM323, RM563, RM413, RM407, RM440, RM262, PAL and P450) were selected from rice data base according to Jensen .et al (2008) used to detect genetic variability under the molecular level among rice

genotypes under study.Genomic DNA was isolated from 0.5 g of three weeks old leaves of the used twelve rice genotypes and only ten extremely lines were strong allelopathic activity from the cross (SK103 / Rikuto Norin 22 and their parents (Sakha103 and Rikuto Norin 22), according to (Murray and Thompson, 1980). The quantification and qualification of the extracted DNA was determined on 0.8 % agarose gel comparing to known concentrations of λ uncut genomic DNA. The concentration of DNA was adjusted to approximately 15 ng/ µl for PCR reaction.

Ten SSR primers were used for studying association allelopathic activity, 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, 12.0 µl of PCR master mix (Ferments) and 10 µl ddH$_2$o.   Thermal cycler was used with the following PCR profile: 95 ^{0}C for 5 min (initial denaturation step) 35 cycles for of extension, 94^{0}C for 1min, 55^{0}C for 1min (primer annealing), primer elongation at 72^{0}C for 2min and 72^{0}C for 7min and stored the last temperature was 4^{0}C.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).

Table (1) list of SSR primer

Locus Name	Primer Name	Forward Primer	Reverse Primer
RM164	RM164	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC
RM439	RM439	TCATAACAGTCCACTCCCCC	TGGTACTCCATCATCCCATG

Statistical Analysis:-
The data were subjected to analysis of variance for randomized complete block design as suggested by Panse and Sukhatme (1954).

RESULTS AND DISCUSSION
Mean performance values of one hundred individual plants for each generation (F3, F4 and F5) were presented in Table (2) .

For radial area , the results showed that, highly differences were found among the populations for each generation during the three

summer seasons. The data on F3 generation showed wide rang between the stronger line and poor line as shown in Table (2) whereas the strong line recorded the highest value (12.66 cm) of radial area comparing to strong allelopathy rice variety (9.33) cm, that meaning, transgressive segregation was found among the F3 lines as well as, the general mean value was 9.33 cm that meaning the selection was directed to strong allelopathy, so, the selected population were nearest to the general mean value. The same trend for the allelopathic activity was obtained from F4 and F5 generations and the selection was directed to strong allelopathy, whereas, the general mean values were 9.50 cm and 10.66 cm recorded for the F4 and F5 lines, respectively in their allelopathic reaction against weeds (*E. crus -galli*).

For weed control %, the results showed that , highly differences were found among the rice genotypes under study whereas, the weed control % values were ranged between, 41.00 % of poor allelopathic activity to 85.00% for the strong allelopathic activity in F3 generation, more over, the selected line recorded the highest values 75.00, 74.50 and 74.66 % respectively, indicating to the direction of dominance was towards to better parent. In F4 and F5 generation the general mean values were 80.33 and 82.50 % indicate to the direction of selection was towards the highest values, moreover the values for selected lines of F4 and F5 were nearest to the general mean value, these results were confirmed with the radial area character that meaning, could be develop new promising lines performed as strong to allelopathic activity.

Table (2) Mean performance of parental lines with their selected progenies in F3, F4 and F5 generations for SK 103X Rikuto Norin22 for some morphological characters during 2012, 2013 and 2014 seasons

Season		Entries SK 103X Rikuto Norin22	line No.	Radial Area (cm)	line No.	Weed control %	line No.	Day to Heading (day)
2012	F3 Generation	P1	---	2.33	---	41.33	--	90
		P2	----	9.33	----	87.00	---	93
		H	3	12.66	3	85.00	17	103
		L	11	6.85	20	41.00	85	96
		S₁	33	9.33	33	75.00	33	101
		S₂	40	9.50	45	74.50	70	101
		S₃	51	9.33	51	74.66	83	102
		X̄	-----	9.33	---	75.33	----	101
2013	F4 Generation	P1	----	2.66	---	38.66	---	91
		P2	----	9.33	----	85.33	---	95
		H	20	12.66	20	88.00	20	102
		L	17	6.40	17	45.00	35	97
		S₁	35	9.66	33	78.00	35	100
		S₂	62	9.33	62	78.33	42	100
		S₃	81	9.33	81	80.66	81	100
		X̄	----	9.50	---	80.33	----	100
2014	F5 Generation	P1	---	3.24	---	40.00	----	91
		P2	---	10.50	---	89.00	----	93
		H	51	12.33	51	88.66	80	102
		L	38	5.21	80	46.00	62	98
		S₁	16	10.13	16	80.00	17	100
		S₂	72	10.33	72	81.00	72	100
		S₃	95	10.33	63	79.00	89	100
		X̄	----	10.66	---	82.50	----	100

P1: The first parent   P2: The second parent

H: The highest value   L: The lowest value   S: The selected lines

For days to heading, narrow differences for days to heading was found for the parental lines, because the two parents were early in heading date, while the produced populations were late for heading date, whereas the selection for this trait was direct to the plants which recorded the lowest days to heading indicating to could be utilize from these populations as donors to transfer these desirable characteristics thought breeding programs. Similar results were obtained for most studied traits by El-Abd and Abdalla (2004); Babu *et al.,(*2006) hammoud,(2005).

Mean performance values of the hybrid combination SK.103 / Rikuto Norin 22 with their parents through F3, F4 and F5 generation for some yield and its components characters during three summer seasons 2012 , 2013 and 2014 are shown in Table (3).

For seed set % , as shown in Table (3) narrow differences of seed set % was found among the parental lines with their progenies for the hybrid combination SK. 103 / Rikuto Norin 22, whereas, the SK 103 recorded 86 % but, the Rikuto Norin 22 recorded 90 % during 2012 season .

Table (3) Mean performance values of the populations produced from the F3, F4 and F5 generation for SK 103X Rikuto Norin22 hybrid and its parents for some yield and its component characters during 2012, 2013and 2014seasons

Season		Entries SK 103X Rikuto Norin22	line No.	Seed set (%)	Line No.	Harvest index %	line No.	Grain yield /plant (gm)
2012	F3 Generation	P1	----	86	---	42.50	----	28.66
		P2	----	90	---	47.50	----	49.33
		H	11	100	3	61.33	3	95
		L	18	80	18	32.50	18	60
		S_1	33	95.33	33	39.50	33	70
		S_2	40	95.66	40	40.33	40	70.33
		S_3	100	95.33	100	40.33	90	70.66
		$\bar{X}$	---	95	----	40.23	---	70.33
2013	F4 Generation	P1	-----	83	---	39.50	------	30.33
		P2	-----	92	----	45.23	------	57.50
		H	20	100	20	63.33	20	99.80
		L	70	85	17	31.50	17	53
		S_1	35	95.50	35	45.50	35	75
		S_2	51	95.33	51	45.66	62	75.33
		S_3	59	95.11	65	44.33	65	75.66
		$\bar{X}$	----	95.33	---	45.50	----	75
2014	F5 Generation	P1	---	88	---	40.33	----	27.50
		P2	----	93	---	45.33	----	52.33
		H	31	100	51	64.23	51	112
		L	18	82	40	37.33	40	45
		S_1	16	95.33	16	49.50	16	80.50
		S_2	60	96.27	60	50.33	72	81.66
		S_3	80	96.33	98	50.33	80	81.33
		$\bar{X}$	----	96.33	---	50.00	----	81.86

P1: The first parent   P2: The second parent
H: The highest value   L: The lowest value   S: The selected lines

On the other hand, wide differences of seed set % was found among all these progenies, the highest value was 100 % of line No (11) and lowest value was 80 % of line No (18) , but , general mean

value was 95 % and selected populations recorded the same value , the results indicate the direction of selection was towards the dominant value and the genetic improvement was 5 % surpassed the better parent , the same trend of results were found in the F4 and F5 generations , whereas the general mean value were 95.33 and 96.33 respectively, during both 2013 and 2014 seasons respectively. That meaning, the direction of selection was toward the highest value , and could be enable the breeders to develop a new promising lines with highest allelopathic activity, seed set % and high grain yield. For Harvest index % as shown in Table (3) wide differences was found among the parental lines with their progenies for the hybrid combination of SK103 / Rikuto Norin 22, whereas , the highest Parent (Rikuto Norin22) recorded value 47.50 % but , the lowest **(**SK 103) parent recorded value 42.50 %. Moreover , the progeny of F3 generation recorded wide differences of mean performance , the highest line recorded value 61.33% and the lowest line recorded value 32.50% while, the general mean value was 40.23% and the selected populations were recorded the similar values, the results indicate to the direction of selection was toward the highest value of Harvest index %. So, could be develop new promising line with high yielding and strong to allelopathic activity, moreover , the general mean values for F4 and F5 were 45.50% and 50.00 %, respectively, these results, indicate that, the direction of selection was toward the highest value of Harvest index % for   F4 and F5 generation, to develop new promising line high Harvest index % with strong to allelopathic activity.

For grain yield / plant (gm) as shown in Table (3) wide range of grain yield / plant (gm) was found among the parental lines with their progenies for the hybrid combination SK103 / Rikuto Norin 22, whereas, the highest parent recorded value 49.33 gm, but , the lowest parent recorded value 28.66 gm. Moreover, the progenies of F3 generation recorded wide rang of mean performance , the highest line recorded value 95 gm / plant and the lowest line recorded value 60 gm / plant, while, the general mean value was 70.33 gm / plant and the selected populations were recorded the similar values, the results indicate to the direction of selection was toward the highest grain yield. So, could be develop new promising line with high yielding and strong to allelopathic activity, moreover , the general mean values for F4 and F5 were 75 and 81.86 gm / plant respectively, these results, indicate, the direction of selection was toward the highest value for grain yield through F4 and F5generation, to develop new promising line with high yielding and strong in allelopathic activity. Same results were obtained for most studied characteristics by Adnan Kanbar *et al* (2011) who found that, In pedigree method, the mean values of grain yield were higher in F4 (18.62 g), F5 (20.89 g) and F6 (23.47 g) generations. In

F5generation, Number of tillers and number of panicles were increased from F3 generation to F5 generation.

The data in Table (4) showed highly differences and significant for the studied characters, the mean square values and heritability in broad sense of one hundred populations for each; F3 , F4 and F5 of the hybrid combination Sakha 103 **X** Rikuto Norin22 for some morphological characters, Radial area, weed control % and days to heading recorded the highest values during 2012, 2013 and 2014 seasons, indicating that these characters were under genetic control and no influences by environmental conditions.

Table (4) Mean square values of the selected genotypes for   F3, F4 and F5 for SK 103X Rikuto Norin22 hybrid for some morphological characters during 2012, 2013 and 2014seasons

S.O.V	d.f	Radial area(cm)			Weed control %			Day to heading (days)		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
Reps.	2	0.012	0.009	0.001	0.06	0.13	0.011	0.023	0.04	0.003
Population	99	21.57**	26.45**	19.79**	289.53**	236.35**	311.55**	85.94**	101.18**	67.36**
Error	198	0.23	0.28	0.39	0.26	0.03	0.70	1.56	2.74	1.49
H.b.s		98.95	98.95	98.06	99.91	99.98	99.77	98.21	97.36	97.83
S		4.64	5.14	4.44	17.01	15.37	17.65	9.27	10.06	8.21
C.V		0.024	0.029	0.036	0.003	0.001	0.008	0.015	0.027	0.015

The data in Table (5) showed the mean square values of one hundred individual plants of each genotypes for F3 , F4 and F5 of the hybrid combination of Sakha 103 X Rikuto Norin22 for some yield and its component characters, seed set %**,** Harvest index %   and grain yield / plant $^{-1}$ during 2012, 2013 and 2014 season**s.** The mean square values for the genotypes were highly significant of all selected lines from all generations and the selection will be effective to get promising line with desirable characters, This mean that, all these characters were under genetic control, in the same time, the heritability in broadsence were more than 90% for all the characters of the three generations, this referred to genetic stability of F4 and F5 generations, finally, the variance and coefficient of variation showed that the studied characters did not affected by the environmental factors. Same results were previously obtained by Han *et al.*, (1995); Hammoud *et al.,* (2012) and El-Malky *et al.,* (2013), they found that, moderate genetic advance along with high heritability indicating the involvement of additive type of gene action in controlling these characters.

Table (5) Mean square values of the selected genotypes for F3, F4 and F5 for SK 103X Rikuto Norin22 hybrid for some yield and its component characters during 2012, 2013 and 2014 seasons

S.O.V	d.f	Seed set %			Harvest index%			Grain yield /plant $^{-1}$		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
Reps.	2	0.011	0.014	0.026	0.021	0.0131	0.003	0.015	0.003	0.004
Population	99	840.55**	798.30**	703.32**	145.62**	178.30**	170.50**	1301.80**	1142.11**	1171.93**
Error	198	2.23	1.58	1.12	1.22	1.03	0.89	5.38	2.83	3.74
H.b.s		99.73	99.80	99.84	99.17	98.07	99.42	99.58	99.75	99.68
S		28.99	28.25	26.52	12.07	13.35	13.06	36.08	33.79	34.23
C.V		0.023	0.016	0.012	0.030	0.023	0.018	0.076	0.038	0.046

There were positive and significant associations between grain yield and allelopathic activity in all populations in three years. High heritabilities were estimated for most studies characteres, heritability values showed that grain yield weight as well as allelopathic activity traits could be used as selection criterion in early generations.

Molecular analysis

 Ten SSR markers were used in this study to analyse genetic variability at molecular level among the studies rice genotypes. Among ten SSR markers only two primers generated polymorphic alleles (RM164 and RM439) while, eight markers (RM323, RM563, RM413, RM407, RM440, RM262, PAL and P450) showed monomorphic allele with all studied genotypes.The detected polymorphism reflects the amount of diversity among the tested genotypes and thus the possibility of genetic improvement for such a set of genotypes could by using in breeding programs since genetic diversity is the prerequisite for successful such programs.

The banding patterns presented in Figures (1 and 2) and Table (6). The obtained results showed clear polymorphism between the two parents Sakha103 and RikutoNorin22 with their respective F_5 populations. Two primers, RM164 and RM439 were be have the point to the strong or poor allelopathy. Hence, the two primers were used to screen the individual components of strong and poor along with their parents.

Figure (1) showed that the primer RM164 demonstrated polymorphic bands (alleles) with size of 296 bp for strong parent (Rikuto Norin22) and ten selected lines strong to allelopathic activity of F_5 lines resulting from the cross sakha103 (P1) x Rikuto Norin22 (P2) while, the band with the size of 254 bp was presented in the poor parent sakha103, these results showed that the primer RM164 was found to be segregating with strong and poor individuals in a co-dominant fashion and indicating to the band296 bp of the primer RM164 may be related to activity to allelopathy while, the band 254 bp

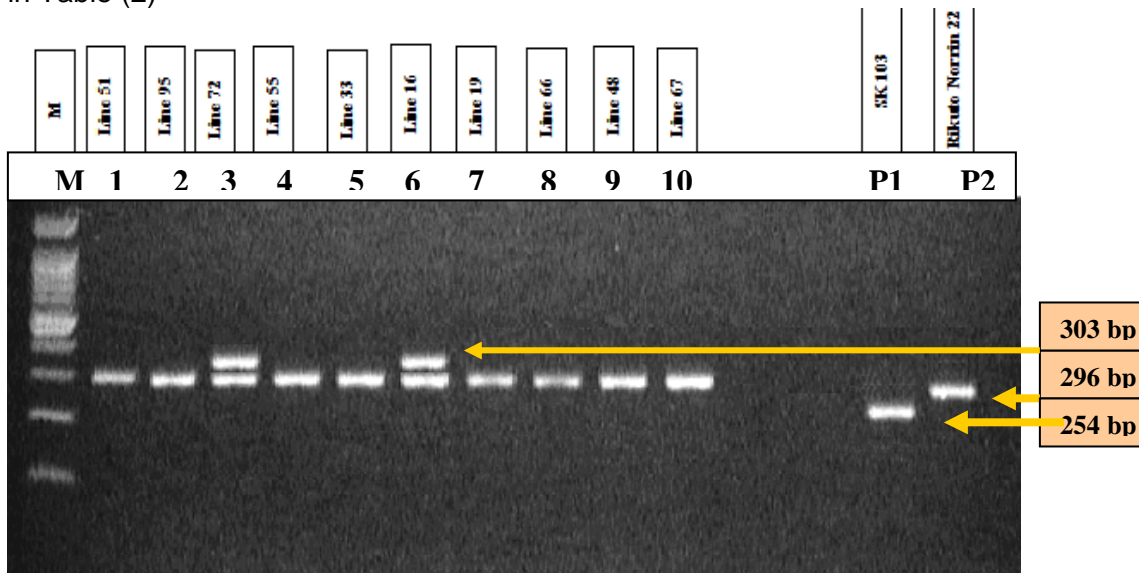of the same primer may be related to suppress to allelopathy as shown in Table (2)

Fig. (1) Agarose gel electrophoresis of PCR productof the ten selected lines, lane 1 to lane 10, P1is Sakha103 ,P2 is Rikuto Norin 22 and M is 100 bp DN Aladder

Lanes 1-10 are ten selected lines of F5 generation according allelopathy activity and yield components. Comparing with both parents, all selected lines showed the associated band with allelopathy activity 296 bp, while the band 254 bp was presented in the parent sk103 and absent in selected lines, indicating to the selection were for allelopathy during F2, F3 and F4. On the other hand, a new allele (303 bp) appear in some lines; lane 3 and 6 , this band may be related to highest grain yield as shown in Table (3).

The conducted DNA banding pattern RM 439 primer showed in Figure (2) polymorphic alleles among all studied genotypes, Rikuto Norin 22 and Sk103 with their F5 generation, strong parent Rikuto Norin 22 showed one distinguish band with molecular mass 576 bp with six lines of F5 generation which was absent in four lines and poor parent Sk103. Primer RM439 gave also an other distinguish band (131 bp) in the parent SK103 and two line; lane 5and 9.
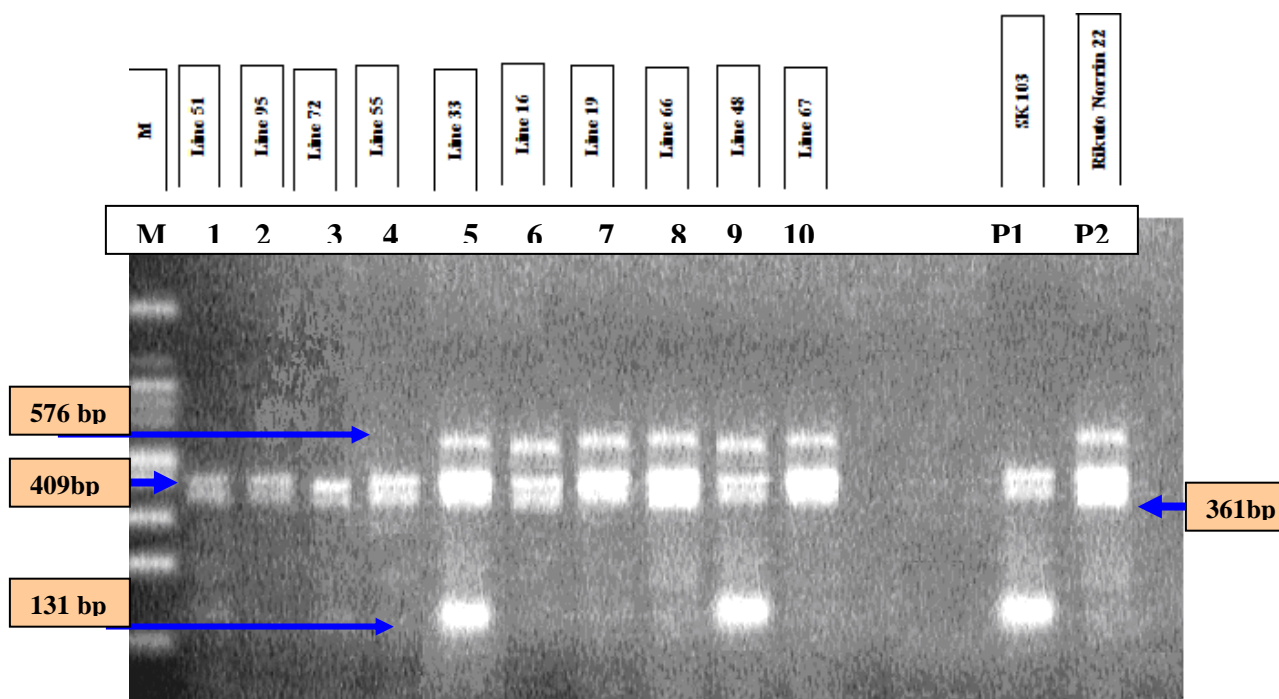
Fig. (2) Agarose gel electrophoresis of PCR productof the ten selected lines, lane1 to lane 10, P1is Sakha103, P2 is Rikuto norin22and M is 100 bp DNA ladder.

Table 6. Showed presence and absence matrix

Marker	alleles	P1	P2	1	2	3	4	5	6	7	8	9	10	M.W
RM164	1	0	0	0	0	1	0	0	1	0	0	0	0	303
	2	0	1	1	1	1	1	1	1	1	1	1	1	296
		1	0	0	0	0	0	0	0	0	0	0	0	254
RM439	1	0	1	0	0	0	0	1	1	1	1	1	1	576
	2	1	1	1	1	1	1	1	1	1	1	1	1	409
	3	1	1	1	1	1	1	1	1	1	1	1	1	361
	4	1	0	0	0	0	0	1	0	0	0	1	0	131

CONCLUSION

In conclusion, this study indicated that presence of genetic diversity among the mean performance of all generations for all studied traits. The genotypic effect was significant in allelopathic activity traits (radial area andweed control %) indicated that genotypic effect played an important role in determining the inheritance of these traits. Our finding with the SSR markers tested pointed to some distingush vans (alleles) and cauld be used as selection tool in early generation. the results, could be concluded also that, the parent Rikuto

Norin 22 recorded a wide radial area and mean values of grain yield, finally, line No.16 and line No.72 for the cross Sakha 103 / Rikuto Norin 22 which similer to their male parent with reaction to the two primer RM164 and RM439 and might be considered as the most superior and prospective genetic materials for most studied characters, especially had the wide radial area to weed control and highest yield / plant**.**

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التحسين الوراثي لنشاط الاليلوباثى في بعض التراكيب الوراثية للأرز

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الملخص العربي

اجريت هذه الدراسه فى مركز البحوث والتدريب فى الارز ، سخا ، كفر الشيخ ومعمل قسم الوراثه كليه الزراعه جامعه طنطا – مصر خلال ثلاث مواسم زراعه 2012 و 2013 و 2014 .تم تقييم صنفى من الارز هما سخا 103 والركتونورين 22 والهجن الناتجه منهم فى دراسه سـابقه وذلك للاجيـال مـن الثالـث حتى الخـامس لمقاومتهـا لحشيشـه الدنيبـه تحت ظروف عـدوى الطبيعي. واظهرت النتائج ان هذه الاصناف تباينت فى مقاومتها للحشائش تحت ظروف العدوى الطبيعية بالحقل وكانت القيم تتراوح من 2.33سم الى 12.6سم للمسافه الخاليه حول النبات وبالنسبه لمقاومـه الحشـائش فكانت القيم تتراوح من 41.3% الـى 87% . ويتضـح مـن ذلك ان التراكيـب الوراثيه المنتخبه تكون مفيده للزراعه المباشره ويمكن الاستفاده بها من خلال برامج التهجين فى نقل صفه المقاومه للحشائش الى الاصناف التجاريه او المنزرعه لتثبيط نمو الحشائش وخفض تلوث البيئه ويرجع معامل التباين الوراثى الى تاثير التركيب الوراثى للصنف والذى يلعب دور مهم فى وراثه الصفات المدروسه وكان نسبه المكافي الوراثى بالمعنى الواسع تتراوح من 98.06%الى 99.98% لنشاط الاليلوباثى . وتم استخدام عشره من المعلمـات الوراثيه(SSR marker) اظهر اثنين فقط تعدد مظهرى للمـاده الوراثيه   DNA وهمـا   RM164 و RM439 حيث اظهر البريمـر RM164 حزمه بوزن جزيئى 296 قاعده وراثيه فى كل التراكيب الوراثية التى لها نشاط اليلوباثى وكانت غائبه فى التراكيب المنخفضه فى النشاط الاليلوباثى . ولذلك يعتبر SSR marker عامل مهم فى التعرف على تتابع ال DNA الذى يساعد مربى النبات فى برامج التربيه.