PRELIMINARY STUDIES ON THE PRODUCTION OF NEAR ISOGENIC LINES RESISTANT TO BLAST DISEASE VIA ANTHER CULTURE IN RICE

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Abstract

The regenerated plantlets obtained from the F1's via anther culture technique in rice, were adapted and grown in the greenhouse to produce seed. Twenty-five near isogenic lines succeeded to produce seeds. These lines were derived from two crosses namely Giza 159 x BL1 (18 lines) and Giza 159 x Pi 4 (7 lines). These near isogenic lines were grown with their respective parents for field evaluation for blast reaction as well as agronomic and yield characters. Three lines of the cross Giza 159 x BL1 and one line of the cross Giza 159 x Pi 4 produced high yields comparable to their parents and exhibited resistance to blast. The entries at seedling stage were evaluated under greenhouse conditions using artificial inoculation by four identified pathotypes of the pathogen (*Pyricularia oryza*). All 18 lines of the cross Giza 159 X BL1 were resistant to all isolates except one line which was moderately resistant to isolate no. 2. Meanwhile, out of seven lines of the cross Giza 159 x Pi 4, one and three lines were resistant to isolates no. 1and no.3, respectively. On other hand, one line was moderately resistant to isolate no. 2.The results showed also that Giza 159 was susceptible for all isolates except isolate no. 3, while both BL1 and Pi 4 genotypes were resistant to all isolates. Thus, we can use both of them as donors to blast resistance in anther culture programs in rice. Further studies are needed to develop anther cultuer technique for increasing the number of regenerated plants to detect more near isogenic lines.

INTRODUCTION

Rice (Oryza sativa L.) is one of the major cereal crops in the world. The properties of its kernels make it the leading cereal for human food. To cope with the rice demands of the fast growing population in Egypt, increasing grain yield of rice is of a national necessity. The increased grain yield per unit area is an urgent goal and might be the most possible way for enhancing total production. This goal could be achieved through growing rice cultivars of high yield potentiality as well as following appropriate management.

Rice blast disease (*Pyricularia oryza*) has long been recognized as the most spread and potentially damaging disease of the rice crop in Egypt. The outbreak of rice blast disease in 1984 season caused tremendous quantitative and qualitative losses of rice crop especially on the variety Reiho cultivated in about four million

feddans (Kamel *et al.* 1986). Yield losses due to blast could be reduced with the increase of cultivated area with high yielding and blast resistant new varieties.

Plant tissue culture is an enabling technology from which many novel tools have been developed to assist plant breeder. These tools can be used to increase the speed of the breeding process to improve the accessibility of existing germplasm and to develop new varieties for crop improvement. They include micropropagation, anther culture, in vitro selection, embryo rescue, somaclonal variation, somatic hybridization and transformation (Karp 1995)

By accelerating the breeding cycle through anther culture techniques, new varieties could be obtained within a short period. Callus induction and regeneration of plants from anther culture media differ from one medium to another (Davoyan *et al.* 1988, Mei *et al.* 1988 and Quinio and Zapata 1990). Selection of effective medium for callus induction and subsequent regeneration of rice plants from anthers in a short period of time with a large number of plants is very important for saving time, effort and money.

Breeding for blast resistance in rice is still one of the major goals of breeding objectives in rice breeding program in Egypt. Different strategies are used in Egypt to breed for blast resistance such as gene accumulation, pyramiding deployment and utilization of partial resistance (Aidy *et al.* 2000). One of the recent applied breeding strategies is using multiline varieties in rice (Yu *et al.* 1991 and Mackill and Onman 1992). An attempt to utilize this strategy in Egypt is needed.

Near isogenic lines (NILs) represent a tool to introduce new genes in a multiline variety which contains more than one near isogenic line for resistance to diseases. They have many advantages over other disease resistance breeding methods such as gene pyrimiding, gene accumulation and gene deployment. NILs can use many resistant genes in a multiline no matter if it is allelic or non-allelic. Another advantage is that a multiline can accept new resistant genes any time, since the new NIL is introduced and the susceptible one can be removed from the multiline. On the other hand, Nakajima *et al.* (1996) found that both blast disease severity and percentage of diseased plants in the mixture were less than that observed in the single line plantings.

As the development of NILs through the backcross technique requires considerable time, anther culture technique can be used to accelerate the breeding cycle and reduce time of varietal improvement. Therefore, the main objectives of this study was to utilize anther culture technique for producing near isogenic lines resistant to blast disease for releasing multiline resistant rice varieties.

MATERIALS AND METHODS

This investigation was carried out at the Rice Research and Training Center, Kafr El-Sheikh, Egypt and the Genetics Dept., Fac. Of Agric., Kafr El-Sheikh, Tanta Univ., during the successive seasons from 1997 to 2000.

Ten local and introduced rice *(Oryza sativa L.)* varieties, with different blast resistant genes, were used in this study. Eight of them (Pi No. 4, BL1, Zenith, Dular, K.3, K59, Akiyutaka and Fukuhikari) were donors for blast resistance genes and used as male parents. The two recurrent genotypes were the Egyptian rice varieties, Giza 159 and Reiho that were used as female parents to produce hybrid seeds of the 16 hybrids (8 donors x 2 recurrents). The hybrid seeds were harvested separately for each cross and prepared for sowing in the next season. In 1998 rice growing season, the hybrid seeds were sown to produce F_1 plants. These F_1s were grown for anther culture experiments.

In 1998 season, anther culture procedure was applied to the 16 F1's to produce the near isogenic lines. According to the data collected on parents with the two callus induction media, Fj medium (Gamborg, 1970) was chosen as the more responsive medium than N6 medium (Chu et al., 1975). About 100 anthers were inoculated in petri dish. The dishes were sealed with parafilm and incubated in darkness at 25 $\pm$ 1 °C. The responded anthers in both media for callus induction were recorded usually for 5-9 weeks. This part of investigation was carried out in a randomized complete block design with three replications. Fifteen petri dishes were plated with the recommended density of anthers to avoid contamination and three plates were randomly chosen for data collection. Furthermore, all petri dishes were used to regenerate the near isogenic lines.

For plant regeneration, the calli forming 2-3 mm in diameters (collected after incubation for 1-2 months) transferred to petri dishes containing plant regeneration MS medium (Murashige and Skoog 1962) and kept in the light (16 hours light and eight hours dark) at 25-27 °C. Shoot development and roots establishment are usually done in regeneration media in large test tubes. Plantlets with vigorous rooting systems were transferred to the culture solution for two weeks under normal day length for their adaptation to the field conditions. Plantlets were individualized and replanted in the culture solution for one more week after which they were finally transplanted in pots in the greenhouse.

Anther culture-derived plants at the beginning of flowering and before the onset of anthesis were bagged to prevent cross pollination with other rice plants.

Seeds were separately collected from the anther culture- derived plants and prepared for seed multiplication for further evaluation of obtained lines.

The regenerated plantlets from the F1's during 1998 season were adapted and grown in the greenhouse to produce seeds. A total of 25 near isogenic lines succeeded to produce seeds. These lines belonged to both crosses i.e., BL 1 x Giza 159 (18 lines) and Pi No. 4 x Giza 159 (7 lines). These lines were grown with their respective parents for field as well as greenhouse evaluation for blast reaction. In 1999 rice growing season, 28 genotypes, including the prospective near isogenic lines (25) and their respective parents (3), were grown in May 15th. After 30 days from sowing, seedlings were transplanted in the experimental field with three rows/ entry. Each row was 5 m long and one seedling/hill with three replications in a completely randomized block design. The recommended field practices were applied as recommended. Data were collected for some agronomic traits, yield and its components and blast reaction characters.

Data collection: for correlation analysis, all characters of parents (10- characters) and some of them for anther culture-derived lines (5 characters) such as: Plant height, number of tillers per plant, grain yield/plant (g), panicle weight (g), one hundred grain weight (g), number of panicles per plant, number of spikelets per panicle, number of filled grains per panicle, panicle length (cm) and number of primary branches per panicle, as well as leaf blast reaction estimated according to IRRI (1996) by using 0-9 standard scale as follows 0 = Highly resistant (HR), 1-2 = Resistant (R), 3 =Moderately resistant (MR), 4-5 = Susceptible (S), and 6-9 = Highly susceptible (HS).

STATISTICAL ANALYSIS

From the analysis of variance, heritability and correlation coefficients among the ten parents and among the AC derived lines for grain yield per plant, blast reaction and yield attributes were computed.

RESULTS AND DISCUSSION

1-Evaluation of Anther culture (AC) derived doubled haploid lines (near isogenic lines) under field conditions

AC derived doubled haploid lines obtained in the present study from the parents and their two F_1 crosses were evaluated in the field for some agronomic and yield characters as well as blast reaction. The mean performance of AC lines, 3 parents and their 25 F_1 hybrids for grain yield, yield attributes and leaf blast reaction characters are presented in Table (1). For number of panicles/plant, the parent

BL1and AC lines No. 1, 3, 4, 6, 7, 9, 11, 12, 14, 15, 16, 17 and 18 from the cross Giza 159 x BLi gave the highest mean value (more than nine tillers/plant) and was resistant to blast reaction. The same trend was obtained for the AC line no. 1 regenerated from the cross Giza 159 x Pi 4. Moreover, five AC lines (No. 1, 3, 4, 12, 18) from the cross (Giza 159 x BLi) and one AC line (No. 1) from the cross (Giza 159 x Pi 4) gave the highest mean values of grain yield/plant (more than 45.58 grams). Also, those lines showed desirable values for panicle weight and 100 grain weight, as well as high resistance to blast reaction. AC line No. 1 obtained from cross (Giza 159 x Pi 4) exhibited the highest value for grain yield trait (53.48 grams) and resistance to blast (Score 1). In spite of Pi 4 parent considers as a good donor for blast reaction, it had lowest value of grain yield (28.85 g). On the other side, AC line No. 10 from the cross (Giza 159 x BL 1) and AC lines No. 2 and No. 7 from the cross (Giza 159 x Pi 4) exhibited mean values of grain yield as 33.83, 42.67 and 42.73 g, respectively, and moderately resistance to blast reaction.

The results demonstrated that through anther culture, it is possible to produce dihaploid lines in a short time. Meanwhile, the possibility of regenerating recombinants with desirable characters such as highest yield and increased resistance to disease from both parents is more favorable. Field evaluation revealed that the three lines (No. 1, 18 and 12) of the cross Giza 159/BLi and one line (No.1) of the cross Giza 159/Pi 4 produced high yields comparable with their parents. Moreover, these lines were evaluated for resistance to blast and recorded 1-2 scores indicating that the obtained lines were resistant to blast. Furthermore, the feasibility of using the multiline of four isogenic lines with different resistance was effective for the control of the disease. These results agreed with those obtained by Miah *et al.*. (1996) who found five lines tolerant to salt at 8-10 ds/m. and gave high yields comparable with the salt tolerant control under saline conditions.

Regarding the similarity among the AC derived lines of the sister lines of the cross Giza 159 x BLi and line number 1 of the cross Giza 159 x Pi 4, to the parent Giza 159, no significant differences among grain yield, yield attributes and blast reaction were detected. According to these results we can consider these lines as near isogenic lines for blast resistance to the parent Giza 159. However, further studies are needed under field conditions to clarify this point.

Table 1. Mean performance of AC derived lines for grain yield, yield contributing traits and blast reaction in 1999

Genotype	No of panicles/plant	Panicle Weight (g)	100-grain weight (g)	Grain yield (g)	Blast reaction
Giza 159	10.92 a-d*	3.74 abc	2.65 a-d	52.84 abc	7
BL1	10.10 c-g	3.86 ab	2.63 a-d	35.17 f-i	2
Giza 159xBL1					
Line 1	10.53 a-c	3.75 abc	2.56 a-d	55.55 a	2
Line 2	9.67 d-h	3.63 a-e	2.96 a	39.26 d-i	2
Line 3	10.33 a-f	3.31 b-e	2.63 a-d	48.19 a-c	1
Line 4	10.93 a-d	3.73 abc	2.52 a-d	45.58 a-f	2
Line 5	8.93 g-k	3.13 def	2.62 a-d	44.39 a-g	1
Line 6	10.93 a-d	3.68 a-d	2.79 abc	41.58 b-h	1
Line 7	11.00 abc	3.73 abc	2.60 a-d	39.17 d-i	1
Line 8	9.87 c-g	3.51 a-c	2.45 a-d	38.04 c-i	1
Line 9	10.10c-g	3.53 a-e	2.55 a-d	43.39 b-g	1
Line 10	11.13 abc	3.27 c-f	2.79 abc	33.83 f-i	3
Line 11	10.53 a-c	3.97a	2.87 ab	32.46 ghi	2
Line 12	10.17 b-g	3.49a-e	2.63 a-d	50.53 a-d	2
Line 13	9.93 c-g	3.57 a-e	2.62 a-d	41.05 c-h	2
Line 14	10.60 a-e	3.87 ab	2.66 a-d	41.79 b-h	2
Line 15	11.40ab	3.99 a	1.72 e	38.98 d-i	2
Line 16	10.87a-d	3.71 abc	2.57 a-d	43.95 a-g	2
Line 17	11.47 a	3.63 a-e	2.59 a-d	41.42 b-h	2
Line 18	10.67a-e	3.82abc	2.67 a-d	51.97 abc	2
Pi 4	8.17ijk	2.27 g	2.43 a-d	28.85 i	1
Giza159 x Pi 4					
Line 1	9.17f-j	3.50 a-e	2.39 a-d	53.48 ab	1
Line 2	7.87k	3.07ef	2.13 d-e	42.67 b-g	4
Line 3	9.57e-h	2.77fg	2.29 bcd	37.55 e-i	5
Line 4	8.00jk	2.51 g	2.52 a-d	36.83 e-i	6
Line 5	9.27 f-I	2.49 g	2.23 cd	51.73 abc	5
Line 6	8.57h-k	2.47 g	2.33 bcd	30.40 h-i	6
Line 7	7.87 k	3.38 b-e	2.30 bcd	42.37 b-g	4

Correlations:

Correlation coefficients were computed among the ten parents (10 characters), as well as AC derived lines (5 characters) for grain yield per plant, blast reaction and yield attributes (Tables 2 and 3, respectively). The results revealed significant and positive correlations among most of traits. These relationships are very important to the plant breeder at different group levels to select for each pair of traits separately.

Several authors such as Mahajan and Mehan (1980), Mehetra *et al.* (1994), Ammar (1997) and Abo Yousef (2001) reported the same trend. They found that there was positive and significant correlation between yield and each of number of panicles/plant and 100 grain weight and significant and positive correlation between plant height and panicle length.

2- Evaluation of near isogenic lines for blast reaction under greenhouse conditions

Eighteen and seven lines were produced from the crosses between Giza 159 x BLi and Giza 159 x Pi 4, respectively. These lines as well as their parents were exposed as 21 day-old seedlings to artificial inoculation under greenhouse condition with four isolates of *P. oryza* (No. 355, 358, 15 and 357) .

Data presented in Table (4) showed that seventeen out of 18 lines form the cross Giza 159 x BLi were resistant to isolate No. 2 (358) and one line was moderately resistant. Meanwhile, all lines were resistant to isolates No. 1, 3 and 4. In contrast, one line only out of seven lines of cross Giza 159 x Pi 4, and three lines were resistant to isolates No.1 and 3, respectively, while, one line was moderately resistant to isolate No. 2 and all other lines were susceptible to the four isolates.

Data presented in Table (4) showed that 17 out of 18 lines for the cross Giza 159 x BLi were resistant to isolate No. 2 (358) and one line was moderately resistant. On the other hand, all lines were resistant to isolates No. 1, 3 and ٤. In contrast, one line only out of seven lines of cross Giza 159 x Pi 4, and three lines were resistant to isolates No.1 and 3, respectively. On the other side, one line was moderately resistant to isolate No. 2 and all other lines were susceptible to the four isolates.

Table 2. Correlation coefficients between grain yield, yield attributes and blast reaction for ten rice parents.

	PH	N.P/PI	PL	PW	N.Sp/P	NFG/P	NPB	100GW	IPGY	BR
Plant height	1	-0.179	0.670*	0.295	0.323	0.563	0.649*	0.289	-0.013	-0.044
No.0 of panicle/plant		1	-0.599*	-0.255	0.364	-0.120	-0.225	0.289	0.352	-0.546
Panicle length			1	0.551*	0.703*	0.730*	0.831**	-0.255	0.003	-0.285
Panicle weight				1	0.597*	0.869**	0.634*	0.678*	0.626*	0.493
No. of spikelets/panicle					1	0.607*	0.865**	0.624*	0.106	0.182
No. of filled grain/panicle						1	0.788**	0.293	0.562*	0.330
No. of primary branches							1	0.743**	0.177	0.190
100- grain weight								1	0.238	-0.122
Individual plant grain yield									1	0.687*
Blast reaction										1

* and ** Significant at 0.05 and 001 levels of probability, respectively.

Table 3. Correlation coefficients between grain yield, yield attributes and blast reaction of the AC derived lines and three parents.

	N.P/PI	PW	100-GW	GY/PI	BR
No. of panicle/plant	1	0.731**	0.296	0.206	- 0.241
Panicle length		1	0.394*	0.497*	- 0.456**
100- grain weight			1	0.004	- 0.258
Grain yield				1	- 0.024
Blast reaction					1

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

The results of AC-derived lines reflected the genetic background for their parents, where Giza 159 was susceptible for all isolates except isolate No. 3. While the other parents, BLi and Pi 4 were resistant to all isolates, thus, we can use both of them as donors to blast resistance in anther culture programs.

In a similar study, Reiffers and Freire (1990) found that eight anther culture derived lines regenerated from two single crosses had low records ranging from 1-3, indicating that these lines were blast resistant. Another study (El-Wahsh 1997) found that three out of five AC lines of Giza 171 (H.S., highly susceptible) were resistant whereas, two lines were susceptible, eight out of 24 AC lines of Giza 176 (H.S) were resistant, while, 16 were susceptible. And five out of eight AC lines of Reiho (H.S) were resistant, while three lines were susceptible. .However, further studies are needed to improve anther culture techniques for increasing the number of regenarated plants to have the chance to produce more near isogenic lines similar to the recurant parent but has doner parent's gene(s).

Table 4. Response of anther culture derived lines and their parents to artificial inoculation with *P. oryza* under greenhouse conditions at seedling stage.

Genotype	Tested lines (no)	Response to infection with *P. oryza*											
		Resistant				Moderate				Susceptible			
		1*	2	3	4	1	2	3	4	1	2	3	4
Giza159x BL1	18	18	17	18	18	-	1	-	-	-	-	-	-
Giza 159x Pi4	7	1	-	3	-	-	1	-	-	6	6	4	7
Total	25	19	17	21	18	-	2	-	-	6	6	4	7
Giza 159	1	-	-	R	-	-	-	-	-	S	S	-	S
BL	1	R	R	R	R	-	-	-	-	-	-	-	-
Pi4	1	R	R	R	R	-	-	-	-	-	-	-	-

* (1) Isolate # 355 (2) Isolate # 358
(3) Isolate # 15 (4) Isolate # 357

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دراسات أولية علي إنتاج سلالات شقيقة من الأرز مقاومة لمرض اللفحة من خلال زراعة المتك

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تمت زراعة نباتات الجيل الأول الناتجة من زارعة المتك بعد أقلمتها في الصوبة الزجاجية لإنتاج البذور. نجحت ٢٥ سلالة شقيقة في إنتاج بذور. وهذه السلالات تتبع هجينين الأول هو جيزة ١٥٩ × BL1 (١٨ سلالة) والثاني هو جيزة ١٥٩ × Pi 4 (٧ سلالات). زرعت هذه السلالات الشقيقة مع آبائها في الحقل لتقييمها من حيث المقاومة لمرض اللفحة بالإضافة إلي الصفات الحقلية والمحصولية.

وقد وجد أن ثلاث سلالات من الهجين جيزة ١٥٩ × BL1 وسلالة واحدة من الهجين جيزة ١٥٩ × Pi 4 تفوقت علي آبائها من حيث المحصول بالإضافة إلي مقاومتها لمرض اللفحة وفي الصوبة تم تقييم هذه السلالات في مرحلة طور البادرة باستخدام العدوى الصناعية لأربع عزلات معروفة من فطر مرض اللفحة.

وقد وجد أن جميع السلالات الثماني عشر الناتجة من الهجين جيزة ١٥٩ × BL1 كانت مقاومة لجميع عزلات الفطر عدا سلالة واحدة كانت متوسطة المقاومة للعزلة رقم ٢.

ينما كانت سلالة واحدة من السبعة سلالات مع الهجين جيزة ١٥٩ × Pi 4 مقاومة للعزلة رقم ١ وثلاث سلالات مقاومة للعزلة رقم ٣ بينما وجدت سلالة واحدة متوسطة المقاومة للعزلة رقم ٢.

وقد أظهرت النتائج أن الصنف جيزة ١٥٩ يعتبر قابلا للإصابة بجميع عزلات الفطر عدا عزلة رقم ٣. بينما كان كل من الأبوين BL1، Pi 4 مقاوما لجميع العزلات. وعليه يمكن استخدام أيهما كأب مانح للمقاومة لمرض اللفحة.

كما أظهر البحث الحاجة إلي مزيد من الدراسة من أجل تطوير تقنيات زارعة المتك من أجل زيادة عدد النباتات المتكشفة من كل هجين بهدف الحصول علي عدد أكبر من السلالات الشقيقة.