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Research Article
Improvement of Blast Resistance and Yield Productivity in Rice (*Oryza sativa* L.) Using Tissue Culture and Combining Ability

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Abstract

Background and Objective: Blast disease is one of the major pathogens affecting crop productivity. Breeding for resistant is economic way to produce resistant variety and tissue culture used as a tool to decrease the period of breeding program. **Materials and Methods:** Seven varieties (Al-Ahsa Type1, Al-Ahsa Type2, Sakha104, Gz6903, Giza178, Giza177 and Sakha105) were used for tissue cultural and crossing through line X tester method. Somaclonal variation and 12 F1 were evaluated and estimated the GCA and SCA under field condition. The MS with 2,4-D (2,4-Dichlorophenoxyacetic acid) 3% sucrose were used for callus induction and plant regeneration. **Results:** For callus induction, the varieties Sakha104, Gz6903, Giza177 and Sakha105 gave 100%, while, varieties Al-Ahsa Type1, Al-Ahsa Type2 gave 85%. In addition, 18 lines produced from Al-Ahsa Type1 results showed that 13 lines were resistant to blast and 5 lines were susceptible. In SC1 family derived from Al-Ahsa Type2 results indicated that ten lines were resistant to leaf blast, while 6 lines were susceptible. As for SC1 family derived from Sakha104, 11 lines were produced and results showed that 6 lines were susceptible to blast and five lines were resistant to leaf blast. In field experiment, 12 F1s were produced through Line×Tester and evaluated with their parents for genotypic variation. The results showed that both general combining ability (GCA) variances were highly significant for all characters studied in F1 generations. **Conclusion:** Tissue culture in traditional plant breeding is one of the most effective means for production new varieties of rice resistant to blast disease as well as improving crop traits. The most promising hybrid combinations were Al-Ahsa Type1×Sakha105, Al-Ahsa Type2×Giza178, Al-Ahsa Type2×Sakha105, Sakha104×Giza177 and Gz6903-1-2-2×Sakha105 for desirable traits and could be utilized in rice breeding program to improve these traits.

Key words: *Magnaporthe grisea*, *Oryza sativa*, rice blast, somaclonal, tissue culture

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop in the world and the world annual production reach[1] to 750 Mt in 2016. Rice has principle food components for all human development stages; therefore, more than two billions of people in the globe depends on rice as a good source of proteins and calories [2-4]. The main obstacles in the production of rice are due to different causes such as attack of insects and disease in addition to the shortage of water. The major diseases caused by fungi is rice blast that caused by fungal pathogen of *Magnaporthe grisea*. Yield losses thought blast epidemic reduce paddy yield globally with an average of 10 Mt annually[5]. In addition, it is estimated that the yield losses by blast disease annually ranged[6] from 24-41%. However, the economic controls for resistance has produced new varieties thought tissue culture techniques and transfer the resistant genes by hybridization methods compared with chemical control method[7]. Somaclonal (SC) variations in rice were used to improve the resistance and agronomic traits after tissue culture, which involve a callus stage[8-10]. Seeds were used to induce callus induction, which has more potential compared to node or tip on nutrient medium containing specific growth chemicals[11-16]. On the other hand, hybridization method is important in any breeding program to transfer the desirable traits[17]. The cross analysis helps to identify the best parents and their combinations. Combining ability analysis, which is derived from Line X Tester mating design is usually the appropriate method for choosing the parents and progenies with high General Combining Ability (GCA) and high Specific Combining Ability (SCA), respectively[18,19]. The present study is based on tissue culture techniques for callus induction and plant regeneration. Evolution of somaclonals as new lines. Estimate General Combining Ability (GCA) and Specific Combining Ability (SCA) of yield and its components for the F_1, which is produced from crossing between different genotypes.

MATERIALS AND METHODS

Tissue culture experiment

Callus induction and maintenance: This study was carried out at the Department of Biotechnology, Faculty of Agriculture and Food Science, King Faisal University with cooperation with experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during 2016-2018 rice-growing seasons. Seven rice genotypes were used in this investigation (Table 1). A total of 10 dehusked mature seeds from each variety were sterilized by submerging in 30% Sodium Hypochlorite for 20 min and washing them three times by sterile distilled water under aseptic conditions in a laminar airflow hood. Three replications were made and used MS[20] supplemented with 100 mg L^{-1} Myo-inositol, 1 mg L^{-1} thiamine-HCl, 3 mg L^{-1} 2,4-D (2,4-Dichlorophenoxyacetic acid) 3% sucrose. The pH was adjusted to 5.7 prior to autoclaving of the medium at 121°C and 15 psi for 20 min. The cultures were incubated in darkness at 25+1°C for 4 weeks to encourage callus initiation and induction. After 4 weeks, the frequencies of callus induction and the mean value of callus fresh weight were recorded.

Plant regeneration: The excellent types of calli were chosen for plant regeneration and they were transferred to regeneration media, which content by 6-Benzile Adenine (BA). Calli regenerated into normal looking rice plantlets were transferred to greenhouse for adaptation.

Somaclonal (SC) variation: Regenerated plantlets, SC0 plants, were placed in small pots for one week and transplanted to grow until maturity. Seed of each panicle were threshed separately to produce a line in the next season. In 2016, each line was cultivated in a separate row with its parent. The quantitative characters were measured on the plants of this season (SC1 plants). The studied characters included, sensitivity to rice blast, duration (day), plant height (cm), number of panicles/plant, panicle length (cm), panicle weight, 1000 grain weight (g), number of grains/panicle, number of unfilled grains/panicle and grain yield/plant (g).

Field experiment: This study was carried out at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during 2016-2018 rice-growing seasons. The materials used were seven rice varieties (*Oryza sativa* L.) included two varieties from Kingdom of Saudi Arabia, namely; Al-Ahsa Type1and Al-Ahsa Type2 and five Egyptian varieties and lines viz. Sakha104, Gz6903, Giza178, Giza177 and Sakha105. A line x Tester mating design was used, where four cultivars/lines; Al-Ahsa Type1, Al-Ahsa Type2, Sakha104 and Gz6903 were used as "Lines" and the three varieties; i.e., Giza178, Giza177 and Sakha105 were stock in the Rice Research and Training Center (RRTC), Agricultural Research Center (ARC), Egypt (Table 1).

Table 1: Parentage, origin and blast disease reaction used for rice genotypes under study

Entries	Parentage	Origin	Blast reaction
Al-Ahsa Type1	Exotic	Saudi Arabia	S*
Al-Ahsa Type2	Exotic	Saudi Arabia	S
Sakha104	Gz4096-8-1 / Gz4100-9-1	Egyptian	S
Gz6903-1-2-2	(GZ 4596/SUWEON 313)	Egyptian	R**
Giza178	Giza175/Milyange49	Egyptian	R
Giza177	(Giza 171/Yu mji No.1//piNo.4)	Egyptian	R
Sakha105	Gz5581 /Gz4316	Egyptian	R

*S: Susceptible, **R: Resistant

A line×tester cross among seven parents was used to produce twelve crosses during 2015 season. F_1 seeds and parental varieties were grown in 2016 in rows at distances of 20×20 cm and the experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications. F_1 plants and the parents were transplanted in 2016 season and nine agronomic traits were recorded. These agronomic traits included sensitivity to rice blast, duration (day), plant height (cm), No. of tiller/plant, panicle length (cm), No. of panicle/plant, panicle weight (g), 1000 grain weight (g), No. of filled grain/panicle, Sterility (%) and grain yield/plant(g).

Statistical analysis: In tissue culture experiments, analysis of variance was used as described by Snedecor[21]. While, Duncans Multiple Range Test (DMRT)[22,23] was used for the analysis of plant produce from tissue culture. Line×tester analysis were subjected to analysis of variances for a randomized complete blocks design as suggested by Panse and Sukhatme[24] and the analysis of variance for line×tester crossing design Kempthorne[25]. The fixed model used to estimate General Combining Ability (GCA) and Specific Combining Ability (SCA) effects is as follows:

$$xij = U+gi+gj+sij+eijk$$

Where:

U = Population mean
gi = GCA effect of ith line parent
gj = GCA effect of jth tester parent
Sij = SCA effect of ijth combination
eijk = Error associated with the observation xijk

The individual effects were estimated as indicated below; The estimates of GCA effects:

- GCA effects of each line were calculated according to the following equation:

$$gi = \frac{Y_{i..}}{tr} - \frac{Y..}{Ltr}$$

Where:

Yi.. = Total of the ith line over testers
Y.. = Grand total
L, t and r = Number of lines, testers and replications, respectively

- GCA effects of testers were calculated as follows:

$$gj = \frac{\sum Y.j.}{Lr} - \frac{Y..}{Ltr}$$

Where:

Y.j. = Total of ith tester over lines

The estimates of SCA effects:

- The values of SCA effects were determined as follows:

$$Sij = \frac{Yij.}{r} - \frac{Yi..}{rt} - \frac{Y.j.}{rL} + \frac{Y..}{L+r}$$

Where:

Yij. = Value of ith line with ith tester

The estimates of Standard Error (SE) pertaining to GCA effects of lines and testers and SCA effect of different combinations were calculated as follows:

$$SE\ (GCA\ for\ lines) = \sqrt{\frac{Me}{rt}}$$

$$SE\ (GCA\ for\ testers) = \sqrt{\frac{Me}{rL}}$$

$$SE\ (SCA\ effects\ for\ combinations) = \sqrt{\frac{Me}{r}}$$

RESULTS

Callus induction and plant regeneration: Callus initiation began with enlargement of the embryo area of the seed and radical started to grow until it reached 2-3 cm long. Radical growth is stopped after 5-7 days and callus started to grow, coming out of the embryo and after 30 days, it was separated from seeds (Fig. 1).

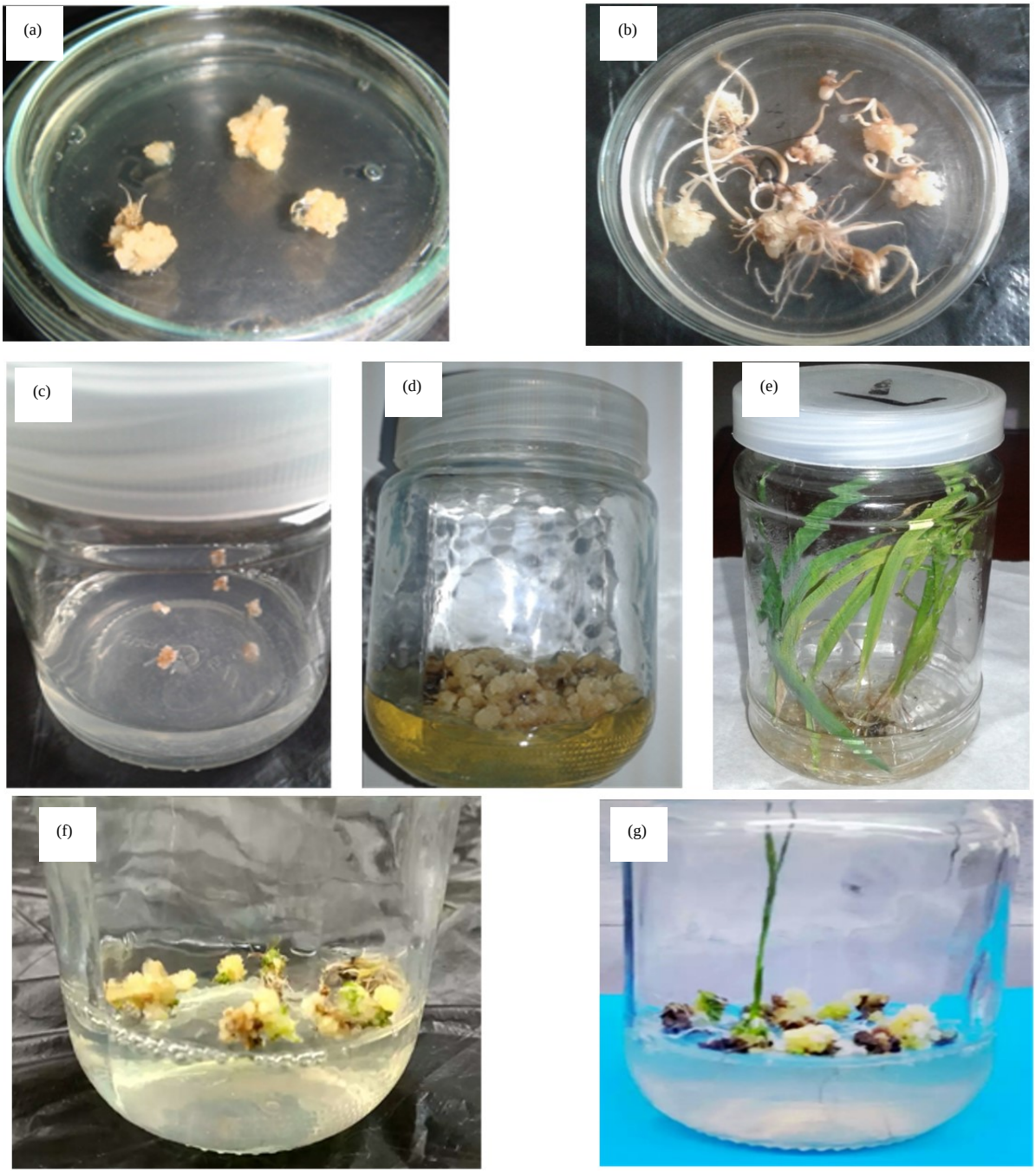

Fig. 1(a-g): (a) Callus inducing emerging from the rice embryo cultured on MS media with 3% (w/v) 2, 4-D (4 weeks), (b) Rhizgenic callus where roots developed out of the callus, (c) Maintenance of callus through sub-culture on MS media with 3% (w/v) 2, 4-D, sub-cultured every 5 weeks, (d) Callus after three cycling sub-culture, (f) Abnormal malformed green structures enveloped from callus and (e, g) Plant regeneration to formed shoots and roots

Subcultures were made every 21-28 days for 7 varieties namely: Al-Ahsa Type 1, Al-Ahsa Type 2, Sakha104, Gz6903, Giza178, Giza177 and Sakha105. The results indicated that there were no significant differences in callus induction percentage among varieties and the callus induction percentage ranged between 75-100% (Table 2). The varieties Sakha104, Gz6903, Giza177 and Sakha105 gave 100%, while the varieties Al-Ahsa Type1, Al-Ahsa Type2 gave 85% in callus induction. On the other hand, Giza 178 which is Indica- Japonica type gave the lowest value (75%). As for initial callus fresh weight, the varieties Al-Ahsa Type1 and Al-Ahsa Type2 gave high weight (0.698 and 0.765), respectively (Table 2), while the lowest value was 0.432 with Sakha106 variety. In addition, after 30 days the varieties Al-Ahsa Type1and Al-Ahsa Type2 recorded the highest value 1.126 and 1.321 in callus fresh weight, respectively, as shown in Table 2. However, excellent and normal type of calli were used in plant regeneration (Fig. 1) and the plants were transferred for adaptation. Success in improving rice plants by inducing somaclonal variation has been accomplished.

Somaclonal variation: The total of produced new lines were 91 plants in the SC1, 18 plant produced from Al-Ahsa Type1, 16 derived from Al-Ahsa Type2, 11 plants derived from Sakha104, 13 plants from Gz6903, 7 plants from Giza178, 15 plants derived from Giza177 and 11 plants derived from Sakha105. The results of SC1 derived from Al-Ahsa Type1 showed that 13 lines were resistant to rice blast (*M. grisea*), while 5 lines were susceptible (Table 3). On the other hand, all the lines were earlier than their parents and the lines 10, 13 and 15 were short duration compared with the parents. For plant height, the lines 1, 3, 10 and 15 were shortest statures. As for yield character, the lines 17, 13, 18, 12 and 6 gave the highest yield/plant (Table 3). In the SC1 family derived from Al-Ahsa Type2 the results indicated that 10 lines showed resistance to rice blast (*M. grisea*), while 6 lines were susceptible (Table 4). As for duration, lines 5, 13, 11 and 9 had short duration and gave 140, 140, 143 and 144 days, respectively, compared with the parents (157 days). Concerning plant height, the lines 5 and 9, 10 and 14 were of short stature compared with the parent (Table 4). For grain yield/plant, the results revealed that all the lines were higher than the parent, the height value with the lines 11, 4, 16, 12 and 8 gave 41, 40, 40, 39.9 and 39 g, respectively (Table 4). About 11 plants were in SC1 family derived from Sakha104 and the results showed that 6 plants were susceptible to *M. grisea*, while five plants were resistant (Table 5). The results for duration trait showed that the lines 11, 5 and 10 were earlier than parents. For plant height and grain yield characters, the results revealed remarkably slight differences in these traits (Table 5). Also, the results of the SC1 family derived from Gz6903 displayed remarkably slight differences from Gz6903 in terms of duration and lines 13, 9, 3, 8 and 12 were a shorter ones (128, 128, 129, 130 and 130 days), respectively, in terms of duration compared with the parent that was 136 days (Table 6). In summary, these lines could be included in earliness breeding programs. According to grain yield/plant, the line 10, 13, 7, 3 and 6 gave the high yield of 48, 47.1, 47, 47 and 48.3, respectively (Table 6). The SC1 family derived from Giza178 displayed remarkably slight differences in from Giza178 in terms of duration and lines 7, 3, 1 and 4 were shorter ones in terms of duration (Table 7). On the other hand, grain yield traits showed that the lines 4 and 5 were higher than the parents and could be used as a donor in breeding program. In addition, the results for the SC1 family derived from Giza177 and Skha105 were slightly different from the parents in all

Table 2: Callus induction percentage, initial callus weight and callus fresh weight after 30 days

Entries	Callus induction (%)	Initial callus weight	Callus fresh weight after 30 days
Al-Ahsa Type1	85.0	0.765	1.321
Al-Ahsa Type2	85.0	0.698	1.126
Sakha104	100.0	0.475	0.9875
Gz6903	100.0	0.432	0.9645
Giza178	75.0	0.564	0.9321
Giza177	100.0	0.476	0.9764
Sakha105	100.0	0.488	0.8790
σ^2	107.14	1.610	2.250
STDEV	10.35	0.127	0.150
SE	3.91	4.800	5.670

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

Table 3: Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Al-Ahsa Type1) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller/plant	No. of panicles/plant	Panicle length
Parent 1	S	159	147	18	14	23.0
Line 1	S	145	133	21	18	24.0
2	R	147	135	19	17	24.2
3	S	150	133	23	22	23.6
4	S	144	137	20	17	24.7
5	R	146	141	22	20	25.0
6	R	151	135	24	23	23.9
7	R	145	139	21	19	24.1
8	S	143	140	19	17	24.5
9	R	148	135	17	15	25.0
10	R	141	132	22	20	23.0
11	R	150	136	24	22	24.7
12	R	144	140	20	19	23.9
13	R	140	142	25	23	22.9
14	R	152	138	18	15	23.8
5	S	141	132	20	19	23.2
16	R	148	137	19	17	24.3
17	R	146	134	17	15	24.0
18	R	150	140	21	20	24.0
σ^2	-	21.029	15.363	5.596	0.418	7.708
STDEV	-	4.585	3.919	2.365	0.646	2.776
SE	-	1.052	0.899	0.542	0.148	0.636

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfilled grains/panicle	Grain yield/plant
Prent 1	2.7	23.6	110	52	32.8
Line 1	3.1	24.2	122	36	34.9
2	2.9	23.9	118	26	35.0
3	3.0	24.0	132	18	36.3
4	3.3	25.2	126	28	37.1
5	3.0	24.7	129	31	35.0
6	2.9	24.2	131	22	38.2
7	2.7	24.0	119	19	31.0
8	3.2	23.8	123	25	37.7
9	3.5	24.1	125	37	35.4
10	3.1	25.0	128	30	37.2
11	2.8	25.7	133	26	38.0
12	3.6	24.4	120	22	39.7
13	2.8	24.6	117	36	40.0
14	3.4	24.0	109	31	34.0
15	2.6	23.8	121	32	37.4
16	3.7	23.9	125	20	36.8
17	3.5	24.2	128	28	41.0
18	3.0	23.7	130	31	39.9
σ^2	0.107	0.306	47.152	63.83	6.685
STDEV	0.327	0.553	6.866	7.989	2.585
SE	7.511	0.126	1.575	1.832	0.593

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

the traits except the grain yield per plant, which was higher than the parents and the lines 9, 6. 15, 5 and 11 were higher ones (Table 8). Also, the same results were found in the SC1 derived from Sakha105 and the lines 10, 7, 11, 4 and 8 gave 48.2, 46.7, 46.5, 46.3 and 45.6, respectively, compared with the parent gave 43.5 g/plant (Table 9) and could be used as a donor in a breeding program for this trait.

Field experiment

Analysis of variance: Using line×tester analysis main squares of crosses were partitioned into lines (females), testers (males) and line×tester interaction for all studied characters. The results in Table 10 revealed highly significant differences among the 19 genotypes (12 hybrid combinations and 7 parents) tested for agronomic characters, blast sensitivity, duration, plant height, No. of

Table 4: Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Al-Ahsa Type2) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller/plant	No. of panicles/plant	Panicle length
Parent 2	S	157	144	20	22.7	16
Line 1	S	150	140	22	23.2	21
2	R	153	138	21	22.9	19
3	R	149	144	24	24.1	22
4	R	14	136	25	23.3	23
5	S	140	135	22	22.8	20
6	S	148	140	21	23.5	18
7	S	151	141	23	24.2	21
8	R	147	142	24	25.2	22
9	R	144	135	23	23.0	19
10	R	14	131	22	24.3	20
11	S	150	138	24	22.7	23
12	R	143	140	21	22.9	18
13	R	140	142	20	23.7	17
14	R	147	134	19	23.2	18
15	S	152	140	23	23.0	22
16	R	148	136	24	23.5	21
σ^2	-	20.441	13.382	2.941	0.471	4.500
STDEV	-	4.521	3.658	1.715	0.686	2.121
SE	-	1.096	0.887	0.415	0.166	0.514

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfiled grains/panicle	Grain yield/plant
Parent 2	2.9	24.3	112	55	34.6
Line 1	3.2	25.5	122	29	38.5
2	3.0	24.9	119	34	35.7
3	3.5	25.1	124	32	38.0
4	3.1	24.8	130	27	40.0
5	2.9	26.0	117	38	37.2
6	3.0	25.7	121	40	36.8
7	3.3	25.9	125	31	34.9
8	3.6	26.0	133	36	34.2
9	3.2	25.8	116	29	39.0
10	3.0	24.8	123	24	37.7
11	2.9	24.0	129	19	41.0
12	3.1	23.9	135	22	39.9
13	2.8	25.3	127	28	38.3
14	3.5	24.9	122	34	35.6
15	3.2	26.0	118	37	35.2
16	3.0	25.7	124	23	40.0
σ^2	5.471	0.489	37.618	71.868	4.589
STDEV	0.233	0.699	6.133	8.477	2.142
SE	5.673	0.169	1.487	2.056	0.519

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

tiller/plant, panicle length, No. of panicle/plant, panicle weight, 1000 grain weight, No. of filled grain/panicle, sterility (%) and grain yield/plant. The parental lines and the crosses showed highly significant differences for all studied characters. Parents vs. crosses mean square indicated that the average heterosis was highly significant in all crosses. On the other hand, the male testers exhibited highly significant differences for all agronomic characters studied. The highly significant mean squares of lines×testers for all agronomic characters indicated that they interacted and produced markedly different combining ability effects and this might be due to the wide genetic diversity of lines and testers. The estimate of variance due to GCA was higher than that due to SCA for plant height suggesting greater importance of additive genetic variance in the inheritance of this trait. These results are in agreement with those obtained by Saleem *et al.*[26], Tiwari *et al.*[27] and El-Malky and Elamawi[28].

General combining ability effects (GCA): Combining ability analysis helps in identifying superior parents to be exploited in rice breeding programs. In this study, the general

Table 5 : Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Sakha104) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller/plant	No. of panicles/plant	Panicle length
Parent 3	S	136	108	24	22	21.4
Line 1	R	133	103	25	23	22.3
2	R	132	105	24	21	23.0
3	R	135	104	26	24	24.4
4	R	132	103	28	25	23.8
5	R	129	105	25	22	23.2
6	R	133	104	24	21	22.9
7	R	131	106	27	24	24.0
8	R	134	103	25	23	23.4
9	R	130	10	29	26	25.3
10	R	129	108	30	27	25.0
11	R	128	105	26	24	24.5
σ^2	-	6.333	3.091	4.083	1.28	3.545
STDEV	-	2.516	1.758	2.02	1.131	1.882
SE	-	0.726	0.507	0.583	0.326	0.543

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfiled grains/panicle	Grain yield/plant
Parent 3	3.9	27.0	135	12	42.5
Line 1	4.4	27.8	142	11	44.5
2	4.2	28.0	140	9	45.0
3	4.5	27.6	139	12	44.9
4	4.3	27.4	136	10	47.0
5	4.2	28.0	144	8	45.3
6	4.0	27.7	141	12	44.0
7	3.9	28.2	137	11	46.2
8	3.8	27.6	146	9	43.8
9	3.6	27.3	141	6	45.4
10	4.2	27.0	143	10	44.0
11	4.1	28.1	138	8	43.7
σ^2	6.205	0.164	11.061	3.768	1.472
STDEV	0.249	0.405	3.325	1.941	1.213
SE	7.191	0.117	0.960	0.560	0.350

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

combining ability (GCA) effects of parents were presented in Table 11. The negative significance is desirable with duration, plant height and sterility percentage as appeared with verities of Sakha104, Gz6903-1-2-2, Giza177 and Sakha105. This indicates that these entries could be considered as good combiners for the improvement of these traits. With respect to grain yield/plant, the rice verities Gz6903, Giza 178 and Sakha 105 showed significant and positive general combining ability effects 4.20, 3.92 and 3.27 respectively, indicating that these entries could be considered as good combiners for the improvement of this trait (Table11).

Specific combining ability (SCA) effects: The specific combining ability (SCA) effects of crosses are presented in Table 12. The results showed that five combination were highly significant and negative (SCA) for duration and plant height. These combinations (Al-Ahsa Type1×Sakha 105, Al-Ahsa Type2×Giza 178, Al-Ahsa Type2×Sakha 105, Sakha 104×Giza 177 and Gz 6903-1-2-2×Sakha 105) are desirable for the improvement of these traits in breeding programs since the low mean values are the target of the breeder. While, the crosses Al-Ahsa Type1×Giza 177, Al-Ahsa Type2×Giza 177, Al-Ahsa Type2×Sakha 105 and Gz6903-1-2-2×Giza178 gave negative significance in plant height and could be utilized in rice breeding programs to improve these traits. Data in Table 12 revealed that three crosses gave highly significant positive estimates of (SCA) effects for grain yield/plant. The best combinations of them were Sakha104×Giza178, Sakha104×Sakha105 and Gz6903-1-2-2×Giza177.

DISCUSSION

Rice blast (*M. grisea*) is a world-wide problem that seriously influences grain production. Increasing human population and global climate change make the situation more serious[29,30]. Genetic improvements of rice for resistance through conventional breeding is slow because

Table 6: Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Gz6903) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller/plant	No. of panicles/plant	Panicle length
Parent 4	R	136	98	21	19	23.3
Line 1	R	133	96	23	22	24.0
2	R	132	96	24	21	25.3
3	R	129	95	26	24	24.4
4	R	134	96	23	21	23.9
5	R	131	94	27	25	24.1
6	R	135	95	25	23	25.0
7	R	132	98	27	24	24.9
8	R	130	94	24	21	25.3
9	R	128	95	26	23	24.7
10	R	131	97	23	20	24.2
11	R	130	96	25	22	23.8
12	R	133	95	27	24	24.5
13	R	128	93	28	26	23.7
σ^2	-	6.11	2.247	4.071	0.378	3.962
STDEV	-	2.471	1.499	2.017	0.614	1.990
SE	-	0.660	0.400	0.539	0.164	0.531

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfiled grains/panicle	Grain yield/plant
Parent 4	4.0	26.4	148	15	43.5
Line 1	4.4	27.3	155	13	45.5
2	4.2	26.8	149	10	44.2
3	4.5	28.0	152	9	47.0
4	4.3	27.2	156	6	43.9
5	4.2	26.9	151	12	44.6
6	4.6	27.0	154	14	46.3
7	4.3	27.2	150	8	47.0
8	3.9	28.0	149	11	45.8
9	4.0	27.6	145	14	44.7
10	4.2	27.2	147	10	48.0
11	4.5	27.0	159	7	43.6
12	3.9	26.8	154	9	43.2
13	4.1	26.5	160	12	47.1
σ^2	5.104	0.227	19.918	7.858	2.474
STDEV	0.225	0.476	4.462	2.803	1.572
SE	6.038	0.127	1.192	0.749	0.420

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

Table 7: Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Giza178) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller-plant	No. of panicles/plant	Panicle length
Parent 5	R	138	98	25	24	22.4
Line 1	R	133	97	27	25	22.5
2	R	135	98	28	26	23.1
3	R	132	95	26	25	22.7
4	R	133	96	28	27	23.2
5	R	135	95	31	27	23.0
6	R	134	95	30	28	22.9
7	R	130	96	28	26	22.7
σ^2	-	5.643	1.643	3.839	4.125	1.714
STDEV	-	2.375	1.281	1.959	0.285	1.309
SE	-	0.839	0.453	0.692	0.100	0.462

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfilled grains/panicle	Grain yield/plant
Parent 5	3.4	16.6	165	11	50.4
Line 1	3.5	21.2	168	9	53.0
2	3.7	22.3	171	7	53.5
3	3.5	21.5	168	10	52.7
4	3.4	23.0	173	8	56.0
5	3.6	22.5	175	11	54.8
6	3.5	22.1	179	6	53.9
7	3.4	23.0	177	10	51.7
σ^2	1.143	2.002	23.714	3.89	3.086
STDEV	0.106	1.415	4.869	1.972	1.756
SE	3.78	0.500	1.721	0.697	0.621

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

Table 8: Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Giza177) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller/plant	No. of panicles /plant	Panicle length
Parent 6	R	124	100	18	18	18.5
Line 1	R	124	97	21	20	20.4
2	R	123	99	19	19	21.0
3	R	124	100	22	21	19.8
4	R	122	97	20	20	21.5
5	R	124	96	18	18	22.0
6	R	123	95	19	19	20.7
7	R	124	100	20	19	21.0
8	R	122	97	22	21	18.9
9	R	124	95	23	20	20.6
10	R	120	100	21	20	21.0
11	R	124	95	19	18	22.4
12	R	122	97	18	17	21.7
13	R	120	94	17	17	20.8
14	R	123	100	21	20	22.0
15	R	124	97	20	19	21.5
σ^2		5.64	1.64	3.83	8.12	1.71
STDEV		2.37	1.28	1.95	0.285	1.309
SE		0.839	0.453	0.692	0.100	0.462

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfilled grains/panicle	Grain yield/plant
Parent 6	4.3	26.5	134	5	40.0
Line 1	4.4	28.5	140	5	43.5
2	4.5	29.0	144	4	44.0
3	4.8	27.4	139	6	42.6
4	4.3	27.8	145	3	41.8
5	4.6	27.6	141	4	44.7
6	4.7	29.0	147	4	45.0
7	4.5	28.5	151	5	44.3
8	4.3	28.0	139	3	42.8
9	4.6	27.9	143	2	46.0
10	4.3	27.6	148	5	43.0
11	4.2	26.9	152	4	44.2
12	4.8	27.6	148	3	41.9
13	4.5	28.1	147	4	40.8
14	4.0	29.2	153	3	42.1
15	4.8	27.3	148	5	44.8
σ^2	1.14	2.0	23.71	3.89	3.08
STDEV	0.106	1.41	4.86	1.97	1.75
SE	3.780	0.50	1.72	0.697	0.621

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

of the low heritability of yield under stress, low inherent variation in the field. Plant tissue culture played an important role in the production of agricultural and ornamental plants and in the manipulation of plants for improved agronomic performance[31]. *In vitro* culture of plant cells and tissue has attracted considerable interest over recent years because it provides the means to study plant physiological and genetic processes in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability. Regenerated plants are expected to have the same genotype as the donor plant; however, in some cases somaclonal variants have been found among regenerated plants[32]. Somaclonal variation can be used as good source for plant breeding via the generation of new desirable clones/variants with better disease resistance and agronomic traits under high phytosanitary conditions[33-35]. However, somaclonal variation is affected by different factors, such as presence of a disorganized growth phase, nature of explant, growth regulator, number of subcultures and propagation methods[36-39]. In this study, callus induction for seven genotypes was successfully induced via cultured immature seeds on the MS medium, supplemented with 3 mg L^{-1} 2,4-D (Table 2). The healthy calluses were regenerated by added growth regulator BAP at 2 mg L^{-1} (Fig. 1). Ullah *et al.*[34] found that the addition of 2,4-D, independently or in combination with BAP, depends mainly

Table 9: Vegetative and yield traits of somaclonal plants of SC1 generation derived from (Sakha105) genotypes

Genotypes	Blast sensitivity	Duration	Plant height	No. of tiller/plant	No. of panicles/plant	Panicle length
Parent7	R	121	105	22	21	23.4
Line1	R	121	103	24	23	24.4
2	R	120	105	23	22	24.7
3	R	121	104	25	23	25.0
4	R	119	102	23	21	24.3
5	R	120	104	24	22	23.8
6	R	120	103	26	24	23.5
7	R	119	105	25	23	24.5
8	R	118	104	27	26	24.0
9	R	119	101	24	23	23.9
10	R	120	100	26	25	25.1
11	R	120	104	23	21	26.2
σ^2	-	0.879	2.606	2.242	0.599	2.515
STDEV	-	0.937	1.614	1.497	0.774	1.585
SE	-	0.27	0.466	0.432	0.223	0.457

Genotypes	Panicle weight	1000 grain weight	No. of grains/panicle	No. of unfilled grains/panicle	Grain yield/plant
Parent7	4.2	27.8	143	6	43.5
Line1	4.5	28.2	148	6	44.2
2	4.3	28.0	151	5	45.0
3	4.2	27.9	144	4	44.7
4	4.6	27.8	145	6	46.3
5	4.3	28.1	147	3	43.8
6	4.1	28.4	145	5	44.6
7	4.4	29.0	143	6	46.7
8	4.7	27.9	148	4	45.6
9	4.3	27.7	152	3	45.0
10	4.2	28.1	142	5	48.2
11	4.6	28.5	149	6	46.5
σ^2	3.697	0.136	10.629	1.453	1.874
STDEV	0.192	0.368	3.26	1.205	1.368
SE	5.551	0.106	0.941	0.348	0.395

σ^2: Variance, STDEV: Standard deviation, SE: Standard error

Table 10: Analysis of variance and mean square from line x testers analysis for the studied traits

S.O.V	d.f	Leaf blast	Duration	Plant height	No. of tiller/plant	Panicle length	No. of panicle/plant
Replication	2	0.07	3.53	1.95	5.28	1.09	3.70
Treatment	18	8.71**	245.72**	618.14**	40.85**	17.98**	39.75**
Parents	6	16.43**	514.05**	1205.86**	40.16**	35.26**	65.41**
Pa. vs Crosses	1	55.82**	592.99**	341.12**	168.42**	14.17**	83.69**
Crosses	11	0.21	67.79**	322.76**	29.63**	8.90**	21.77**
Lines	3	0.25	114.25**	589.52**	18.70**	3.49**	4.62**
Testers	2	0.19	41.69**	427.58**	103.86**	24.30**	91.58**
Lines×Testers	6	0.19	53.25**	154.44**	10.34**	6.48**	7.06**
Error	36	0.14	3.43	6.19	1.56	0.95	2.31

S.O.V	d.f	Panicle weight	1000 grain weight	No. of filled grain/panicle	Sterility (%)	Grain yield/plant
Replication	2	0.04	0.02	0.65	1.14	0.14
Treatment	18	1.00**	21.48**	1191.87**	519.22**	83.06**
Parents	6	2.14**	38.16**	1553.11**	1235.21**	140.85**
Pa. vs Crosses	1	0.78**	0.60**	4802.01**	1195.04**	58.16**
Crosses	11	0.40**	14.28**	666.63**	67.24**	53.80**
Lines	3	1.03**	5.01**	1261.81**	111.44**	22.94**
Testers	2	0.60**	38.40**	1496.78**	33.78**	198.73**
Lines x Testers	6	0.01	10.88**	92.33**	56.30**	20.92**
Error	36	0.03	0.42	20.45	11.37	3.24

S.O.V: Source of variance, d.f: Degree of freedom

on the genotypes. In this investigation the results relate to the SC1 family derived from Al-Ahsa Type1, Al-Ahsa Type2, Sakha104, Gz6903, Giza178, Giza177 and Sakha105 varieties were differences between the lines and their parents. However, these differences were remarkably slight compared with the parents and these lines, which could be used in

breeding programs as a genetic resource or good donor in these traits. In the present study, ANOVA for combining ability revealed significant differences among all genotypes for all the traits studied and amongst the treatments. In addition, it showed highly significant line × tester interaction, which might be due to wide genetic diversity among lines and testers[40-42]. In addition, the genotype with negative GCA values is preferred for earliness traits and considered as a good combiner for earliness traits[43,44]. The estimates of general combining ability GCA effects among lines for blast

Table 11: General combining (GCA) ability for each lines for studied traits

GCA	Leaf blast	Duration	Plant height	No. of tiller/plant	Panicle length	No. of panicle/plant
Al-Ahsa Type1	-0.14	4.19**	4.83**	0.72	-0.41	0.47
Al-Ahsa Type2	0.08	1.53	8.72**	-0.72	0.16	-0.31
Sakha104	-0.14	-1.92	-5.17**	1.61	-0.56	0.69
Gz6903-1-2-2	0.19	-3.81*	-8.39**	-1.61	0.81	-0.86
S.E (gca for line)	0.36	0.79	0.91	0.65	0.57	0.71
S.E (gl-gj) line	0.42	0.93	1.08	0.77	0.68	0.85
LCD 0.05	1.02	2.26	2.61	1.85	1.64	2.04
0.01	1.85	4.09	4.74	3.36	2.97	3.71
Giza178	-0.14	2.14	0.42	-0.44	1.25	-0.83
Giza177	0.03	-1.28	-5.75**	-2.69*	-1.55*	-2.25
Sakha105	0.11	-0.86	-6.17**	3.14*	0.29	3.08*
S.E (gca for tester)	0.33	0.73	0.85	0.60	0.53	0.66
S.E (gt-gj) tester	0.39	0.87	1.01	0.71	0.63	0.79
LCD 0.05	1.08	2.38	2.76	1.96	1.73	2.16
0.01	2.07	4.57	5.29	3.75	3.31	4.14

GCA	Panicle weight	1000 grain weight	No. of filled grain/panicle	Sterility (%)	Grain yield/plant
Al-Ahsa Type1	-0.04	0.19	-7.53**	5.25*	0.68
Al-Ahsa Type2	-0.41	-0.89	-7.81**	-1.31	-2.38*
Sakha104	0.04	0.90	-3.25	-2.19	1.08
Gz6903-1-2-2	0.42	-0.19	-6.47**	-1.75	4.20*
S.E (gca for line)	0.24	0.47	1.23	1.06	0.77
S.E (gl-gj) line	0.29	0.55	1.46	1.26	0.92
LCD 0.05	0.70	1.34	3.52	3.04	2.22
0.01	1.27	2.43	6.39	5.52	4.04
Giza178	-0.22	-2.01*	11.06**	0.22	3.92*
Giza177	-0.02	1.60*	0.22	-1.78	1.20
Sakha105	0.23	1.41*	11.28**	1.56	3.27*
S.E (gca for tester)	0.23	0.43	1.14	0.99	0.72
S.E (gt-gj) tester	0.27	0.52	1.36	1.17	0.86
LCD 0.05	0.74	1.41	3.72	3.22	2.35
0.01	1.42	2.71	7.13	6.16	4.50

Table 12: Estimates of specific combining ability (SCA) effect for studied characters

SCA	Leaf blast	Duration	Plant height	No. of tiller/plant	Panicle. length	No. of panicle/plant
Al-Ahsa Type1 X Giza178	0.14	3.64**	3.25*	-0.22	0.08	-0.06
Al-Ahsa Type1 X Giza177	-0.36	-1.28	-4.42**	-0.31	-1.49*	-0.64
Al-Ahsa Type1 X Sakha105	0.22	-2.36*	1.17	0.53	1.41*	0.69
Al-Ahsa Type2 X Giza178	-0.08	-2.36*	9.69**	-1.78*	0.97	-0.94
Al-Ahsa Type2 X Giza177	0.08	3.39**	-3.97*	2.81**	1.24*	2.47*
Al-Ahsa Type2 X Sakha105	-0.58**	-41.83**	-37.53**	-8.53**	-8.15**	-8.08**
Sakha104 X Giza178	-0.13	8.96**	0.31	3.58**	1.49*	2.82**
Sakha104 X Giza177	0.31	-3.83**	5.25**	-0.53	-0.14	-0.86
Sakha104 X Sakha105	-0.11	6.08**	2.50	-0.69	0.82	-0.19
Gz6903-1-2-2 X Giza178	0.14	0.97	-5.19**	0.78	-0.38	-0.06
Gz6903-1-2-2 X Giza177	-0.03	1.72	3.14*	-1.97*	0.39	-0.97
Gz6903-1-2-2 X Sakha105	-0.11	-2.69*	2.06	1.19	-0.02	1.03
S.E (sca effects) =	0.22	1.07	1.44	0.72	0.56	0.88
S.E (Slj- Skl) =	0.31	1.51	2.03	1.02	0.80	1.24
LCD 0.05	0.46	2.26	3.03	1.52	1.19	1.85
0.01	0.63	3.06	4.11	2.06	1.61	2.51

Table 12: Continue

SCA	Panicle weight	1000 grain weight	No. of filled grain/panicle	Sterility (%)	Grain yield/plant
Al-Ahsa Type1×Giza178	0.02	-0.28	1.39	6.67**	0.62
Al-Ahsa Type1×Giza177	-0.02	0.58	2.56	-1.33	-0.96
Al-Ahsa Type1×Sakha105	0.00	-0.30	-3.94	-5.33*	0.34
Al-Ahsa Type2×Giza178	-0.05	1.80**	-7.94**	-1.78	0.94
Al-Ahsa Type2×Giza177	0.02	-3.21**	2.89	1.56	0.23
Al-Ahsa Type2×Sakha105	-1.82**	-9.20**	-51.33**	-3.89	-15.39**
Sakha104×Giza178	0.29**	1.33**	21.74**	-0.68	5.08**
Sakha104×Giza177	-0.06	0.97*	-5.33	-1.56	-2.79*
Sakha104×Sakha105	0.06	-0.58	-0.50	2.78	3.27**
Gz6903-1-2-2×Giza178	0.03	-1.13**	0.72	-3.67	-1.08
Gz6903-1-2-2×Giza177	0.06	1.66**	-0.11	1.33	3.51**
Gz6903-1-2-2×Sakha105	-0.09	-0.53	-0.61	2.33	-2.43*
S.E (sca effects) =	0.10	0.38	2.61	1.94	1.04
S.E (Slj- Skl) =	0.15	0.53	3.69	2.75	1.47
LCD 0.05	0.22	0.79	5.51	4.11	2.19
0.01	0.29	1.08	7.47	5.57	2.97

resistant traits showed that the rice genotypes Gz6903-1-2-2, Giza178, Giza177 and Sakha105 were the best for blast and grain yield traits. The result was similar with Dalvi and Patel[45] and Saidaiah *et al.*[46]. Finally, the best genotypes which showed desirable SCA effects were Al-Ahsa Type1×Sakha105, Al-Ahsa Type2×Giza178, Al-Ahsa Type2×Sakha105, Sakha104×Giza177 and Gz6903-1-2-2×Sakha105 for duration and plant height traits. Similar conclusion was drawn by Asfaliza *et al.*[47], Surek and Korkut[48] and Hassan *et al.*[49]. The current study highlighted the importance of producing improved rice genotypes to increase rice production. Plant tissue culture techniques were approved as an effective methodology for the induction of somaclonal variation in rice. This technique was applied for the production of new varieties of rice relatively short time compared with the traditional methods of rice breeding.

CONCLUSION

Tissue cultural and classical breeding are effective means to produce new varieties and improve agronomic traits. In this study the best genotypes for yield character, the lines 17, 13, 18, 12 and 6 gave the highest yield/plant and resistant for blast, which produced from Al-Ahsa Type1 and the lines 11, 4, 16, 12 and 8 produced from Al-Ahsa Type2. On the other hand, the best combinations of them were Al-Ahsa Type1×Sakha105, Al-Ahsa Type2×Giza178, Al-Ahsa Type2×Sakha105, Sakha104×Giza177 and Gz6903-1-2-2×Sakha105. Finally, these materials could be used in the breeding program to improve this trait and crossing to transfer these characters to the progeny.

SIGNIFICANCE STATEMENT

In this study, tissue culture used to produce somaclonals from mature embryo, which gave the desirable variation. The progeny is homogeneity compared with traditional breeding took about 5-6 generation for homogeneity. However, selection of best characters is useful to start improvement and produce new variety. Finally, this study may help the researchers to discover the critical area of the complex qualitative and quantitative characters like resistant and grain yield per plant that many researchers were not able to explore. Thus, a new theory on these combinations traits and possibly other combinations may arrive.

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