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Grain Quality of Doubled Haploid Lines in Rice (*Oryza sativa* L.) Produced by Anther Culture

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Abstract

Genetic devlopment to imrove genotypes with high quality is the most important approach of rice. Thus, anther culture technique is one of straight forward approaches for improvement of rice cultivars with good grain quality. Therefore, this investigation aimed to develop some doubled haploid lines (DH) through anther culture technique and evaluate them along with their five parents including two check varieties for some nutritional characteristics. The results indicated that the three mineral element contents (Zn, Mn and Fe) of rice grain were clearly different among genotypes (DH), which implied that genotypic variations might provide opportunities to select for higher mineral element content. Analysis of variance revealed that the differences among genotypes were significant for all studied traits. Highly significant positive correlations were recognized among the studied characteristics. Accordingly, Rice lines with the high nutritional values will use as donors for this trait in rice breeding programs for exploitation and in hybridization.

Keywords: anther culture, correlation coefficient, rice quality

1. Introduction

Rice is (*Oryza sativa* L.) an important crop that feeds more than half of the world's population. Rice is the major source of carbohydrate to millions of people world over, particularly in Asia (Peng et al., 2009). Rice is the staple food of more than half of the world's population and is mostly consumed as cooked rice as the primary dietary source of carbohydrate and energy. It is rich in carbohydrates, contains a moderate amount of protein, and is a source of the B vitamins thiamin, riboflavin, and niacin (Hu et al., 2004; Fresco, 2005; Denardin et al., 2007). Rice occupies conspicuous position in the predominately agricultural economy of Egypt and this attention is required to improve its yield, quality characters and quality of elements nutrition (Abo-Youssef, 2015).

To achieve food security in our country, there is an urgent need to improve the rice productivity by introducing new cultivars of rice characterized by high yield and good grain quality. With the development of rice functional genomics, new technology platforms have been proved success in improving complex agronomic traits such as yield, grain quality and disease resistances, which are inefficiently selected by using traditional breeding methods (Liu et al., 2003). Many varieties of rice genotypes produced by using tissue culture techniques have improved some of the characteristics of shape, nutritional quality, pest resistance and the harsh conditions of the environment (Sun et al., 1992).

Production of double haploids through anther culture is a rapid approach to homozygosity that shortens the time required for the development of new rice cultivars as compared to conventional methods (Xa & Lang, 2011). Anther culture is an actively developed method of great potential. It shortens the breeding term and increases selection efficiency by producing doubled haploid plants from hybrids (Yan et al., 1996). Production of double haploids through anther culture is a rapid approach to homozygosity that shortens the time required for the development of new rice cultivars as compared to conventional methods, which require at least 6-7 generations.

Haploids are also valuable for the detection and repair of desirable recessive traits introduced through mutation (Chen et al., 2001). However, it is important to enhance the efficiency of anther culture. The frequency and period of plant regeneration can be increased by the concentration and combination of plant growth regulators (Yi et al., 2003; Chung & Sohn, 1996; Yamamoto et al., 1995). Using anther culture technique, several lines as donors for different traits can be developed and these selected doubled haploid lines could possess high yield potentiality, better grain quality, nutritional value, blast resistance and also stem borer resistance (Draz, 2004). With keeping the above points in view, the objective of the current investigation was to develop new rice genotypes with high nutritional value by using anther culture technique.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

This study was conducted at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafrelsheikh, Egypt during three successive seasons (2007-2010) as well as the biotechnology lab., located at this center. Black rice variety (Jiegnou 9601) and four Egyptian rice varieties (*Oryza sativa* L.) naming Giza 177, Sakha 101, Sakha 102 and Sakha 104 were utilized in this study. Thirty days old seedlings of the mentioned genotypes were transplanted in five sowing dates with 15 days intervals for flowering synchronization in the growing season of 2007. Line x tester mating system was carried out for the five parents and their four crosses were performed among them. In the flowering period, bulk emasculation method was applied according to Butany (1961) by using hot water method (42-44 °C for 10 min) for the emerged panicles to kill the pollen grains of the mother plants without any harmful effect to their stigmas. The hybrid seeds born on the female parents were harvested separately for each cross and properly packed for sowing in the following season. F_1-seeds of the four crosses were grown in a greenhouse during the winter season of 2008 to produce F_1 plants. Anthers born on F_1 plants grown in the greenhouse through the winter period to develop the doubled haploid lines (DHLs) using anther culture technique, as described by Abd El-Khalek (2001). After that the plantlets were transferred to pots containing bitmoss and clay with 2:1 ratio in the air-conditioned greenhouse. At the beginning of flowering the plants were bagged to prevent cross pollination with other rice plants and maintain the homozygosity of lines.

2.2 Plant Sampling and Measurements

The seeds born on the doubled haploid plants were harvested separately and every plant was considered as double haploid line (DHL). Sixty six doubled haploid lines (DHLs) were obtained, thirty nine DHLs derived from cross No. 1, fifteen DHLs derived from cross No. 2, four DHLs derived from cross No. 3, eight DHLs derived from cross No. 4 and prepared for seed multiplication and evaluation. The seeds of the doubled haploid plants were sown for seed multiplication and evaluation studies in a randomized complete block design (RCBD) with three replications. Seedlings were transplanted into 20 × 20 cm spacing in 5 meters rows and the recommended field practices were applied.

Table 1. Crosses and derived doubled haploid lines (DHLs) resulted by using anther culture technique

Cross no.	Cross Parents	Doubled haploid lines produced	No. of lines
1	Black Rice × Giza 177	DHL1-DHL39	39
2	Black Rice × Sakha 101	DHL40-DHL54	15
3	Black Rice × Sakha 102	DHL55-DHL58	4
4	Black Rice × Sakha 104	DHL59-DHL66	8
	Total		66

Five grain quality and nutritional value traits i.e. hulling %, milling %, Zn, Mn and Fe contents of the grain were studied. Analysis of variance was computed by IRRISTAT program. Correlation coefficients (r) among all studied traits were computed using SPSS statistical package according to (K. A. Gomez & A. A. Gomez, 1984).

3. Results and Discussion

3.1 Grain Quality and Nutritional Value Traits in the Tested Rice Genotypes

Hulling %, milling %, Zn, Mn and Fe contents of the grain were studied and the mean performance of these characteristics in the sixty six studied rice genotypes are listed in Table 2. As it can be seen that the data from the performance Table 2, showed the hulling trait in the variety Giza 177 was the best with a mean value (81.9%) and the check variety Jiegnou 9601 was the lowest one in this trait (72.9%). Among doubled haploid lines, DHL

23 which resulted from cross No. 1 recorded the highest mean value of hulling trait (94.6%) followed by DHL 14 which recorded (81.5%) mean value and these two lines were derived from cross No. 1. On the other hand, DHL 42 out from cross No. 2 revealed the lowest mean value of this trait (54.3%).

According to the milling percentage, trait the check variety Giza 177 scored the highest mean value of this trait (75.2%), while the check Jiegnou 9601 revealed the lowest mean value of the trait (71.0%). Among the doubled haploid lines, DHL 23 which was obtained from cross No. 1 recorded the highest mean value of milling trait (89.5%), followed by DHL 46 with mean values (77.4%), which was resulted from cross No. 2. Meanwhile, the lowest mean value of this trait was obtained by DHL 42 (50.6%), it was derived from cross No. 2.

Table 2. Mean performance of grain quality and nutritional value of rice genotypes

No.	Genotypes	Hulling %	Milling %	Mn Mill. (ppm)	Zn Mill. (ppm)	Fe Mill. (ppm)
1	Jiegnou 9601 (P1)	72.9	71.0	42.9	52.5	341
2	Giza 177 (P2)	81.9	75.2	37.5	33.6	546
3	Sakha 101 (P3)	81.6	68.2	35.9	28.6	187
4	Sakha 102 (P4)	78.4	72.6	27.2	30.1	215
5	Sakha 104 (P5)	78.6	71.9	28.6	23.8	199
Lines from cross No. 1 (P1 × P2)						
1	DHL 1	78.1	76.6	35.7	43.5	415
2	DHL 2	77.8	71.6	31.7	34.6	178
3	DHL 3	75.9	73.3	33.8	30.5	195
4	DHL 4	75.7	72.6	33.3	27.4	222
5	DHL 5	77.2	74.8	31.4	28.7	138
6	DHL 6	76.3	65.9	32	32.3	198
7	DHL 7	74.5	70.6	28.6	27.7	325
8	DHL 8	80.9	73.3	33.3	27.9	167
9	DHL 9	76.8	73.2	32	29.4	176
10	DHL 10	78.4	75.7	31.6	32.2	331
11	DHL 11	81.3	71.6	24.9	26.9	416
12	DHL 12	75.8	66.7	30.5	44.1	170
13	DHL 13	79.1	71.1	30.9	50.3	303
14	DHL 14	81.5	76.9	22.1	40.3	304
15	DHL 15	80.9	71.9	27	33.1	177
16	DHL 16	77.2	71.4	29.3	31	301
17	DHL 17	78.6	61.3	22.6	40.3	207
18	DHL 18	77.2	70.2	25.4	36.5	320
19	DHL 19	78.4	73.4	33.3	31.7	339
20	DHL 20	76.5	68.1	23.8	25.3	270
21	DHL 21	78.3	73.7	34.3	26.6	199
22	DHL 22	75.1	72.8	30.3	35.4	171
23	DHL 23	94.6	89.5	24.6	44.1	180
24	DHL 24	73.9	68.1	19.4	27.3	547
25	DHL 25	77.7	70.2	29.6	29.4	409
26	DHL 26	77.2	73.1	32.3	32.4	192
27	DHL 27	78.1	75.1	26.6	25.3	297
28	DHL 28	79.4	76.6	28.5	28.2	202
29	DHL 29	75.7	71.6	32.7	33.9	315
30	DHL 30	79.1	69.2	28.3	28.2	665
31	DHL 31	79.3	72.8	29.1	28.5	482

32	DHL 32	79.4	74.5	24	27.7	205
33	DHL 33	79.0	71.6	25.6	33.2	366
34	DHL 34	76.5	69.9	25.6	24.8	194
35	DHL 35	77.3	68.9	34.2	24	166
36	DHL 36	76.6	75.2	23.5	28.7	471
37	DHL 37	76.1	71.4	28.3	40	501
38	DHL 38	79.3	71.8	27.1	33.1	189
39	DHL 39	75.7	70.8	29.9	23.7	261

Table 2. Continued

No.	Genotypes	Hulling %	Milling %	Mn Mill. (ppm)	Zn Mill. (ppm)	Fe Mill. (ppm)
Lines from cross No. 2 (P1 × P3)						
40	DHL 40	80.2	71.4	24.4	42.5	196
41	DHL 41	74.8	70.9	30.6	43.9	201
42	DHL 42	54.3	50.6	24	42.9	521
43	DHL 43	73.9	70.2	31.4	23.7	330
44	DHL 44	77.2	68.7	25.6	30.7	240
45	DHL 45	76.6	71.2	27.2	30.6	187
46	DHL 46	81.3	77.4	28.6	31.9	317
47	DHL 47	79.2	71.4	32.4	32.7	195
48	DHL 48	77.1	73.1	24.3	26.5	291
49	DHL 49	77.2	75.6	32.3	35.5	510
50	DHL 50	78.3	73.9	34	24.4	189
51	DHL 51	75.6	68.4	34.7	29.9	191
52	DHL 52	74.8	73.9	28	41.1	386
53	DHL 53	76.9	70.1	29.5	53.2	401
Lines from cross No. 3 (P1 × P4)						
54	DHL 54	78.2	70.2	21.6	37.4	260
55	DHL 55	75.9	71.6	37.9	34.4	336
56	DHL 56	74.5	72.1	29.6	35.5	433
57	DHL 57	74.0	72.3	33.6	34.3	276
58	DHL 58	74.8	73.3	30.5	33.9	179
Lines from cross No. 4 (P1 × P5)						
59	DHL 59	75.9	72.9	24.5	31.2	260
60	DHL 60	61.6	59.8	30.6	33.9	217
61	DHL 61	69.6	65.9	25.3	33.3	217
62	DHL 62	74.4	70.6	36.3	21.5	133
63	DHL 63	77.9	74.1	31	24.9	163
64	DHL 64	78.4	71.7	24	25.2	193
65	DHL 65	74.4	71.4	30.6	26.9	295
66	DHL 66	67.7	67.0	31.5	33	317
Mean		76.823	71.571	29.49	32.564	282.9
LSD 0.05		2.203	2.355	2.379	2.350	29.71
LSD 0.01		2.912	3.113	3.149	3.110	39.32

The check variety Jiegnou 9601 showed the best mean value of Mn concentration (42.9 ppm) in milled rice, while the variety Sakha 102 gave the lowest mean value (27.2 ppm). Among doubled haploid lines, DHL 55 scored the highest mean value of Mn conc. (37.9 ppm), it resulted from cross No. 3, while DHL 24 which resulted from cross No. 1 revealed the lowest mean value of Mn conc. (19.4 ppm). For Zn concentration in milled rice, the check variety Jiegnou 9601 recorded the highest mean value (52.5 ppm), while the variety Sakha 104 revealed the lowest mean value of Zn conc. (23.8 ppm). Among doubled haploid lines, DHL 53 which resulted from cross No. 2 scored the highest mean value of Zn conc. (53.2 ppm), while DHL 62 revealed the lowest mean value (21.5 ppm), resulted from cross No. 4.

Concerning Fe concentration in milled rice, the check Giza 177 recorded the highest mean value of Fe conc. (546 ppm), while the variety Sakha 101 showed the lowest mean value (187 ppm). Among doubled haploid lines, DHL 30 and DHL 24 scored the highest mean values of Fe conc. (665 and 547 ppm), respectively, these two lines resulted from cross No. 1, they were followed by DHL 42 with a mean value (521 ppm), it was derived from cross No. 2. Meanwhile, DHL 62 which resulted from cross No. 4 revealed the lowest mean value of Fe conc. (133 ppm).

3.2 Analysis of Variance for Grain Quality and Nutritional Value Traits

Analysis of variance of five grain quality and nutritional value characters exhibited in Table 3.

Table 3. Mean square estimates for grain quality and nutritional value characters

(SOV)	d.f	Hulling %	Milling %	Mn Mill. (ppm)	Zn Mill. (ppm)	Fe Mill. (ppm)
Replication	2	5.23455	4.265	14.886	50.992	223.6
Genotypes	70	64.746**	62.830**	57.392**	144.511**	41423.6**
Error	140	1.857	2.122	2.173	2.119	338.7

Note. **: Significant at 0.01 level.

The data revealed that wide range of variations in mean performances was observed for hulling and milling percentage. In addition, least significant differences showed highly diversified genotypes for their performance. These results agreed with what found before by Binodh et al. (2007) and Anis et al. (2016), they reported that grain quality has always been an important consideration in rice variety selection and development and it would help in quick realization of better selection for quality rice.

A wide range of variations in mean performances of all the studied nutritional value characteristics was observed. It was also noticed that genotypes were highly diversified for their performance and this high level of variation might help for selecting in nutritional value breeding program. These results are in agreement with the previously published results of Zhang et al. (2004) and Jiang et al. (2007). They found that indirect selection of grain characteristics may be one of breeding methods to select for Fe, Zn and Mn content in black pericarp rice. Thus, it is important to improve understanding of the relationships between mineral nutrients and rice quality, and to select rice genotypes appropriate for breeding program.

3.3 Correlation Coefficients for Nutritional Value and Grain Quality Characters

Correlation coefficients between all pairs of variables for nutritional value and grain quality characteristics are shown in Table 5. The loss of mineral elements content was quite different among all the three mineral elements during milling process as agreed with the results reported by Zeng et al. (2003). In this study, highly significant positive correlations were recognized between hulling and milling. The visible positive correlation was observed between Mn and Zn content in milled rice (Table 4). These results are similar with those accomplished by Wang et al. (2002). Data revealed positive correlation of Zn content with Fe and Mn content in milled rice. Similar results were also reported earlier by Abilgos et al. (2002) in rice.

Table 4. Correlation coefficients for nutritional value and grain quality traits of rice

Trait	Hulling %	Milling %	Mn/mi	Zn/mi	Fe/mi
Hulling %	1.010	0.991**	-0.060	-0.064	-0.157
Milling %		1.000	-0.053	-0.058	-0.145
Mn/mi			1.000	0.049	-0.125
Zn/mi				1.000	0.187
Fe/mi					1.000

Note. *, **: Significant at 0.05 and 0.01 level, respectively.

4. Conclusion

From these results, it can be concluded that several rice lines may be developed with high grain quality and nutritional values. The differences among genotypes were significant for all studied traits as well as significant positive correlations were recognized among the studied characteristics. Consequently, rice lines with the high nutritional values will use as donors for this trait in rice breeding programs for exploitation and in hybridization.

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