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The Agronomical Performances of Doubled Haploid Lines of Rice (*Oryza sativa* L.) Derived from Anther Culture

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Abstract

Anther culture technique offers great opportunities to accelerate breeding progress. The current study aimed to develop some good doubled haploid lines (DH) through anther culture technique and to evaluate them along with their five parents including two check varieties for yield and yield related traits. Analysis of variance revealed that varietal differences were signified and wide variability present among the genotypes with respect to all the characteristics studied. Correlation analysis revealed that grain yield was highly positive correlated with panicle length. The information on the inter association among the yield attributing characters showed the nature and extent of relationship with each other. Consequently, this will help in the improvement of different characters along with yield in breeding programmes.

Keywords: doubled haploid, correlation analysis, rice

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population. In Egypt, rice is one of the major cereal crops with annual growing area of about 600,000 hectares and with a production of 5926 million tons of paddy rice. The average yield (9.88 t/ha) is considered one of the highest average yield for rice in the world (RRTC, 2012). Rice is one of the most important cereals, providing carbohydrate sources for over half of the world's population (Cassman, 1999; Khush, 2005). To meet the demand of increasing population and to maintain self-sufficiency the present rice production needs to be increased by 30% by the year 2020 (Hossian, 1997). Considerable efforts are being directed towards improvement of existing cultivars for combined tolerance to biotic and abiotic stress which substantially decrase rice productivity. This can be achieved by gene stacking and pyramiding. The employment of doubled haploid technique and marker assisted selection (MAS) can enhance the stacking process. The production of doubled haploids via anther culture represents an alternate tool for the traditional crop improvement programs (Kaushal et al., 2015a).

Maintaining stable rice production is extremely important for the nutrition of constantly growing human population. Anther culture is one of the biotechnological tools for the traditional plant breeding with numerous advantages: shortening breeding cycle by immediate fixation of homozygousity, increasing selection efficiency, widening of genetic variability through the production of gametoclonal variants and allowing early expression of recessive genes (Zapata, 1992). Using anther culture technique, several lines as donors for different traits can be developed and these anther culture derived lines could possess high yield potentiality, better grain quality, higher nutritional value, blast resistance and stem borer resistance (Draz, 2004). Development of high yielding rice varieties through tissue culture viz. anther culture accelerates the breeding cycle by reducing the generation needed to fix a population (above F7-F9 generations) in a short period of time (F1 or advanced breeding lines) (Lapitana et al., 2009). Anther culture, an unconventional approach, could be a complementary technique along with conventional breeding for rice improvement (De-Filippis & Ahmed, 2014). Recent advances in plant tissue culture and its related disciplines opened an avenue that greatly facilitated the doubled haploid breeding scheme, and this enables the extraction of instant homozygous lines in a single generation (Baenziger et al., 1989; Wu et al., 2012). Production of doubled haploid plants through anther culture together with gene stacking for multiple agro-morphological and nutritional value traits is an attractive approach to fix these traits. More than 280 varieties have been produced with the use of doubled haploid technique in several crops (Kaushal et al., 2015a). Despite the practical use of the technique in rice breeding, there is still a limited understanding of the potential for cultivar development via anther culture because of its inherent factors, such as genotypic dependence of androgenesis (Kaushal et al., 2015b). With keeping the above points in view, the current research aimed to develop some good doubled haploid lines (DH) through anther culture technique and to evaluate them along with their five parents including two check varieties for yield and its component traits.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

The study was conducted at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Egypt. Rice variety Jiegnou 9601 and four Egyptian rice varieties were utilized in this study and they were listed in the Table 1. Thirty days old seedlings were transplanted into the experimental field in five planting dates with 15 days intervals for flowering synchronization. Line × tester mating system was carried out for the five parents. During the flowering period, bulk emasculation method was applied according to Butany (1961) by using hot water (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 from the female parents were harvested separately for each cross and properly packed for sowing in the following season. F_1-plants from the F_1-seeds of the four crosses were grown in a greenhouse. The anthers from F_1 plants grown in the greenhouse were used to develop the doubled haploid DH lines using anther culture technique, as described by Abd El-Khalek (2001). The doubled haploid plantlets derived from anther culture were transferred to pots containing bitmoss and clay with 2:1 ratio in the air-conditioned greenhouse.

Table 1. Origin, pedigree and type for the studied varieties

No	Parents	Origin	Pedigree	Type
1	Jiegnou 9601	IRRI	Chinese line	Japonica
2	Giza 177	Egypt	Giza171/Yomji No 1//Pi No 4	Japonica
3	Sakha 101	Egypt	Giza176/Milyang79	Japonica
4	Sakha 102	Egypt	Giza 177/GZ 4096-7-1	Japonica
5	Sakha 104	Egypt	GZ4096-8-1/GZ 4100-9-1	Japonica

2.2 Plant Sampling and Measurements

At the beginning of flowering, inflorescences of each doubled haploid plant werebagged to prevent cross pollination and to maintain the homozygosity of derived lines. The seeds from plants were harvested separately and every plant was considered as double haploid (DH). Sixty six lines were obtained as shown in Table 2.

Table 2. Hybrid parents and doubled haploid lines (DHLs) obtained from them by using anther culture technique

Cross no.	Cross Parents	Doubled produced	No. of DH 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

The seeds of the doubled haploid plants were sown for seed multiplication. The DH lines were evaluated using a randomized complete block design (RCBD) with three replications. Seedlings were transplanted with a 20 × 20 cm spacing and the recommended field practices were applied. Studied characters were days to heading, plant height (cm), number of panicles/plant, panicle length (cm) and grain yield/plant. Analysis of variance was carried out by using IRRISTAT program. Correlation coefficients (r) among all studied traits were computed using SPSS statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) according to (K. A. Gomez & A. A. Gomez, 1984).

3. Results and Discussion

3.1 Mean Performance for Yield and Its Component Traits

The results of variance analysis (ANOVA) on the data related to yield and yield components were presented in the Table 3.

Table 3. Mean square estimates for yield and yield components under study

(SOV)	d.f	DH	PH	PL	NP	GY/P
Replication	2	3.695	12.22	0.627	139.24**	2.303
Genotype	70	58.504**	669.70**	21.901**	26.31**	316.905**
Error	140	2.381	10.22	1.497	11.49	6.473

Note. **: Significant at 0.01 level; DH: Days to heading (day); PH: Plant height; PL: Panicle length; NP/P: No. of Panicles; GY/P: Grain yield/Plant.

As seen in the Table 3, the differences within replications were not significant but wide variability was observed among the genotypes. Comparisons of the genotypes by LSD-test revealed that there were significant differences among the genotypes in all studied characters as well as this high level of variation strongly increases the efficiency of selection in breeding program. Similar results were also reported previously by El-Kady et al. (1990); El-Hity and El-Keredy (1992), Rasheed et al. (2002) and Anis et al. (2016a) in some rice genotypes. The check variety Jiegnou 9601 scored the lowest period to heading (84.7 days), while Sakha 101 scored the highest period (106.7 days) (Table 4). Among the doubled haploid lines, DH 53 revealed the shortest period to heading (84.3 days), it resulted from cross No. 2. DH 66 recorded the longest period to heading (103 days), this line resulted from cross No. 4.

Table 4. Mean values of yield and yield components performance of yield and its component of the for studied rice genotypes

No.	Genotypes	DH	PH	PL	NP/P	GY/P
1	Jiegnou 9601 (P1)	84.7	87.5	15.2	16.3	27.7
2	Giza 177 (P2)	92	97	19.4	23	43.8
3	Sakha 101 (P3)	106.7	99	22.4	24	54.6
4	Sakha 102 (P4)	94	106	21.2	24.7	46.5
5	Sakha 104 (P5)	95	104	22	23	47.2
Doubled Haploid Lines from cross No. 1 (P1 × P2)						
1	DHL 1	95	80.4	14.3	14.7	44.8
2	DHL 2	96.7	75.7	14.2	17.3	35.4
3	DHL 3	96	76.5	14.5	16.7	39.1
4	DHL 4	94	76.6	14.4	18.7	22.5
5	DHL 5	98	73.9	14.5	14	30.9
6	DHL 6	97.7	74.8	14.5	15	33.1
7	DHL 7	96.7	77.1	15	14	28.3
8	DHL 8	96.8	74.8	15.4	15.7	35.7
9	DHL 9	96	76.8	15	18.7	37.7
10	DHL 10	91	73.2	13.8	16.7	22
11	DHL 11	91.3	74.2	16.2	10.7	34.4
12	DHL 12	90.7	72.2	13.3	16.3	26.7
13	DHL 13	92.3	73.1	14.6	19.3	46.3
14	DHL 14	95.3	70.9	14.2	19	25.7

15	DHL 15	88.7	73	14.5	17.3	40.4
16	DHL 16	98.3	72.4	14.2	18.7	32.4
17	DHL 17	89	88.8	19.9	18	35.9
18	DHL 18	89.3	94.1	17.8	18.3	26.6
19	DHL 19	89.3	91	20.2	22.7	26.1
20	DHL 20	89	72.7	18.2	16.7	30.6
21	DHL 21	91.7	80.4	14.5	17	27.9
22	DHL 22	91	100.6	18.1	18.3	43.9
23	DHL 23	90.7	102.9	18	18	40.5
24	DHL 24	91.7	101.1	19.3	17	40.2
25	DHL 25	91	105.6	17.8	13.3	41.3
26	DHL 26	90.7	101.3	17.6	15.7	54.9
27	DHL 27	91	99.9	18.3	19.3	54.8
28	DHL 28	89	98.6	18.4	21.3	38.6
29	DHL 29	84.7	89.4	16.7	20.7	39.5
30	DHL 30	88.7	78	16.1	21	53.9
31	DHL 31	90.7	103.4	18.9	19.3	54.6
32	DHL 32	92	102.1	17.7	21.3	57.9
33	DHL 33	92	99.6	17.8	16	46
34	DHL 34	92.7	99.3	17.3	16.3	56.7
35	DHL 35	91.3	90.6	17.4	19	21.3
36	DHL 36	94.7	98.5	17.6	18.3	54.1
37	DHL 37	89	98.6	18.4	15.3	49.5
38	DHL 38	93.3	103.1	17.6	17	42.8
39	DHL 39	91.3	96	18.2	21.7	54.3
Doubled Haploid Lines from cross No. 2 (P1 × P2)						
40	DHL 40	94.7	80	17.7	14.3	52.7
41	DHL 41	87.3	85.2	20.4	12.7	46.3
42	DHL 42	87.7	84	18.7	11.7	35.8

Note. DH: Days to heading (day); PH: Plant height; PL: Panicle length; NP/P: No. of Panicles; GY/P: Grain yield/Plant.

Table 4. Continued. Yield and yield related characteristics of the studied rice genotypes

No.	Genotypes	DH	PH	PL	NP	GY/P
43	DHL 43	99	92.2	21.1	14.3	38.4
44	DHL 44	92.7	84.3	18	15.7	47.6
45	DHL 45	92.7	80.8	17.3	19	47.1
46	DHL 46	93.3	81.5	17.7	17	35.1
47	DHL 47	92.3	80.2	17.8	16.7	35.8
48	DHL 48	95.7	77.8	17.3	18.7	33.3
49	DHL 49	92.7	84.4	17	19	34.7
50	DHL 50	94.7	81.5	17.2	17.7	56.5
51	DHL 51	100.7	90.4	23.6	22	45.5
52	DHL 52	84.7	78.8	19.4	11.7	35.8
53	DHL 53	84.3	78	18.7	13.3	42.3
54	DHL 54	98.7	91.2	20.4	20	23
Doubled Haploid Lines from cross No. 3 (P1× P4)						
55	DHL 55	88	70	15.7	17.7	34.5

56	DHL 56	91	72	15.6	19.3	33.3
57	DHL 57	95.3	67	15.2	16.3	30.1
58	DHL 58	97.3	94.5	24	16	45.7
Doubled Haploid Lines from cross No. 4 (P1× P5)						
59	DHL 59	97.7	119.1	21.5	15	55.6
60	DHL 60	102.6	86.8	19.1	12.7	36.9
61	DHL 61	96.7	121.7	22.3	15	55.1
62	DHL 62	98	95.8	18	18	29.6
63	DHL 63	97.3	122.2	24	18	51.1
64	DHL 64	96.3	122	22.4	17	55.2
65	DHL 65	98.7	126.3	21.3	19	47.8
66	DHL 66	103	129.8	22.7	17	55.1
Mean		93.29	89.87	17.889	17.47	40.67
LSD 0.05		2.491	5.161	1.9750	5.472	4.107
LSD 0.01		3.297	6.831	2.614	7.242	5.436

Note. DH: Days to heading (day); PH: Plant height; PL: Panicle length; NP/P: No. of Panicles; GY/P: Grain yield/Plant.

Jiegnou 9601 was the shortest parent for plant height (87.5 cm), while Sakha 102 was the longest parent for this trait with a mean value of 106 cm (Table 4). Among doubled haploid lines, the shortest plant height was recorded for DH 57 which was derived from cross No. 3 by mean value of 67 cm, followed by DH 55 (70 cm), which resulted from the same cross and DH 14 (70.9 cm) from cross No. 1. Meanwhile, the highest mean value was recorded for DH 66 (129.8 cm) and then by DH 65 (126.3 cm), these two lines were obtained from cross No. 4.

Considering panicle length, the longest panicle (22.4 cm) was observed on the variety Sakha 101, whereas Jiegnou 9601 produced the shortest panicle (15.2 cm). Among the doubled haploid lines, DH 58 and DH 63 showed the longest panicle length with a mean value of 24 cm, DH 58 derived from the cross No. 3 and DH 63 resulted from cross No. 4, followed by DH 51 which resulted from cross No. 2 (23.6 cm). Meanwhile, DH 12 revealed the shortest panicle (13.3 cm), it resulted from the cross No. 1.

It was found that, the variety Sakha 102 recorded the highest mean value for number of panicles per plant (24.7), while the check variety Jiegnou 9601 scored the lowest mean value for this trait (16.3). Among the doubled haploid lines, DH 19 and 51 scored the higher mean values (22.7 and 22, respectively). They were obtained from cross No. 1 and cross No. 2, respectively. On the other hand, DH 11 revealed the lowest mean value for number of panicles per plant (10.7), it was obtained from cross No. 1. It has been seen that the highest mean value for grain yield per plant (54.6 g) was scored by Sakha 101, while the lowest mean value (27.7 g) was scored by the check variety Jiegnou 9601. Among doubled haploid lines, DH 32 derived from the cross No.1 was the best line with highest mean value (57.9 g), followed by DH 34 from the same cross with mean value (56.7 gm). Whereas, the lowest mean value (21.3 g), was obtained from DH 35 which was a result also from cross No. 1.

3.2 Correlation Coefficients for Yield and Its Components

Correlation coefficients among all pairs of variables for the studied characteristics are shown in Table 5. Regarding days to heading, it showed positive correlation but not significant with all traits, except panicle length which showed positive significant association. These results agreed with those reported by Ramakrishnan et al. (2006). The remained traits showed highly significant negative correlations. Plant height showed a highly positive significant correlation with panicle length and the correlation between these two traits suggested that taller plants would bear longer panicles. Plant height also showed a highly significant positive correlation with grain yield per plant. These results are similar with those reported by El-Kady et al. (1990), Cristo et al. (2000), Abo Youssef (2001) and Anis et al. (2016b), they found highly significant and positive correlation between plant height and panicle length. Highly positive significant correlations were found for panicle length with grain yield per plant and plant height. Also it showed positive significant correlation with days to heading. For number of panicles, it recorded positive correlation with grain yield per plant and other traits. In the present study, correlation coefficients of grain yield were positive with all the traits. The highly positive significant associations of grain yield were found with panicle length. These results are similar with those published by Chaudhary et al. (1980), Kaushik and Patil (1982), Kumar and Rangasamy (1986), Mirza et al. (1992), Babar et al. (2007) and Anis et al. (2016b). The information on the inter association among the yield attributing characteristics showed the nature and extent of relationship with each other. This will help in the simultaneous improvement of different characteristics along with yield in breeding programmes.

Table 5. Correlation coefficients for yield and its component traits

Trait	DH	PH	PL	NP/P	GY/P
Days to heading	1.00	0.205	0.279*	0.115	0.142
Plant height		1.00	0.760**	0.174	0.590**
Panicle length			1.00	0.186	0.478**
No. of panicles/plant				1.00	0.123
Grain yield/plant					1.00

Note. *, **: Significant at 0.05 and 0.01 level, respectively; DH: Days to heading (day); PH: Plant height; PL: Panicle length; NP/P: No. of Panicles; GY/P: Grain yield/Plant.

4. Conclusion

Anther culture technique could be used to obtain the dubled haploid lines with promising agronomical characteristics in a short time. The information on the inter associations among the yield attributing characters showed the nature and extent of relationship with each other. Accordingly, this will help in the improvement of different characters along with yield in breeding programmes.

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