

USING MORPHOLOGICAL AND MOLECULAR METHODS TO DETERMINE THE GENETIC PURITY OF HYBRID SEEDS OF THE EGYPTIAN HYBRID RICE CULTIVAR NO.1

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ABSTRACT

The present investigation was carried out at the farm of Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt, during 2007 and 2008 rice growing seasons by using morphological and molecular methods to determine the genetic purity of hybrid seeds of the Egyptian hybrid rice cultivar No.1.

Hybrid rice seeds were produced during 2007 summer season by using IR 69625 A line (cytoplasmic male sterile line), as a female parent, with the open pollinated male parent, Giza 178 R line, as a restorer line on a large scale under isolated conditions. After harvesting, the seeds, of female line, were cleaned and kept in eight different lots, each lot was about six tons. Samples were taken, randomly, from each lot and tested in the lab. during January 2008. These seeds were sown in the grow out test experiment during summer season in a randomized complete block design, with three replications. All cultural practices were done as recommended.

The results showed that there were no differences between the data obtained in the laboratory and those obtained in the field regarding the genetic purity and agricultural values. The lot, which possessed high genetic purity gave the highest grain yield (14.250 t / ha), while, that possessed poorer genetic purity gave the lower grain yield (9.96 t / ha). Phenotypic simple correlation coefficient suggested that genetic purity percentage could be improved by selecting uniform seeds, based on grain shape, as well as germination percentage. Moreover, lot No. 6 showed the highest similarity value of genetic purity (98 %), comparing with lot No. 5, which gave the lowest similarity value (90.8 %), while, lot No. 4 gave 96.5 % similarity value.

INTRODUCTION

Rice (*Oryza sativa* L.) is considered as one of the most important cereal crops, not only in Egypt, but also, all over the world. The increase in the cultivated area with hybrid rice will be the main factor of increasing the global productivity of rice in the coming decade.

Hybrid rice is a proven technology with a yielding potential of 20 % above the existing high yielding cultivars (Jiming and Leng Peng, 2007). Improvement of seed production technology led to increase grain yield as a result of using high pure seeds, which might decrease the cost of hybrid rice .

The success of hybrid rice planting depends on the success of hybrid rice seed production program, which enables seed producers to produce high quality of seeds at an economical price. The seeds of A lines with, 100 percentage of purity are required to achieve the maximum grain yield of hybrid rice (Ingale and Waghmode, 2005 and Ahmed *et al.* , 2007) .

The PCR technique can be used as one of the indicator for predicting the genetic purity of CMS lines, based on polymorphism among parental lines. This characteristic has been used in China to perform preliminary evaluation of the purity of hybrid rice seeds. Many scientists studied the

correlation of the PCR bands with purity in female parent, on the bases of a number of bands with high activity. It was concluded that the genetic purity of hybrid seeds could differ from place to another under a large scale, depending on the synchronization of flowering for parental lines and the seed purity of the female line (CMS).

The present study was aimed to use morphological and molecular methods to determine the genetic purity of hybrid seeds of the Egyptian hybrid rice cultivar no,1.

MATERIALS AND METHODS

Two field experiments were carried out at the experimental farm of Sakha Agricultural Research Station, Egypt, during 2007 and 2008 summer seasons to study the effect of hybrid seed purity on the productivity of hybrid rice cultivar no 1. In 2007 rice growing season, seeds of the hybrid combination, IR 69625 A / Giza 178 R, was produced on a large scale and harvested as certified seeds. Moreover, the seeds were cleaned and kept in different lots (1 – 8). Each lot was six tons and random samples (2 Kg) were taken from each lot for testing in the lab. in 2007 winter season. These samples were tested in the field in 2008 summer season in grow out test experiment. However, each lot was planted in 7 rows in a randomized complete block design with three replications, at a rate of one seedling/hill. All cultural practices, up to harvesting, were performed as recommended.

Studied characters :

- 1- Heading period (days): It is the number of days from panicle exertion to complete heading.
- 2- Plant height (cm): Seven days before harvesting, plant height was measured for five random plants from soil surface up to the top panicle of the main stem.
- 3- Genetic purity (%) : After heading, the offtype plants were counted in each plot and the genetic purity was estimated as follows :

$$\text{Genetic purity (\%)} = \frac{\text{Number of types}}{\text{Total plants in unit area}} \times 100 .$$

- 4- Grain yield and its components: Five days before harvesting, ten random hills from each plot were collected to determine the number of panicles/hill and five panicles characters (panicle length , panicle weight, number of filled grains / panicle, seed set (%) and 1000- grain weight).

Five square meters of each plot was manually harvested, then left four days for air drying and mechanically threshed and weighed to determine grain and straw yields. Grain and straw yields were adjusted to 14 % moisture content and converted to tons / ha .

- 5- Harvest index (%): It was estimated according to the following equation:
Grain yield (t / ha)

$$\text{Harvest index (\%)} = \frac{\text{Grain plus straw yields (T/ ha.)}}{\text{Grain plus straw yields (T/ ha.)}} \times 100 .$$

Molecular techniques :

DNA isolation and purification were carried out, using CTAB (Cetyl-tetramethyl ammonium bromide) method (Murray and Thompson ,1980).

The PCR thermocycler machines, from Perkin-Elmer, Gene Amp. 2400 were used in this study.

RM 212, Primer sequences :

Forward 5' AAGGTCAAGGAAACAGGGACTGG 3'

Reverse 5' AGCCACGAATTCCACCACTTTCAGC 3'

Gene Ruler (50 bp) DNA ladder

RESULTS AND DISCUSSION

Genetic purity of parental lines and hybrids is of crucial importance, as one percent reduction in purity of hybrid seeds results in a reduction of about 100 kg/ha. In the yield of a commercial crop, traditional genetic purity is tested by Grow Out Tests (GOT), based on morphological assay. The seed production technology has been improved, resulting in a significant increase of the seed yield and a decrease of its cost. The yield of hybrid rice will decrease by 0.8 percent when its seed purity is less than 90 percent (Long Ping *et al.* 2003). The effective values of seed quality, on the productivity of the tested hybrid rice cultivar are presented in Tables 1 and 2.

Table (1): Mean performance of the seed lots in the Egyptian hybrid rice No. 1 for some vegetative traits during 2008 season.

Lot No.	Germination percentage (%)	Genetic purity in the lab.(%)	Agri. value (%)	Heading period (days)	Genetic purity in the field (%)	Plant height (cm)
1	88.50	99.90	85.60	9.00	98.10	97.00
2	87.30	99.80	88.50	10.33	97.00	95.00
3	88.30	99.80	88.30	9.66	97.70	96.50
4	87.00	99.90	88.40	11.33	96.80	95.00
5	87.90	98.80	89.70	10.00	97.50	96.00
6	89.90	99.80	88.60	8.00	98.50	99.00
7	87.00	98.20	86.70	12.33	96.50	94.00
8	86.50	97.30	86.50	13.00	96.00	92.00
L.S.D. (0.05)	3.284	N.S	3.12	0.931	N. S	4.98
(0.01)	3.838	N.S	3.731	1.27	N.S	5.63

N.S. = Not significant.

The genetic purity percentage was estimated as uniformity to the seeds in the lab. (Table 1). The genetic purity in the lots was very high, which was ranged from 97.3 %, for lot no. 8 to 99.9 % for lot no. 1, although the differences were not significant. While, for the germination percentage ranged between 86.5 for lot no. 8 and 89.9 for lot no. 6 and the differences were significant. Moreover, the agricultural value ranged between 85.6 %, for lot no. 1, and 89.7 % for lot no. 5 with significant differences. Sowing with high purity seeds, showed the shortest heading period (eight days, lot no. 6),

but, the poorer quality seeds showed the longest heading period (13 days, lot no. 8), indicating that the population with high uniformity showed the shorter period (eight days) to complete heading. These data coincided with the genetic purity percentage under field conditions in grow out test experiment, where, the number of off-type plants increased with the poor quality seed lots during 2008 summer season .

Furthermore, significant differences were found among the lots for the number of panicles per plant and its values differed from 19.00, in lot no. 6, to 29.00 in lot no. 1 (Table 2). For panicle length, lots no.1,3,4 and 6 gave the highest mean values, which ranged between 24.00 to 25.00cm. While, lots no. 2,5,7 and 8 gave the lowest mean values for this trait and ranged from 22.00 to 23.00 cm. Besides, regarding panicle weight, lots no.1, 3 and 6 gave the highest mean values, being 4.90, 4.90 and 5.00 g, respectively. While, lots no. 7 and 8 gave the lowest mean values (3.90 and 3.80 grams , respectively). With respect to seed set, the highest mean values were obtained from lots no. 1 and 6 (96.0 %). The lowest mean values were obtained from lot no.8 (90.70 %).

For 100- grain weight, as shown in Table (2) , there were significant differences among the lots. The highest value was that of lot no. 8 (2.45 g), whereas, the lowest value was that of lot no. 1 (2.20 g).

Table (2): Mean performance of the seed lots in the Egyptian hybrid rice No. 1 for grain yield and its components during 2008 season.

Lot (no.)	No. of panicles/plant	Panicle length (cm)	Panicle weight (gm)	Seed set (%)	100-grain weight (gm)	Grain yield / (t/ha)	Harvest index (%)
1	29.00	24.00	4.90	96.00	2.20	13.75	58.00
2	21.00	23.20	4.10	93.00	2.35	11.00	52.00
3	25.00	24.00	4.90	95.00	2.40	13.00	56.50
4	27.00	24.00	4.00	92.00	2.38	9.55	48.00
5	29.00	23.50	4.40	94.30	2.30	11.20	55.00
6	21.00	25.50	5.50	96.00	2.30	14.25	59.50
7	20.00	23.00	3.90	91.00	2.40	9.65	46.50
8	19.00	22.00	3.80	90.70	2.45	9.47	42.90
L.S.D. (0.05)	1.53	1.31	0.23	4.53	0.17	0.85	1.98
(0.01)	1.93	1.53	0.27	4.93	0.32	1.16	2.35

The data in Table (2) further showed that the highest value for the number of panicles/plant was 29.00, but, the lowest value was 19.00. For the panicle characters, the desirable mean values were 25.50, 5.50 and 96.00 % for lot no. 6 for panicle length, panicle weight and seed set percentage, respectively. Moreover, lot. no. 6, with high genetic purity percentage in the field, gave the highest values of grain yield (14.25 t/ha.) and harvest index percentage (59.50 %). On the other hand, lot no. 8, with poor genetic purity percentage, gave the lowest values of grain yield (9.47 t / ha) and harvest index (42.90 %). These results suggested that the genetic purity and germination percentages under lab. testing could be used as indicators to seedling vigor in early growth stages. However, for determining the heterosis

- in hybrid grain yield, the grow out test experiment, under the field condition, might be used, and this might need one year before planting. But, using PCR technique, based on the use of specific marker for the parental lines and F₁ hybrid, it could take shorter time (several weeks).

These results were similar to those obtained by Ingale and Waghmod (2005) who reported that the maximum yield potential was obtained from using 100 % pure seeds of the parental lines. Also, Yamauchi (1994) showed that the major limitations to the large scale adoption of hybrid in grain rice technology in the tropics were the inadequate level of standard heterosis of grain yield.

Phenotypic simple correlation coefficient:

The phenotypic simple correlation coefficients were highly significant between genetic purity (%) in the field and each of genetic purity in the lab. and germination percentages in 2008 (Table 3). Moreover, the correlation coefficient was significant between germination percentage with each of genetic purity in the lab. and agricultural value, as shown in Table (3).

Table (3): Phenotypic simple correlation coefficients among some vegetative traits during 2008.

Characters	Genetic purity in the lab.(%)	Germination percentage(%)	Agric. value
Genetic purity in the lab.(%)	-		
Germination percentage (%)	0.584 *	-	
Agric. value	0.462 *	0.598 *	-
Genetic purity in the field.(%)	0.719 **	0.961 **	0.586 *

*, ** Significant at 0.05 and 0.01 levels, respectively.

These results suggested that, improving the genetic purity, could be found through selection of uniform hybrid rice seeds and high germination percentage under the lab., testing conditions in the present study.

Table (4): Phenotypic simple correlation coefficients between grain yield and its studied components.

Characters	No. of panicles / plant	Panicle length	Panicle weight	Seed set	Grain yield
No. of panicles / plant					
Panicle length(cm)	0.455 *				
Panicle weight (g)	0.543 *	0.863 **			
Seed set	0.511 *	0.783 **	0.932 **		
Grain yield (t/ha)	0.489 *	0.765 **	0.968 **	0.961 **	
Harvest index (%)	0.480*	0.811 **	0.918 **	0.986 **	0.939 **

*, ** :Significant at 0.05 and 0.01 levels, respectively.

In addition, the simple phenotypic correlation coefficients were either significant or highly significant among the number of panicles / plant, grain yield and harvest index. The correlation coefficient was highly significant between grain yield with each of panicle weight, seed set percentage and harvest index percentage, as shown in Table (4). The results indicated that

the seed set percentage played an important role in increasing the grain yield (t / ha). There were, also, similar results among the other traits (Table 4). These results were confirmed with those obtained by El, Shamey (2008).

Molecular techniques .

Detection of genetic purity for the hybrid seeds, which produced from the hybrid combination, IR69625A/G178R, on a large scale, with open pollination by using molecular marker, was an attempt to detect the off-type plants in hybrid seeds, based on molecular level. The RM 212 primer was used for DNA amplification to determine the genetic purity in three lots, which were randomly selected out of the eight lots. Moreover, five gels. of each lot were made and each gel contained ten individual seeds. The results showed that lot No. 6 showed the highest similarity value of genetic purity 98 %, compared with lot No. 5, which gave the lowest similarity value (90.8 %). While, lot No. 4 gave 96.5 % similarity value, on the basic number of bands of individual plants, where, the hybrid seeds contained two bands, but, the off-type seeds contained only one band, as shown in Figs. 1, 2 and 3. These results indicated that the genetic purity of hybrid seeds could differ from place to another under a large scale open pollination, depending on the synchronization of flowering for parental lines and the seed purity of the female line (CMS).

Lot	No. of samples	No. of pure lines	Purity (%)	Off -type	Impurity(%)
5	50	45	90	5	10

1 2 3 4 5 6 7 8 9 10



Figure 1. The hybrid seed purity in lot no.5. Samples no. 2, 3, 4, 5, 6 ,8, 9 and 10 were hybrid seeds, where samples no 1 and 7 are off type seeds.

Lot	No. of samples	No. of pure lines	Purity (%)	Off -type	Impurity (%)
4	50	48	96	2	4

1 2 3 4 5 6 7 8 9 10 11 12



Figure 2. The hybrid seed purity in lot no.4. Samples no. 1, 2, 3, 4, 5, 6 , 8 , 9, 10, 11 and 12 were hybrid seeds, where sample no 8 was off -type seeds.

Lot	No. of samples	No. of pure lines	Purity (%)	Off -type	Impurity (%)
6	50	49	98	1	10




Figure 3. The hybrid seed purity in lot no.6. Samples no 1, 2, 3, 4,5, 6, 7,

8, 9 and 10 were hybrid seeds, without off -type seeds

Finally, the results suggested that the primer, RM 212, could be used to identify the genetic purity for hybrid seeds , since it could distinguish between A, R and F₁ plants in a fast time, which could be used as one of the supporting data to the grow out test experiment. In this respect, Garg *et al.* (2006) reported that the assessment of genetic purity of hybrid seeds, based on unlinked markers, might not be fully reliable.

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إستخدام الطرق المورفولوجية والجزئية لتحديد نقاوة الوراثية في تقاوى الأرز
لصنف هجين مصري ١
محمود إبراهيم أبو يوسف ، عبد الله عبد النبي عبد الله ، سعيد محمد شحاتة و
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أجريت هذه التجربة بالمزرعة البحثية بسخا خلال موسمي ٢٠٠٧ و ٢٠٠٨م لاستخدام
الطرق المورفولوجية والجزئية في تحديد نقاوة الوراثية في تقاوى الأرز لصنف هجين مصري
١. في موسم ٢٠٠٧م أنتجت تقاوى هجين بتلقيح السلالة " IR 69625 A " المفتوحة التلقيح
مع الأب "جيزة ١٧٨" والمنزرع على النطاق التجارى تحت ظروف العزل و بعد حصاد تقاوى
السلالة الأم تم تنظيفها وتقسيمها إلى ثمانية لوطات ، كل لوط أحتوى على ستة أطنان . وقد تم
أخذ العينات من كل لوط وتم اختبارها في المعمل خلال شهر يناير من عام ٢٠٠٨م وتمت
زراعة التجربة في الموسم الصيفي بالمزرعة البحثية باستخدام القطاعات الكاملة العشوائية فى
ثلاث مكررات وتم تطبيق كل التوصيات الفنية اللازمة للمعاملات الزراعية .
و بمقارنة البيانات المتحصل عليها من المزرعة البحثية مع البيانات المعملية أوضحت
النتائج أن اللوط الذي أعطى نقاوة وراثية ودرجة تماثل عالية هو الذى أعطى قيمة عالية
لمحصول حبوب (١٤,٢٥٠ طن / هكتار) ودليل الحصاد (٥٩ %) ،بينما اللوط الذى أعطى
نقاوة وراثية ودرجة تماثل أقل هو الذى أعطى قيمة منخفضة من محصول الحبوب (٩,٩٦٠
طن / هكتار) ودليل الحصاد (٤٢ %). وأظهرت البيانات الناتجة من تحليل التلازم البسيط أن
النسبة المئوية للنقاوة الوراثية يمكن تحسينها بانتخاب التقاوى المتماثلة وذات نسبة الإنبات العالية
في المعمل. كما أن اللوط رقم ٦ أعطى أعلى قيم تماثل وراثي (٩٨ %) ،بينما اللوط رقم ٥
أعطى أقل قيم تماثل وراثي (٩٠,٨ %) واللوط رقم ٤ أعطى (٩٦,٥ %) قيمة تماثل وراثي

قام بتحكيم البحث

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خارجي

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