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Discovering of new rice genotypes for restoration maintenance ability and their relationship to hybrid rice seed production

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THE EXPLOITATION of hybrid rice technology has been made possible by the identification of restorers and maintainers as parental lines. Four CMS lines of rice viz., E2015A, G46A, CCG5A, and E0034A were crossed with 24 diverse testers during the growing seasons of 2022 in Experimental Farm of RRTC, Sakha, Egypt. Ninety-six hybrid combinations were generated to identify the effective restores and maintainers from elite local and exotic rice genotypes under test cross nursery and determine the best heterotic combinations in terms of yield and its components during 2023 seasons. The F1 hybrids expressed different fertility reactions. Out of 96 test crosses the results of this investigation revealed that sixteen rice genotypes of tested 24 rice parents were identified as effective restorers (R) which can easily restore four different sources of CMS lines (Pollen fertility and spikelet fertility more than 83%) with heterotic morphological attributes. On the contrary, three rice genotypes of 24 rice parents (BN-19, BN-45, and Winixyan) were identified as maintainers (M) for the same aforementioned CMS lines. Among the fourteen F1 hybrids, assessment of standard heterosis based on the standard check (Giza 178) of five effectively restored cross combinations showed higher useful heterosis than the standard check variety. Three hybrid combinations i.e. E0034A/SR-86 (26.41%) and E0034A/GZ10848 (25.51%) recorded more than 25% yield advantage over the check variety Giza 178. Restorers and maintainers identified illustrate the possibility of the development of new rice hybrids and CMS lines in the future.

Keywords: Hybrid rice, CMS lines, Restorers, Maintainers.

Introduction

Rice is an important source of food, and more than 50% percent of people rely on rice to fulfill their hunger needs globally (Maurya *et al.,* 2022). Rice production and consumption is perhaps the most critical economic activity, and it is believed that nearly every day, rice food is taken by half of the population globally at least once (Wang *et al.,* 2023). Moreover, hybrid rice is important for improving food security and enhancing crop production. Keeping this in view, more work is required to increase its productivity to combat the food scarcity issue (Durand-Morat *et al.*,2011). The task is quite challenging and the options available are very limited given the plateauing trend of yield in high-productivity areas, decreasing and degrading land, and scarcity of water (Santhanalakshmi *et al.,* 2010). The improvement in the production of hybrid rice is the result of joining certain factors such as advances in breeding technologies, improved restorer lines with desirable floral traits and genetic mechanisms, and better agronomic practices. Rice breeders can continue to improve the yield, quality, and sustainability of hybrid rice production, ensuring astable food supply for the growing global population (He *et al,* 2019; Huang *et al,* 2022 Ashraf *et al.*,2024).

Developing new restorer lines is an important pre-request for the successful production of hybrid varieties. Identification of effective maintainers and restorers is of great importance in any hybrid rice breeding program based on cytoplasmic male sterility (CMS) (Bijral *et al.,* 1991). It is a common phenomenon in higher plants characterized by sterile, nonfunctional pollen grains due to the alterations in the mitochondrial genome. The sterility can be restored by crossing CMS lines with the restorer lines containing fertility restorer genes (*Rf*). In rice, wild abortive CMS (WA-cms) source has been utilized extensively in commercial hybrid rice seed production due to its stability, excellent outcrossing potential, and the availability of a broader genetic base for the restorer lines (Virmani *et al,* 1994). Restorer lines play an important role in successful hybrid rice development. They are detected conventionally through test cross procedure by crossing rice germplasm lines (male parents) with the sterile CMS lines (female parents) and the F1's are evaluated for pollen and spikelet fertility.

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Hybrid rice production in Egypt faces some problems such as low-quality traits to some extent in addition to the small number of restorer lines with desired grain yield (El-Mowafi *et al.,* 2005). Therefore, it is necessary to search for new sources capable of restoring the ability of new cytoplasmic male sterility lines developed under Egyptian conditions with desirable floral traits to increase the rate of cross-pollination, which in turn is reflected in increasing the percentage of seed set in hybrid rice fields. This study was conducted for the purpose of studying the maintenance and restoring ability of the pollen parents over different cytoplasmic sources for a group of promising and locally bred lines in addition to a group of imported lines to identify the ability of any of these lines a restoring ability to the new CMS lines, as well as to obtain the best hybrids with high yielding and desired quality traits. With this objective, a set of 24 locally adapted varieties and elite promising lines were evaluated to identify the restorer and maintainer lines with four CMS lines to develop new rice hybrids under Egyptian conditions.

Materials and Methods:

Plant materials

The experiment was conducted at the Experimental Field of Rice Research and Training Center (RRTC) at Sakha, Kafr El-Sheikh, Egypt during the rice-growing seasons of 2022 and 2023. The experimental materials consist of four CMS lines E2015A, G46A, CCG5A, and E0034A derived from different sterile sources and twenty-four rice genotypes (local and introduced genotypes) as shown in Table 1.

Table 1. Background information of the studied rice genotypes evaluated in the study.

Cato.	Genotypes	Cytoplasmic source/Type	Origin	Remarks
CMS lines	**E2015A**	Kalinga	Egypt	New CMS line
	G46A	Gambiaca	China	Commercial line
	CCG5A	Wild Abortive (WA)	Egypt	New CMS line
	E0034A	Wild Abortive (WA)	Egypt	New CMS line
Tester lines	**GZ11190-3-3-1-1**	Indica-Japonica	Egypt	Promising line
	GZ11190-3-13-4-1	Indica-Japonica	Egypt	Promising line
	GZ11663-4-8-10-17	Indica-Japonica	Egypt	Promising line
	GZ12021-4-1-1-6	Indica-Japonica	Egypt	Promising line
	GZ12021-7-8-13-4	Indica-Japonica	Egypt	Promising line
	GZ12089-3-11-2-1	Indica-Japonica	Egypt	Promising line
	GZ11504-1-3-2-4	Indica-Japonica	Egypt	Promising line
	DLD-2-1	Indica-Japonica	Introduced	-------------
	BN-19	Indica-Japonica	Introduced	-------------
	BN-45	Indica-Japonica	Introduced	-------------
	Winixyan	Indica-Japonica	Egypt	Promising line
	GZ12021-13-14-22-2	Indica-Japonica	Egypt	Promising line
	SR-52	Indica	Introduced	-------------
	SR-61	Indica	Introduced	-------------
	SR-86	Indica	Introduced	-------------
	CIV-5	Indica	Introduced	-------------
	CIV-6	Indica	Introduced	-------------
	POP-112-75	Indica-Japonica	Egypt	Promising line
	POP-112-101	Indica-Japonica	Egypt	Promising line
	GZ12103-3-4-6-7	Indica-Japonica	Egypt	Promising line
	GZ12176-5-1-1-2	Indica-Japonica	Egypt	Promising line
	SAGC-03	Indica-Japonica	Introduced	-------------
	GZ10848-1-2-2-1	Indica-Japonica	Egypt	Promising line
	Giza 178	Indica-Japonica	Egypt	Promising line

Plant methods

A set of 96 hybrids was generated in line x tester mating design to identify the parental lines maintainer and restorer as well as the best hybrid rice combinations. During 2022, cytoplasmic male sterile lines (4 CMS) were planted on three successive sowing dates at fifteen-day intervals to overcome the differences in flowering date for each tester. To facilitate hybridization at the flowering stage, several CMS plants were transferred to pots pre-filled with water. Clipping of CMS panicles was done in the evening and hand-pollinated the next morning with pollen dust from twenty-four selected rice varieties. The clipped and crossed panicles were tagged and bagged properly with white papers. The packing process continued after hybridization to avoid unwanted pollination. After maturity, the filled spikelets from 96 hybrids were collected from panicles and properly packed for planting in the following season. A total of 96 F1 hybrids were grown with their pollen parents during 2023 in three rows of 5 meters long and seedlings were transplanted at 20 × 20 cm spacing in the main field. Recommended packages of practices such as fertilization, irrigation, weed, and pest control were adopted. Pollen studies were carried out to assess the fertility/sterility status of F1 hybrids. For this purpose, 10-15 spikelets from just emerged panicles of F1 plant in a vial containing 70% ethanol for pollen viability test. In the laboratory, one drop of 1% Iodine Potassium Iodide (IKI) stain was put on a glass slide. The anthers of five spikelets were taken out with forceps and placed on a glass slide. Anthers were crushed gently by using a needle to release pollen grains. After removing debris, pollen fertility status was observed under a compound microscope. The entire slide was scanned and pollen fertility was counted in three random fields. The pollen grains of F1 plants were classified as follows based on the extentof pollen sterility by Virmani et al 1997 (Table2).

Table 2.Classification of test lines into maintainers and restorers.

Category	Pollen fertility (%)	Spikelet fertility (%)
Maintainer	0 – 1	0
Partial maintainer	1.1 – 50	0.1 – 50
Partial restorer	50.1 – 80	50.1 – 75
Restorer	> 80	> 75

Round well filled and deeply stained pollen grains were counted as fertile and shriveled and lightly stained were counted as sterile. Pollen fertility and spikelet fertility percentages were recorded to identify maintainers and restorers of CMS lines. The following formulae were used to calculate pollen fertility and spikelet fertility (%).

$$\text{Pollen fertility \%} = \frac{\text{No. of fertile pollens}}{\text{Total No. of pollens}} \times 100$$

$$\text{Spikelet fertility \%} = \frac{\text{Filled grain per panicle}}{\text{Total spikelets per panicle}} \times 100$$

Statistical analysis

The observations and mean values were recorded and subjected to statistical analysis. Analysis of variance was computed for the genotypes under study are random. The descriptive statistical analysis and analysis of variance (ANOVA) of yield and its component traits were worked out using SPSS software (version 20.0). The estimates of genotypic variance (GV), phenotypic variance (PV), phenotypic coefficient of variations (PCV%), and genotypic coefficient of variations (GCV%) were computed according to the formula suggested by Burton (1952). Broad sense heritability was calculated using the formula suggested by Hanson et al (1956). The estimates of genetic advance were obtained and categorized as high, medium, and low as suggested by Johnson et al. (1955). Standard heterosis was determined as the increase of the mean of F1 hybrid over the check variety Giza 178, these were proposed by Mather (1949) and Mather and Jinks (1982) as follows:

$$\text{Standard heterosis \% (SH)} = \frac{F1 - \text{check variety}}{\text{Check variety}} \times 100$$

Appropriate L.S.D values were calculated to test the significance of the heterosis effects for better parent and check variety according to the following formula, suggested by Wynne et al. (1970):

$$\text{L.S.D. for check variety} = t.\ {}^{0.05}_{0.01} \sqrt{\frac{2\ MSE}{r}}$$

The check variety used in the present investigation was the best commercial variety Giza 178.

Results and Discussion

Classification restorer and maintainer genotypes depending on the percentage of pollen fertility and spikelet fertility were classified into four categories; Maintainers (M), Partial Maintainers (PM), Partial Restorer (PR) and Restorer (R) according to the scale of Virmani et al., 1997, and presented in Tables 3 and 4. It's clear that 15 lines of tested 24 rice parents were identified as effective restorers (R) which can easy restore four different sources of CMS lines E2015A, G46A, CCG5A and E0034A (Pollen fertility and spikelet fertility more than 83%).

Table 3. Pollen fertility (%) and spikelet fertility (%) of F_1 crosses of an A/R cross combination for different CMS lines.

Genotypes	E2015A			G46A		
	PF%	SF%	Categories	PF%	SF%	Categories
GZ11190-3-3-1-1	85.33	82.20	R	92.70	89.33	R
GZ11190-3-13-4-1	95.83	93.00	R	96.20	94.00	R
GZ11663-4-8-10-17	91.00	90.50	R	87.00	85.10	R
GZ12021-4-1-1-6	10.50	16.70	PM	69.00	63.33	PR
GZ12021-7-8-13-4	86.83	84.00	R	91.50	88.20	R
GZ12089-3-11-2-1	34.10	30.16	PM	11.00	8.10	PM
GZ11504-1-3-2-4	93.16	92.00	R	94.83	92.00	R
DLD-2-1	26.33	24.50	PM	15.10	10.50	PM
BN-19	0.00	0.00	M	0.00	0.00	M
BN-45	0.50	0.00	M	0.00	0.00	M
Winixyan	0.00	0.00	M	0.33	0.00	M
GZ12021-13-14-22-2	89.33	88.16	R	93.83	91.10	R
SR-52	85.00	84.83	R	89.20	85.33	R
SR-61	83.83	80.16	R	94.66	93.00	R
SR-86	90.16	89.50	R	95.10	92.33	R
CIV-5	84.66	80.20	R	Absent	Absent	---
CIV-6	88.00	82.70	R	90.50	87.16	R
POP-112-75	69.33	61.16	PR	58.00	53.10	PR
POP-112-101	94.83	93.50	R	96.20	94.33	R
GZ12103-3-4-6-7	87.16	85.66	R	85.66	83.00	R
GZ12176-5-1-1-2	20.00	18.33	PM	71.20	56.16	PR
SAGC-03	91.66	90.00	R	93.50	92.00	R
GZ10848-1-2-2-1	97.33	96.10	R	98.00	96.83	R
Giza 178	96.50	95.00	R	97.50	95.16	R

Table 4. Pollen fertility (%) and spikelet fertility (%) of F_1 crosses of an A/R cross combination for different CMS lines.

Genotypes	CCG5A			E0034A		
	PF%	SF%	Categories	PF%	SF%	Categories
GZ11190-3-3-1-1	91.20	89.00	R	89.50	85.10	R
GZ11190-3-13-4-1	94.70	93.16	R	96.33	94.00	R
GZ11663-4-8-10-17	82.00	80.50	R	88.20	81.83	R
GZ12021-4-1-1-6	73.16	70.00	PR	80.70	77.10	R
GZ12021-7-8-13-4	86.50	85.66	R	89.33	80.00	R
GZ12089-3-11-2-1	13.00	9.00	PM	58.66	53.00	PR
GZ11504-1-3-2-4	90.83	89.20	R	95.33	93.10	R
DLD-2-1	Absent	Absent	---	32.00	28.50	PM
BN-19	0.00	0.00	M	0.33	0.00	M
BN-45	0.00	0.00	M	0.00	0.00	M
Winixyan	0.00	0.00	M	0.00	0.00	M
GZ12021-13-14-22-2	89.83	85.00	R	90.00	86.20	R
SR-52	83.50	79.10	R	86.50	82.00	R
SR-61	88.20	86.70	R	90.00	87.70	R
SR-86	93.10	91.00	R	96.16	94.50	R
CIV-5	89.33	85.20	R	87.50	88.00	R
CIV-6	90.00	87.33	R	94.00	90.20	R
POP-112-75	64.00	60.83	PR	59.16	55.00	PR
POP-112-101	94.66	90.10	R	95.00	93.70	R
GZ12103-3-4-6-7	87.16	85.00	R	92.16	91.00	R
GZ12176-5-1-1-2	30.50	27.33	PM	17.00	15.83	PM
SAGC-03	88.83	86.16	R	90.50	87.00	R
GZ10848-1-2-2-1	97.00	95.66	R	95.70	93.16	R
Giza 178	95.33	92.00	R	93.83	91.20	R

At the same time, two of these 15 lines (GZ11190-3-13-4-1 and GZ10848-1-2-2-1) recorded the highest restoring ability for the different CMS sources which give more than 90% pollen and spikelet fertility, indicating that these restorers contain major fertility restoration Rf genes. These results indicated that the identification of these high-yielding restorer lines could be effectively utilized in hybrid rice breeding programs to develop new hybrid rice varieties. These results were in general agreement with those reported by El-Keredy et al., 2007. They found that the pollen parents under study varied significantly regarding to restoring ability and the CMS line G46A appeared to be easy restorability. Anis et al, (2024) confirmed the same results and found that GZ10848 was identified as a restorer and including five fertility restorer genes Rf3 and Rf4, Rf5, Rf6, and Rf1. Contrarily, three lines of 24 rice parents (BN-19, BN-45, and Winixyan) were identified as maintainers (M) for the same aforementioned CMS lines. While for the CMS E2015A, one tester was identified as a partial restorer (PR), also two testers were identified as partial restorers for the two CMS lines CCG5A and E0034A, and three testers for G46A.

On the other hand, there are ten combinations were identified partial maintainers (PM), they were four testers (GZ12021-4-1-1-6, GZ12089-3-11-2-1, DLD-2-1 and GZ12176-5-1-1-2) with the new CMS E2015A, two testers (GZ12089-3-11-2-1 and DLD-2-1) with the CMS G46A, two testers (GZ12089-3-11-2-1 and GZ12176-5-1-1-2) with the new CMS CCG5A and two testers (DLD-2-1 and GZ12176-5-1-1-2) with the new CMS E0034A (Figure 2 and Table 5). However, some of these testers were identified as partial maintainers with some cytoplasmic male sterility lines but with others were identified as partial restorers. This result leads to the source of cytoplasmic male sterile may be playing important role in restoring ability for any testers. These combinations were identified as either partial maintainers or partial restorers which have less interest in hybrid rice breeding programs.

Table 5. Potential restorers and maintainers for four CMS lines.

CMS lines	Restorers	Maintainers
E2015A	GZ11190-3-3-1-1, GZ11190-3-13-4-1, GZ11663-4-8-10-17, GZ12021-7-8-13-4, GZ11504-1-3-2-4, GZ12021-13-14-22-2, SR-52, SR-61, SR-86, CIV-5, CIV-6, POP-112-101, GZ12103-3-4-6-7, SAGC-03, GZ10848-1-2-2-1, Giza 178	BN-19, BN-45, Winixyan
G46A	GZ11190-3-3-1-1, GZ11190-3-13-4-1, GZ11663-4-8-10-17, GZ12021-7-8-13-4, GZ11504-1-3-2-4, GZ12021-13-14-22-2, SR-52, SR-61, SR-86, CIV-5, CIV-6, POP-112-101, GZ12103-3-4-6-7, SAGC-03, GZ10848-1-2-2-1, Giza 178	//
CCG5A	GZ11190-3-3-1-1, GZ11190-3-13-4-1, GZ11663-4-8-10-17, GZ12021-7-8-13-4, GZ11504-1-3-2-4, GZ12021-13-14-22-2, SR-52, SR-61, SR-86, CIV-5, CIV-6, POP-112-101, GZ12103-3-4-6-7, SAGC-03, GZ10848-1-2-2-1, Giza 178	//
E0034A	GZ11190-3-3-1-1, GZ11190-3-13-4-1, GZ11663-4-8-10-17, GZ12021-4-1-1-6, GZ12021-7-8-13-4, GZ11504-1-3-2-4, GZ12021-13-14-22-2, SR-52, SR-61, SR-86, CIV-5, CIV-6, POP-112-101, GZ12103-3-4-6-7, SAGC-03, GZ10848-1-2-2-1, Giza 178	//

Previous studies showed that among eleven out of 60 crosses derived from 10 CMS lines and 6 testers exhibited spikelet fertility more than 80% (El-Degwy, 2000). Also, 239 rice genotypes tested for their status in the hybrid rice gene pool, 12 restorers and 16 maintainers were identified. Most of the genotypes were found to be partial restorers and partial maintainers (Akhter et al., 2008). There are considerable variations in the frequency of maintainers for different CMS lines. The average frequency was 5% over the tested crosses. Considerable variability was found in the frequency of restorers over different CMS lines and with lines from different sources (Eusebio et al., 2002). Types of pollen grains of the F1 hybrids after staining with IKI are presented in Figure 1.

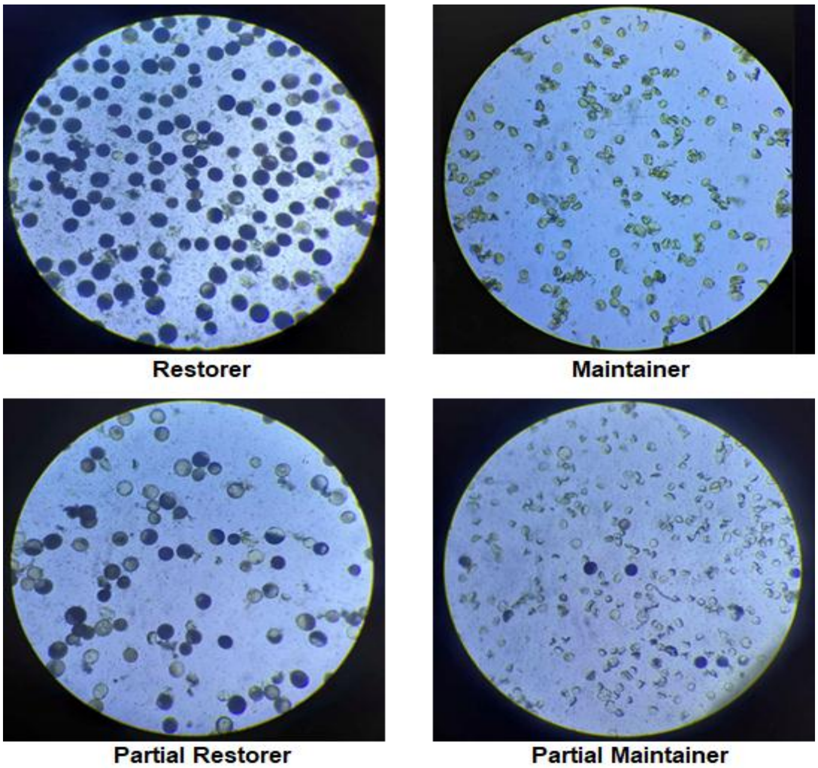

Fig. 1. Types of pollen grains of F_1 combinations (Crosses) after IKI stain examination. Round dark stained- fertile pollen and light stained with irregular shape- sterile pollen.

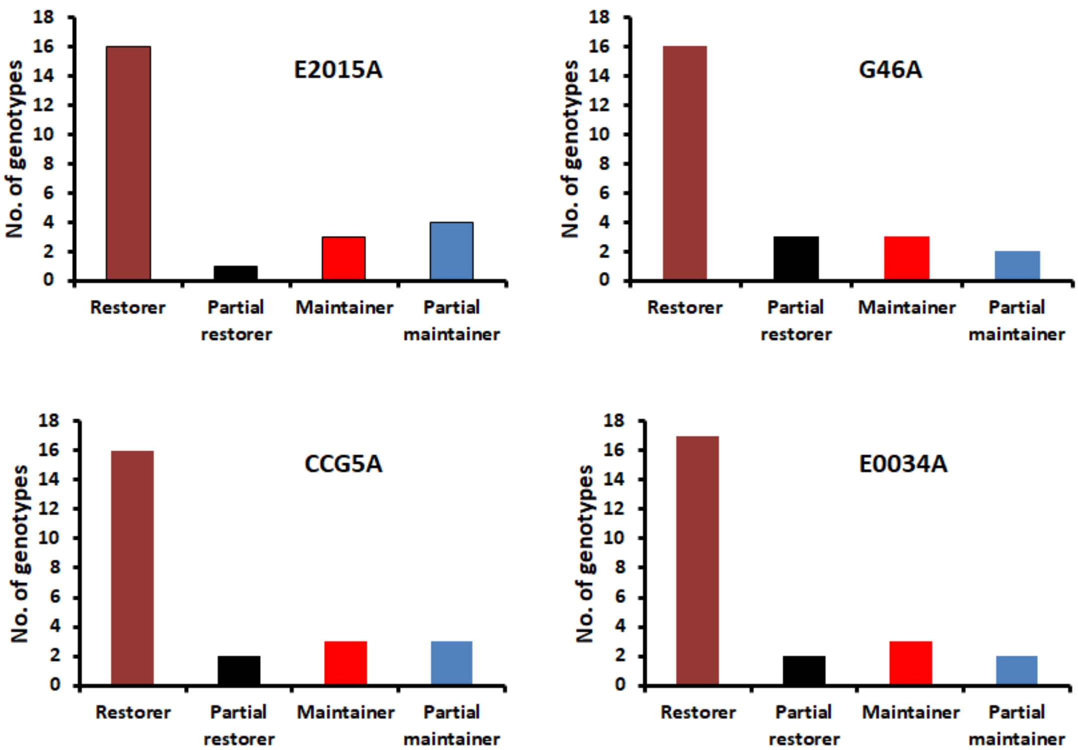

Fig. 2. Number and frequency distribution of restorers, maintainers, partial restorers and partial maintainers for the four CMS lines.

Analysis of variance.

The analysis of variance revealed that significant differences existed among the genotypes for all the studied traits. The grand mean, variance, phenotypic and genotypic coefficients of variation, heritability, and genetic advance as a percentage of the mean are presented in Table 6.

Table 6. Mean squares for six characters studied of fourteen hybrid rice combinations.

Source	d.f	Days to maturing	Number of panicles/plant	Panicle length	Spikelet fertility %	1000-grain weight	Grain yield/plant
Replications	2	7.929	7.929	10.047	11.342	6.761	9.529
Genotypes	13	43.509**	5.929**	10.323**	15.559**	3.740**	18.236**
Error	26	0.108	0.159	0.071	0.104	0.121	0.147

Mean performance and standard heterosis

The average number of days to maturing was 125.4 days with a range of 118.3 to 133.0 days (Table 7). The hybrid combination E0034A/Pop-112-101 observed the earliest hybrid as 118.3 days, while G46A/GZ11504-1-3-2-4 was recorded as the latest hybrid as 130.7 days compared with the check variety (133.0 days). The negative estimates of heterosis for days to maturity are performed to develop earliness new rice hybrids. All crosses exhibited desirable highly significant negative standard heterotic values towards earliness for check variety (Giza 178). The highest negative value was recorded for the cross combination E0034A/Pop-112-101 (-11.05%). On the contrary, the crosses G46A/GZ11504-1-3-2-4 and G46A/SR-86 gave the lowest values for standard heterosis -1.73 % and -2.26 %, respectively. These findings indicated that heterosis can be used to get earliness in rice hybrids. In general, the results are in agreement with those reported by El-Mowafi (2001) and El-Degwy (2005). Regarding to No. of panicles/plant, data presented in Table 7 showed that only five among the best forteen rice crosses detected significant or highly significant heterosis effects with positive values, such estimates ranged from 18.3 for G46A/GZ11190-3-13-4-1 to 23.0 panicles for G46A/SR-86. As shown in Table (7) the highest estimate was detected for G46A/SR-86 (9.52 %), while the lowest estimate was recorded for E0034A/GZ11504-1-3-2-4 (3.33 %). Positive heterosis effects for the number of panicles/plant have previously been obtained by El-keredy et al., (2003a), Abd Allah (2008), Hadifa (2021), and Mahrous (2022). The hybrid combination CCG5A/Pop-112-101 registered the lowest panicle length with a value of 20.2 cm while the hybrid G46A/GZ10848 (Giza 183) registered the highest panicle length with 27.9 cm. For panicle length, as evident from Table (7) highly significant positive heterotic effects were estimates for all cross combinations for the standard heterosis except CCG5A/Pop-112-101 which recorded insignificant value. The highest estimates were detected for the crosses G46A/GZ10848 (Giza 183) with a value of 37.44 %, while the hybrid combination CCG5A/GZ11190-3-13-4-1 was recorded as 10.34 %. These results are in general agreement with those reported by El-Degwy (2014) and El-Mowafi et al., (2021).

Concerning spikelet fertility %, the maximum spikelet fertility % was noted by the hybrid combination CCG5A/GZ11190-3-13-4-1 (94.3 %), while E0034A/Pop-112-101 was observed as 88.0 % (Table 8). Data showed that only two crosses exhibited highly significant positive heterosis over check variety Giza 178. The highest estimate was detected for the cross CCG5A/GZ11190-3-13-4-1 (1.18 %) and the lowest value was 0.97 % for E2015A/GZ11190-3-13-4-1. Out of fourteen crosses, twelve hybrid combinations exhibited highly significant and negative heterosis over the check variety. In this connection, El-Keredy et al., (2003b) found that most of F1 hybrids (derived from CMS lines) were significantly higher than their parents in sterility percentage. The reports of Abd Allah (2008) and Mahrous (2022) also revealed a high level of negative and positive heterosis for the spikelet fertility trait. The average 1000-grain weight was observed as 28.8 gm. Among the hybrid combinations the minimum 1000-grain weight was recorded by G46A/GZ11190-3-13-4-1 (27.5 gm) and the maximum was recorded by E2015A/GZ10848 (Giza 183) (31.7 gm) followed by E0034A/GZ10848 (Giza 183) (30.0 gm). All the hybrid combinations showed highly significant positive heterosis over check variety for this trait. The highest estimates were detected for E2015A/GZ10848 (Giza 183) (50.95 %), while the lowest value was 30.95 % for this hybrid G46A/GZ11190-3-13-4-1. Superiority for 1000-grain weight trait was also detected by Borah et al., (2017) and Anis et al., (2024). For grain yield/plant, the lowest grain yield was recorded by G46A/GZ11190-3-13-4-1 (48.3 g) while the hybrid combination E0034A/SR-86 registered the highest grain yield/plant with 56.0 g. As shown in Table (8) heterosis over check variety was highly significant and positive in all crosses for grain yield/plant trait. The highest value (26.41 %) was recorded for the cross E0034A/SR-86 and the lowest (9.03 %) for the cross G46A/GZ11190-3-13-4-1. Positive heterosis effects for grain yield/plant have previously been obtained by El-Keredy (1994), Abd Allah (2008), Hassan et al., (2017), El-Mowafi et al., (2021), Mahrous (2022), and Anis et al., (2024).

Table 7. Mean performance and standard heterosis for the best selected rice hybrids from the testcross against check variety (Giza 178).

Genotypes	Days to maturing (day)		Number of panicles/plant		Panicle length (cm)	
	Mean	SH%	Mean	SH%	Mean	SH%
E2015A/B	112	---	13	---	19.7	---
G46A/B	125	---	19	---	22.8	---
CCG5A/B	132	---	17	---	21.5	---
E0034A/B	121	---	14	---	20.0	---
GZ11190-3-13-4-1	124	---	21	---	23.1	---
GZ11504-1-3-2-4	122	---	20	---	21.2	---
SR-86	130	---	16	---	26.5	---
Pop-112-101	115	---	18	---	20.6	---
GZ10848 (Giza 183)	125	---	21	---	22.7	---
E2015A / GZ11190-3-13-4-1	124.3	-6.54**	21.0	0.00	23.5	15.76**
G46A / GZ11190-3-13-4-1	129.0	-3.01**	18.3	-12.86**	23.8	17.24**
CCG5A / GZ11190-3-13-4-1	122.3	-8.05**	22.3	6.19**	22.4	10.34**
E0034A / GZ11190-3-13-4-1	123.0	-7.52**	20.7	-1.43 ns	22.9	12.81**
G46A / GZ11504-1-3-2-4	130.7	-1.73**	19.0	-9.52**	24.4	20.20**
CCG5A / GZ11504-1-3-2-4	127.7	-3.98**	21.0	0.00	24.3	19.70**
E0034A / GZ11504-1-3-2-4	125.0	-6.02**	21.7	3.33*	23.6	16.26**
G46A / SR-86	130.0	-2.26**	23.0	9.52**	25.5	25.62**
E0034A / SR-86	123.0	-7.52**	22.0	4.76**	26.0	28.08**
CCG5A / Pop-112-101	120.3	-9.55**	20.0	-4.76**	20.2	-0.49ns
E0034A / Pop-112-101	118.3	-11.05**	22.3	6.19**	23.4	15.27**
E2015A / GZ10848 (Giza 183)	125.3	-5.79**	19.0	-9.52**	25.9	27.59**
G46A / GZ10848 (Giza 183)	130.0	-2.26**	20.0	-4.76**	27.9	37.44**
E0034A / GZ10848 (Giza 183)	127.0	-4.51**	20.7	-1.43ns	25.0	23.15**
Giza 178 (Check variety)	133.0	---	21.0	---	20.3	---
LSD 0.05 %	***0.457***		***0.556***		***0.372***	
0.01 %	***0.665***		***0.807***		***0.541***	

Table 8. Mean performance and standard heterosis for the best selected rice hybrids from the testcross against check variety (Giza 178).

Genotypes	Spikelet fertility %		1000-grain weight (gm)		Grain yield/plant	
	Mean	SH%	Mean	SH%	Mean	SH%
E2015A/B	88.2	---	29.0	---	35.4	---
G46A/B	87.3	---	27.6	---	40.0	---
CCG5A/B	95.6	---	24.3	---	38.2	---
E0034A/B	89.7	---	29.5	---	43.4	---
GZ11190-3-13-4-1	96.5	---	26.5	---	45.1	---
GZ11504-1-3-2-4	94.0	---	25.8	---	42.2	---
SR-86	82.4	---	27.6	---	43.0	---
Pop-112-101	91.8	---	25.0	---	39.6	---
GZ10848 (Giza 183)	94.9	---	26.2	---	44.0	---
E2015A / GZ11190-3-13-4-1	94.1	0.97**	28.3	34.76**	54.6	23.25**
G46A / GZ11190-3-13-4-1	88.6	-4.94**	27.5	30.95**	48.3	9.03**
CCG5A / GZ11190-3-13-4-1	94.3	1.18**	28.3	34.76**	55.1	24.38**
E0034A / GZ11190-3-13-4-1	92.4	-0.86**	29.5	40.48**	50.2	13.32**
G46A / GZ11504-1-3-2-4	86.2	-7.51**	28.7	36.67**	49.5	11.74**
CCG5A / GZ11504-1-3-2-4	91.9	-1.39**	27.9	32.86**	51.1	15.35**
E0034A / GZ11504-1-3-2-4	92.4	-0.86**	29.5	40.48**	53.0	19.64**
G46A / SR-86	91.1	-2.25**	28.0	33.33**	54.3	22.57**
E0034A / SR-86	91.5	-1.82**	29.3	39.52**	56.0	26.41**
CCG5A / Pop-112-101	92.4	-0.86**	27.8	32.38**	50.2	13.32**
E0034A / Pop-112-101	88.0	-5.58**	28.7	36.67**	52.2	17.83**
E2015A / GZ10848 (Giza 183)	90.4	-3.00**	31.7	50.95**	52.9	19.41**
G46A / GZ10848 (Giza 183)	90.9	-2.47**	28.1	33.81**	54.3	22.57**
E0034A / GZ10848 (Giza 183)	89.7	-3.76**	30.0	42.86**	55.6	25.51**
Giza 178 (Check variety)	93.2	---	21.0	---	44.3	---
LSD 0.05 %	***0.450***		***0.484***		***0.533***	
0.01 %	***0.654***		***0.704***		***0.775***	

Estimates of genetic parameters.

Analysis of variance showed a wide range of variability among studied rice genotypes for all studied characters, where mean squares were highly significant. Therefore, the selection would be effective among the rice genotypes for these characters. Similar results were obtained by Sedeek et al. (2009). Data in Table (9) exhibited that the phenotypic coefficient of variance was generally higher than the genotypic coefficient of variance for all studied traits indicating the environmental influence on the expression of these traits. Still, the biggest portion of P.C.V was contributed by the genotypic component, less than by the environmental component. The highest phenotypic and genotypic variances were observed in the days to maturing. The highest values of the phenotypic coefficient of variability and genotypic coefficient of variability were recorded for panicle length and number of panicles/plant.

Table 9. Estimates of genotypic and phenotypic variance, phenotypic coefficient of variance, genotypic coefficient of variability, heritability in broad sense and genetic advance % for studied traits.

Genetic Parameters	Days to maturing (day)	Number of panicles/plant	Panicle length (cm)	Spikelet fertility %	1000-grain weight (gm)	Grain yield/plant
Grand Mean	125.4	20.8	24.2	91.0	28.8	52.7
Variance	65.36	119.25	6.61	51.44	36.35	76.13
G . V.	14.467	1.923	3.417	5.152	1.206	6.030
P . V.	14.575	2.082	3.489	5.256	1.328	6.176
G.C.V	3.032	6.672	7.638	2.495	3.813	4.663
P.C.V	3.044	6.943	7.717	2.520	4.000	4.719
E . V	0.108	0.159	0.071	0.104	0.121	0.147
h b	99.26	92.35	97.95	98.1	90.87	97.62
Δ g	7.80	2.74	5.60	3.20	3.74	4.36
Δ g %	6.22	13.20	23.16	3.52	12.99	8.29

Broad sense heritability percentage is estimated as the ratio between the genotypic variance and the total phenotypic variance. Heritability in a broad sense was high for all studied characters with values of 99.26 % for days to maturing and 90.87% for 1000-grain weight (Table 9). High heritability coupled with high genetic advance was observed for panicle length, number of panicles/plant, and 1000-grain weight. This indicates that the selection process for these traits would certainly bring improvement in the genotypes. Previous study showed that the expected genetic advance from selection was high for fertility percentage (41.47) and number of filled grains/panicle (El-Degwy, 2013). Burton (1952) and Dhakal et al. (2020) concluded that the genotypic coefficient of variance, together with heritability estimates would give a clear image of the value of advance expected from the selection. Thus, the expected gain from selection would be a better indication of the selection response. All the traits showed relatively high G.C.V with high heritability estimates in a broad sense (Elbadawy et al., 2024). Accordingly, the genetic advance (g%) from selection appeared to be effective for these characters. The highest genetic advance was recorded for days to maturing and panicle length, while the lowest values of genetic advance were observed for spikelet fertility %. The genetic advance % was exhibited for panicle length, number of panicle/plant, and 1000-grain weight while the lowest value was those of spikelet fertility %.

Conclusion.

The exploitation of hybrid rice technology has been made possible by the identification of restorers and maintainers as parental lines. Five effectively restored cross combinations showed higher useful heterosis than the standard check variety. Three hybrid combinations i.e. E0034A/SR-86 (26.41%) and E0034A/GZ10848 (25.51%) recorded more than 25% yield advantage over the check variety Giza 178. Restorers and maintainers identified illustrate the possibility of the development of new rice hybrids and CMS lines in the future.

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All authors declare their consent for publication.

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