

*Egypt. J. Plant Breed.* 27(1):61–76 (2023)

## **OUT-CROSSING RATE AS INFLUENCED BY OPTIMIZING THE METHOD OF PARENTAL LINES SYNCHRONIZATION FOR HYBRID RICE SEED PRODUCTION**

**H.Sh. Hamad, El.E. Gewaily, A.M. Elmoghazy\* and M.A. Elsayed**

Rice Research and Training Center, Field Crops Research Institute, ARC, 33717 Sakha,  
Kafr Elsheikh, Egypt.

\*Corresponding author: [drashrafmoghazy@gmail.com](mailto:drashrafmoghazy@gmail.com)

### **ABSTRACT**

*Hybrid seed set on the female line depends on its flowering synchronization with the restorer line (R), therefore, the sowing of male and female lines must be planned properly. During 2016 and 2017 summer seasons, a preliminary investigation was carried out to study the leaves number, days to heading and effective accumulated temperature as principle methods to determine the synchronization of the CMS line and the restorer line. Besides, an experiment was carried out during 2018 and 2019 to investigate the effect of synchronization method on flowering synchronization on hybrid rice seed production. Results indicated that the method of flowering synchronization between CMS lines and R lines had highly significant effects on the traits: panicle weight, seed set percentage, harvest index and seed yield of hybrid rice. The highest seed yields were 1.760 and 1.870 t ha<sup>-1</sup>. They were recorded from the number of leaves method and the hybrid combination of Giza 178R × IR69625A, respectively. The number of leaves is the best time for the synchronization of hybrid rice lines and the hybrid combination Giza 178R × IR69625A may be recommended for better performance. It was noticed that planting restorer line rows at three different dates with four times of supplemental pollination resulted in the highest number of fertile panicles hill<sup>-1</sup>, panicle weight, number of filled grains panicle<sup>-1</sup> and hybrid seed yield.*

Key words: *Oryza sativa*, Parental Lines, Synchronization, Hybrid Rice, Seed Production, CMS.

### **INTRODUCTION**

Rice (*Oryza sativa* L.) is an important food crop and the primary food source for more than half of the global population. Rice is cultivated in Egypt at 660 thousand hectares, with an annual production of about 4.6 million tons of paddy, with an average productivity of 10 tons per hectare (Abbas *et al* 2021). In Egypt hybrid rice production is an innovative technology to increase rice productivity, resulting in food security and poverty reduction in Egypt (Gaballah *et al* 2021a and Gaballah *et al* 2022). This technology could be used to boost current yield levels in rice, where conventional cultivar yield levels have stabilized. In the context, hybrid rice reported yield advantages of 15–20% over traditional varieties (Gramaje *et al* 2020 and Gaballah *et al* 2021a). The heterosis advantage of hybrid rice may be expressed by superiority over inbred varieties in vigour, number of productive tillers, panicle size, number of spikelets panicle<sup>-1</sup> and seed yield. The three-line and two-line breeding methods are commonly used to create hybrid varieties. Meanwhile, in the three-line method, three lines were involved: a cytoplasmic male sterile (CMS) source (A line), a maintainer (B

line), and a restorer (R line). This method is extensively used in rice hybrid production (Manonmani *et al* 2012). Several factors influence the multiplication of cytoplasmic male sterile (CMS) and hybrid seed production, such as seeding time, field conditions, planting pattern, weather conditions at flowering, synchronization of flowering in the parental lines, supplementary pollination techniques, and GA<sub>3</sub> application (Hamad *et al* 2021).

Synchronization of flowering between the parental lines of a hybrid is one of the most critical considerations in hybrid seed production. Female parent seed production depends on pollen supplied by the male parent during the flowering period. Thus, failure to achieve proper synchronization results in very poor, or no, seed set. This problem might be considered as the most commonly experienced problem in hybrid seed production (Kader *et al* 2017). Female parents and the pollinator parents flower simultaneously by synchronizing flowering, even though they may have different growth durations. Pollen grains from the B and R lines should be available to the CMS line throughout its flowering period, so synchronization of flowering is essential. Adjusting the sowing dates of the parental line in the seedbed is one of the strategies to obtain flowers in both lines simultaneously in the field.

The out-crossing between the R line and cytoplasmic male sterility lines assumes greater significance because the amount of pollen supplied by the male parent during the flowering period determines the amount of seed set on the female parent (El Sayed *et al* 2021). The most common issue in CMS line multiplication is failing to earn proper synchronization, resulting in a poor or no seed set. It is critical to understand the flowering behavior of the parental lines, which varies with sowing dates, to determine the exact difference in days to flowering between the parental lines. If there is a flowering gap, the problem of non-synchronization could be solved by staggered sowing of the male parent based on the days to flowering information.

After observing the difference at the primordial development stage, it is critical to adjust the flowering of parental lines. The application of urea could be used to manipulate the difference in flowering to some extent by

the application of urea and potassium as a foliar spray. In adverse conditions, non-flowering synchronization among parental lines could be predicted. In such conditions, some techniques should be adapted to adjust flowering synchronization as possible. Since the parents of each hybrid behave differently in diverse locations and different situations to flowering at other sowing dates, there is a need to take up studies to find out the effectiveness of different techniques to achieve the out-crossing (Anis *et al* 2019). The methods used for determining seeding intervals were growth duration differences (GDD), based on the difference between the CMS and B lines in terms of days from seeding to initial heading (10%), the number of leaves differences (LND) based on the difference between the parental lines in terms of leaf growth and emergence rate. The spikelets open for a brief period only. Hence, pollen dispersal needs to be high when spikelets are open. Supplementary pollination is essential to increase pollen dispersal and subsequently out-crossing in A-lines. The frequency and time of supplemental pollination are imperative. It is done at the peak of anthesis (Elshamey *et at* 2021).

There is a need to take up studies to find out effectiveness of different techniques to achieve synchronization of flowering, with this background, the present investigation was carried out to test some techniques to get suitable synchronization of flowering between parental lines for enhancing the seed yield in hybrid rice seed production plot. Therefore, the present study aimed to determine the suitable methods of synchronization between parental lines to be used in hybrid rice seed production.

## **MATERIALS AND METHODS**

Three experiments were carried out at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh, Agricultural Research Center, Egypt.

### **First experiment**

The first experiment was carried out during 2016 and 2017 seasons using a randomized complete design with three replications to determine the leaves number, days to heading, and effective accumulated temperature as principle methods to determine the optimum synchronization between the

CMS line and restorer line. The materials included the parental lines of Giza 178R as a restorer line and IR69625A and G46A as female cytoplasmic male sterile (CMS) lines. The number of leaves on the main culm of parental lines was recorded as leaf just emerged (0.2), leaf half-opened (0.5), leaf almost opened (0.8) according to (Gao *et al* 1992). Days to heading (day) was determined from seeding to initial heading or 50% flowering. The Effective Accumulated Temperature (EAT) was calculated according the equation of Mei *et al* 2015 as follows:  $EAT = T-H-L$  where, T, mean daily temperature, H, temperature over upper limit ( $T-27\text{ }^{\circ}\text{C}$ ) and L, temperature of lower limit ( $12\text{ }^{\circ}\text{C}$ ).

### **Second experiment**

The second experiment was carried out during 2018 and 2019 seasons in a split-plot design with three replications. The main plots were devoted to CMS lines and the sub-plots were devoted to methods of synchronization (Number of leaves, days to 50% heading and effective accumulated temperature) to identify the best method of synchronization among the parental lines used. The CMS line IR69625A was sown on May 1<sup>st</sup> and the restorer line Giza 178R was sown in three sowing dates. To get a proper synchronization of flowering, the first sowing date (S1) when the number of leaves for CMS line was two leaves. The second sowing date (S2) when the number of leaves for CMS line was 3.5 leaves. The third sowing date (S3) when the number of leaves for CMS line was 5 leaves. For G46A line, it was sown when the first date of Giza 178R was four leaves. The G46A line was sown at May 20<sup>th</sup> and Giza 178R was sown at three dates, namely, May 6<sup>th</sup>, May 10<sup>th</sup> and May 14<sup>th</sup>. EAT was 1435 and 1450 $^{\circ}\text{C}$  for IR69625A and was 1040 and 1050 $^{\circ}\text{C}$  for G46A. While EAT for Giza 178R was 1329.11 and 1335.76 $^{\circ}\text{C}$  in 2016 and 2017 seasons, respectively, so the CMS line IR69625A was sown on May 1<sup>st</sup>, G46A was sown at June 4<sup>th</sup> and Giza 178 was sown at the three dates, May 12<sup>th</sup>, May 17<sup>th</sup> and May 22<sup>th</sup>. The traits studied for CMS lines were panicle weight (g), seed set (%), seed yield ( $\text{t ha}^{-1}$ ), and harvest index (%) in 2018 and 2019 seasons.

### **Third experiment**

The third experiment was carried out during 2020 and 2021 in a split split-plot design with three replications. The main plots were devoted to

CMS lines, while the subplots were laid out at three sowing dates and the number of times supplementary pollination occurred was devoted to the sub-sub plots that carried out depending on leaves number as a method of flowering synchronization among the parental lines. To study the out-crossing rate of the two cytoplasmic male sterile lines to produce F<sub>1</sub> hybrid seeds, the CMS lines IR69625A and G46A were sown on May 1<sup>st</sup> and May 20<sup>th</sup>, respectively. The restorer line Giza 178R was sown at three sowing dates, to get a proper synchronization of flowering. The first sowing date (S1) was done when the average number of leaves was 2.5, the second sowing date (S2) when the average number of leaves was 3.5 and the third sowing date (S3) when the average number of leaves was 5 for CMS line IR69625A. The G46A line was sown when the first date of Giza178R was four leaves. The traits studied of CMS lines were: days to 50% heading, days to complete leaf number, days from complete leaf number to heading, leaf area index, plant height (cm), panicle length (cm), panicle exertion (%), spikelet opening angle, duration of spikelet opening (min), panicle weight (g), filled grain, seed set (%), seed yield (t ha<sup>-1</sup>), and harvest index (%).

Rice seeds at the rate of 20 kg ha<sup>-1</sup> (15 kg from the CMS lines and 5 kg from the R line) were soaked in fresh water for 24 hrs., then drained and incubated for 48 hrs., to hasten germination. The field was well flowed and dry-leveled, and then the field was irrigated to make the soil puddle. The phosphorous fertilizer in the form of Mono-super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) at a rate of 240 kg ha<sup>-1</sup> was added during field preparation. Nitrogen fertilizer at a rate of 165 kg N ha<sup>-1</sup> was added in the form of Urea (46.5% N). Two-thirds of N was applied as a basal application, and the other third was top-dressed at 30 days after transplanting (DAT). Zinc fertilizer (24% ZnSO<sub>4</sub>) at a rate of 48 kg ha<sup>-1</sup> was added before seedling transplanting. Thirty-day-old seedlings of the CMS (A) and restorer (R) lines were transplanted by 3 and 2 seedlings per hill, respectively. The rowing direction was perpendicular to the wind direction. The row ratio for each combination was 2 R: 10 A. The rice production procedures recommended by the Rice Research Department program were used as follows: Two times of GA3 foliar application, the first spray (40% of GA3) when A line was at 15-20% heading, and the second spray (60% of GA3) was applied when A line was at 35-40%

heading (five days after heading). Shaking the pollen parents (R line) with bamboo sticks provided supplementary pollination. This operation was carried out across ten days between 9 a.m. and 11.30 a.m. The crop was harvested when 80% of the grains became golden yellow. Grains were sun-dried and adjusted at 14% moisture content to estimate grain yield. Panicle exertion % and seed set % was estimated according to the following formulas:

Panicle exertion % = (Exerted panicle length, cm) / (Panicle length, cm) × 100

Seed set % = (Number of filled grains panicle<sup>-1</sup>) / (Total spikelets number panicle<sup>-1</sup>) × 100

#### **Data analysis**

The data were collected according to the Standard Evaluation System (SES) of International Rice Research Institute, (IRRI 2014) for all the studied traits. Data were statistically analysed according to (Gomez and Gomez, 1984) using COSTAT Computer Program. The differences among treatments were compared *via* Duncan's Multiple Range Test (Duncan, 1955).

## **RESULTS AND DISCUSSION**

### **Results**

The development of hybrid rice seed technology and adoption of hybrid rice cultivars to Egyptian environments offer one approach to the problem of matching food supply to expected demand. The perfect number of times of supplementary pollination of the parental lines is crucial in producing F<sub>1</sub> hybrid seeds with a high out-crossing rate. Two CMS lines with their restorer line were chosen and used as parental lines to study the perfect number of supplementary pollinations and sowing dates of restorer line, stability, floral traits, and for some yield and yield components.

#### **First experiment**

Table 1 represents the results for methods of synchronization for parental lines. The CMS line IR69625A gave the highest values of 18.49 and 18.90 for number of leaves, 105.30 and 104.90 days for days to 50% heading and 1435 and 1450°C for EAT during the two seasons, 2016 and 2017, respectively.

**Table 1. Means of number of Leaves, days to 50% heading and EAT for parental lines used in the experiment.**

CMS lines	Number of Leaves		Days to 50% Heading		Effective accumulated temperature (EAT)	
	2016	2017	2016	2017	2016	2017
IR69625A	18.49a	18.90a	105.30a	104.90a	1435a	1450a
G46A	16.30c	16.50c	85.96c	86.18c	1040c	1050c
Giza 178R	17.5b	17.19b	98.50b	99.05b	1329.11b	1335.76b
<b>F-test</b>	**	**	**	**	**	**

\*\* Significant at the 1% probability level.

While, the lowest values of 16.30 and 16.50 for number of leaves, 85.96 and 86.18 for days to 50% heading and 1040 and 1050°C for EAT were obtained for the CMS line G46A in 2016 and 2017 seasons, respectively.

### **Second experiment**

Effect of synchronization methods and CMS lines as well as their interaction on panicle weight, seed set, seed yield and harvest index characters are presented in Table (2).

### **Cytoplasmic male sterile line effects**

The cytoplasmic male sterile (CMS) line had highly significant effects on panicle weight, seed set, seed yield and harvest index. The CMS line IR69625A line showed the highest panicle weight, seed set, seed yield and harvest index during both seasons. In contrast, the CMS line G46A gave the lowest panicle weight, seed set, seed yield and harvest index during both seasons (2018 and 2019).

### **Synchronization method effects**

The synchronization method had highly significant effects on panicle weight, seed set, seed yield and harvest index. Number of leaves method gave the highest panicle weight, seed set, seed yield and harvest index during both seasons. While, EAT method gave the lowest panicle weight, seed set, seed yield and harvest index during 2018 and 2019 seasons.

**Table 2. Effect of synchronization method, CMS line, and their interaction on yield traits during 2018 and 2019 seasons.**

Main effect and interaction	Panicle weight (g)		Seed set (%)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
<b>CMS lines (L)</b>								
IR69625A x Giza 178R	2.77a	2.66a	35.23a	36.29a	1.583a	1.691a	18.69a	19.84a
G46Ax Giza 178R	2.53b	2.46b	32.41b	33.43b	1.480b	1.588b	18.01b	19.16b
F-test	**	**	**	**	**	**	**	**
<b>Synchronization method (S)</b>								
Number of Leaves	2.75a	2.73a	35.89a	37.47a	1.722a	1.830a	19.31a	20.46a
Days to 50% Heading	2.72a	2.55b	33.90b	34.54b	1.572b	1.680b	18.78b	19.93b
EAT	2.49b	2.42c	31.67c	32.56c	1.301c	1.409c	16.96c	18.11c
F-test	**	**	**	**	**	**	**	**
<b>Interaction</b>								
L × S	**	**	**	**	**	**	**	**

\*\* Significant at the 1% probability level.

### Interaction effect

The effect of interaction between CMS lines and synchronization method was highly significant for panicle weight, seed set, seed yield and harvest index in 2018 and 2019 seasons (Table 3).

**Table 3. Panicle weight, seed set, seed yield and harvest index as affected by the interaction between method synchronization and CMS lines**

CMS Lines	Synchronization methods	Panicle weight (g)		Seed set (%)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
		2018	2019	2018	2019	2018	2019	2018	2019
IR69625A	Number of leaves	2.90a	2.92a	37.47a	38.76a	1.760a	1.870a	19.76a	20.91a
	Days to 50% heading	2.60b	2.84b	35.06b	36.05b	1.640c	1.750c	19.22a	20.37b
	EAT	2.48d	2.55c	33.18d	34.07c	1.340e	1.450e	17.09e	18.24e
G46A	Number of leaves	2.54c	2.58c	34.31c	36.18b	1.670b	1.780b	18.86c	20.02c
	Days to heading	2.50d	2.60c	32.74d	33.05d	1.500d	1.610d	18.34d	19.49d
	EAT	2.35e	2.43d	30.17e	31.05e	1.260f	1.360f	16.84f	17.99f

The number of leaves with the CMS line IR69625A gave the highest values for panicle weight, seed set, seed yield and harvest index during seasons, 2018 and 2019. While, EAT method with the CMS line G46A



produced the lowest panicle weight, seed set, seed yield and harvest index in both seasons.

### Third experiment

Effect of CMS lines, sowing dates of restorer line and number of times of supplementary pollination as well as their interactions on number of leaves, days to 50% heading, days to complete leaf number, days from whole leaves number to heading, and leaf area index are presented in Table (4).

**Table 4. Growth characteristics of CMS lines as affected by sowing dates of restorer lines and number of times of supplementary pollination as well as their interactions during 2020 and 2021 seasons.**

Main effect and interaction	Number of leaves		Days to 50% heading		Days to complete leaf number		Days from complete leaves number to 50% heading		Leaf area index	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
<b>CMS lines (L)</b>										
IR69625A	19.63a	19.82a	105.16a	104.08a	99.41a	97.91a	5.43b	6.15b	29.31b	29.48b
G46A	17.25b	17.95	88.26b	87.48b	81.24b	79.97b	7.13a	7.49a	32.21a	33.28a
F-test	**	**	**	**	**	**	**	**	**	**
<b>Sowing dates of restorer line (S)</b>										
Date 1	18.43	18.78	96.75	95.82	90.38	88.94	6.30	6.88	30.31	31.46
Date 2	18.55	18.87	96.75	95.69	90.38	88.92	6.26	6.77	30.30	31.38
Date 3	18.34	18.81	96.66	95.83	90.22	88.96	6.26	6.82	30.17	31.29
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Number of Time of supplementary pollination (T)</b>										
Control	18.38	18.75	96.75	95.62	90.35	88.91	6.35	6.84	30.26	31.41
T1	18.46	18.79	96.72	95.93	90.39	88.90	6.31	6.83	30.28	31.39
T2	18.45	18.81	96.69	95.80	90.28	88.80	6.26	6.88	30.31	31.39
T3	18.40	18.79	96.68	95.67	90.29	88.85	6.23	6.79	30.29	31.32
T4	18.50	18.80	96.70	95.67	90.31	88.95	6.24	6.78	30.17	31.40
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Interaction</b>										
L × S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L × T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S × T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L × S × T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*\* Significant at the 1% probability level, NS = not significant.

### **Cytoplasmic male sterile lines effects for vegetative traits**

The differences between the two CMS lines significantly affect the number of leaves, days to 50% heading, days to complete leaf number, days from complete leaf number to heading, and leaf area index. CMS line IR69624A gave the highest values of 19.63 and 19.82 for the number of leaves, the most extended duration of 105.16 and 104.08 for days to 50% heading, and the most extended duration of 99.41 and 97.91 days to complete leaf number during the 2020 and 2021 seasons, respectively. While the CMS line G46A gave the highest values of days from complete leaf number to 50% heading (7.31 and 7.49) and the highest values of leaf area index (32.21 and 33.28) in both seasons. In contrast, the CMS line G46A gave the lowest values (17.25 and 17.95) for the number of leaves, the shortest duration (88.26 and 87.48 days) for days to 50% heading, and (81.24 and 79.97 days) for days to complete leaf number during both seasons. At the same time, the CMS line IR69624A gave the lowest values (5.43 and 6.15) for days from complete leaf number to 50% heading and (29.31 and 29.48) for leaf area index during both seasons. Most of the variation among the growth characteristics of CMS lines might be due to genetic background differences. Sowing dates of restorer line, the number of times of supplementary pollination, and their interaction had no significant effect on the studied traits as mentioned above during both seasons (Table 3).

The effects of CMS lines, sowing dates of restorer line, and number of times of supplementary pollination as well as their interaction on plant height, panicle length, panicle exertion, duration of spikelet opening, and spikelet opening angle characteristics are given in Table (5).

### **Cytoplasmic male sterile lines effects on floral traits**

The effect of CMS lines has a highly significant effect on panicle length, panicle exertion, spikelet opening angle, and duration of spikelet opening time. CMS line IR69624A gave the tallest plants (115.91 and 117.69 cm), the most extended panicle length (23.48 and 24.19 cm), and the highest panicle exertion percentage (73.21 and 75.18%) during the 2020 and 2021 seasons, respectively.

**Table 5. Means of plant height, panicle length, panicle exertion, spikelet opening angle, and duration of spikelet opening of CMS lines as affected by sowing dates of restorer line, number of times of supplementary pollination as well as their interactions during 2020 and 2021 seasons.**

Main effect and interaction	Plant height (cm)		Panicle length (cm)		Panicle exertion (%)		spikelet opening angle (°)		duration of spikelet opening (min)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
<b>CMS lines (L)</b>										
<b>IR69625A</b>	115.91a	117.69a	23.84a	24.19a	73.21a	75.18a	24.46b	26.29b	157b	160.51b
<b>G46A</b>	110.82b	112.63b	24.50a	22.35b	69.73b	71.74b	27.16a	28.26a	168a	170.48a
<b>F-test</b>	**	**	**	**	**	**	**	**	**	**
<b>Sowing dates of restorer line (S)</b>										
<b>Date 1</b>	113.11	115.08	22.84	23.25	71.01	73.46	25.80	27.23	162.69	165.65
<b>Date 2</b>	113.5	115.83	22.84	23.29	71.20	73.44	25.84	27.29	162.18	165.54
<b>Date 3</b>	113.44	115.01	22.85	23.23	71.21	73.49	25.79	27.31	162.79	165.45
<b>F-test</b>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Times of supplementary pollination (T)</b>										
<b>Control</b>	113.44	115.23	22.84	23.28	71.28	73.42	25.80	27.27	162.74	165.59
<b>One</b>	113.45	115.20	22.85	23.25	71.21	73.47	25.82	27.26	162.75	165.62
<b>Two</b>	113.50	115.29	22.87	23.23	71.16	73.48	25.79	27.31	162.76	165.57
<b>Three</b>	113.21	115.01	22.50	23.25	71.18	73.47	25.80	27.27	162.77	165.49
<b>Four</b>	113.24	115.03	22.82	23.27	71.20	73.47	25.84	27.27	162.77	165.46
<b>F-test</b>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Interaction</b>										
<b>L × S</b>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>L × T</b>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>S × T</b>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>L × S × T</b>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*\* Significant at the 1% probability level, NS = not significant.

While the CMS line G46A gave the highest values of spikelet opening angle ( $27.16^{\circ}$  and  $28.26^{\circ}$ ) and the most extended duration of spikelet opening (165 and 170.48 min) during both seasons. On the other hand, CMS line G46A gave the lowest values (110.82 and 112.63 cm) of plant height, panicle length (24.50 and 22.35 cm) and panicle exertion percentage (69.73 and 71.74%) during the 2020 and 2021 seasons, respectively. In contrast, CMS line IR69624A gave the lowest values (24.46 and 26.29) of spikelet opening angle and duration of spikelet opening (157 and 160.51 min) during both seasons. The variation among the CMS lines as shown in Table (5) might be due to the differences in CMS lines genetic background. The results in Table (5) showed that plant height, panicle length, panicle exertion, duration of spikelet opening, and spikelet opening angle were not significantly affected by the sowing dates of the restorer line, the number of times of supplementary pollination as well as their interactions during the 2020 and 2021 seasons.

The effects of CMS lines, sowing dates of restorer line, and number of supplementary pollinations as well as their interaction on the number of panicles hill<sup>-1</sup>, panicle weight, number of filled grain panicle<sup>-1</sup>, seed set, seed yield t ha<sup>-1</sup> and harvest index are given in Table (6).

#### **Cytoplasmic male sterile lines effects for yield traits**

The effect of the planting date of rows of restorer line had a highly significant effect on the number of panicles hill<sup>-1</sup>, panicle weight, number of filled grains panicle<sup>-1</sup>, seed set, seed yield, and harvest index. The date of planting of parents gave the highest values of panicles hill<sup>-1</sup> (15.34 and 16.01), the heaviest panicle weight (2.28 and 2.38 g), the highest values (39.86 and 42.81) of filled grains panicle<sup>-1</sup>, the highest values (17.90 and 19.60%) of seed set percentage, the highest values (1.323 and 1.388) of seed yield ha<sup>-1</sup>, during 2020 and 2021 seasons. The planted rows of restorer parent on the same date (S<sub>1</sub>) yielded the lowest values of panicles hill<sup>-1</sup> (12.40 and 13.11), panicle weight (1.59 and 1.65 g), number of filled grain panicles<sup>-1</sup> (27.72 and 30.72), seed set (12.42 and 14.02%), seed yield (1.027 and 1.092 t ha<sup>-1</sup>) and harvest index percent (13.41 and 14.11%).

**Table 6. Effect of CMS lines, sowing dates of restorer line, and number of times of supplementary pollination as well as their interactions on yield and yield characters during 2020 and 2021 seasons.**

Main effect and interaction	Number of panicles hill <sup>-1</sup>		Panicle weight (g)		No. of filled grains panicle <sup>-1</sup>		Seed set (%)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
<b>CMS lines (L)</b>												
IR69625A	15.27a	16.02a	1.96a	2.04a	36.36a	39.36a	16.21a	17.87a	1.183a	1.247a	15.32a	15.95a
G46A	12.69b	13.32b	1.87b	1.94b	31.04b	34.04b	14.02b	15.66b	1.128b	1.192b	14.311b	15.26b
F-test	**	**	**	**	**	**	**	**	**	**	**	**
<b>Sowing of restorer line (S)</b>												
S 1	12.40c	13.11c	1.59c	1.65c	27.72c	30.72c	12.42c	14.02c	1.027c	1.092c	13.41c	14.11c
S 2	14.18b	14.87b	1.87b	1.95b	33.56b	36.52b	15.03b	16.68b	1.114b	1.179b	15.05b	15.84b
S 3	15.34a	16.02a	2.28a	2.38a	39.86a	42.81a	17.90a	19.60a	1.323a	1.388a	15.98a	16.86a
F-test	**	**	**	**	**	**	**	**	**	**	**	**
<b>Times of supplementary pollination (T)</b>												
Control	8.09e	8.86e	1.25e	1.33e	11.87e	14.87e	5.32e	6.78e	0.827e	0.892e	11.65e	12.43e
One	13.31d	13.94d	1.62d	1.70d	27.89d	30.89d	12.51d	14.12d	1.070d	1.135d	13.80d	14.52d
Two	14.99c	15.71c	1.96c	2.04c	33.06c	36.20c	14.90c	16.56c	1.177c	1.242c	15.05c	15.79c
Three	16.22b	16.76b	2.28b	2.36b	42.95b	45.59b	19.09b	20.81b	1.309b	1.374b	16.18b	17.04b
Four	17.28a	18.06a	2.45a	2.55a	52.95a	55.90a	23.77a	25.58a	1.390a	1.455a	17.39a	18.25a
F-test	**	**	**	**	**	**	**	**	**	**	**	**
<b>Interaction</b>												
L × S	**	**	**	**	NS	NS	NS	NS	**	**	**	**
L × T	**	**	**	**	**	**	**	**	**	**	**	**
S × T	**	**	**	**	**	**	**	**	**	**	**	**
L × S × T	**	**	**	**	**	**	**	**	**	**	**	**

### **Times of supplementary pollination effects**

The effect of the number of times supplementary pollination had a highly significant effect on the number of panicles hill<sup>-1</sup>, panicle weight, number of filled grains panicle<sup>-1</sup>, seed set, seed yield, and harvest index. Four times of supplementary pollination gave the highest values for panicles hill<sup>-1</sup> (17.28 and 18.06), the heaviest (2.46 and 2.55 g) panicle weight, the number of filled grains panicle<sup>-1</sup> (52.295 and 55.95), seed set (23.77 and 25.58%), seed yield (1.390 and 1.455 t ha<sup>-1</sup>) and harvest index (17.39 and 18.25%) during the 2020 and 2021 seasons, respectively. However, the control (no supplementary pollination) gave the lowest values (8.09 and 8.86) for number of panicle hill<sup>-1</sup>, panicle weight (1.26 and 1.33 g), number of filled grains panicle<sup>-1</sup> (11.87 and 14.87), seed set (5.32 and 6.78 %), seed yield (0.827 and 0.892 t ha<sup>-1</sup>) and harvest index (11.165 and 12.43 %) during the 2020 and 2021 seasons, respectively.

### **Interaction effects**

Almost all the interactions had highly significant effects on the number of panicle hills<sup>-1</sup>, panicle weight, number of filled grain panicles<sup>-1</sup>, seed set, seed yield t ha<sup>-1</sup> and harvest index. Only the interactions between CMS lines and planting dates of row of the restorer line had no significant effects on the number of filled grain panicle<sup>-1</sup> and seed set during both seasons (Table 6).

The results in Table (7) showed that the interaction between CMS lines and sowing of restorer lines had a highly significant effect on the number of fertile panicles per hill<sup>-1</sup>, panicle weight (g), seed yield (t ha<sup>-1</sup>), and harvest index in both seasons. During both seasons, the CMS line IR69625A with three different planting dates of rows of restorer line produced the highest values of panicles hill<sup>-1</sup> (16.54 and 17.07), panicle weight (2.34 and 2.45g), seed yield (1.368 and 1.433 t ha<sup>-1</sup>) and harvest index (16.43 and 17.26%). In addition, CMS line G46A with planted rows of restorer line on a single date recorded the lowest values of number of panicles hill<sup>-1</sup> (10.84 and 11.29), panicle weight (1.56 and 1.62g), seed yield (1.011 and 1.076 t ha<sup>-1</sup>) and harvest index (12.88 and 13.83%) during both seasons.

**Table 7. Effects of interaction between CMS lines and sowing dates of restorer line on yield characteristics during 2020 and 2021 seasons.**

Lines (L)	Sowing date	Number of fertile panicles hill <sup>-1</sup>		Panicle weight (g)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
		2020	2021	2020	2021	2020	2021	2020	2021
IR69625A	S 1	13.96c	14.92c	1.62e	1.68e	1.043e	1.108e	13.94e	14.39e
	S2	15.29b	16.06b	1.92c	2.00c	1.136c	1.202c	15.58b	16.22c
	S3	16.54a	17.07a	2.33a	2.45a	1.368a	1.433a	16.43	17.26a
G46A	S1	10.84e	11.29a	1.56f	1.62f	1.011f	1.076f	12.88f	13.83f
	S2	13.08d	13.67d	1.82d	1.90d	1.092d	1.156d	14.52d	15.47d
	S3	14.15c	14.96c	2.23b	2.31b	1.277b	1.342b	15.53c	16.48b

The results in Table (8) showed that the interaction between the CMS lines and the number of times of supplementary pollination showed a highly significant effect on number of fertile panicles hill<sup>-1</sup>, panicle weight, number of filled grains panicle<sup>-1</sup>, seed set, seed yield and harvest index in both seasons. During both seasons, the CMS line IR69625A with four times supplementary pollination produced the highest values of fertile panicles hill<sup>-1</sup> (18.58 and 19.44), panicle weight (2.52 and 2.62 g), number of filled grains panicle<sup>-1</sup> (56.39 and 59.39), seed set (25.13 and 26.94%), seed yield (1.430 and 1.495 t ha<sup>-1</sup>) and harvest index (17.92 and 18.69%). The lowest values of number of fertile panicles hill<sup>-1</sup> (7.49 and 8.16), panicle weight (1.23 and 1.30 g), number of fertile panicles hill<sup>-1</sup> (10.52 and 13.52), seed set (4.73 and 6.19%), seed yield (0.809 and 0.871 t ha<sup>-1</sup>) and harvest index (11.29 and 12.24%) during both seasons, was obtained with the CMS line G46A without supplementary pollination.

**Table 8. Effect of interaction between the CMS lines and number of times of supplementary pollination on yield characteristics during 2020 and 2021 seasons.**

Line	No of times of supplementary pollination	Number of panicles hill <sup>-1</sup>		Panicle weight (g)		No. of filled grains panicle <sup>-1</sup>	
		2020	2021	2020	2021	2020	2021
IR69625A	Control	8.68h	9.56i	1.27i	1.35i	13.22h	16.22h
	Once	14.98e	15.77e	1.65g	1.73g	30.54f	33.53f
	Two	16.57c	17.22c	2.06e	2.09e	35.64e	38.64e
	Three	17.51b	18.12b	2.23c	2.43c	46.00c	49.00c
	Four	18.58a	19.44a	2.51a	2.62a	56.39a	59.39a
G46A	Control	7.49i	8.16j	1.23j	1.30j	10.52i	13.52i
	One	11.64g	12.11h	1.59h	1.67h	25.24g	28.24g
	Two	13.41f	14.19g	1.92f	1.98f	30.76f	33.76f
	Three	14.93e	15.42f	2.23d	2.28d	39.18d	42.18d
	Four	15.98d	16.67d	2.38b	2.47b	49.51b	52.51b
Line	No of times of supplementary pollination	Seed set (%)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
		2020	2021	2020	2021	2020	2021
IR69625A	Control	5.89h	7.37h	0.847i	0.912i	12.01i	12.64i
	Once	13.64f	15.25f	1.096g	1.161g	14.37g	14.85g
	Two	15.91e	17.56e	1.197e	1.262e	15.62e	16.15e
	Three	20.49c	22.22c	1.343c	1.408c	16.68d	17.45c
	Four	25.13a	26.94a	1.430a	1.495a	17.92a	18.69a
G46A	Control	4.73i	6.19i	0.809j	0.871j	11.29j	12.24j
	One	11.39g	12.97g	1.044h	1.109h	13.24h	14.19h
	Two	11.39g	15.52f	1.157f	1.222f	14.48f	15.43f
	Three	17.70d	19.40d	1.274d	1.339d	15.68d	16.43d
	Four	22.43b	24.22b	1.350b	1.415b	16.86b	17.81b

Results in Table (9) showed that the interaction between plant dates of rows of restorer line and the number of times of supplementary pollination showed a highly significant effect on the number of fertile panicles per hill<sup>-1</sup>, panicle weight, number of filled grains per panicle<sup>-1</sup>, seed set, seed yield and harvest index during both seasons.



**Table 9. Effect of interaction between sowing dates of restorer line and time(s) of supplementary pollination on yield characteristics during 2020 and 2021 seasons.**

Sowing Date (S)	Times of Supplementary pollination	No. of fertile panicles hill <sup>-1</sup>		Panicle weight (g)		No. of filled grain panicle <sup>-1</sup>	
		2020	2021	2020	2021	2020	2021
S1	Control	7.02l	8.01l	1.19m	1.25n	10.45n	13.45n
	One	11.77i	12.25i	1.50j	1.56k	23.04k	26.04k
	Two	13.24h	14.26h	1.63i	1.67j	28.56i	31.56i
	Three	14.48f	15.04g	1.74g	1.81h	34.05g	37.05g
	Four	15.51e	15.98e	1.91f	1.97f	42.46d	45.46d
S2	Control	8.20k	8.96k	1.23l	1.28m	11.82m	14.82m
	One	13.62g	14.20h	1.65h	1.72i	27.81j	30.81j
	Two	15.44e	16.04e	1.75g	1.83g	33.32h	36.32h
	Three	16.40d	17.13c	2.26e	2.37e	40.81e	43.81e
	Four	17.27c	18.01b	2.47d	2.55d	53.85b	56.85b
S3	Control	9.04j	9.61j	1.34k	1.45l	13.33l	16.33l
	One	14.53f	15.37f	1.73g	1.81gh	32.82h	35.81h
	Two	16.29d	16.81d	2.53c	2.61c	37.72f	40.72f
	Three	17.76b	18.13b	2.85b	2.89b	52.91c	55.91c
	Four	19.04a	20.18a	2.99a	3.13a	62.53a	65.53a
Sowing Date (S)	Times of Supplementary pollination	Seed set (%)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
		2020	2021	2020	2021	2020	2021
S1	Control	4.68m	6.14m	0.774n	0.839o	10.96n	11.79l
	One	10.32j	11.88j	0.987l	1.052l	12.29l	13.03j
	Two	12.80i	14.41i	1.068j	1.133j	13.26j	13.92i
	Three	15.26g	16.91g	1.131i	1.196i	14.37i	15.01g
	Four	19.03d	20.75d	1.176h	1.241h	16.16f	16.81e
S2	Control	5.29l	6.75l	0.381m	0.894n	11.66m	12.47k
	One	12.48i	14.08i	0.615j	1.108k	13.96j	14.67h
	Two	14.98gh	16.62gh	1.043k	1.251f	15.30g	15.89f
	Three	18.29e	19.99e	1.186f	1.293e	16.71d	17.59d
	Four	24.13b	25.94b	1.283c	1.348c	17.46c	18.59b
S3	Control	5.98k	7.46k	0.877m	0.942m	12.32l	13.05j
	One	14.74h	16.38h	1.180g	1.245g	15.15h	15.87f
	Two	16.92f	18.60f	1.278d	1.343d	16.59e	17.55d
	Three	23.73c	25.24c	1.567b	1.632b	17.46c	18.52c
	Four	28.16a	30.05a	1.710a	1.775a	18.38a	19.35a

Three sowing dates of restorer line ( $S_3$ ) with four times of supplementary pollination produced the highest value of fertile panicles hill<sup>-1</sup>, panicle weight, number of filled grains per panicle<sup>-1</sup>, seed set, seed yield and harvest index. On the other hand, the lowest number of fertile panicles per hill, panicle weight, number of filled grains per panicle, seed set, seed yield and harvest index during both seasons were obtained under one planting date of restorer line without supplementary pollination.

The results in Table (10) shows that the interaction among CMS lines, plant dates of rows of restorer line, and the number of times of supplementary pollination had a highly significant effect on number of fertile panicles hill<sup>-1</sup>, panicle weight, number of filled grains panicle<sup>-1</sup>, seed set, seed yield and harvest index during both seasons. CMS line IR69625A with three planting dates of restorer line ( $S_3$ ) under four times supplementary pollination produced the highest values of number of fertile panicles hill<sup>-1</sup> (20.22 and 21.38), panicle weight (2.99 and 3.27 g), number of filled grains panicle<sup>-1</sup> (67.25 and 70.25), seed set (30.01 and 31.92%), seed yield (1.804 and 1.869 t ha<sup>-1</sup>). Meanwhile, the lowest values of number of fertile panicles hill<sup>-1</sup> (6.53 and 7.26), panicle weight (1.19 and 1.25 g), number of filled grains panicle<sup>-1</sup> (9.24 and 12.24), seed set (4.16 and 5.61 %), seed yield (0.761 and 0.826 t ha<sup>-1</sup>) and harvest index (10.61 and 11.56 %) during 2020 and 2021 seasons were recorded with CMS line G46A when rows of restorer line were planted on the same date ( $S_1$ ) without supplementary pollination.

## **DISCUSSION**

Climate change has an impact on a wide range of plants in many ways. Climate change is affecting the ranges of both CMS lines and restorer. This discrepancy is evident in recent studies on flowering parental lines and great attention has been paid to change in flowering time. In contrast, little study has been done on direct physiological impacts, despite the fact that these effects are expected to have significant implications for parental lines interaction. Rice production should be increased to cope with continuing population growth and the threat of environmental pressures. In that context cytoplasmic male sterility (CMS) system is a beneficial

approach for commercial exploitation of heterosis and producing high-yielding hybrid rice.

**Table 10. Effect of interaction between CMS lines, sowing dates of restorer line, and number of time(s) of supplementary pollination on yield characteristics during 2020 and 2021 seasons.**

Lines (L)	Sowing date (S)	Times of supplementary pollination	No. of fertile panicles hill <sup>-1</sup>		Panicle weight (g)		No. of filled grains panicle <sup>-1</sup>	
			2020	2021	2020	2021	2020	2021
IR69625A	S1	Control	7.5r	8.76p	1.21u	1.25v	11.66p	14.66p
		One	13.62k	14.42i	1.52p	1.58q	26.00l	29.00l
		Two	15.08ij	16.50e	1.66n	1.70o	31.50j	34.50j
		Three	16.30gh	17.02d	1.78l	1.86l	38.00h	41.00h
	S2	Four	17.33e	17.93c	1.95i	2.01j	44.00e	47.00e
		Control	8.62p	9.90n	1.26t	1.31u	13.50o	16.50o
		One	15.28ij	16.03f	1.69m	1.75mn	30.25jk	33.25jk
		Two	16.87f	17.12d	1.81k	1.86l	35.50i	38.50i
	S3	Three	17.44e	18.25c	2.32g	2.46h	44.50e	47.50e
		Four	18.22c	19.01b	2.55d	2.59e	57.93b	60.93b
		Control	9.93o	10.03n	1.36r	1.50s	14.50n	17.50n
		One	16.04h	16.85d	1.76l	1.86l	31.50j	38.35i
S3	Two	17.75de	18.04c	2.55d	2.66d	39.94g	42.94g	
	Three	18.77b	19.07b	2.94b	2.97b	55.51c	58.51c	
	Four	20.22a	21.38a	3.05a	3.27a	67.25a	70.24a	
	Control	6.53s	7.26r	1.17v	1.25v	9.24q	12.24q	
G46A	S1	One	9.92o	10.09n	1.46q	1.55r	20.09m	23.09m
		Two	11.39n	12.02m	1.61p	1.61p	25.63l	28.63l
		Three	12.67l	13.05k	1.70m	1.76mn	30.11k	33.11k
		Four	13.68k	14.04j	1.86j	1.93k	40.93f	43.93f
	S2	Control	7.77r	8.03q	1.22u	1.26v	10.15q	13.15q
		One	11.95m	12.37l	1.62o	1.70o	25.37l	28.37l
		Two	14.01k	14.97h	1.71m	1.78m	31.15jk	34.15jk
		Three	15.37i	16.01f	2.21h	2.28i	37.13h	40.13h
	S3	Four	16.33gh	17.01d	2.37f	2.50g	49.76d	52.76d
		Control	8.16q	9.20o	1.31s	1.40t	12.16p	15.16p
		One	13.02l	13.89j	1.69m	1.77m	30.27jk	33.27jk
		Two	14.84j	15.58g	2.45e	2.56f	35.51i	38.51i
S3	Three	16.76fg	17.19d	2.78c	2.81c	50.31d	53.31d	
	Four	17.93cd	18.98b	2.92b	2.99b	57.82b	60.82b	

**Table 10. Cont.**

Lines (L)	Sowing date (S)	Times of supplementary pollination	Seed set (%)		Seed yield (t ha <sup>-1</sup> )		Harvest index (%)	
			2020	2021	2020	2021	2020	2021
IR69625A	S1	Control	5.20o	6.67o	0.787v	0.852v	11.32v	12.03u
		One	11.60l	13.18l	1.003q	1.068q	12.74q	13.26p
		Two	14.06k	15.68k	1.084o	1.147o	13.87n	14.25n
		Three	16.96i	18.63i	1.153m	1.218m	14.95l	15.27k
		Four	19.64f	21.36f	1.191j	1.255j	16.83f	17.18f
	S2	Control	6.02n	7.48n	0.851t	0.916t	12.08s	12.75s
		One	13.51k	15.13k	1.083o	1.147o	14.65m	15.11l
		Two	15.87j	17.53j	1.204i	1.269i	15.95i	16.19i
		Three	19.87f	21.59f	1.251h	1.316h	17.21d	18.04e
		Four	25.73c	27.55c	1.294f	1.359f	18.02b	19.02b
	S3	Control	6.47n	7.95n	0.904s	0.968s	12.62r	13.14q
		One	15.81j	17.46j	0.903s	1.267j	15.71j	16.19i
		Two	17.80h	19.48h	1.202i	1.369e	17.05e	18.01e
		Three	24.64d	26.44d	1.625b	1.690b	17.88c	19.04b
		Four	30.01a	31.92a	1.804a	1.869a	18.91a	19.89a
G46A	S1	Control	4.16p	5.61p	0.761w	0.826w	10.61x	11.56v
		One	9.04m	10.58m	0.972r	1.037r	11.84u	12.78s
		Two	11.54l	13.13l	1.052t	1.117p	12.66r	13.61o
		Three	13.66k	15.18k	1.108n	1.173n	13.81o	14.75m
		Four	18.43g	20.14g	1.161l	1.226l	15.50k	16.45h
	S2	Control	4.56p	6.02p	0.807u	0.872u	11.25w	12.20t
		One	11.46l	13.05l	1.003q	1.068q	13.28p	14.23n
		Two	14.08k	15.72k	1.168k	1.226l	14.65m	15.60j
		Three	16.71i	18.39i	1.206i	1.271i	16.20g	17.15fg
		Four	22.54e	24.33e	1.273g	1.337g	17.23d	18.18d
	S3	Control	5.48o	6.96o	0.852t	0.916t	12.02t	12.96r
		One	13.66a	15.30k	1.151m	1.221m	14.59m	15.54j
		Two	16.05j	17.73j	1.252h	1.317h	16.14h	17.09
		Three	22.83e	24.64e	1.510d	1.575d	17.04e	17.99e
		Four	26.41b	28.18b	1.616c	1.681c	17.86c	18.81c

The period synchronization of flowering between the female and restorer line is crucial for an efficient and higher seed yield in multiplication cytoplasmic male sterile lines for hybrid seed production programmers (Bastawisi *et al* 1998). The leaf number diverged from 15.07 to 18.63 and from 17.05 to 19.89 for the CMS and R lines, respectively. The EAT ranged from 1069 to 12910°C for the CMS line, and 1174 to 13,510°C for the R

lines. The growth duration differences between CMS and R lines ranged from 1 to 19 days. Similarly, the leaf number difference between CMS and R lines ranged from 0 to 4.82. The seeding differences between CMS and R lines in terms of EAT were from 13.50 to 282.20 °C.

Development of flowering can be influenced to some extent by factors such as energy supply, humidity, nutrients and water stress; it is principally controlled by temperature and photoperiod. For the optimal synchronization of flowering, the CMS parent should flower two to three days earlier than the R parent. Note that CMS and R lines have the same growth duration. Thus, methods of flowering synchronization between CMS lines and R lines has highly significant effects on flowering and agronomic characteristics of hybrid seed production.

The number of leaves provided the highest seed yield (1.722 and 1.830 t ha<sup>-1</sup>), and the hybrid combination of IR69625A x Giza 178R produced the highest seed yield of 1.583 and 1.691 t ha<sup>-1</sup> in the 2020 and 2021 seasons, respectively. The interaction between synchronization of flowering methods and CMS lines was highly significant. Number of leaves with the CMS line IR 69625A x Giza 178R gave the highest values of seed yield (1.760 and 1.870 t ha<sup>-1</sup>) in both seasons. Elshamey *et al* (2021) studied floral features because of their impact on cross-pollination efficiency and hybrid seed production. As a result, floral components are critical for identifying suitable parents, which might be used in a hybrid seed production program.

The CMS lines and restorer showed significant variation for spikelet opening time, panicle exertion, and spikelet opening angle. The considerable variances revealed that the CMS lines functioned differently depending on which restorer was crossed. In addition, the vast range of variances demonstrated significant genetic heterogeneity among the employed parents and crosses, which might be used in hybrid rice breeding programs. Anis *et al* 2019, Suvi *et al* 2021 and Gaballah *et al* 2021b have all demonstrated genetic variability for floral characteristics.

Seed set percentage on the female line depends on supplementary pollination by the restorer line at flowering in the production plot. The determination of suitable seeding dates is the primary and foremost step in

the successful synchronization of the parental lines (Huang *et al* 1994). However, seasonal and weather fluctuations had a significant impacts on the flowering of female and restorer line, as well as seed setting. Therefore, the seeding interval may not be the same for all environments. Actual practices for synchronization of flowering would have to be standardized for each hybrid and the location selected for multiplication of cytoplasmic male sterile lines for hybrid seed production. Sowing dates of male parents were found to have highly significant effect for seed yield, indicating the importance of seeding date in grain yield and related traits, and appropriate seeding time improved seed setting percentage and adjusted synchronization between multiplication of cytoplasmic male sterile lines for hybrid seed production lines CMS/B (Schmitz and Ransom 2021).

To achieve good synchronization, different sowing dates of restorer line depend on many factors, *viz.*, temperature degree, humidity percentage and soil fertility (Elshamey *et al* 2021). Regardless of the application, CMS systems face some challenges, such as limited B line germplasm resources, low genetic diversity between CMS lines and R line, male sterility instability under weather conditions, the negative effect of aberrant mitochondrial genes on hybrid performance, and the difficulty of incorporating new traits into parental lines (Mertes *et al* 2021). CMS hybrid rice could increase CMS source variety while lowering the risk of using a single CMS source. Casco *et al* 2021 pointed out that supplementary pollination should be done by shaking the pollen parent with ropes or sticks so that the pollen is shed effectively on A-line plants to increase seed set percentage. Supplementary pollination should be performed 3–4 times at 20–30 minute intervals and should continue for 10–12 days during the flowering period (Elshamey *et al* 2021). Hybrid seed yield can be significantly increased by improving parent management and using effective supplementary pollination. Hybrid seed yield can be increased significantly (Hamad *et al* 2021 and Spielman *et al* 2021). It has been reported that shaking the panicles of pollen parents with a rope between two people or stirring the canopy layer of pollen parents with a bamboo stick can increase the horizontal dispersal of pollen grains during anthesis (supplementary pollination). Cao *et al* (2021) discovered that when seedlings are kept in the

nursery beds for an extended period, primary tiller buds on the lower nodes of the main Culm degenerate, resulting in decreased tiller production. Tillering begins about a fortnight after direct seeding if the seed is not planted too deeply. Tillering starts early in about a fortnight from sowing in the case of direct seeding. However, transplanted rice takes a little longer to begin tillering because it needs more time to recover from transplant shock. Tillering and growth proceed normally when rice seedlings are transplanted at the appropriate age (Huang *et al* 2021) and only a few tillers are produced during the vegetative period, resulting in a low yield if transplanting is delayed.

To multiply CMS lines and produce hybrid seeds, we should be planting restorers and R lines, staggered to achieve optimized synchronization between CMS and R line. Meanwhile, the key to increasing CMS/R seed production is to optimize synchronization at sowing date differences. Moreover, it increased seed yield remarkably compared to other sowing dates. The CMS parent should bloom two to three days before the B parent. The CMS and R lines grow at the same rate; however, in all panicle developmental stages, the CMS line flowers one to two days earlier than the R line (Dhillon *et al* 2021 and Huang *et al* 2021). The R line should behave one stage earlier than the CMS line through the first three panicle development stages. Once the CMS line is longer than the R line in terms of growth duration, the CMS line should be two to three days ahead of the R line during the first three panicle development stages.

#### **CONCLUSION**

Synchronization of flowering between the restorer (R) line and cytoplasmic male sterile (CMS or A) line in hybrid rice seed production is very necessary to get higher hybrid seed yield. Through this study, it is clear that the leaves number method was the most accurate method, followed by the days to heading method. As for the method of effective accumulated temperature, it is affected by the temperature difference from year to year, which affects the flowering synchronization between the parental lines and leads to a reduction in out-cross in hybrid rice seed production.

## REFERENCES

- Abbas, A., Y. Ping, S. Lianping, Y. Zhengfu, C. Daibo, C. Shihua and C. Liyong. (2021). Exploiting genic male sterility in rice: from molecular dissection to breeding applications. *Frontiers in Plant Science*, 12, 220.
- Anis, G., H. Hassan, A. El-Sherif, H. Saneoka and A. EL Sabag. (2019). Evaluation of new promising rice hybrid and its parental lines for floral, agronomic traits and genetic purity assessment. *Pakistan Journal of Agricultural Sciences*, 56(3): 567-576.
- Bastawisi, A. O., I. R. Aidy, H. F. El-Mowafy and M. A. Maximos. (1998). Research and development for hybrid rice technology in Egypt. *Advances of hybrid rice technology. Proceedings of the 3rd International Symposium of rice.*
- Cao, J., L. Xiaoxiao, W. Fei, H. Jianliang and P. Shaobing. (2021). The Responses of yield performance to seedling ages with varied seeding or transplanting dates of middle-season rice in central China. *Journal of Plant Growth Regulation*, 1-16.
- Casco, V. V., R. T. Tapic, J. R. Undan, A. M. Latonio, S. Lourdes, R. R. Suralta and N. L. Manigbas. (2021). Combining ability, floral biology, and seed producibility of promising cytoplasmic male-sterile (CMS) lines for hybrid rice development. *CABI Agriculture and Bioscience*, 2(1): 1-10.
- Dhillon, B. S., G. Kaur and G. S. Mangat. (2021). Influence of seedling age on growth, productivity and heat use efficiency of rice genotypes in North West India. *Journal of Agrometeorology*, 23(1), 30-37.
- Duncan, D.B. (1955). Multiple Range and Multiple F-Test. *Biometrics*, 11: 1-5.
- El Sayed, M. A., A. S. Kheir, F. A. Hussein, E. F. Ali, M. E. Selim, A. Majrashi and E. A. El Shamey,. (2021). Developing new lines of Japonica rice for higher quality and yield under arid conditions. *Peer J*, (14)9: e11592, DOI: 10.7717/peerj.11592.
- ElShamey, E. A., E. F. Ali, M. E. Selim, M. A. ElSayed, M. Ahmed, F. A. Alotaibi and A. M. Kheir. (2021). Water deficit induced physiological and amino acid responses in some rice varieties using NMR-metabolic analysis. *Agron. J.* 113(6): 4690-4704.
- Gaballah, M., H. Hamad, A. Bamagoos, H. Alharby, A. Sharif, A. Ismail and A. EL Sabagh,. (2021a). Flowering Synchronization in Hybrid Rice Parental Lines at Different Sowing Dates. *Sustainability*, 13(6), 3229.
- Gaballah, M., A. F. EL-EZZ, A. M. Ghoneim, B. Yang and L. Xiao. (2021b). Exploiting heterosis and combining ability in two-line hybrid rice. *Acta Agriculturae Slovenica*, 117/1, 1–16.
- <http://dx.doi.org/10.14720/aas.2021.117.1.1847>.
- Gaballah, M., K. A. Attia, A. M. Ghoneim, N. Khan, A. F. EL-Ezz, B. Yang, L. Xiao, E. I. Ibrahim and A. A. Al-Doss. (2022). Assessment of genetic parameters and gene action associated with heterosis for enhancing yield characters in novel hybrid rice parental lines. *Plants*, 11, 266. <https://doi.org/10.3390/plants11030266>.



- Gramaje, L. V., J. D. Caguiat, J. O. Enriquez, D. Quirino, A. Reneth, J. E. Carampatana and D. A. Tabanao. (2020).** Heterosis and combining ability analysis in CMS hybrid rice. *Euphytica*, 216(1): 1-22.
- Gomez, K. A. and A. A. Gomez. (1984).** Statistical Procedures for Agricultural Research. 2<sup>nd</sup> Edition, John Wiley and Sons, New York, 680 p.
- Hamad, H., E. Gewaily, A. Ghoneim, M. Shehab and N. El-Kholly. (2021).** Improvement ability of male parent by gibberellic acid application to enhancing the outcrossing of cytoplasmic male sterility rice lines. *Acta agriculturae Slovenica*, 117(3): 1-11.
- Huang, L., F. Wang, Y. Liu, X. Tian and Y. Zhang. (2021).** Can optimizing seeding rate and planting density alleviate the yield loss of double-season rice caused by prolonged seedling age? *Crop Science*, 61(4): 2759-2774.
- Huang P., K. Maruyama, H. L. Sharma and S. S. Virmani. (1994).** Advances in hybrid rice seed production technology. *Hybrid rice technology: new developments and future prospects*, 63.
- IRRI (2014).** Standard evaluation system for rice (SES), 5<sup>th</sup> edition. Los Banos, philippines, International Rice Research Institute. pp.19-20.
- Kader, M. d., H. Abdul Khaleque, Md. H. Monzur, K. Tapas and Md. Maksudul. (2017).** Determination of seeding interval of most promising parental lines of hybrid rice (*Oryza sativa* L.). *Haya Saudi J. Life Sci.*, 2: 1-5.
- Manonmani, S, Umadevi, M, Pushpam, R, Robin, S, Rajeswari, S, & Thiyagarajan, K. (2012).** Evaluation of Floral and Morphological Traits in CMS Lines of Hybrid Rice. *Madras Agricultural Journal*, 99(1/3), 5-7.
- Mei, S., P. Xie, F. Primeau, J. C. McWilliams and C. Pasquero. (2015).** Northwestern Pacific typhoon intensity controlled by changes in ocean temperatures. *Sci. Adv.*, 1 Article e1500014.
- Mertes, C., I. F. Scheller, V. A. Yépez, H. Muhammed, Y. Liang, L. S. Kremer and J. Gagneur. (2021).** Detection of aberrant splicing events in RNA-seq data using FRASER. *Nature communications*, 12(1): 1-13.
- Schmitz, P. K. and J. K. Ransom. (2021).** Seeding Rate Effects on Hybrid Spring Wheat Yield, Yield Components and Quality. *Agronomy*, 11(6), 1240. <https://doi.org/10.3390/agronomy11061240>.
- Spielman, D. J., P. S. Ward and D. E. Kolady. (2021).** Challenges and Opportunities for Hybrid Rice in Bangladesh. *Securing food for all in Bangladesh*, 62. [https://doi.org/10.2499/9789845063715\\_02](https://doi.org/10.2499/9789845063715_02).
- Suvi, W. T., H. Shimelis, M. Laing, I. Mathew and A. T. Shayanowako. (2021).** Determining the combining ability and gene action for rice yellow mottle virus disease resistance and agronomic traits in rice (*Oryza sativa* L.). *Agronomy*, 11(1): 12. <https://doi.org/10.3390/agronomy11010012>.

## تأثر معدل التلقيح الخلطي بالطريقة المثلى لمزامنة تزهير السلالات الأبوية لإنتاج تقاوي الأرز الهجين

حسن حماد، السيد الجويلي، أشرف المغازي\* و محمود السيد

قسم بحوث الأرز، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، ٣٣٧١٧، سخا، كفر الشيخ، مصر

يعتمد إنتاج الحبوب الهجينية في السلالة الأم على تزامن تزهيرها مع السلالة المعيدة للخصوبة، وعليه، فيجب تخطيط مواعيد زراعة السلالات الأبوية بصورة مناسبة. خلال موسمي الزراعة الصيفيين ٢٠١٦ و ٢٠١٧ تم إجراء دراسة مبدئية لتحديد عدد الأوراق وعدد الأيام حتى التزهير والحرارة المتراكمة الفعالة كطرق أساسية لتحديد تزامن التزهير بين السلالة العقيمة والأب المعيد للخصوبة. كما تم إجراء تجربة خلال موسمي ٢٠١٨ و ٢٠١٩ لدراسة تأثير طرق التزامن المختلفة على تزامن التزهير في إنتاج تقاوي الأرز الهجين. وقد أوضحت النتائج أن طرق تزامن التزهير كان لها تأثير معنوي على وزن السنبل والنسبة المئوية لتكون الحبوب ولبيل الحصاد ومحصول الحبوب من تقاوي الأرز الهجين. وقد سجل أعلى محصول تقاوي حبوب والذي قدر بـ ١,٧٦ و ١,٨٧ طن للهكتار من استخدام صفة عدد الأوراق والهجين *Giza 178R × IR69625A*، على التوالي. وتعتبر صفة عدد الأوراق أفضل طريقة لحساب وقت التزامن بين السلالات الأبوية في إنتاج أعلى محصول من تقاوي الأرز الهجين وكذلك الهجين *Giza 178R × IR69625A*. ويعتبر زراعة الأب المعيد للخصوبة في ثلاث مواعيد مختلفة مع أربع مرات من التلقيح الإضافي أفضل طريقة للحصول على أعلى عدد من السنابل الخصبة في الجورة ووزن السنبل وعدد الحبوب الممتلئة في السنبل ومحصول الحبوب.

المجلة المصرية لتربية النبات ٢٧(١): ٦١ - ٧٦ (٢٠٢٣)