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Development of Two Novel Photoperiod and Thermo-Sensitive Genic Male Sterile Lines in Rice (*Oryza sativa* L.)

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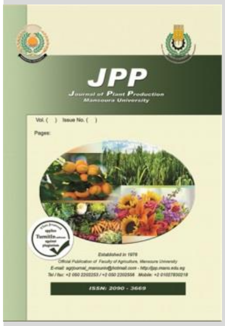

ABSTRACT

Developing hybrid rice by two lines system under Egyptian conditions requires high performance lines. The promising Photoperiod and Thermo-sensitive Genic Male Sterile (PTGMS) lines A001S and G001S were developed by crossing, continuously inbred for six generations,and the selection depended onduration and tillering ability. F_2 populations were segregated and fitted well to the ratio of 15:1 for pollen grains fertility trait, indicating the presence of digenic inheritance and epistatic dominant duplicate gene action control. The PTGMS line G001S had the most desirable characteristics for the hybrid rice breeders, with values of 19cm panicle length,13 primary branches panicle^{-1}, 8.5mm grain length, 2.9mm grain width, 1.9mm grain thicknessand 25g for 1000-grain weight. Both A001S and G001S were superiors of most studied traits to be good PTGMS lines for two-line hybrid seed production systems. Both linesexhibited a high rate of panicle *exertion* with values of 90.70 % and 94.30 % for A001S and G001S, respectively. The two lines were completely male sterile when sowing from 1st to 20th April. Still, their fertility alternated from completely sterile to partially fertile in late July when the temperature decreased and the day length was long. A001S x GSR1 and G001S x GSR9 new hybrid combinations were derived from the newlydeveloped PTGMS lines; A001S and G001S, with short duration, semi-dwarf and high tillering ability with a high yield potential of 80gplant^{-1}. For the panicle and grain traits, both hybrids exhibited good characteristics.Both hybrids showed favorable positive significant, and highly significant standard heterosis.

Keywords: Rice, Breeding, Hybrid, PTGMS, Gene action.

INTRODUCTION

Hybrid rice technology is one of the effective ways to increase rice production leading to self-sufficiency and facing overpopulation in Egypt. With the release of conventional cultivars, rice yield levels have reached a plateau that can be broken with this technique (Abd El-Aty *et al*., 2022). Commercial hybrids often produced between 15 to 20% more than the most acceptable inbred varieties grown in comparable conditions, which were attributed to "hybrid vigor" or "heterosis" from mating the two parents (El Sayed *et al*., 2021; Melandri *et al*., 2021). The superiority of hybrid varieties over inbred varieties in terms of grain output, vigor, panicle weight, number of spikelets per panicle, and number of productive tillers are examples of how hybrids can benefit from heterosis (Abd El-Aty *et al*., 2022).

Two main breeding techniques, the three-line, and the two-line breeding procedures are typically used to create hybrid types. Commercial rice hybrids are frequently produced using the three-line cytoplasmic genetic male sterility system, consisting of a CMS source, a maintainer, and a restorer (Elshamey *et al*., 2022a; Gaballah *et al*., 2022a). In the two-line system, certain lines, according to temperature and day length, so-called S lines, can either be male sterile (functionally female) or male (produces viable pollen). S lines are crossed as females to fertile inbred lines under one set of temperature/day length conditions, producing hybrid seed. Under a different set of temperature/day length conditions, the same lines are let to self-pollinate, producing viable seeds to preserve a source of the line. The main objectives of this investigation are to evaluate two new promising PTGMS lines under Egyptian field conditions. In addition to two hybrid combinations derived from the two-line system for some agronomic, yield and its component traits may be used for commercial exploitation of two-line hybrid rice breeding.

MATERIALS AND METHODS

Plant materials

The present investigation was carried out at the Sakha Agricultural Research Station experimental farm, Kafr El Sheikh, Egypt (Latitude: 31 05 '12", Longitude: 30 56 '49") during the three rice growing seasons of 2019-2021. A001S and G001S as new photo-Thermo sensitive genic male sterile lines were selected from two different rice crosses. A001S was developed from a cross between PTGMS-38 and PR78, whereas; G001S was developed from PTGMS-38 and GZ8479. The Egyptian PTGMS-38 was used as a donor for *ptgms* genes, presented by El-Mowafi *et al*., 2012. Green Super Rice-1 (GSR-1) and Green Super Rice-9 (GSR-9) are good lines for male parents that crossed with A001S and G001S.

Breeding methodology

In this study, we tried to develop new PTGMS lines with high floral traits and better agronomic traits to increase yields of hybrid seed production. A001S and G001S were developed as follows: the F_1 hybrids of the two crosses were continuously inbred for six generations. In our ongoing breeding program from F_2 to F_5, we use panicles bagging and pollen fertility tests to examine the background of the selected lines and select the complete sterile plants. In conjunction with the experiment, during F_2 generation, the heredity of

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fertility was studied (Figure 1). All segregate generations were sown successfully on only one sowing date on 15th April. The negative plants (fertile plants) were removed or used in inbreed rice program according to their characteristics. Seed multiplication of the sterile plants in all generations (F_2 to F_5) was done using the ratoon method in Sakha Research Station Farm during optimum day length and temperature. The new PTGMS lines A001S and G001S were selected from nine and thirteen rice lines, respectively (Figure 1). These two lines were selected and used for further phenotypic evaluation and fertility alteration tests during the 2019 and 2020 seasons. During the 2020 growing season, GSR-1 and GSR-9 were used for crossing with A001S and G001S, respectively, to produce two F_1 hybrids and evaluated them in the 2021 growing season.

Climatic conditions

The monthly temperature, humidity percentage, and daily day length data for the three years of the study (2019 – 2021) were provided by the Division of Meteorology (RRTC) at Sakha (31°05'36.4"N 30°55'45.6"E and 4m elevation), Kafr El-Sheikh, Egypt (Table 1).

Field experiments

A001S and G001S lines were sown at different stages during 2019 and 2020. The sowing dates began on 1st April, with 15 days intervals, and altogether were five dates. In 2020, the genetic materials (A001S and G001S) were used as females and crossed with two new super rice lines as males to check the genetic behavior of these lines. The F_1 crosses were grown with their parents and standard check Egyptian Hybrid 1. After 30 days, the seedlings were transplanted individually in three rows five meters long and 20 x 20 cm spacing. All standard cultural practices were applied as recommended by RRTC (RRTC, 2013). Measurements and observations were recorded using the Standard Evaluation System (SES, IRRI 2014).

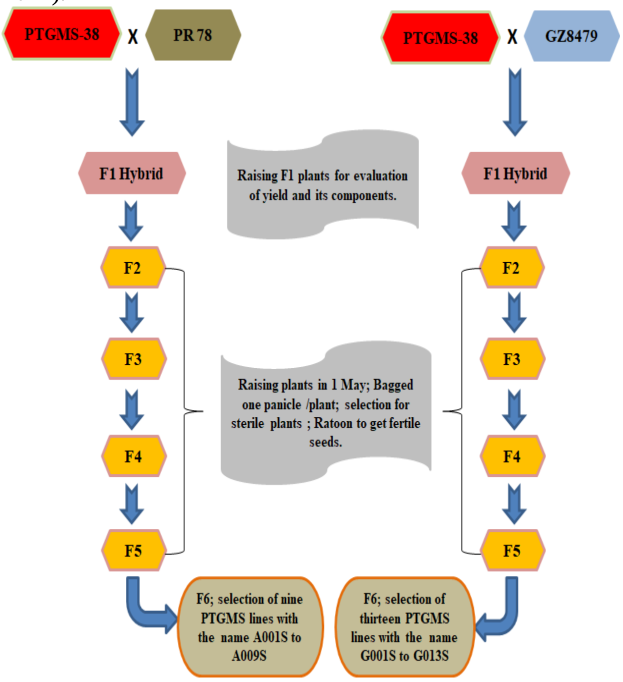

Figure 1. The scheme showing the development of new PTGMS lines by genetic background of *ptgms* genes (donor parent: PTGMS-38).

Table 1. Day length and the average temperature degrees (c°) for the growing seasons day and night.

Months	Day length (h)	2019		2020		2021	
		Day	Night	Day	Night	Day	Night
April (1st-15th)	12:43	22.0	12.0	23.0	14.0	24.0	15.0
April (16th-30th)	13:08	26.0	17.0	26.0	18.0	29.0	19.0
May (1st-15th)	13:31	33.0	22.0	32.0	23.0	30.0	22.0
May (16th-31st)	13:49	34.0	25.0	34.0	24.0	32.0	24.0
June (1st-15th)	14:01	33.0	25.5	34.0	24.0	34.0	26.0
June (16th-30th)	14:05	33.5	27.0	33.0	26.0	35.0	26.0
July (1st-15th)	14:01	35.0	27.0	34.0	26.0	33.0	27.0
July (16th-31st)	13:50	33.5	26.0	33.0	26.0	33.0	25.5
August (1st-15th)	13:24	33.0	25.0	32.5	25.0	34.0	24.0
August (16th-31st)	13:01	31.0	24.0	32.0	24.0	34.0	24.0
Sept. (1st-15th)	12:33	30.0	20.0	30.0	23.0	31.0	24.0
Sept. (16th-30th)	12:09	26.0	19.0	27.5	22.0	30.0	23.0

Flowering studies

Investigations were conducted on the properties of stigma exertion, the length of a spikelet's blooming period, and the distribution of spikelet's flowers on a single panicle.

Cytological observations

Seeding rice at various times and monitoring the pollen fertility percentage under natural settings during five sowing dates, the features of fertility change of the A001S and G001S lines were experimentally evaluated. For each line, 10 – 15 anthers were removed from spikelets at the tops of the panicles during the first heading date, and the proportion of pollen grains dyed with 1% of iodine potassium iodide solution (i_2ki) and examined under an optical microscope was used to calculate the pollen fertility. While usual irregularly shaped, round, yellowish, and light brown colored pollen grains were classified as sterile, all round and dark stained pollen were scored as normal fertile. On each slide, pollen grains from randomly selected fields were analyzed, and pollen fertility was reported as a percentage in accordance with ElShamey *et al*. 2022b formula. Based on obtained data, standard heterosis was calculated for the two hybrid combinations compared with the check variety Egyptian Hybrid 1according to Mather and Jinks, 1982.

RESULTS AND DISCUSSION

Evaluation of Pollen and Spikelet Fertility in F_2 Populations

Physiological and environmental variables affect spikelet fertility; an increase in spikelet fertility increases yield. Therefore, a pollen fertility value in the F_2 population is typically utilized to gather information on the number of genes and their functions and activities in regulating fertility. During the 2012 season, the crossing between PTGMS-38 and PR78 and GZ8479 lines was done to study The F_2 genetic behavior in 2014. Table 2 demonstrates a Chi-square analysis of spikelet fertility and pollen grains fertility traits in two crossing combinations. Spikelet fertility tests F_2 populations resulting from a cross of PTGMS-38/PR78 showed 79 fertile plants and 25 sterile plants. Out of 104 F_2 plants, this did not differ substantially from the predicted ratio of 3 fertile: 1 sterile (χ^2 = 0.051, P 0.05); this suggests that single gene inheritance is present, the complete dominance action controlling fertility in this background.

The segregation pattern observed in the F_2 population derived from PTGMS-38/GZ8479 revealed 122 fertile plants and 9 sterile plants of 131 total tested plants. Table 3 indicated no statistically significant difference from the predicted ratio of 15 fertile: 1 sterile (P = 0.05); this suggests that digenic inheritance and epistatic dominant duplicate gene activity are at play spikelet fertility in these cross. The pollen grains test revealed 114 fertile and 6 fully sterile plants in the F_2 population of PTGMS-38/PR78, and it suggested a good match to 15 fertile: 1 sterile (χ^2 = 0.152, P= 0.05); this suggests that there were two distinct dominant genes in the genetic background and that one of the two genes had a duplicate impact that demonstrated higher expression than the other gene and could, by itself, reveal the fertility in the pollen grain. The PTGMS-38/GZ8479 F_2 population cross-segregated and fit well to 15 fertile and 1 sterile ($\chi2$ = 1.374, P 0.05). Such a segregation pattern across people of varied genetic backgrounds demonstrated the presence of two redundant epistatic dominant genes in regulating. The results are consistent with those mentioned by (Hasan *et al*., 2015; Sattari *et al*., 2008; Seesang *et al*., 2014; and Abd El-Mageed *et al*., 2022).

Table 2. Pattern and segregation for spikelet fertility and pollen fertility in F2 populations derived from PTGMS-38 and each of PR78 and GZ8479 as normal male fertile parents.

Crosses	Trait	No. of plants					genetic ratio	χ^2 test
		total	Fertile		Sterile			
			observed	Expected	observed	Expected		
PTGMS-38	SF	104	79	78	25	26	$3:1^{ns}$	0.051
/PR78	PF	120	114	113	6	7	$15:1^{ns}$	0.152
PTGMS-38	SF	131	122	123	9	8	$15:1^{ns}$	0.133
/GZ8479	PF	106	102	99	4	7	$15:1^{ns}$	1.374

^{ns}, not significant at 5% statistical level; SF, Spikelet Fertility; PF, Pollen Fertility.

Morphological characterization of PTGMS

Two of 22 promising PTGMS lines were selected depending on duration and tillering ability. The evaluation was conducted under normal conditions for five sowing dates and was confirmed in two years. A001S and G001S lines were sown at different stages during the 2019 and 2020 seasons. The sowing dates startedon1st April, with a 15-days interval, for five growing dates. The PTGMS A001S showed a 137-day growing duration from seed to seed with 74cm plant height, 37panicles plant^{-1},and gave 20-g seeds plant^{-1} due to out-crossing ability (Fig. 2). While the PTGMS G001S had 129 days growing duration from seed to seed with 73cm plant height, 35 panicles plant^{-1} and gave 25g seeds plant^{-1} due to out-crossing ability. El-Namaky and van Oort, 2017 and Abbas *et al*., 2021 presented similar results for hybrid seed production and reported that; short duration, dwarfism, high tillering, and high rate of seed set percentage in female lines are helpful for hybrid seed production.

Panicle and grains characterization

PTGMS A001S line scored 19-cm panicle length with 11 primary branches panicle-1, while their grains were characterized as 9 mm grain length, 2.9 mm grain width, 1.7 mm grain thickness, and 24.9 g for 1000-grain weight. The second PTGMS line, G001S, had similar panicle and grains traits with values of 19-cm panicle length and 13 primary branches panicle-1. Regarding grains, traits showed the values of 8.5 mm grain length (G.L), 2.9 mm grain width (G.W), 1.9 mm grain thickness (G.T), and 25 g for 1000-grain weight (Figure 3).

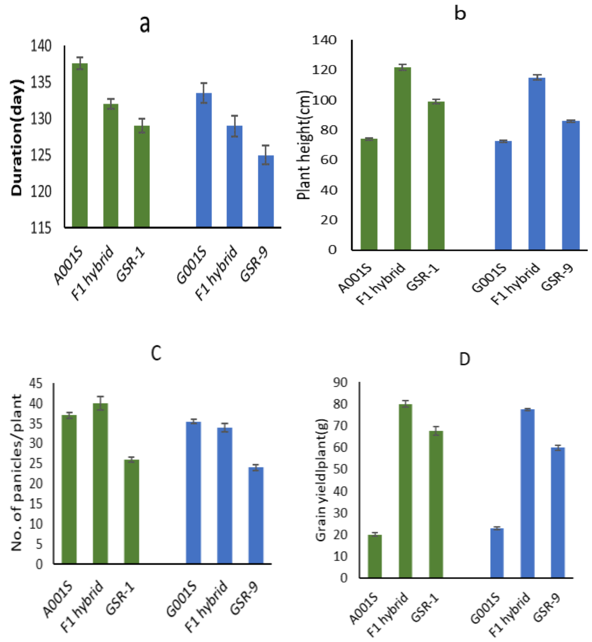

Figure 2. The performance of the two promising PTGMS lines and the two F_1 hybrids for some morphological traits and grain yield.

Both A001S and G001S were superiors of most studied traits to be good PTGMS lines for two-line hybrid seed production due to their dwarfism, tillering ability, and 1000-grain weight. While some agronomic applications like Gebbrilic acid treatments could improve the out-crossing rate as reported by Elshamey *et al*., 2022a; Rahman *et al*., 2010. Virmani (2003) reported that different methods developed several EGMS lines, some derived from hybridization and pedigree selection. Due to the importance of Panicle and Grain characters, which are considered yield components' main traits, as Anis *et al*., 2017; Elgamal 2019 and Gaballah *et al*., 2022b; Saleh *et al*., 2019 reported the yield potential related positively to high panicle weight and 1000-grain weight.

Flowering Characteristics

Mean values of floral traits for PTGMS lines are presented in Figure 4. For both PTGMS lines, the duration of open florets was prolonged. Line A001S was discovered to remain open for the most significant duration of time (65 min), whereas line G001S was determined to have the least period of floret opening (57 min) (figure4e). The angle of open floret in A001S (23.5º) was wider than in G001S (21.5º). A wide angle in PTGMS lines is desirable because it would help cross-pollination (Abdel Megeed *et al*., 2022; Abdelmegeed and Elshamey, 2022 and Liu *et al*., 2021). Stigma length is vital for PTGMS lines because a long stigma facilitates the out-crossing rate.

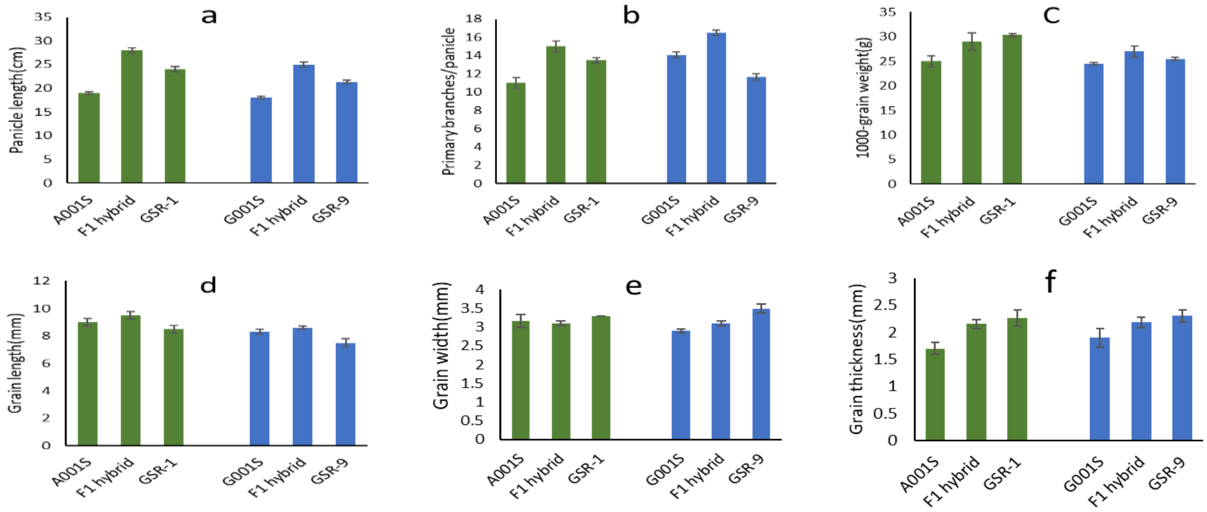

Figure 3. The performance of the two promising PTGMS lines and the two F_1 hybrids for some panicle traits (a and b) and grain characters (c to f).

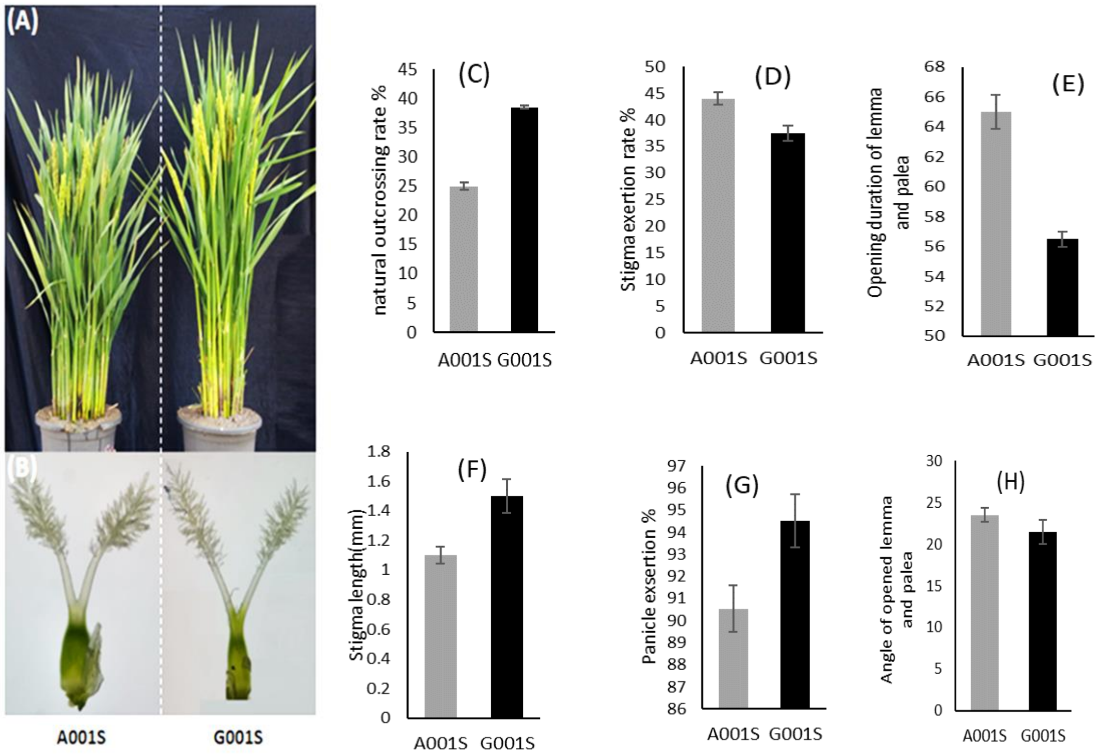

Figure 4. The performance and main differences of two promising ptgms lines (A001S and G001S) for tillering and plant height (a) and floral traits (b-h).

Stigma length did not exceed high significantly between both new PTGMS. The longest stigma was recorded in G001S (1.4 mm), while the short stigma was recorded in A001S (1.1 mm). (Hasan *et al*., 2015) stated that stigma length (>1 mm) had a pronounced influence on the out-crossing rate of a CMS line. The new PTGMS line A001S and G001S exhibited a high rate of panicle exertion with values of 90.70 % and 94.30 %, respectively. In hybrid rice, stigma exertion is a crucial characteristic that helps with seed output. Stigma assertion rates were high for both A001S and G001S. However, A001S had a more significant rate of stigma exertion (44%) than G001S did (37.5%) (Figure 4d), thus indicating that A001S was more conducive to hybrid seed production. Regarding the out-crossing rate, line G001S performed a higher rate of natural out-crossing than line A001S; the values were 36% and 25 %, respectively. We can conclude from the presented results that the new PTGMS lines are promising for hybrid rice production as a female parent. Under Egyptian conditions, many PTGMS were developed by (El-Mowafi *et al*., 2012; El-Mowafi *et al*., 2021).

Fertility-Sterility Alteration Pattern under field conditions

Generally, due to Egyptian weather conditions during rice growing seasons from 2019 to 2021, there are no significant differences between seasons for temperature average and day length, which are presented in table 1. Dynamic pollen grain fertility expression of A001S and G001S was observed in the summer of 2019 and 2020 under natural field conditions on five sowing dates, starting on 1st April, with 15 days between each sowing date. Between 10th June and 25th July, daily pollen microscopy outcomes showed no fertile pollen, implying complete abortion (Figure 5). These critical periods synchronize with the first and second sowing dates; the critical stage (sterility stage) meets with a high average of temperature degrees >(33D/24N) and a day length longer than 14 h/ day (Table 1). Between 25th July and 5th August, the pollen abortion type was typical abortion, with a small amount of round abortion (partial fertility) Figure 5. This stage met with the temperature degrees still high, but day length less than 13.75 h/day, which is considered a translocation stage between sterility and fertility stages. From 6th August to the end of September, synchronized with the last two sowing dates, the pollen gradually normalized, and the high fertility rate (fertility stage) met with short days and low average temperature degrees D/N. These results indicate that the new two PTGMS lines, A001S and G001S, were completely male sterile (pollen sterility surpassing 99.5%) when sowing from 1stto 20th April. The two PTGMS fertility alternated from completely sterile to partially fertile in late July when the temperature decreased, and the day length in late August became shorter (Figures 5 and 6). For Fertility-sterility alternation, characterization uses two main methods, the first under normal field conditions depending on temperature variation and daylength changing throughout the growing season as previously applied by El-Mowafi *et al*., 2021; Li *et al*., 2020. While the second conducting under controlled conditions in growth chambers, as reported by Liu *et al*., 2021; Yang *et al*., 2021.

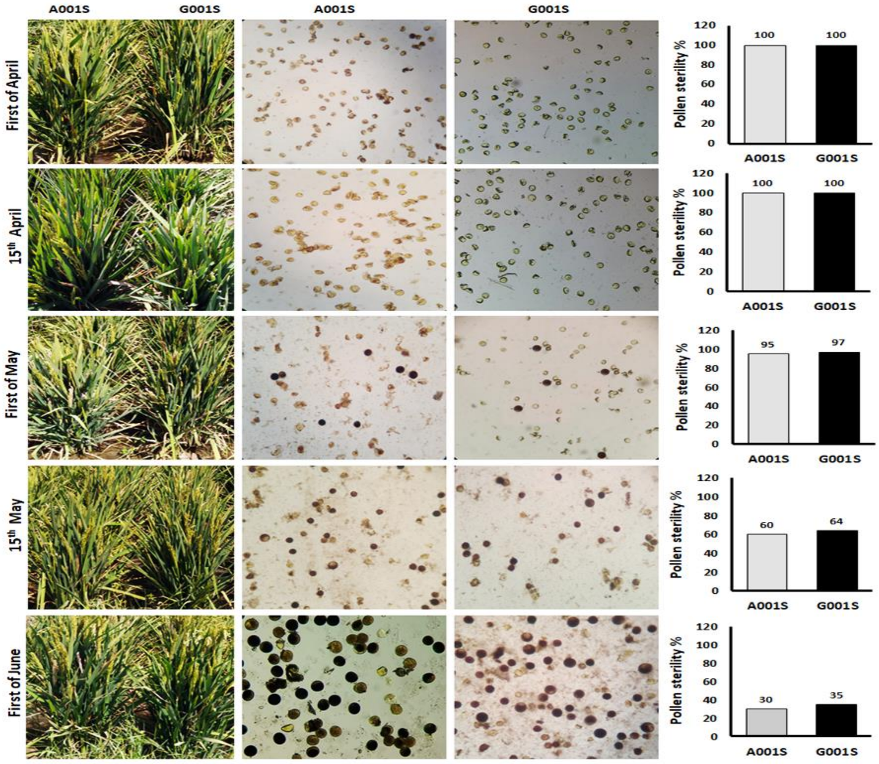

Figure 5. Morphological and Cytological observation of pollen morphology and sterility percentages around five growing dates for A001S and G001S lines.

The field Performance and grain characterization of Derived Hybrid combinations

Two hybrid rice combinations were derived from the new PTGMS linesA001S and G001S. The two combinations derived from A001S x GSR1 and G001S x GSR9 demonstrated satisfactory luxuriance with short duration, Simi dwarf, high tillering ability, and the yield potential reaching 80g/plant (Figure 2d). For the panicle and grain traits, both the two hybrids exhibited good characteristics; the first combination was superior to the second combination for panicle length (28cm), grain length (9.5mm), and 1000-grain weight (29.1g). At the same time, the second combination scored the highest number of primary branches (16) Figure 3. The most crucial target of PTGMS lines is to produce an appropriate high-yielding hybrid variety, so many hybrid rice breeders worldwide as Anis *et al*., (2017); Abebrese *et al*., (2018); Sakran *et al*., (2020) and ElShamey *et al*., (2021), work on this target during their research and agreed with our vision to increase the hybrid rice cultivated area with new hybrid rice varieties.

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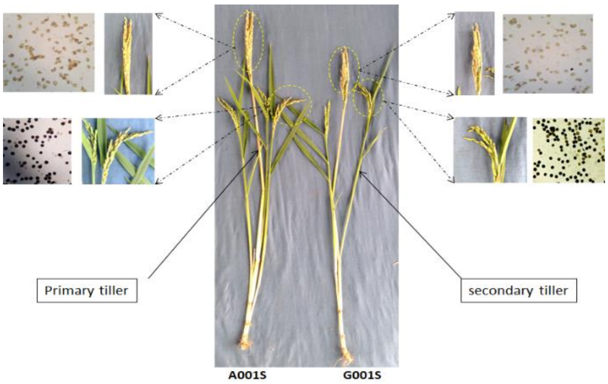

Figure 6. The sterility and fertility alternation from primary tiller to secondary tillers.

Heterosis

A phenomenon known as heterosis occurs when F_1 hybrids with varied parental backgrounds outperform their parents in terms of vigor, yield, panicle size, number of spikelets per panicle, productive tiller count, etc. Three methods express heterosis, depending on the benchmark used to evaluate a hybrid's performance. The improvement or deterioration of a hybrid's performance is known as standard heterosis compared with the standard check hybrid or a variety of regions. Egyptian Hybrid 1 was checked in this study. Estimates of heterosis relative to standard heterosis for all the traits under investigation are presented in Table (3). The standard heterosis of all studied features for the first hybrid A001S/GSR-1 was favorable positive significant, and highly influential, except for duration, Primary branches/ panicle, Grain width, and Grain thickness traits were not significant.

Table 3. Standard heterosis of the two hybrids A001S/GSR-1 and G001S/GSR-9 comparing with Egyptian Hybrid 1 as a check variety.

Traits	Standard heterosis %		Mean values of Egyptian Hybrid 1	LSD 0.05 %
	A001S/ GSR-1	G001S/ GSR-9		
Duration (day)	-2.22^{ns}	-4.74*	135.0	4.24
Plant height (cm)	12.15*	-6.54*	107.0	6.74
No. of tillers/plant	46.43**	42.86**	28.0	1.69
No. of panicles/plant	43.21**	25.93*	27.0	4.24
Spikelet fertility %	-1.08**	3.34**	86.9	0.24
Grain yield/plant	17.39**	10.14**	69.0	0.98
Panicle length (cm)	18.26*	0.83^{ns}	24.1	4.22
Primary branches/panicle	7.69^{ns}	15.38*	13.0	2.92
1000-grain weight (g)	8.30**	3.02**	26.5	0.34
Grain length (mm)	14.63*	3.66^{ns}	8.2	1.01
Grain width (mm)	3.33^{ns}	1.00^{ns}	3.0	0.24
Grain thickness (mm)	5.00^{ns}	5.00^{ns}	2.0	0.24

***, ** is significant and highly significant at probability 0.05 and 0.01, respectively**

Meanwhile, all the traits revealed favorable positive significant, and highly significant standard heterosis in the second hybrid G001S/GSR-9 except for duration and plant height traits with desirable negative significant heterosis. While, the panicle length, grain length, grain width, and grain thickness traits were not significant heterosis. Anis *et al*., 2017, El-Mowafi *et al*., 2021 and Gaballah *et al*., 2022a and manyother rice breeders agreed with the importance of heterosis to increase the yield potential.This increasearound 20% forthree-line systems and approximately 40 % for two-line systems due to the hybrid vigor in the progeny of diverse varieties of species or crosses between species exhibit greater biomass, yield and fertility than both parents.

CONCLUSION

From the results of this study, it could be concluded that, the two newly developed PTGMS rice lines A001S and G001S were promising and exhibited very good characteristics. Both lines could be very important to produce new high yielding hybrid rice varieties.

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إستنباط سلالتين جديدتين ذات عقم ذكري وراثي حساس للضوء والحرارة في الأرز

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الملخص

تطوير الأرز الهجين بنظام السلالتين تحت الظروف المصرية يستوجب سلالات ذات صفات جيدة. السلالتين A001S و G001S أستنبطتا بواسطة التهجين والانتخاب لمدة ستة أجيال متتابعة إعتمادا علي الشكل المظهري. عشيرة الجيل الثاني لكل منها إنعزلت مطابقة للنسب الوراثية 15:1 لصفة عقم حبوب اللقاح، دليل علي مشاركة كل من السيادة والتفوق في التحكم في هذه الصفة حيث يتحكم بها زوج من الجينات. السلالة G001S تملك معظم الصفات المرغوبة بالنسبة لمربي الأرز الهجين من 19 سم طول سنبلة، 13 فرع أولي لكل سنبلة، 8.5 مم طول الحبة، 2.9 عرض الحبة، 1.9 مم سمك الحبة الشعير ووزن الألف حبة 25 جم. كلا السلالتين تميزتا في معظم الصفات المدروسة لتكونا سلالات عقم ذكري وراثي بيئي لإنتاج الأرز الهجين بنظام السلالتين إعتمادا على صفاتهم المتميزة. زاوية تفتح السنيبلة واسعة لإمكانية المساعدة على التلقيح الخلطي، الميسم الريشي من الصفات المهمة التي تساعد على التلقيح الخلطي فالميسم الطويل يزيد من فرصة التلقيح الخارجي كما لوحظ فروق غير كبيرة لطول الميسم بين السلالتين. علي الجانب الأخر السلالتي أظهرتانسبة عالية لخروج السنبلة من الغمد بقيم 90.70 و 94.30. السلالتين أظهرتا عقم ذكري كامل عند الزراعة بين 1 – 20 أبريل وتحولتا من حالة العقم التام إلي الخصوبة الجزئية تدريجيا حتي نهاية يوليو عندما تقل درجات الحرارة ويقصر النهار. الهجن الناتجة عن التراكيب A001S/GSR-1 , G001/GSR-9 كانت مميزة من حيث التبكير ومعدل التفريع العالي مع قدرة محصولية عالية تصل إلى 80 جرام للنبات الفردي بالإضافة إلى صفات حبوب جيدة. تميز الهجين الأول A001S/GSR-1عن الهجين الثاني G001/GSR-9 في طول السنبلة وطول الحبة ووزن الـ1000حبة. كلا الهجينين أظهرا معنوية موجبة ومرغوبة لقوة الهجين القياسية.