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Original article

Characterization of fertility alteration and marker validation for male sterility genes in novel PTGMS lines hybrid rice

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ABSTRACT

Photoperiod and thermosensitive genetic male sterile (PTGMS) lines have become one of the main sources of global rice production increasing. This study was conducted to evaluate the fertility alteration and validate the male sterility genes using validation markers in novel Egyptian *Indica* and *Japonica* PTGMS lines under natural conditions. The study revealed that the new genetic male sterile lines belong to the type of photo–thermosensitive genetic male sterility (PTGMS). The fertility alteration of these lines has influenced by photoperiod and temperature interaction. The new PTGMS lines have three sensitive periods of fertility alteration; transformation, sterility, and fertility period. Furthermore, the sensitive stage of fertility transformation might be from secondary branch primordial to pollen mother cells (PMC) meiosis. Under the natural Sakha condition, the new PTGMS lines were stable sterile under the condition of day length upper 13,75 h and temperature over 25 °C, while its convert to fertile under day length under 13 h, and temperature lower than 24 °C. The co-dominant markers identified the *pms3* and *tms5* genes in the new PTGMS lines, indicated that the fertility alteration in these lines controlled by photoperiod and thermosensitive stages.

1. Introduction

Hybrid rice has already become an effective tool to promote rice yield. It is the most ideal application of hybrid-vigor in agriculture, which is referred to as "The Second Green Revolution" (Su et al., 2012; Lin and Yuan, 1979; Cheng et al., 2007). It is easy to obtain more than 25% higher yield advantage over conventional pure rice varieties (Virmani, 1996). This technology has been successful in China and recently in some other Countries including Egypt (Yuan, 1994a; El-Mowafi et al., 2009). In hybrid seed production mainly used three-line or two-line systems (Luo et al., 2013; Zhang et al., 2013). Since the widely used 3-line system is extremely time-consuming and costly, the discovery of a photosensitive genetic male sterile (PGMS) line laid the new strategy for hybrid rice production from a 3-line to a 2-line system (Shi, 1985). This 2-line system is an efficient breeding method for producing hybrid seeds (Yuan, 1993). Two-line system has been widely applied especially in China due to its many advantages comparing with the 3-line system (Huang et al., 2015). Two-line system is based on photoperiod and/or thermosensitive genetic male sterile (P/TGMS) lines as female parents to produce hybrid seeds (Li et al., 2007). The PTGMS line is the most important component of the 2-line system hybrid rice. The PTGMS lines are sterile at long day (LD) with high temperature (HT) and become fertile at the short day (SD) with low temperature (LT) (Zhou et al., 2012; Ding et al., 2012). During the sterile phase, these lines can produce hybrid seeds by crossing with restorer (pollinator fertile male variety), while, during the fertile phase, these lines can propagate

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itself. Fertility alteration (FA) in PTGMS lines is starting from PMC (pollen mother cell) formation stage to the meiotic division stage (Xiao et al., 1997). This FA is a consequence of an interaction between photoperiod and temperature. However, the temperature has the major contribution in promoting the FA (Zhang et al., 1992; Yuan, 1992). When the temperature becomes above a certain point (calling critical sterility temperature CST) the PTGMS lines become sterile, while, when it reaches under a certain point (calling critical fertility temperature CFT), these lines convert to fertile (Cheng et al., 1996). The span between these two critical points is the temperature range in which the FA is regulating by photoperiod conditions (Zhang et al., 1992). The photosensitive stage of the FA in PGMS lines occurs in the period from the secondary rachis branches differentiating stage to the PMC, which is about 5 days after panicle initiation (or 25 –15 days before heading) (Mou et al., 2001). The FA of PTGMS lines in a year classified generally to three stages; fertility stage, fertility transformation stage, and sterility stage based on the environmental factors conditions (Attia et al., 2003; Yuan et al., 2003). Nongken-58S rice line was the first PGMS mutant found in China (Shi, 1985). Many of PTMGS lines have developed using sterility source of Nongken-58S (Ali et al., 1995). Male sterility controlling genes are possible to introduce to rice varieties to generate new variable PTGMS lines (Yuan, 1990; Si et al., 2011).

The fertility alteration of PTGMS lines is mostly regulated by multi-genes in different environmental conditions. Many of these PTGMS genes have identified, mapped, and functionally characterized (Subudhi et al., 1997; Wang et al., 2003; Lee et al., 2005; Pitnjam et al., 2008; Qi et al., 2014; Qi et al., 2017). Some of these genes regulated only the photoperiod-sensitive stage, while, some other regulated the thermosensitive stage only, and others governed both photo-thermo sensitive stages (Fan and Zhang, 2018; Muhammad et al., 2020). The *Pms3* gene was found to be regulated both Photoperiod and thermosensitive genic male sterility and it has been cloned and mapped on Chromosome 12 (Zhou et al., 2012; Ding et al., 2012). This gene regulates the fertility alteration in Nongken-58S and Pei'ai-64S through a point mutation. This point mutation is the substitution of one base of G-C on a long non-coding RNA (lncRNAs) causing loss-of-function of 21-nucleotides small RNA encoded by that gene (Zhou et al., 2012; Ding et al., 2012; Xiao et al., 1997; Zhang et al., 1992; Yuan, 1992; Cheng et al., 1996; Zhang et al., 1992; Mou et al., 2001; Attia et al., 2003; Yuan et al., 2003; Ali et al., 1995; Yuan, 1990; Si et al., 2011; Subudhi et al., 1997; Wang et al., 2003; Lee et al., 2005; Pitnjam et al., 2008; Qi et al., 2014; Qi et al., 2017). The *tms5* gene controls thermo sensitive male sterility has been identified and mapped on chromosome 2 (Yang et al., 2007). In China, the widely used PTGMS lines are harboring *tms5* and *pms3* genes (Huang et al., 2015; Zhou et al., 2014; Zhang and Yang, 2014). Recently, an Egyptian Scientist Professor EL-Mowafi H.F. at Rice Research & Training Center (RRTC), Sakha, Egypt, developed ten PTGMS lines (El-Mowafi et al., 2012). Therefore, the current study was designed to study the fertility alteration characteristics and marker validation of *tms5* and *pms3* genes in new PTGMS lines for developing new Egyptian Hybrid-Rice-Varieties.

2. Material and methods

2.1. Plant materials

Ten Egyptian rice PTGMS lines developed by Professor EL-Mowafi H.F. (El-Mowafi et al., 2012) were used in this study (Table 1). Three of these PTGMS lines were *Indica* type with donor sterility coming from Pei'ai-64S (Chinese TGMS), while, the other seven were *Japonica* with sterility source from Nongken-58S (Chinese PGMS). Two PTGMS lines; one *Japonica* (PTGMS-23) and one *Indica* (PTGMS-7) were selected based on their best flowering habits to study fertility alteration characteristics. The plants of PTGMS lines were grown in the isolated field under normal condition at the experimental farm of Sakha Research Station, Sakha, Egypt.

2.2. Sowing and transplanting of PTGMS lines

The PTGMS lines were sown successively at different stages during the year of 2017 and 2018 and the experiments were conducted at the Experimental Farm of Sakha Research Station, where the summer is typically hot and humid. The sowing dates began from April 1th, with 7 days interval. Transplanting was done 30 days after sowing date and seedlings were transplanted individually.

2.3. Testing of pollen fertility/sterility

At initial heading stage ten anthers were taken from spikelets of each line and fixed them in fixing solution and then stained them with 1% I.KT solution. Simultaneously, the single heads of the initial heading were bagged to evaluate the filled spikelet percentage after maturity. The examination of the pollen was done under an optical microscope. All-round and dark stained pollens were scored as normal fertile, while typical irregular–shaped, round yellowish, and light brown colored pollen grain were scored as sterile. Around 200 pollen grains chosen randomly from each slide were evaluated and pollen fertility was expressed in percentage according to this formula: Pollen fertility % = Dark dyed pollen/ total number of counted pollen × 100.

2.4. Determination of seed set rate (%)

Thirty days after start of heading, the bagged and un-bagged of the PTGMS lines were harvested. Seed set rate (%) was determined separately for bagged and un-bagged panicles to count the seed set rate (%) and spikelet fertility (%) as this formula: Seed set rate % = Number of seed set per panicle / total number of glumes per panicle × 100. The correlation coefficients and linear regression using SAS program were counted between the fertile pollen rate and the daily temperature of the period from 15 to 25 days before blooming.

2.5. DNA extraction and validation markers analysis

Genomic DNA was isolated from young leaves from ten plants of each genotype using CTAB method (Murray and Thampson, 1980). The concentration and quality of DNA was assessed with 0.8% agarose gel electrophoresis using diluted uncut lambda phage

Table 1
List of ten PTGMS lines with their sterility source used in the study.

No.	Lines	Rice-type	Sterility-Source
1	PTGMS-3	*Indica*	Pei'ai-64S
2	PTGMS-4	*Indica*	Pei'ai-64S
3	PTGMS-7	*Indica*	Pei'ai-64S
4	PTGMS-10	*Japonica*	Nongken-58S
5	PTGMS-11	*Japonica*	Nongken-58S
6	PTGMS-14	*Japonica*	Nongken-58S
7	PTGMS-20	*Japonica*	Nongken-58S
8	PTGMS-21	*Japonica*	Nongken-58S
9	PTGMS-23	*Japonica*	Nongken-58S
10	PTGMS-38	*Japonica*	Nongken-58S

Table 2
Sensitive Periods of Fertility Alteration Phases of New PTGMS lines under natural daylength and minimum temperature before heading in Sakha, Egypt (2017–2018).

Fertility Alteration Phases	Line	Type	DS (m/d)	Year	DH (m/d)	SP (m/d)	DDL (h/d)	D Min T(° c)	Fertility%	
									PF %	SPF %
Fertility Transformation Period	PTGMS-23	*Japonica*	April 1–29	2017	8/3–8/12	7/9–7/28	13.93	25.21	1.95	1.68
			April 1–29	2018	8/4 – 8/12	7/10–7/25	13.95	25.40	5.38	4.30
	PTGMS-7	*Indica*	April 1–29	2017	7/14–7/29	6/19–7/14	14.13	25.45	2.13	2.19
			April 1–29	2018	7/17–7/31	6/22–7/16	14.01	25.39	1.25	1.62
Sterility Period	PTGMS-23	*Japonica*	May 6–13	2017	8/14–8/16	7/20–8/1	13.78	28.89	0	0
			May 6–13	2018	8/15–8/18	7/21–8/3	13.77	28.51	0	0
	PTGMS-7	*Indica*	May 6–27	2017	8/2–8/14	7/7–7/30	13.94	29.17	0	0
			May 6–20	2018	8/5–8/12	7/11–7/25	13.87	28.83	0	0
	PTGMS-23	*Japonica*	June 3–24	2017	8/22–8/27	7/28–8/12	13.53	24.87	79.32	79.48
Fertility Period			June 3–24	2018	8/23–8/29	7/29–8/14	13.42	22.61	77.74	77.15
	PTGMS-7	*Indica*	June 17–24	2017	8/27–9/2	8/2–8/18	13.33	24.63	73.33	75.28
			June 17–24	2018	8/31–9/6	8/6–8/22	13.21	23.19	72.87	76.23

Note: DS: date of sowing; DH: date of heading; SP: sensitive period; DDL: daily day-length during sensitive period (average); D Min T: daily minimum temperature during sensitive period; PF%: Pollen fertility percentage; SPF%: Spikelet fertility%.

Table 3
Fertility performance of new PTGMS lines under natural conditions in Sakha during summer season of 2017 and 2018.

Line	Type	Year	Sterile period	Day	Observed plants	Sterile plant rate (%)	Sterile pollen (%)	Fertile period
PTGMS-23	*Japonica*	2017	7/20–8/1	14	100	100	95.1–100	7/28–8/12
		2018	7/18–8/3	17	100	100	94.3–100	7/29–8/14
PTGMS-7	*Indica*	2017	7/7–7/30	24	100	98.2	93.6–100	8/2–8/18
		2018	7/11–7/28	18	100	97.0	92.8–100	8/6–8/22

Table 4
Correlation coefficient between pollen, spikelet fertility %, and temperature of the PTGMS lines in Sakha during 2017 and 2018.

Line	Temperature (°C)	Pollen fertility		Spikelet fertility	
		2017	2018	2017	2018
PTGMS-23	DM.T	0.155	−0.111	0.140	−0.117
	DMax.T	0.078	−0.652*	0.067	−0.658*
	DMin.T	0.236	0.660*	0.219	0.658*
PTGMS-7	DM.T	0.084	−0.041	0.072	−0.055
	DMax.T	0.089	0.047	0.086	0.064
	DMin.T	0.064	−0.333	0.089	−0.332

Note: DM.T; daily mean temperature; D Max.T: daily maximum temperature; DMin.T: daily minimum temperature; * and ** significant at 0.05 and 0.01 probability levels, respectively.

Table 5
Correlation coefficient between the pollen and spikelet fertility percentage and daylength of the Egyptian PTGMS.

PTGMS line	Pollen Fertility %		Spikelet fertility %	
	2017	2018	2017	2018
JPTGMS-23	−0.917**	−0.934**	−0.919**	−0.932**
IPTGMS-7	−0.926**	−0.956**	−0.929**	−0.963**

** and * significant at 0.01 and 0.05 probability level.

DNA as size standard and adjusted up-to 50 ng/μl. A polymerase chain reaction (PCR) was conducted in a 20 μl volume consisting of 50 ng of genomic DNA, 0.5 μM of each primer, and 10 μl of 2X GoTaq Green Master Mix (Promega, USA). The PCR amplification was performed using the following reaction program: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, followed by a final extension step at 72 °C for 7 min. The DNA amplification products were analyzed by electrophoresis on 1.5% agarose gels in 1X Tris borate EDTA (TBE) buffer. The DNA was visualized using a UV transilluminator. Co-dominant marker dpms3-54 was used to identify *pms3* gene (Qi et al., 2017), while SSR marker RM5862 was used for validation *tms5* gene (Yang et al., 2007) (Table 6).

3. Results

3.1. Fertility alteration characteristics of the new PTGMS lines under natural daylength and temperature

Data shown in (Tables 2) revealed that during the two years there was a significant difference in the heading stage of the PTGMS lines, which was earlier during the year 2017 than that of year 2018. This difference may be related to the difference of the weather conditions during these two years. The temperature during months of July, August and September was lower during year 2018 than that of year 2017 (Table 2). The results showed that the new PTGMS lines have three sensitive periods of fertility alteration; transformation, sterility, and fertility period (Table 2).

Table 6
Gene specific markers Sequences for Photo-thermosensitive genic male sterility.

Trait	Gene	Marker	Ch.	Primer Sequence	AT (°C)	Band Size	PIC Value
PTGMS	*pms3* (*ptms12-1)*	dpms3-54	12	F-5′-GAATGCCATCTAAACACT-'3 R-5′-ATTTTACTCTTGATGGATGGTC-'3	57	376	0.28
TGMS	*tms5*	RM5862	2	F: 5′-TTAGTACCTCATCATAGCTG-'3 R: 5′-TCTAATCTTCTCTCATTATCA-'3	57	223	0.73

In the transformation period (the pollens changing from fertile to sterile), the PTGMS lines exhibited difference among themselves in this period during the two years. The PTGMS-23 line had transformation period ranged from July 9th to July 28th during year 2017, and it was from 10th to 25th of July during 2018 (Table 2). On the other hand PTGMS-7 line had a transformation period ranged from 19th of June to 14th of July during year 2017, while, it was from 22 of June to 16th of July during year 2018, which, appeared earlier than that of the PTGMS-23 line. The mean daily minimum temperature of fertility transformation was below 30 °C during the year 2017 and below 26 °C during the year 2018. It was ranged from 28.89 °C (PTGMS-23) to 29.17 °C (PTGMS-7) during the year 2017, whereas, it was varied between 25.22 °C (PTGMS-23) to 25.44 °C (PTGMS-7) during year 2018. However, the results showed that the day-length was ranged from 13.93 h to 13.95 h for PTGMS-23 lines, and from 14.07 h to 14.13 h for PTGMS-7 in the fertility transformation period (Table 2). In the sterility period (the pollens were completely abortive) the mean of daily minimum temperature of new PTGMS lines was the lowest (29 °C) during year 2017 and lowest (26 °C) during 2018 (Table 2). There was a little difference between studied lines of daily minimum temperature during the two years. The day length of the sterile period was 13.78 h for PTGMS-23 and over 13.90 h for PTGMS-7 during the two years (Table 2). In the fertility period of the new PTGMS lines the mean daily minimum temperature was below 29 °C for PTGMS-23 line and below 30 °C for PTGMS-7 line during year 2017. While, it was below 26 °C during year 2018, which was higher in PTGMS-23 line than that of PTGMS-7 line (Table 2). The day-length of fertility period of the PTGMS lines was shorter than 13.56 h in the both years, which was ranged from 13.56 h (PTGMS-23) to 13.42 h (PTGMS-7), and from 13.52 h (PTGMS-23) to 13.32 h (PTGMS-7) during 2017 and 2018, respectively (Table 2). This indicated that the influence of low temperature and day-length on fertility alternation of new PTGMS lines was varied in different years and also various among the lines in the same year. Also the results suggested that not only the temperature but also the daylength affected on the fertility alteration characteristics of the PTGMS lines.

3.2. Fertility performance of PTGMS lines during 2017–2018

3.2.1. Sterile stage

Fertility performance of the new PTGMS lines during the two years is presented in Table (3). The results showed that the sterile stage between the PTGMS lines was different during the two years. In the PTGMS-23 line the sterile stage began from July 20th to August 1th during year 2017 and from July 18th to August 3th during year 2018 with sterility period of 14 and 17 days, respectively. However, the PTGMS-7 was the earliest during the two years with sterile stage started from July 7th to July 30th with sterility period of 24 day during 2017 and from July 11th to July 28th with sterility period of 18 days during 2018 (Table 3). Comparing the PTGMS-7 with PTGMS-23, we found a difference between them in the sterility period which was longer of PTGMS-7 (24 and 18 days) than that of PTGMS-23 (14 and 17 days) during the two years, respectively. The results demonstrated that this difference might be related to the temperature and genotype differences.

3.2.2. Fertility stage

According to data shown in (Table 3) there was a little difference in fertile stage between the two PTGMS lines during the two years. Fertile stage of PTGMS-7 line was earlier during year 2017 than that of year 2018. While, fertile stage was the same in PTGMS-23, and was earlier than that of PTGMS-7 line during the two years (Table 3).

3.3. Thermosensitive stage of fertility alteration of new PTGMS lines

To evaluate the thermosensitive stage of the PTGMS lines in this investigation, we estimated the correlation coefficient between pollen and spikelet fertility percentage and daily temperature (mean, maximum and minimum) starting from day 15 to day 25 before heading during the two years (Table 4). The results showed that there was insignificant correlation between fertility and daily temperature of PTGMS lines in 2017. However, the correlation coefficient between fertility (pollen and spikelets) and daily temperature (DMaxT, and DMinT) was significant for *Japonica* PTGMS-23 and insignificant for *Indica* PTGMS-7 in year 2018 (Table 4). According to this analysis, the thermosensitive stage of the *Japonica* and *Indica* PTGMS lines was significantly different during the two years. The Fig. 1 revealed the linear regression relationship between the pollen and spikelet fertility percentage and daily minimum temperature before heading (15–25 days) in PTGMS-23 line during year 17 (Fig. 1, A, B) and year 2018 (Fig. 1, C, D). The results demonstrated that there was a difference due to the effecting of daily minimum temperature on the fertility of PTGMS-23 line at their sensitive phases on the same initial heading date. In terms of PTGMS-7 line, the linear regression relationship between the pollen and spikelet fertility percentage and daily minimum temperature before heading (15–25) was also different during year 17 (Fig. 2, A, B) than that of year 2018 (Fig. 2, C,D). These results revealed that there was no specific critical sterility point (CSP) for thermosensitive stage of those new PTGMS lines but was fluctuation of the temperatures influenced on critical sterility point for thermosensitive stage of those PTGMS lines.

3.4. Photosensitive stage of fertility alteration of new PTGMS lines

In order to evaluate the photosensitive stage of fertility alteration of the new PTGMS lines, the correlation coefficient was also estimated between the pollen and spikelet fertility percentage of the PTGMS lines and the daily daylength from the period 15–25 days before heading during the two years (Table 5). The results suggested that there was a quite significant difference among the PTGMS lines in their photosensitive stage response to the daily daylength during the two years. In order to detect CSP for photosensitive stage of the PTGMS lines, the linear regression relationship between the pollen and spikelet fertility % and daily daylength of PTGMS lines was estimated in the two years. Data showed that in July 20 and July 18, the critical sterility point (CSP) of photosensitive stage of PTGMS-23 line was 13.80 h and 13.84 h during 2017 (Fig. 3, A, B) and 2018 (Fig. 3, C, D), respectively. On the other hand, the PTGMS-7 line had a CSP of the photosensitive stage 14.07 h during 2017 (Fig. 4, A, B) and 14.03 h during 2018 (Fig. 4, C, D). The results revealed that the there was

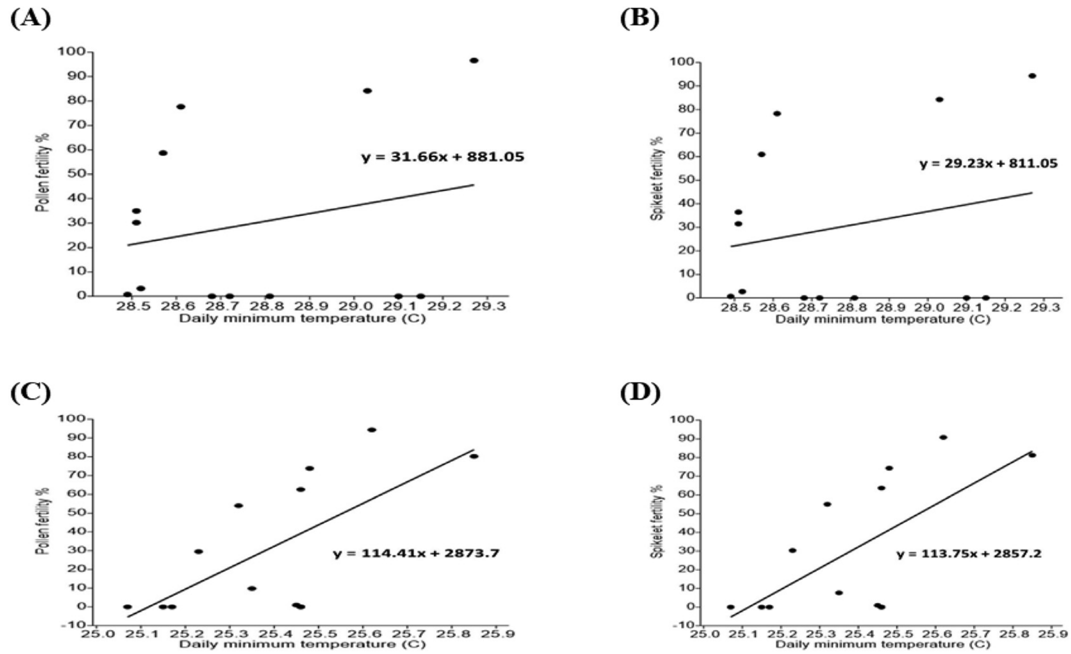

Fig. 1. The linear regression relationship between the pollen and spikelet fertility % and daily minimum temperature of the *Japonica* PTGMS-23 lines during 2017 (A and B) and 2018 (C and D).

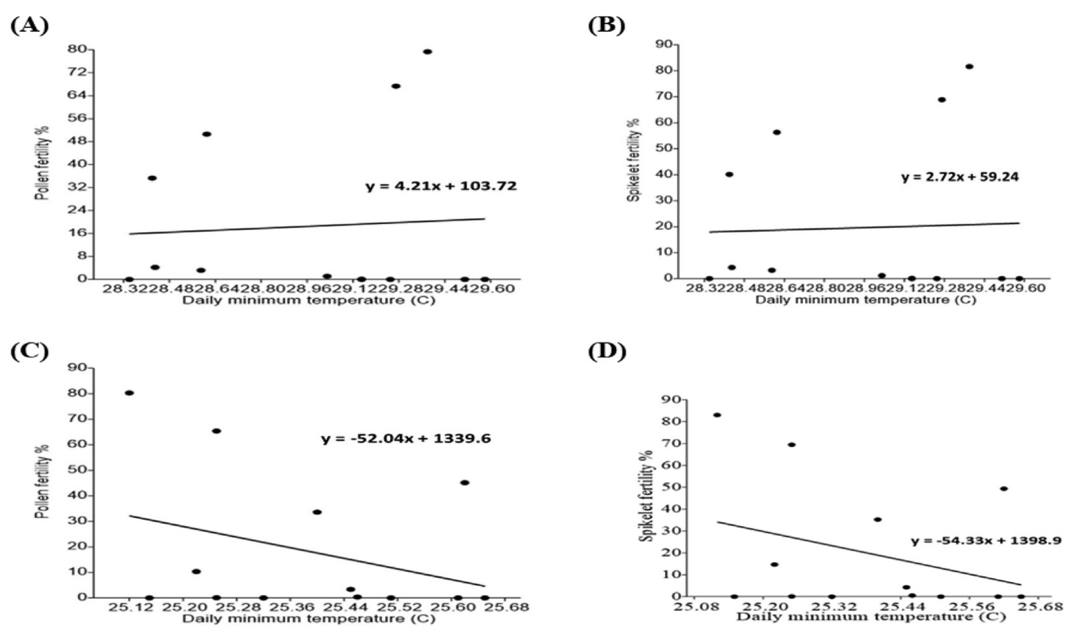

Fig. 2. The linear regression relationship between the pollen and spikelet fertility % and daily minimum temperature of the *Indica* PTGMS-7 lines during 2017 (A and B) and 2018 (C and D).

a significant difference of daily daylength affecting on the PTGMS lines at their photosensitive stage during the two years with different initial heading date. Furthermore, the results suggested that the PTGMS lines have also responded to daylength in their fertility alternative, and the CSP of their photosensitive stage ranged from 13.80 h to 14.07 h.

3.5. Validation markers for sterility genes in PTGMS lines

In order to identify the male sterility genes in the new PTGMS lines, co-dominant dpms3-54 marker (Qi et al., 2017) and RM5862 maker (Yang et al., 2007) were used to identify *pms3* and *tms5* genes, respectively. Data showed that around 400 bp band was identified in tested PTGMS lines using dpms3-54 marker (Fig. 5, upper image). On the other hand, the RM5862 marker recorded around 250 bp in the PTGMS lines (Fig. 5, lower image). Such data indicated that the new PTGMS lines controlled by photo-thermosensitive genes.

4. Discussion

Photo-thermosensitive genetic male sterility is the main resource for 2-line system hybrid rice and plays an essential role in rice heterosis utilization (Normile, 2008; Lin and Yuan, 1979; Cheng et al., 2007; Su et al., 2012; Virmani, 1996; Yuan, 1994a;

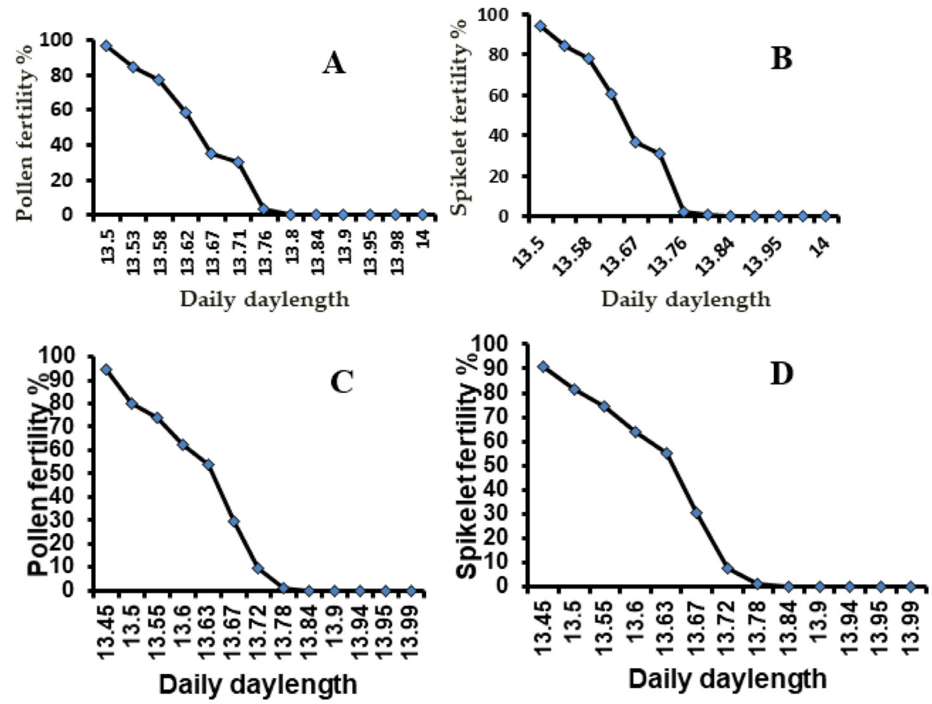

Fig. 3. Linear regression relationship between the pollen and spikelet fertility % and daily day length of the *Japonica* PTGMS-23 lines during 2017 (A, B) and 2018 (C, D).

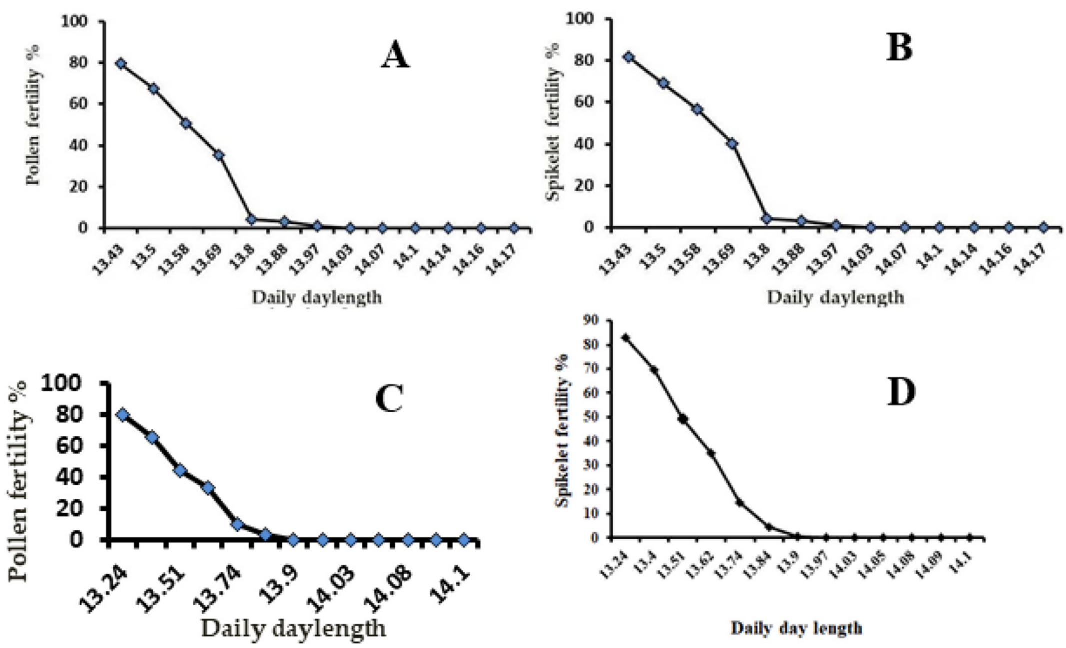

Fig. 4. Linear regression relationship between the pollen and spikelet fertility % and daily day length of the *Indica* PTGMS-7 lines during 2017 (A,B) and 2018 (C,D).

El-Mowafi et al., 2009; Luo et al., 2013; Zhang et al., 2013; Shi, 1985; Yuan, 1993; Huang et al., 2015; Li et al., 2007). The current study revealed that PTGMS-23 and PTGMS-7 lines had fertility alteration characteristics under the natural condition. The new PTGMS lines showed stable sterile under the condition of day length upper 13,75 h and temperature over 25 °C, while, they were fertile under day length below 13 h, and temperature lower than 24 °C. This demonstrated that the fertility alteration of these lines mainly controlled by both photoperiod and temperature; thus, these new genetic male sterile lines belong to the type of PTGMS. It has been reported that the PTGMS lines with low critical sterility points for temperature are required for the purity of hybrid seeds (Lei et al., 2014). There are many of the previously published results about fertility alternation of PTGMS lines derived from Nongken-58S (Zhang et al., 2017; Sun et al., 1991; Tan et al., 2018). The results demonstrated that the effects of temperature factors on pollen fertility differed between *Japonica* PTGMS-23 and *Indica* PTGMS-7 lines and minimum temperature was most important to PTGMS lines. Similarly, it has reported that the longest thermosensitive stage occurred at the daily minimum temperature and daily average temperature (Deng and Fu, 1998; Attia et al., 2001). On the other hand, other previous studies reported

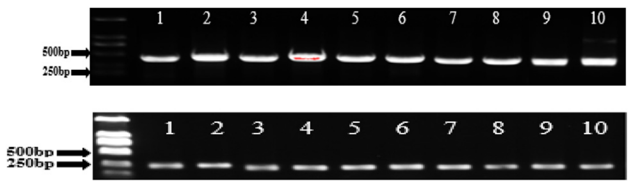

Fig. 5. Validation of *pms3*gene using co-dominant marker dpms3-54 (upper image), and *tms5* gene using SSR marker RMAN7 (lower image) in ten PTGMS lines. Number from 1 to 10 are the PTGMS names according to the Table 1.

that the fertility of PTGMS lines was negatively correlated with the daily mean and minimum temperature of the thermosensitive stage (Shahid et al., 2012; Wu et al., 2015). The current study suggested that the sensitive stage of fertility transformation might be from secondary branch primordial to pollen mother cells (PMC) meiosis. This result is in agreement with previous studies reported that the most sensitive stage of temperature phase inducing fertility was around PMC formation stage (Zhang et al., 1992; Mou et al., 2001; Attia et al., 2003; Yuan et al., 2003; Ali et al., 1995; Yuan, 1990; Si et al., 2011; Subudhi et al., 1997; Wang et al., 2003; Lee et al., 2005; Pitnjam et al., 2008; Qi et al., 2014; Qi et al., 2017; Fan and Zhang, 2018; Muhammad et al., 2020; Yang et al., 2007; Zhou et al., 2014; Zhang and Yang, 2014; Huang et al., 2015; El-Mowafi et al., 2012; Murray and Thampson, 1980; Normile, 2008; Lei et al., 2014; Sun et al., 1991; Tan et al., 2018; Zhang et al., 2017; Deng and Fu, 1998; Attia et al., 2001; Shahid et al., 2012; Wu et al., 2015; Cai et al., 2007). It is possible that at these stages, the temperature changes the mutant activates of some physiological processes, which produce fertile pollen grains instead of sterile pollen grains. Similarly, it was reported that the critical sensitive period in some TGMS lines was in the period of PMC formation to the early monokaryon stage (Chen et al., 1993; Mou et al., 1998). Another study indicated that the stage from secondary rachis-branch and spikelet primordial differentiation to PMC formation in the process of panicle development is the photoperiod sensitive stage of fertility alteration induced (Zhang et al., 1993).

The critical day length for fertility alteration and the intensity of the interaction between photoperiod and temperature are the main factors in controlling the adaptability of aliens for hybrid rice seed production (Yuan, 1987; Guo et al., 2017). The ideal sterile line would have a low critical temperature point for sterility induction, a wide temperature range of photoperiod sensitivity, and a strong supplementary effect between photoperiod and temperature (Zhang et al., 1992). Our finding revealed that under natural Sakha condition, the fertility alteration of the new PTGMS lines was stable sterile under the condition of day length upper 13,75 h with temperature over 25 °C, while its became fertile under day length below 13 h, with temperature lower than 24 °C. These findings indicated that these lines had photo–thermosensitive character statics of their fertility alteration and are suitable for hybrid seed production.

The differences in critical sterility points for photoperiod and temperature are associated with different PTGMS genes background. Many molecular markers have been developed to identify the PTGMS genes (Zhang et al., 1994; Qi et al., 2017; Fan and Zhang, 2018; Muhammad et al., 2020; Yang et al., 2007). In the current study, the PTGMS genes of PTGMS-7 line came from Pei'ai-64S, which carried the *tms5* mutation, that controlling thermosensitive genic male sterility in rice (Zhou et al., 2014). Whereas, the sterility genes of PTGMS-23 line derived from Nongken-58S, which is conferred by *pms3* (Zhou et al., 2012; Ding et al., 2012). Our results showed that the co-dominant marker dpms3-54 identified the *pms3* gene, while the RM5862 marker identified the *tms5* gene in the new PTGMS lines, which indicated that the new PTGMS lines harboring both type of photo-thermo sensitivity genes for sterility induction. These findings were consistent with previous studies reported by many researchers (Ding et al., 2012; Xiao et al., 1997; Zhang et al., 1992; Yuan, 1992; Cheng et al., 1996; Zhang et al., 1992; Mou et al., 2001; Attia et al., 2003; Yuan et al., 2003; Ali et al., 1995; Yuan, 1990; Si et al., 2011; Subudhi et al., 1997; Wang et al., 2003; Lee et al., 2005; Pitnjam et al., 2008; Qi et al., 2014; Qi et al., 2017; Lei et al., 2014; Sun et al., 1991; Tan et al., 2018; Guo et al., 2017; Zhang et al., 1994).

In conclusion, the findings of this investigation manifested that the new Egyptian PTGMS lines are stable sterile lines for hybrid rice seed production for enhancing the rice yield through two-line system hybrid rice.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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