Thoughts and Practice on Some Problems about Research and Application of Two-Line Hybrid Rice

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Abstract: The main problems about research and application of two-line hybrid rice were reviewed, including the confusing nomenclature and male sterile lines classification, the unclear characteristics of photoperiod and temperature responses and the unsuitable site selection for male sterile line and hybrid rice seed production. In order to efficiently and accurately use dual-purpose genic male sterile lines, four types, including PTGMS (photo-thermo-sensitive genic male sterile rice), TGMS (thermo-sensitive genic male sterile rice), reverse PTGMS and reverse TGMS, were proposed. A new idea for explaining the mechanism of sterility in dual-purpose hybrid rice was proposed. The transition from sterile to fertile was involved in the cooperative regulation of major-effect sterile genes and photoperiod and/or temperature sensitive ones. The minor-effect genes with accumulative effect on sterility were important factors that affected the critical temperature of sterility transfer. In order to make better use of dual-purpose lines, the characterization of responses to photoperiod and temperature of PTGMS should be made and the identification method for the characterization of photoperiod and temperature responses of PTGMS should also be put forward. The optimal ecological site for seed production could be determined according to the historical climate data and the requirements for the meteorological conditions during the different periods of seed production.

Key words: two-line hybrid rice; dual-purpose genic male sterile line; photoperiod and temperature characteristics; seed production; seed propagation

Great success has been achieved in the application of two-line hybrid rice in China. Many excellent dual-purpose genic male sterile lines were released by the Provincial Crop Variety Approval Committee and lots of two-line hybrid rice with practical application values were bred over thirty years. To the end of 2007, a total of 78 dual-purpose genic male sterile lines have been applied to production and 243 two-line hybrid rice combinations have been released in various rice-growing regions (Yang et al, 2009). In China, the total plant area of two-line hybrid rice was more than 3 million hectares in 2008, accounting for approximately 25% of the total rice planting area. The planting area of two-line hybrid rice was even larger than three-line hybrid rice in Hunan, Hubei and Anhui provinces in China. The new two-line hybrid rice combinations and the innovation, application and confirmation of two-line hybrid rice technologies will play important roles in bringing a plentiful grain harvest in China.

The seed production of two-line hybrid rice requires strict environmental conditions. Although the two-line hybrid rice technology has already matured, some of the major issues on two-line hybrid rice research and production have not been fully understood yet. In particular, a part of staff in seed companies lack the knowledge of some key issues in application of two-line hybrid rice, which results in some serious problems in recent years (He et al, 2004; Lei et al, 2009). According to incomplete statistics, about 6 700 hectares of two-line hybrid rice failed to seed production because of the continued low temperature (24°C) in Jiangsu, Anhui and Sichuan provinces, China in 2009. The direct economic loss was nearly 100 million Yuan. What’s worse, this further caused the reduction of planting area of two-line hybrid rice combinations for more than 1.3 million hectares in 2010. The indirect economic loss was incalculable. Moreover, the propagation of dual-purpose genic male sterile lines failed in consecutive three years because of abnormal low or high temperatures during the growing season from 2007 to 2009 since there is a narrow difference between the critical sterility and fertility temperatures for dual-purpose genic male sterile lines. This resulted in the reduction of excellent two-line hybrid rice seed
production and a large economic loss. Also, it broke the balance of the hybrid rice seed market. Furthermore, the critical sterility-inducing temperature of dual-purpose genic male sterile lines would gradually increase because of fertility drift or imperfect seed production technology, which seriously affects the purity of two-line hybrid seeds and the safety of seed production (Xiao et al, 2000; Xiao and Zhou, 2000).

The insecurity of two-line hybrid seed production has influenced food security in China. In addition, some of the theories in previous research cannot match with current seed production, and many problems arise in practice, which is difficult to be explained by original theories. Therefore, we should strengthen the basic research on two-line hybrid rice, deepen the analysis of new problems, find solutions to these problems and achieve sustainable development of two-line hybrid rice. The main problems on two-line hybrid rice research and application are described below based on the experience in two-line hybrid rice breeding.

Application and classification of male sterile lines in two-line hybrid rice

At the early stage of the two-line hybrid rice research, the genic male sterile line was named as PGMS (photoperiod-sensitive genic male sterile rice) whatever the source was; further study found that PGMS was not only affected by photoperiod but also by temperature, therefore, it was also named as PTGMS (photo-thermo-sensitive genic male sterile rice). With a more detailed study, it was found that different TGMS (thermo-sensitive genic male sterile rice) lines had different critical sterility-inducing temperatures. These sterile lines with high critical sterility-inducing temperature were called HTGMS, while those with low critical sterility-inducing temperature were called LTGMS. Furthermore, another two completely different types were found, in which one was sterile due to high temperature and the other was sterile induced by low temperature, and they were also called HTGMS and LTGMS, respectively. The name is the same as above but has far different meanings. In addition, the male sterile lines were also called as photoperiod genic male sterile rice, photoperiod sensitive male sterile rice or temperature sensitive male sterile rice and so on (Yuan et al, 1990; Cheng et al, 1996; Xie et al, 1997). These appellations cannot cover the photoperiod and temperature characters of the male sterile lines. So far, the dual-purpose genic male sterile line which is only affected by photoperiod has not been found, so it is inaccurate to name as a photoperiod sensitive male sterile line or a photoperiod genic male sterile rice. Currently, it is found that there are two different types of male sterile lines: long photoperiod and high temperature male sterile line, and short photoperiod and low temperature male sterile line. Thus, it is improper if they are both called photoperiod and temperature sensitive male sterile lines. Therefore, we should regularize the appellation of male sterile line on two-line hybrid rice system. The author considers that ‘dual-purpose genic male sterile line’ is the most appropriate appellation.

However, the dual-purpose genic male sterile line is a general appellation, it is necessary to categorize the dual-purpose genic male sterile lines. We grouped the dual-purpose genic male sterile lines into four types, including PTGMS, TGMS, reverse PTGMS and reverse TGMS (Chen, 2001).

PTGMS and TGMS can be classified by different photoperiods. The difference of the critical fertility transfer temperature under long photoperiod and short photoperiod exceeds 0.5°C, it belongs to PTGMS or reverse PTGMS, otherwise, it is TGMS or reverse TGMS. The classification is based on the demand of regional test, which requires the critical sterility-inducing temperature should not exceed 23.5°C. The critical sterility-inducing temperature of the dual-purpose genic male sterile lines is 24°C if it ascends 0.5°C under short photoperiod conditions, which is safe for seed production in autumn in Guangdong and Guangxi provinces, China.

Sterility mechanism and photo-thermo characters of dual-purpose genic male sterile lines

Sterility mechanism of photoperiod/thermo-sensitive genic male sterility in rice

The sterility mechanism of dual-purpose genic male sterile lines can be divided into two aspects. The first aspect is the transition from sterile to fertile
which is involved in the cooperative regulation of major-effect sterile genes with photoperiod and/or temperature sensitive genes. The so-called pgms gene does not exist in dual-purpose genic male sterile lines. There is only one or two major-effect sterile genes which are like the common nuclear male sterile gene. The sterility character of dual-purpose genic male sterile lines is the result of the cooperative regulation of major-effect sterile genes with photoperiod and/or temperature sensitive genes. The induction of photoperiod and/or temperature sensitive genes to male sterile genes should undergo a process from quantitative changes to qualitative changes, which determines the time of the transition from sterile to fertile. The second aspect is the minor-effect genes, which exhibit accumulative effect for sterility.

**Photo-thermo characters of fertility in dual-purpose genic male sterile lines**

PTGMS is the most complex dual-purpose genic male sterile line because its critical photoperiod and temperature indices are dynamic. Most previous reports indicated that the fertility of PGMS is determined by its critical photoperiod and critical temperature (Zhang et al, 1992). In fact, the critical photoperiod and temperature of sterility transfer is only available on a small region. Numerous studies showed that the fertility of PTGMS was changed by photoperiod and temperature (He et al, 2007). The photo-thermo relationship is just like a U-tube with water: the water on one side will rise when pressure is increased on the other side. The critical sterility-inducing temperature will decrease when the day length is prolonged, otherwise it will slowly increase when the day length gradually shortened. Therefore, the critical sterility-inducing photoperiod and temperature for PTGMS identified in a specific place cannot guide two-line hybrid rice seed production well unless we clearly understand the interaction effect of photoperiod and temperature.

The critical sterility-inducing temperature of TGMS is basically stable, and the day length has no or little impact on its fertility. However, it is affected by daily mean temperature and the duration of low temperature. For example, the critical sterility-inducing temperature of a TGMS line is 23.5°C, and the seed production can not consider to be safe when the daily mean temperature is above 23.5°C during the fertility sensitive period. Usually, the critical sterility-inducing temperature is obtained after the temperature treatment for 3 d or 4 d. In practice, the fertility of TGMS will change if it is subjected to low temperature for more than 4 d, though the low temperature is a little higher than its critical sterility-inducing temperature. Long duration of low temperature is considered to be one of the most important factors that result in the failure of two-line hybrid seed production in Jiangsu Province in 2009. The fertility of TGMS will change if it reaches a certain low temperature accumulation. The more the low temperature accumulation is, the greater change the fertility takes place, which is similar with the propagation of male sterile line. If the range of the optimum fertility temperature for a TGMS is 20–22°C, the fertility rate is not high under the condition of 20–22°C for 3–4 d during the fertility sensitive period, but it will be higher under 20–22°C for more than 10 d. Therefore, we should consider the duration of low temperature on evaluation of the fertility of TGMS.

The fertility of PTGMS was also affected by the duration of low temperature, but the effect is less compared with TGMS because the photoperiod also plays an important role on the fertility of PTGMS. For the reverse PTGMS and reverse TGMS, the principle is the same as PTGMS and TGMS.

**Evaluation on photo-thermo characters of fertility in dual-purpose genic male sterile lines**

The photo-thermo characters of dual-purpose genic male sterile lines, especially some widely used PTGMS, are not understood now, which is the main reason for the failure of two-line hybrid rice seed production. The evaluation of the fertility of PTGMS is under the local long day length condition in most provinces. For production application, it is necessary to combine the photoperiod and temperature on evaluation of the fertility of male sterile lines. There have been some methods for evaluation of the fertility of dual-purpose genic male sterile lines, but most of them cannot connect with actual production. The identification method for the characterization responding
to photoperiod and temperature of P(T)GMS is put forward as follows.

Three photoperiods and six temperatures were designed to evaluate the fertility of PTGMS lines (Table 1). The three photoperiods were 13.67 h, 13.00 h and 11.33 h, which are available for seed production in the central area of China in summer, in Guangdong or Guangxi provinces in autumn and in Hainan Province, China under the short day length, respectively. The six temperatures were 22.0°C, 22.5°C, 23.0°C, 23.5°C, 24.0°C and 26.0°C. The photo-sensitive period of PTGMS starts earlier and ends later than the thermo-sensitive period, therefore, the photoperiod treatment should be conducted earlier than the temperature treatment. The durations of the photoperiod and temperature treatments were 12 d and 6 d, respectively, and the last 6 d photoperiod treatment was overlapped with the temperature treatment.

Three temperatures and three photoperiods were set to evaluate the fertility of TGMS lines. The three temperatures were the critical sterility-inducing temperature, minus and add 0.5°C of the critical sterility-inducing temperature, respectively. The three photoperiods were 3 d, 6 d and 12 d, respectively (Table 1). The detailed treatments were as follows. Three plants in each pot with two duplications were prepared for each treatment. The materials were exposed to low temperature at the fertility sensitive stage (the distance between the pulvinus of flag leaf in the main-clum to the next leaf is about 2 cm), then moved out after the treatment was finished (mark the tillers with the distance between the pulvinus of flag leaf in the main-clum and the next leaf is about 1 cm). The pollen fertility was observed under microscope for continuous three days.

**Propagation of dual-purpose genic male sterile line**

There are three kinds of PTGMS lines: strong, medium and weak photo-sensitive PTGMS lines. The dual-purpose genic male sterile line with strong photo-sensitivity can get high and stable yield in Hainan Province in winter. In practice, the propagation of dual-purpose genic male sterile lines is usually conducted by continuous irrigation with cold water from the reservoir or under natural conditions (Zhou et al, 1993; Luo, 2000; Fu et al, 2004). However, there are many constraints in propagation of dual-purpose genic male sterile lines with cold water. That is, the water temperature and the existing irrigation facilities cannot always meet the propagation requirements. We often use the natural condition to propagate male sterile lines in Hainan Province in winter and some high altitude areas. Since the range of fertile temperature is very narrow (20–22°C), it is difficult to meet the climate condition in propagation. We analyzed the temperature according to the meteorological data in Hainan Province, and found that the safety coefficient for the propagation of PTGMS lines was less than 30% (Xiao et al, 2007).

Then, how to ensure high and stable yield on the propagation of dual-purpose genic male sterile lines? Firstly, we should find more cold water irrigation bases and improve the irrigation conditions. Secondly, we can use the method of main plus ratoon cropping pattern with cold water irrigating, which has been patented by the author. Thirdly, we can propagate dual-purpose genic male sterile lines in Hainan Province or low latitude area and supplemented with cold water irrigation, which can reduce irrigation cold water, expand seed propagation area, save cost and obtain high and stable yield of seed production.

**Seed production of dual-purpose genic male sterile lines**

The critical sterility-inducing temperature for dual-purpose genic male sterile lines can be affected by minor-effect genes. The more minor-effect sterility genes pyramided, the more difficult to stabilize the fertility of dual-purpose genic male sterile lines. When the minor-effect gene is homozygous, the critical sterility-inducing temperature would be lowered and

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<th>Temperature (°C)</th>
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Table 1. The composition of photoperiod and temperature for fertility identification of PTGMS rice lines.
the fertility of dual-purpose genic male sterile lines would be stable (Liao and Yuan, 1996; Tao et al, 2003; Chen et al, 2007). Based on this principle, a new method for seed production of dual-purpose genic male sterile lines was applied. This method can reduce breeding generations and seed production cost, and also guarantee the seed purity. Firstly, select 50 typical plants from the basic seed production field and grow in 50 lines. Choose 6 plants (2 pots, 3 plants of each pot) from each line and move them to an artificial climate chamber or water temperature procession system (the temperature should be 0.2°C lower than the critical sterility-inducing temperature of the dual-purpose genic male sterile line) at the young panicle differentiation IV stage. Move the materials to natural condition and grow in 50 lines after 5 d treatment. Secondly, observe the pollen fertility rate under the microscope for three days and eliminate the lines with low fertility; regenerate the high fertility lines and self-fertilized seeds can be obtained by consecutively irrigation with cold water using the temperature procession system. Finally, harvest the standard seeds in a mixture, and these seeds can be multiplied to the parent seed used in the two-line hybrid seed production.

**Choosing the best time and place for seed production**

Although great progress on two-line male sterile lines and hybrid rice breeding have been achieved in China, however, the breeders pay little attention on two-line hybrid rice seed production, especially for selection of suitable ecological region for seed production.

The selection of the best site for seed production plays an important role in ensuring the safety and high purity of hybrid seed production. Usually, breeders determine the critical sterility-inducing temperature of TGMS lines according to the weather conditions, and arrange hybrid rice seed production at local place when the hybrid combinations are bred. In fact, this way is not scientific. If the place for two-line hybrid rice seed production is arranged in Guangdong, Guangxi, Fujian and the south of Hunan and Jiangxi provinces, China, where the average temperature is usually higher than 23.5°C in summers and autumns, then the security of two-line hybrid rice seed production would be greatly enhanced. In addition, it is also demanded that the unusual high temperature, continuously low temperature and rainy weather do not occur during the flowering period (Chen, 2007; Chen et al, 2007; Ding et al, 2008).

The climate decision-making support system was designed according to the 50 years climate data and the historical meteorological requirements of two-line hybrid seed production (Lei et al, 2009). It indicated that the safety of two-line hybrid seed production has a great difference between different regions. For example, the dual-purpose genic male sterile lines with the critical sterility-inducing temperature of 23°C should be arranged in the early August in Jiangsu Province, China, and those with the critical sterility-inducing temperature of 24°C can be arranged from early July to the end of August in Baishe City, Guangxi Province, China. However, it is hard to find a safe area for two-line hybrid rice seed production in Mianyang City, Sichuan Province, China, even the critical sterility-inducing temperature is 22°C. Therefore, to improve the security of two-line hybrid rice seed production, we should strengthen new male sterile line breeding and breed elite dual-purpose genic male sterile lines with low critical sterility-inducing temperature, so as to widen the selection range of seed production area. Moreover, the scientific selection of hybrid seed production place and reasonable layout of the production schedule would minimize the risk of hybrid seed production and increase the economic efficiency.

**Recommendations**

**Increase financial input on research and application of two-line hybrid rice**

The two-line hybrid rice technology is a key scientific achievement at the advanced stage worldwide, and it is also the most effective technical approach to ensure national food security and exhibit high ratio of output to input among the national scientific research. So, it is necessary to increase the investment and set up major scientific projects for collaborative research. The following aspects about two-line hybrid rice research should be considered: applied and basic
research on two-line hybrid rice; breeding of safe dual-purpose genic male sterile lines; the method for safety, high yield and economic efficiency of hybrid seed production and the techniques for high and stable yield of seed multiplication and basic seed production.

According to the result of screening the best seed production and propagation places, more than $3.3 \times 10^4$ hm$^2$ of two-line hybrid rice seed production base and more than 330 hm$^2$ dual-purpose genic male sterile line propagation base were suggested. The government should invest in the construction of seed production base and it can be implemented by the research institutes or company which has male sterile lines widely used in the production.

**Stricter certification procedures and standards on dual-purpose genic male sterile lines**

The dual-purpose genic male sterile line is the key factor for the development of two-line hybrid rice. Nowadays, only a few provinces in China request the dual-purpose genic male sterile lines to pass the regional test. We recommend that our government should organize experts to formulate certification procedures and standards on the dual-purpose genic male sterile lines. The dual-purpose genic male sterile lines are suggested to pass the national regional test before their hybrid combinations.

**Comprehensively identify photo-thermo characteristics of dual-purpose genic male sterile lines**

A long-run mechanism for monitoring the fertility change and seed quality of dual-purpose genic male sterile lines should be established. It’s necessary to have a comprehensive authentication for the photo-thermo characteristics of dual-purpose genic male sterile lines used in the production. Also, it is needed to randomly identify the photo-thermo characteristics of dual-purpose genic male sterile lines in the production. Some large seed companies can complete this work by themselves, and other companies are suggested to send samples to research institutions to complete identification.

**ACKNOWLEDGEMENTS**

This study was supported by the National High Technology Research and Development Program of (Grant No. 2010AA101304); the Transformation Fund for Agricultural Science and Technology Achievements (Grant No. 2007GB2D200226); the Natural Science Foundation of Hunan Province, China (Grant No. 10JJ4012).

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