Advances in Two-Line Heterosis Breeding in Rice via the Temperature-Sensitive Genetic Male Sterility System

Jauhar Ali, Madonna Dela Paz, and Christian John Robiso

Abstract Hybrid rice technology is a viable strategy to increase rice production and productivity, especially in countries with limited cultivable land for agriculture and irrigation water, along with costlier chemical inputs. The three-line hybrid rice technology adoption rate is slowing down because of restricted heterosis per se, the availability of better combining ability in cytoplasmic male sterile lines, lower hybrid seed reproducibility, and limited market acceptability of hybrids. Two-line heterosis breeding could overcome these shortcomings. However, the wide-scale adoption and use of two-line hybrid rice technology are possible through systematic research and breeding efforts to develop temperature-sensitive genetic male sterile (TGMS) lines with low (<24 °C) critical sterility temperature point, which is discussed in this chapter. Research on the genetics, breeding, grain quality, and resistance to insect pests and diseases for TGMS line development and physiological characterization is also discussed. In addition, the identification and validation of natural sites for TGMS self-seed multiplication and hybrid rice seed production through GIS mapping and climatic data analytical tools are also tackled. The development of high-yielding two-line rice hybrids and improvement in hybrid rice seed reproducibility could help in their wide-scale adoption.

Keywords Temperature-sensitive genetic male sterile (TGMS) lines · Critical sterility temperature point (CSTP) · Physiological characterization · Genetics · Rice

J. Ali (✉) · M. Dela Paz · C. J. Robiso
Rice Breeding Platform, International Rice Research Institute, Los Baños, Philippines
e-mail: j.ali@irri.org

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1 Introduction

Global rice production in 2018 was 782 million tons from 167.1 million hectares with an average productivity of 4.68 t/ha (FAOSTAT 2020). However, production needs to keep pace with the increasing food demand in the coming decades, especially when the global human population is predicted to reach 9.73 billion by 2050 (Worldometer 2020). Increasing rice production under declining resources such as cultivable land and irrigation water and costlier agricultural inputs will become a great challenge in the coming decades. Furthermore, climate change is going to increase the pressure on stable and sustainable rice production.

Hybrid rice technology is a viable approach to increase rice production under limited resources and climate change. This technology took roots as early as 1964 in China, and around the same time, international scientific communities were discussing its prospects, especially in India, the United States, and the Philippines (Carnahan et al. 1972; Swaminathan et al. 1972; Athwal and Virmani 1972). However, it was China under Professor Yuan Longping that demonstrated hybrid rice technology on a commercial scale in 1976 with requisite cytoplasmic male sterile (CMS), maintainer, and restorer lines. This early success led China to expand hybrid rice significantly to reach 16.7 million ha, accounting for 57% of the country's rice area. Hybrid rice now accounts for more than 65% of China's total national rice production. In recent years, the average productivity of rice in China has been 6.45 t/ha: 7.50 t/ha for hybrid rice and 6.15 t/ha for conventional rice. The increased production of hybrid rice each year provides food for more than 70 million people (Yuan 2014). The International Rice Research Institute (IRRI) made a significant effort to deploy hybrid rice technology outside China by sharing the requisite hybrid rice parental lines directly to both the public and private sectors. Parental lines developed by IRRI have been used quite extensively in the release of several commercial hybrids from the private and public arenas in India, Nepal, Pakistan, Vietnam, the Philippines, Bangladesh, and Indonesia. IRRI has directly released 17 hybrids in the Philippines alone.

Despite the enormous research and extension efforts that have gone into hybrid rice from the early 1990s, especially in Asia, hybrid rice area is growing slowly. Among the major reasons for the slow growth is, first, the available level of heterosis or hybrid rice yield advantage over the best checks is from 15% to 20%. Second, hybrid rice seed reproducibility is still below 2 t/ha for most hybrids outside China, besides being cumbersome and expensive, which is not attractive to the private seed industry to adopt the technology on a wide scale. Third, hybrids do not possess the required amount of disease and insect pest resistance in the target regions. Fourth, the grain quality of hybrids does not meet market needs, and decreased head rice recovery is keeping farmers from adopting hybrid rice. In addition, the rapid rise in labor wages in India and China is causing the seed industry to look for alternative approaches to decrease the cost of hybrid rice seed and make it more efficient based on parental line improvement to entice farmers to adopt hybrid rice and benefit. In this regard, the Hybrid Rice Development Consortium (HRDC) at IRRI is consider-

ing these factors and developing market-oriented parental materials. Ongoing hybrid rice research at IRRI seeks to improve the levels of outcrossing and hybrid seed reproducibility, especially by developing newer CMS lines. The HRDC has been sharing these improved materials with both the public and private sectors in an aggressive manner since 2016. Currently, the area of hybrid rice outside China is approximately 8 million ha, and pushing hybrid rice technology is vital to overcome its shortcomings. In this context, it is crucial to revisit other alternative technologies such as two-line hybrid rice technology for efficient seed production and increased heterosis.

2 The Emergence of Two-Line Hybrid Rice Technology with a Historical Perspective

Two-line hybrid breeding began with the discovery of a photoperiod-sensitive genic male sterile (PGMS) mutant, Nongken 58S, in Hubei Province, China, which remains male sterile under long-day conditions (>13.45 h) or fertile under shorter day (<13 h) conditions (Shi 1981, 1985; Shi and Deng 1986). Likewise, the discovery of thermosensitive genic male sterility (TGMS) that renders the plant male sterile at higher mean temperatures and reverts it to fertility at lower mean temperatures allowed significant development of the technology. Several TGMS sources of spontaneous or induced origin were discovered such as Annong S-1 and Anxiang S (Tan et al. 1990; Lu et al. 1994) in China, Norin PL 12 (Maruyama et al. 1990, 1991) in Japan, IR32364 at IRRI (Virmani and Voc 1991), and SM 5, F61, and SA 2 in India (Ali 1993; Ali et al. 1995; Hussain et al. 2012; Reddy et al. 2000) (Table 1). Moreover, photo-thermosensitive genic male sterility systems were also discovered, for which researchers found the interaction of photoperiod and temperature that governs male sterility-fertility alteration. Based on these three male sterility-fertility alteration systems involving photoperiod, temperature, and photo-thermo interactions, Yuan (1987) put forward a new strategy of hybrid rice breeding that did not involve a maintainer line, and it was called the two-line method. Any fertile line with a dominant gene for this trait could be used as a pollen parent to develop rice hybrids (Lu et al. 1994). Two-line hybrid rice technology has several advantages over the three-line system, including a wider range of germplasm resources as pollen parents, thus allowing opportunities to exploit higher heterosis and simpler procedures for breeding and hybrid seed production (Ali et al. 2018; Chen et al. 2020).

In tropical conditions, day length differences are marginal, and therefore, the TGMS system is more useful than the PGMS and PTGMS systems. Consistent temperature differences are found at different altitudes and over different seasons in the same location or region, which could be exploited for two-line hybrid rice development. However, successful exploitation of this novel male sterility system relies on knowledge of the fertility behavior of TGMS lines (Chandirakala et al. 2008).

Table 1 Origin and fertility-sterility transformation behavior of photoperiod-thermosensitive and temperature-sensitive male sterile sources in rice

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
Photoperiod-thermosensitive genic male sterile (PTGMS) (interaction of h and °C)								
02428 S	–	NK58S	JAAS					Li (2009)
108 S	–	NK58S/9022	NAAS					Li (2009)
1541 S	L-*japonica*		YIAS	13.75–14.00	28.0	22.0		Lu et al. (1994); Li (2009)
1647 S	–		BAAS					Li (2009)
2177 S	*indica*		AGAI					
26 Zhai Zao	*indica*	Induced (R), China		12.00–14.00	23.0–25.0			Shen et al. (1994)
3008 S	*japonica*	NK58S/	HAC					Li (2009)
31111 S	L-*japonica*	NK58S/31111	HAU	14.00–14.75	28.0	22.0		Li (2009)
31301-1S					28.0	24.0		Zhang et al. (1994)
3502 S	L-*japonica*	7001S/Pecos	AAAS	14	22.6			Li (2009)
3516 S	L-*japonica*	N5047S/(7001S/Zhao107)	AAAS	14	23.5			Li (2009)
4008 S	L-*japonica*	7001S/Reyan 2	AAAS	14	24.0			Li (2009)
5021 S	–	SDL of MS type. S.mutant	NAU and JAAS					Li (2009)
5047 S					30.0	26.0		Zhang et al. (1994)
6334 S	L-*japonica*		HNU	13.75–14.00	24.0–30.0			Li (2009)
7001 S	E-*japonica*	NK58S/917 (HuXuan19/IR661//C57)	AAAS	13.50–14.0	30.0	22.0		Lu and Wang (1988); Lu et al. (1994); Zhang et al. (1994); Mou et al. (2003); Li (2009)
8087 S	E-*japonica*	7001S/Zhao 107	AAAS	14	23.0			Li (2009)
8801 S	*indica*		HXAU					

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
8902 S	*indica*		WU	13.25–13.45	27.0–30.0			
8906 S	*indica*		WU	13.25–13.45	26.0	24.0		Zhang et al. (1994)
89-7S					30.0	24.0		Zhang et al. (1994)
8912 S	*indica*		WU	13.25–13.45	30.0	26.0		Zhang et al. (1994)
9044 S	*japonica*		HAA		32	28.0		Zhang et al. (1994)
916 S	–	NK58S	JAAS					Li (2009)
AB0195	*japonica*		WDAU					
C407S	*japonica*	Eyi MR	CAAS					Li (2009)
CIS 28-10S	*indica*	S. mutant, China		12.00–14.00				Huang and Zhang (1991)
Double 8-2S	L-*japonica*	NK58S/Double 8–2	WU	14.00–14.25				Li (2009)
EGMS	*japonica*	Induced (C), USA		13.00–14.00				Rutger and Schaeffer (1989)
HN5-2S	*indica*				24.0	24.0		Zhang et al. (1994)
HS-1	*indica*	HPGMR	FU					Lu et al. (1994)
HS-3	*indica*	NK58S	FAU	12.5	23.0[c]			Mou et al. (2003)
J-3S	–	NK58S	JAAS					Li (2009)
K14 S	*indica*		GAU					
K7 S	*indica*		GAU					
K9 S	*indica*		GAU					
Liuqianxin S	–	NK58S	JAAS					Li (2009)
M 201	*japonica*	Induced (C), USA		12.00–14.00				Oard and Hu (1995)
M105 S	–	60 Coγ radiating 105	WU					Li (2009)

(continued)

Table 1 (continued)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
M901 S	*indica*				26.0	24.0		
MSr 54A(B)	*japonica*	S. mutant, China		13.00–14.00				Lu and Wang (1988)
N422 S	*japonica*	7001S/lun hui 422	HHRRC, CAU					Li (2009)
N5047 S	L-*japonica*	NK58S/5047	HAAS	14.00–14.25	26.0–30.0			Lu et al. (1994); Li (2009)
N5088 S	L-*japonica*	NK58S/Nonghu26	HAAS	13.50–14.00	22.0–30.0	22.0		Zhang et al. (1994); Lu et al. (1994)
N95076 S	L-*japonica*	5088S/7001S	HAAS		24.0			Li (2009)
N9643 S	L-*japonica*	NK58S/9643	HAAS	>14.00	24.0			Li (2009)
Nongken58S	L-*japonica*	S. mutant from NK58, China	Hubei	13.75–14.00	30.0	24.0		Shi and Deng (1986); Zhang et al. (1994)
Pei'ai64S	*indica*	NK58S-derived, China	HHRRC	13.00–13.30	24.0	22.0		Yang et al. (2002)
Shuanggung S	*japonica*		HHRRC		32.0	28.0		Zhang et al. (1994)
Shuguang612S	*indica*	NK58S	SAU	12.5	23.5[c]			Lu et al. (1994) (T), Mou et al. (2003) (P)
W6154 S	*indica*		HAAS	13.00–13.30	26.0	24.0		
W7415 S	*indica*		HAAS	13.00–13.30	26.0	24.0		
W91607S					26.0	24.0		Zhang et al. (1994)
W9593 S	*indica*	NK58S	HAAS	13	23.5[c]			Mou et al. (2003)
WD 1S	L-*japonica*	NK58S/WD1	WU	14.00–14.50				Li (2009)
Wuxiang S (WXS)	*indica*							
X 88	*japonica*			>13.75			10–25	Lu et al. (1994)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
Zhenong 1S	L-*japonica*	NK58S	ZAAS					Li (2009)
Temperature-sensitive male sterile (TGMS) (°C)								
9201	*indica*	560 S	FU					Lu et al. (1994)
1103 S	*indica*	HPGMR	WU					Lu et al. (1994)
1356 S	*indica*	Annong S-1	HHRR	–	24.5[c]			Mou et al. (2003)
3418 S	*indica*	HPGMR	AAAS					Lu et al. (1994)
545 S	*indica*		Hunan					
5460 S	*indica*	Induced (R), China	Fujian		28.0–26.0			Yang et al. (1990)
6442S	*indica*	HPGMR	JAAS					Lu et al. (1994)
810 S	*indica*	AnnongS-1	AJAU,	–	24.0[c]			Mou et al. (2003)
Annong S-1	*indica*	S. mutant	Hunan		30.2–27.0			Tan et al. (1990)
Anxiang S	*indica*	Annong S	HHRRC					Lu et al. (1994)
ATG-1	*indica*							
C815 S	*indica*							
Dianxin 1A	*japonica*	CMS	Yunan		20.0–23.0			Lu et al. (1994)
DRR 1S			DRR		30.0			Ramakrishna et al. (2006)
F 61	*indica*	Induced mutation (C) India	IARI		22.0–30.9		19	Ali et al. (1995)
GD 2S	*indica*	HPGMR	GDAAS					Lu et al. (1994)
Guangzhan63S	*indica*	NK58S-derived, China						
H 89-1	*japonica*	Induced (R), Japan			31.0–28.0			Maruyama et al. (1991)

(continued)

Table 1 (continued)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
Hengnong S-1	*indica*	Cross breeding, China	Hunan		29.0–30.0			Lu et al. (1994)
ID24					29.5–25.9		10–14	Sanchez and Virmani (2005)
IR32364-20-1-3-2B	*indica*	Induced (R), IRRI	IRRI		32.0–24.0			Virmani and Voc (1991)
IR38949	*indica*	Introgression from Norin PL12	IRRI		30.0–24.0			Virmani (1992)
IR68298		Introgression from Norin PL12	IRRI		31.5–27.1		11–17	Sanchez and Virmani (2005)
IR68935		Introgression from Norin PL12	IRRI		32.4–27.7		5–14	Sanchez and Virmani (2005)
IR68945	*indica*	Introgression from Norin PL12	IRRI		30.0–24.0		15–21	Virmani (1992); Sanchez and Virmani (2005)
IR71018		Introgression from Norin PL12	IRRI		32.2–27.4		12–24	Sanchez and Virmani (2005)
IR73827-23S			IRRI		35.9		19	Ramakrishna et al. (2006)
IR72093		Introgression from Norin PL12	IRRI		30.4–26.3		8–16	Sanchez and Virmani (2005)
IV A	*indica*	Cross breeding, China			24.0–28.0			Zhang et al. (1991)
J207S	*indica*	S. mutant, China			31.0- > 31.0			Jia et al. (2001)
JP 2	*indica*	S. mutant, India	IARI		23.0–33.9		19	Ali et al. (1995)
JP 24A	*indica*	CMS, India	IARI		23.0–33.8			Ali (1993)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
JP 8-1A-12	*indica*	Breeding population, India	IARI		20.0–30.9	20.0–24.0	23	Ali et al. (1995)
KS 1S	*indica*	HPGMR	GAAS					Lu et al. (1994)
N8 S	*indica*		Hunan					
Norin PL12	*japonica*	Irradiation with 20 kr of gamma rays, Japan			21.4–29.4		9–16	Lopez et al. (2000); Sanchez and Virmani (2005)
R 59TS	*indica*	Induced (R), China	Fujian					Yang et al. (1990)
SA 2	*indica*	Induced mutation (C) India	IARI		20.0–31.7		17	Ali et al. (1995)
SE21S	*indica*	NK58S	FAU	–	23.0[c]			Mou et al. (2003)
SM 3	*indica*	S. mutant, India			22.0–32.0		22	Ali et al. (1995)
SM 5	*indica*	S. mutant, India			22.0–32.3		24	Ali et al. (1995)
TianfengS	*indica*	AnnongS-1	GZAAS,	–	24.5 c			Mou et al. (2003)
TGMS 74S	*indica*		TNAU		34.2	23.0		Rajesh et al. (2017)
TGMS 81S	*indica*		TNAU		32.9	24.2		Rajesh et al. (2017)
TGMS 82S	*indica*		TNAU		32.9	24.2		Rajesh et al. (2017)
TGMS 91S	*indica*		TNAU		34.2	22.7		Rajesh et al. (2017)
TGMS 92S	*indica*		TNAU		34.2	24.2		Rajesh et al. (2017)
TGMS 93S	*indica*		TNAU		34.2	24.2		Rajesh et al. (2017)
TGMS 94S	*indica*		TNAU		34.2	24.2		Rajesh et al. (2017)
TNAU 19S	*indica*		TNAU		20.0–30.0	24.0–26.0		Manonmani et al. (2016)
TNAU 27S	*indica*		TNAU		25.95	25.83	26-Jan	Sasikala et al. (2015)

(continued)

Table 1 (continued)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
TNAU 39S	*indica*		TNAU		20.0–30.0	24.0–26.0		Manonmani et al. (2016); Kadirimangalam et al. (2017)
TNAU 45S	*indica*		TNAU		20.0–30.0	24.0–26.0		Manonmani et al. (2016); Kadirimangalam et al. (2017)
TNAU 60S	*indica*		TNAU		20.0–30.0	24.0–26.0		Manonmani et al. (2016); Kadirimangalam et al. (2017)
TNAU 95S	*indica*		TNAU		20.0–30.0	24.0–26.0		Manonmani et al. (2016); Kadirimangalam et al. (2017)
TS 09 12	*indica*		TNAU		26.45	25.78	26-Jan	Sasikala et al. (2015)
TS 09 15	*indica*		TNAU		25.80	25.45	26-Jan	Sasikala et al. (2015)
TS 09 25	*indica*		TNAU		26.73	26.58	26-Jan	Sasikala et al. (2015)
TS6	*indica*	Spontaneous mutant	TNAU		26.7	25.5		Latha et al. (2005)
TS16 (IR68945-433-4-14)	*indica*	Norin PL 12	IRRI		24.8	24.6	11-Jan	Latha et al. (2005, 2010)
TS18 (IR68949-11-5-31)	*indica*	Norin PL 12	IRRI		24.2	24.0	18-Jan	Latha et al. (2005, 2010)
TS29	*indica*	Spontaneous mutant	TNAU		25.6	25.3	11-Jan	Latha et al. (2005, 2010)
TS46 (IR68942-1-6-13-13-4)	*indica*	Norin PL 12	IRRI		25.4	25.3	26-Jan	Latha et al. (2005, 2010)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
TS47 (IR68298-11-16-3 B)	*indica*	Norin PL 12	IRRI		35.3	25.2	26-Jan	Latha et al. (2005, 2010)
W6111 S	*indica*		Hubei					
W91607 S	*indica*	HPGMR	HAAS					Lu et al. (1994)
W9451 S	*indica*	HPGMR	HAAS					Lu et al. (1994)
Xiang125S	*indica*	Annong S-1	HHRRC	–	23.5[c]			Mou et al. (2003)
Xiangling628S	*indica*							
Xianquang	*indica*	Breeding population, China			24.0–30.0			Cheng et al. (1995)
XinanS	*indica*							Si et al. (2012)
Y58S	*indica*							
Zhu1S	*indica*							Yang et al. (2000)
Reverse thermo-sensitive genic male sterile (rTGMS)								
JP38	*indica*	S. mutant, India	IARI		24.0–30.5			Ali (1993)
Dianxin 1A	*japonica*	China	Yunnan		22[c]			Yiming (1988); Zhang et al. (1991)
IVA	*indica*	China	Yunnan		24[c]	27.0		
26 Zhaizao	*india*	Mutant, China			>23[c]			Shen et al. (1994)
J207S								Jia et al. (2001)
Reverse photoperiod-sensitive genic male sterile (rPGMS)								
YiD1S		B3/Hongjiang	China					Gao (1991)

(continued)

Table 1 (continued)

Source	Ecotype	Origin of gene	Place of development	Critical temp. and photoperiod for inducing sterility		CFTP	Sensitive stage (days before heading)	References
				(h)	(°C)	(°C)		
IVA	*indica*	From cross breeding	Yunnan					Zhang et al. (1991); Virmani et al. (2003)
N10S								Li et al. (1991)
N13S								Li et al. (1991)
go543S								Yang and Zhu (1996)
DiannongS-2								Jiang et al. (1997)
D38S								Joseph et al. (2011)
D52S								Joseph et al. (2011)

GAAS Guangxi Academy of Agricultural Sciences, *GDAAS* Guangdong Academy of Agricultural Sciences, *GZAAS* Gangzhou Academy of Agricultural Sciences, *TNAU* Tamil Nadu Agricultural University, *HAAS* Hubei Academy of Agricultural Sciences, *JAAS* Jiangxi Academy of Agricultural Sciences, *AAAS* Anhui Academy of Agricultural Sciences, *HHRRC* Hunan Hybrid Rice Research Center, *IARI* Indian Agricultural Research Institute, *WU* Wuhan University, *SAU* Sichuan Agricultural University, *FU* Fujian University, *FAU* Fujian Agricultural University, *GAU* Guangxi Agricultural University, *AJAU* An-Jiang Agricultural University, *HAU* Huazhong Agricultural University, *HAC* Hubei Agricultural College, *AGAI* Anhui Guangde Agricultural Institute, *YIAS* Yichang Institute of Agricultural Sciences, *ZAAS* Zheijiang Academy of Agricultural Sciences, *L* late, *E* early, *S* spontaneous, *SDL* short day length, *CDL* critical day length, *CSP* critical sterility point, *CFP* critical fertility point, *R* irradiation, *C* chemical mutagens. Several introgressed forms from Nongken 58S and Annong S-1 developed by Yang (1997) and Mou et al. (1998) not included here

In this regard, to address tropical Asian markets, IRRI is refocused on developing two-line hybrid rice technology with usable TGMS parental lines. The two-line hybrid rice approach via TGMS holds great promise as it does away with one step of outcrossing of parental line production, thus directly bringing down seed costs. Although the two-line system is well established, especially in Vietnam and the Philippines, expansion to other regions remains a challenge because of the lack of TGMS lines with a low critical sterility temperature point (CSTP) of 24 °C. Such low CSTP of TGMS lines could be a game changer in tropical Asia vis-à-vis earlier discovered TGMS lines with CSTP of >27 °C. Currently, the annual planting area of two-line hybrid rice in China has surpassed 5 million ha, while fully exploiting heterosis in rice (Chen et al. 2020). With recent research advances, TGMS-based two-line hybrid rice breeding is poised to replace three-line hybrid rice technology over the next decade (Ali et al. 2018).

3 Advantages and Disadvantages of the TGMS System in the Tropics

The TGMS-based two-line system has several advantages over the three-line system. First, hybrid seed production is less cumbersome as TGMS does not require maintainers and seed can be self-multiplied under fertility-conducive low-tempera-

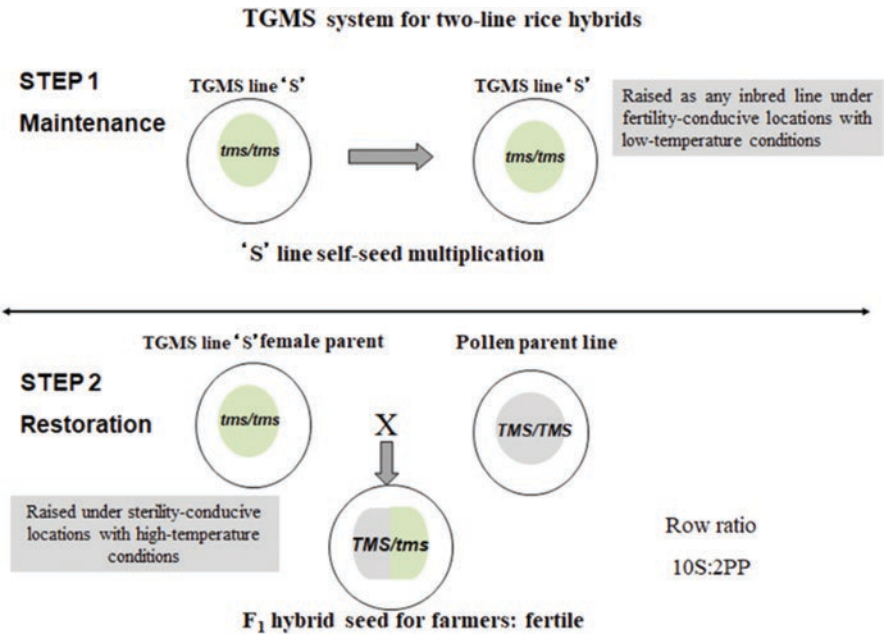

Fig. 1 TGMS system for the production of two-line rice hybrids

ture conditions (Fig. 1). Second, there is a higher probability of identifying the heterotic pool and market-oriented hybrids, as any nonTGMS parent is a potential pollen parent. Third, the current CMS three-line system is primarily based on a single source of wild abortive (WA) cytoplasm that continues to pose a constant threat because of the adverse effects associated with it. However, the two-line approach also has certain shortcomings, such as the adverse effect of low-temperature fluctuations due to sudden/unforeseen weather changes that could trigger self-seeds in hybrid seed production plots. In addition, the higher temperature fluctuations in self-seed multiplication plots could result in lower self-seed yields of the TGMS lines. Therefore, the right choice of locations based on historical agrometeorological data is essential to identify ideal places for hybrid rice seed production and self-seed multiplication.

4 Physiological Characterization of the TGMS Trait

Homozygous and true-breeding TGMS lines need to be physiologically characterized, especially for CSTP and CFTP, besides determining the temperature-sensitive stage for sterility-fertility alteration. The deployment of TGMS lines needs to match the target location requirements. Furthermore, precise information on these two indices is essential for choosing an appropriate source for the development of two-line hybrids (Ali et al. 1995).

4.1 Determination of CSTP and CFTP

The determination of CSTP and CFTP is essential for characterizing TGMS lines for their proper exploitation in target regions. CSTP pertains to the lowest mean temperature among the temperatures inducing sterility, while the highest mean temperature causing fertility is considered as the CFTP (Chandirakala et al. 2008; Latha and Thiyagarajan 2010; Sasikala et al. 2015; Kadirimangalam et al. 2017). The tracking technique (Ali et al. 1995) was used to identify the CSTP and CFTP based on the sensitive stage of a line. Using this method, the CSTP is determined by obtaining the lowest among the maximum temperatures of the three tracking dates coinciding with the sensitive stage of the three panicles that caused complete pollen sterility. At the same time, the CFTP is the temperature range in which the plants produced a higher proportion of fertile and unaborted sterile (partially stained) pollen. Further studies by Vinodhini et al. (2019) considered the lowest value of the mean maximum temperature during the sensitive stage to determine the CSTP of a TGMS line. Viraktamath and Virmani (2001) proved that the maximum temperature is what influences the expression of fertility-sterility alteration of TGMS lines in tropical countries. Moreover, Kadirimangalam et al. (2017) identified TGMS lines with a CSTP at a mean temperature of above 29 °C. It is essential to understand that

a given TGMS gene varies for its CSTP and CFTP when transferred to different genetic backgrounds (Sasikala et al. 2015). The fertility of PTGMS rice lines is affected by both temperature and light duration. Usually, PTGMS rice lines tend to produce low purity of hybrid seeds because of selfing at a low temperature (23–24 °C) in seed production. The spikelets of PTGMS lines during anthesis could not normally open at high temperature (HT, ≥35 °C), thereby severely decreasing hybrid seed yields (Chen et al. 2020). This, along with other factors, makes PTGMS unfavorable for use in tropical conditions. However, PTGMS materials may still be useful in temperate conditions where day length is more crucial.

4.1.1 Characterization Under Controlled-Temperature Screening Conditions

Sterile single-plant selections identified in a mutation population of the M_2 generation or selections from segregating materials derived from TGMS × pollen parent (PP) crosses need to be stubbled and screened at low temperature to check for fertility reversion in the new emerging panicles. The crosses need to be bagged, and the generations correctly advanced under low-temperature facilities. At IRRI, the focus is on TGMS traits with low CSTP; thus, screening of the stable mutants and fixed materials is done under a phytotron in three mean temperature treatments (23, 24, and 25 °C) to determine their critical temperature for sterility/fertility induction (Fig. 2). This helped in identifying several TGMS lines with sterility at 24 °C and above and fertility at 23 °C. A few sterile plants were also identified in all three temperature conditions and are currently being evaluated for fertility reversion at <22 °C. Wongpatsa et al. (2014) carried out a similar study using two TGMS lines (KU-TGMS1 and KU-TGMS3) screened at the panicle initiation stage under growth chambers using day/night temperature parameters of 26/22 °C, 26/20 °C, 24/18 °C, and 22/20 °C, along with 11.5 h light/12.5 h dark periods and 75% relative humidity. Their results suggest that night temperatures of 18–22 °C induced maximum pollen viability and seed set. Furthermore, the highest seed rate was observed for KU-TGMS3 under 24/18 °C, peaking at 33.63%. In conclusion, this revealed that night temperature has a more significant effect on pollen viability than day temperature.

4.1.2 Field Screening Through Sequential Seeding

The physiological characterization of fixed TGMS lines can also be carried out through continuous seeding or sequential sowing. Sequential seeding is done in such a way that flowering is observed throughout the year at the candidate target sites to study pollen sterility and spikelet sterility (bagged and unbagged conditions). Such studies help in evaluating the stability of promising TGMS lines and determining the sterile phase window for hybrid rice seed production. Based on the tracking method (Ali et al. 1995), one can determine the CSTP and the sensitive

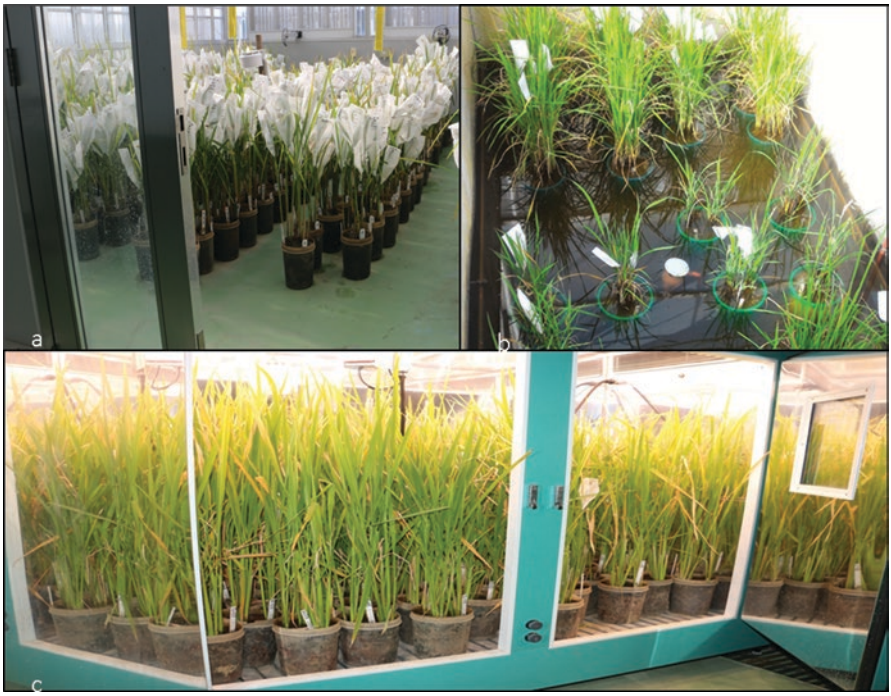

Fig. 2 Physiological characterization of TGMS lines in (**a**) plant growth facility bay, (**b**) cold-water facility, and (**c**) reach-in chamber

stage for sterility. A study done by Ramakrishna et al. (2006) observed six TGMS lines planted in three staggered sowing at intervals of 10 days. The lines were seen over two different seasons, postrainy 2002 (October–December) for fertility reversion with lower temperature range (25.5/16.1 °C) and prerainy 2003 (February–April) for sterility reversion with higher temperature range (35.7/23.8 °C), especially during the panicle initiation stage (Ramakrishna et al. 2006). Shuttle breeding of the selected sterile plants from segregating materials and their stubbles then transfers them to low-temperature conditions for obtaining self-seeds to advance the generations under low-temperature conditions. It would help to identify suitable TGMS lines for such environments. At IRRI, the sterile plant stubbles are sent to Lucban and Benguet in the Philippines for self-seed multiplication and generation advancement. Likewise, researchers at Tamil Nadu Agricultural University, India, evaluated TGMS lines in two sterility-inducing environments, Coimbatore and Sathiyamangalam, during rabi season starting in December 2013 and 2014. The same lines were stubble-planted and evaluated for pollen sterility in pollen fertility-inducing environments during kharif season in July 2013 and 2014 at the Hybrid Rice Evaluation Centre, Gudalur, a high altitude (1500 masl) with colder climate (Manonmani et al. 2016). Latha and Thiyagarajan (2010) also recommended a high-altitude area such as Gudalur for TGMS self-seed multiplication of lines such as

TS29, which was observed to have only 16 days of fertile phase during December in Coimbatore. In Gudalur, TS29 had more than 60% pollen fertility and seed set when the mean temperature was 22 °C (28/17 °C) and below from June to November.

4.2 Determination of the Critical Stage for Fertility-Sterility Alteration

The critical stages of panicle development sensitive to temperature could be determined from the stages exhibiting a significant correlation with pollen sterility (Chandirakala et al. 2008). The stamen pistil primordial stage, which is 15–24 days before heading, was considered as the sensitive stage (Ali et al. 1995; Salgotra et al. 2012). Furthermore, Viraktamath and Virmani (2001) found 4–8 days after panicle initiation as the most sensitive stage. For the lines that Latha and Thiyagarajan (2010) had examined, those were sensitive to temperature from stamen pistil primordial differentiation to pollen ripening except for two lines that were sensitive from the meiotic division of the pollen mother cell to pollen ripening. The sensitive stages observed to vary with the four TGMS lines (TNAU 27S, TS 09 12, TS 09 15, and TS 09 25) showed a significant amount of positive correlation between pollen sterility and maximum and mean temperatures (Sasikala et al. 2015). The period of partial sterility was considered as the phase of fertility transition (Ali et al. 1995; Latha and Thiyagarajan 2010). Sanchez and Virmani (2005) observed differentiation of secondary branch primordium and the filling stage of pollen, that is, 24 to 5 days before heading was considered a sensitive stage for temperature. The results showed that the critical stage for most of the TGMS lines occurred during panicle developmental stages and approximately 26 to 5 days before heading (Kadirimangalam et al. 2017). Based on all these studies, we can demarcate the critical stage for sterility expression from 5 to 26 days before heading that coincides with the differentiation of secondary branch primordium and the filling stage of pollen. These sensitive days before heading also varied with early-, medium-, and late-duration TGMS lines and depending on the synchronous flowering habit.

4.3 Evaluation of TGMS Lines for Sterility-Fertility Alteration in Different Environments

TGMS-based two-line breeding programs require natural sites with low temperatures in higher altitudes in the tropics that are essential for advancing generations of selected TGMS lines. However, it will be worthwhile to select sterile plants with low CSTP in the range of 23–25 °C as they are stable under high-temperature conditions (28–30 °C) for sterility. A recent discovery at IRRI of A07 with low CSTP of 24 °C is an excellent example of this type of TGMS line (Ali et al. 2018). Regular

self-seed multiplication of TGMS lines is carried out for their use in hybrid rice seed production plots under high-temperature conditions. IRRI has two locations (Lucban and Benguet) for self-seed multiplication in the Philippines. Multilocation trials for two-line hybrid rice seed reproducibility trials are essential for understanding the stability of the TGMS parental lines and their outcrossing features.

4.4 Improvement of Outcrossing Traits in TGMS and Pollen Parental Lines

Outcrossing is directly correlated as a function of floral morphology and flowering behavior for the male-sterile parental line (Oka and Morishima 1967). The wider angle of lemma and palea correlated with greater exsertion and surface area of the stigma, leading to higher seed-set percentage. Visual phenotypic selection can be used efficiently to identify higher seed-set potential (Ramakrishna et al. 2006; Salgotra et al. 2012). According to the standards set by Chen et al. (2010), female parents should possess a panicle exsertion rate of >70%, along with an excellent outcrossing rate, early and short flowering span, and well-closed lodicules and lemmas after pollination. On the other hand, pollen parents should exhibit large anthers and pollen quantity, pollen vigor, and vigorous growth ability (Chen et al. 2010).

Better panicle exsertion from the sheath in male-sterile lines would help increase the number of spikelets for outcrossing than lines with incomplete panicle exsertion (Rahul Roy and Kumaresan 2019; Abeysekera et al. 2003; Virmani 1994). The lines with higher panicle exsertion percentage coupled with higher seed set and higher spikelet fertility percentage influence outcrossing ability and could be well exploited for the development of hybrid rice (Arasakesary et al. 2015).

Many of the traits for outcrossing in CMS, such as greater glume opening angle and more stigma exsertion, lead to higher seed setting (Mahalingam et al. 2013), which could be used as well for TGMS breeding. Outcrossing of relevant traits, especially the longer feathery stigma protrusion on either side of the lemma-palea and full glume opening, is highly attractive for increased pollination reception, germination, and seed set. Developing synchronous flowering habits in TGMS lines is essential for successful seed production. At IRRI, a few long feathery stigma-protruding types of TGMS lines with synchronous flowering patterns were successfully identified (Fig. 3) (Ali et al. 2018). Similarly, at TNAU, the TGMS lines developed through pedigree breeding, mutation breeding, and identification of spontaneous mutants in the breeding material were addressing the market requirements for medium duration, better agronomic characteristics, and excellent floral traits and requirements such as high stigma exsertion, wider glume opening, and acceptable grain quality characteristics such as medium slender grain type, etc. (Manonmani et al. 2016). However, the outcrossing traits translating into higher hybrid seed yields need to be verified under hybrid seed production geographies.

Fig. 3 Newly developed TGMS line with long feathery stigma

The floral traits of the pollen parents are also equally important to obtain higher seed setting. The pollen parents need to be highly diverse from the TGMS parental lines. At the same time, they need to possess floral traits similar to those of a restorer in the three-line system, especially in terms of plant height, profuse tillering, heavy pollen load, and pollen dehiscence. Moreover, the pollen parents should possess a staggered flowering habit to provide good pollen dehiscence during hybrid seed production. Consideration should be given to the synchrony of the timing of pollen dehiscence of pollen parents. It should match the TGMS parent's spikelet opening, and stigma receptivity is essential. In addition, pollen parents need to possess all the market-required traits such as appropriate grain shape and quality, abiotic stress tolerance, and insect pest and disease resistance.

5 Genetics of TGMS Lines

The recent discovery of new low-CSTP TGMS lines that showed complete sterility at a mean temperature of 24 °C has sparked renewed interest in two-line hybrid rice technology. The genetics of the TGMS trait is essential for the exploitation of this technology.

5.1 Identification of Genes Governing the TGMS Trait

A single recessive nuclear gene governs the TGMS trait in TGMS lines (Hussain et al. 2012). So far, 13 TGMS genes and their alleles (*tms1, tms2, tms3, tms4, tms5, tms6, tms6(t), tms7(t), tms8, tms9, tms9-1, tms10,* and *tmsX*) found in 5460S, Norin PL 12, IR32364, SA 2, Annong S-1, SoKcho-MS, 0A15-1, UPRI-95-140TGMS, F61, Zhu1S, Hengnong S-1, *japonica* cv. 9522, and Xian S, respectively, have been identified based on their allelic relationship as well as molecular marker studies. (Wang et al. 1995; Subudhi et al. 1997; Yamaguchi et al. 1997; Reddy et al. 2000;

Table 2 Molecular markers associated with EGMS genes in rice (modified from Ali et al. 2018)

Trait	Gene	Source	Chromosome	Closest flanking markers	Distance (cM)	Reference	
TGMS	tms_1	5460S	8	RZ562-RG978	6.7	Wang et al. (1995)	
	tms_2	Norin PL12	7	R643A-R1440 (D24156)	0.3	Yamaguchi et al. (1997)	
		Norin PL12	7	RM11-RM2	5.0, 16.0	Lopez et al. (2003)	
		KDML105	7	0s7g2690	15.4, 16.9	Pitnjam et al. (2008)	
	tms_3	IR32364S	6	$OPAC3_{640}$-$OPAA7_{550}$	7.7, 10.0	Subudhi et al. (1997)	
		IR32364S	6	F18F, F18RM, F18FM/F18RM	2.7	Lang et al. (1999)	
	tms_4	TGMS-VN1	2	$E5/M12_{600}$	3.3	Dong et al. (2000)	
		SA2	9	RM257, EAA/MCAG	6.2, 5.3	Reddy et al. (2000)	
	tms_5	Annong S-1	2	RM174, R394	0, 2.5	Jia et al. (2000)	
		Annong S-1	2	C365-1, G227-1	1.04, 2.08	Wang et al. (2003)	
		M105S	2	RM174	0	Nas et al. (2005)	
		Annong S-1 and Y58S	2	4039-1 and 4039-2	–	Yang et al. (2007)	
		103S	2	RM3294, RM6378, RM7575 and RM71	–	Hien and Yoshimura (2015)	
		Annong S-1	2	dCAPS-172	–	Song et al. (2016)	
		IR68301S	2	RM12676, 2gAP0050058		Khlaimongkhon et al. (2019)	New
	tms_6	Sokcho-MS	5	RM3351, E60663	0.1, 1.9	Lee et al. (2005)	
	$tms_{6(t)}$	0A15-1	3	S187-770	1.3	Wang et al. (2004)	
		UPRI 95-140TGMS	3	–	–	Li et al. (2005)	
		G20S	10	RM3152, RM4455	3.0, 1.10	Liu et al. (2010)	
	$tms_{7(t)}$	UPRI 95-140TGMS	7	–	–	Li et al. (2005)	
	tms_8	F61	11	RM21, RM224	4.3, 3.0	Hussain et al. (2012)	
	tms_9	Zhu1S	2	Indel 37, Indel 57	0.12, 0.31	Sheng et al. (2013)	
		Zhu1S	2	Indel 91, Indel 101	–	Sheng et al. (2015)	
	$tms_{9\text{-}1}$	HengnongS-1	9	QY-9-19, QY-9-27	0.22, 0.07	Qi et al. (2014)	
	tms10	*japonica* cv. 9522	2	Os02g18320		Yu et al. (2017)	New
	tmsX	XianS	2	RMAN81, RMX21	–	Peng et al. (2010)	

Trait	Gene	Source	Chromosome	Closest flanking markers	Distance (cM)	Reference	
PGMS/PTGMS	*pms*$_1$	32001S	7	RG477-RG511, RZ272	3.5–15.0	Zhang et al. (1994)	
				RG477/R277, R1807	0.1, 6.0	Liu et al. (2001)	
	pms$_{1(t)}$	Pei'ai64S	7	RM21242, YF11	0.2, 0.2	Zhou et al. (2011)	
	pms$_2$	32001S	3	RG348, RG191	10.6, 7.0	Zhang et al. (1994)	
	pms$_3$	Nongken 58S	12	RZ261/C751, R2708	5.5, 9.0	Mei et al. (1999)	
		Nongken 58S	12	LJ47 and LJ265	0	Lu et al. (2005), Ding et al. (2012)	
	pms$_4$	Mian 9S	4	RM6659, RM1305	3.0, 3.5	Huang et al. (2008)	
	p/tms$_{12\text{-}1}$	Pei'ai64S	12	PA301, PAIDL2	–	Zhou et al. (2012)	
	ptgms$_{2\text{-}1}$	Guangzhan63S	2	S2-40, S2-44	0.08, 0.16	Xu et al. (2011)	
rTGMS	*rtms*$_1$	J207S	10	RM239-RG257	3.6, 4.0	Jia et al. (2001)	
rPGMS	*rpms*$_1$	YiD1S	8	RM22980, RM23017	0.9, 1.8	Peng et al. (2008)	
	rpms$_2$	YiD1S	9	RM23898, YDS926	0.9, 0.9	Peng et al. (2008)	
	rpms$_{3(t)}$	D52S	10	RM5271 and RM244	6.6, 4.6	Joseph et al. (2011)	
	csa					Zhang et al. (2013)	

Jia et al. 2000; Wang et al. 2004; Lee et al. 2005; Li et al. 2005; Peng et al. 2010; Hussain et al. 2012; Sheng et al. 2013; Qi et al. 2014; Yu et al. 2017) (Table 2).

The identified *tms* genes could be further exploited for developing TGMS pyramiding lines by using two to three *tms* genes for improving stability during the sterility phase. However, only a few studies have been attempted on the pyramiding of these alleles, studying them for improving the stability of the TGMS lines (Nas et al. 2005). So far, 13 *tms*, seven *pms,* and three *rtms* genes have been identified governing the EGMS trait that is spread across all 12 rice chromosomes.

The TGMS trait is governed by a single major gene and could have several modifier genes that exist in different backgrounds. Therefore, it is crucial to characterize the TGMS lines physiologically before their commercial exploitation. The TGMS trait is much easier to transfer to other backgrounds through the marker-assisted backcross (MABC) approach, and one has to take care of modifier genes as well that may influence trait expression. In this context, it is essential to understand the molecular function of the TGMS trait (Ding et al. 2012; Zhou et al. 2012; Wang et al. 2013; Pan et al. 2014; Kim and Zhang 2017; Mishra and Bohra 2018).

Earlier studies on TGMS focused on the physiological aspects and how the gene is phenotypically expressed in the population. However, the first genetic study to confirm the location of the TGMS gene was begun by Wang et al. (1995) using an F_2 cross from a mutant TGMS line (5460S) and Hong Wan 52. Bulk segregant analysis and QTL mapping using RAPD markers identified the first TGMS gene as *TGMS1.2*, located within chromosome 8 (Wang et al. 1995). Succeeding genetic studies are all compiled and given in Table 2 with the corresponding molecular markers.

5.2 Molecular Mechanisms of the TGMS Trait

With the advent of new technologies in the field of genomics and transcriptomics, Luo et al. (2020) confirmed the location of the *tms* gene, which was begun by Wang et al. (1995), for the identification of *tms1* on chromosome 8 using RFLP markers. This transition from RFLP to SSRs and more recently with transcriptomics in confirming the *tms1* loci led to the unraveling of the mechanism behind *tms* genes (Luo et al. 2020). Furthermore, Pan et al. (2014) showed that, in line TGMS-Co27, male sterility is based on the cosuppression of a UDP-glucose pyrophosphorylase gene (*Ugp1*), and the underlying molecular mechanisms need to be unraveled. Zhou et al. (2014) uncovered the molecular mechanism of rice *tms5,* which functions in RNase ZS1-mediated UbL40 mRNA regulation during pollen development. Under permissive (low) temperature conditions, the level of UbL40 mRNAs remains low in the *tms5* mutant plants, allowing the production of normal pollen. However, at restrictive (high) temperature, UbL40 mRNAs are not processed by RNase ZS1, which leads to their high-level accumulation, causing male sterility (Zhou et al. 2014). Wang et al. (2019) carried out a comparative quantitative proteomic analysis of the anthers of TGMS line Annong S-1 grown at permissive (low) (21 °C) and restrictive

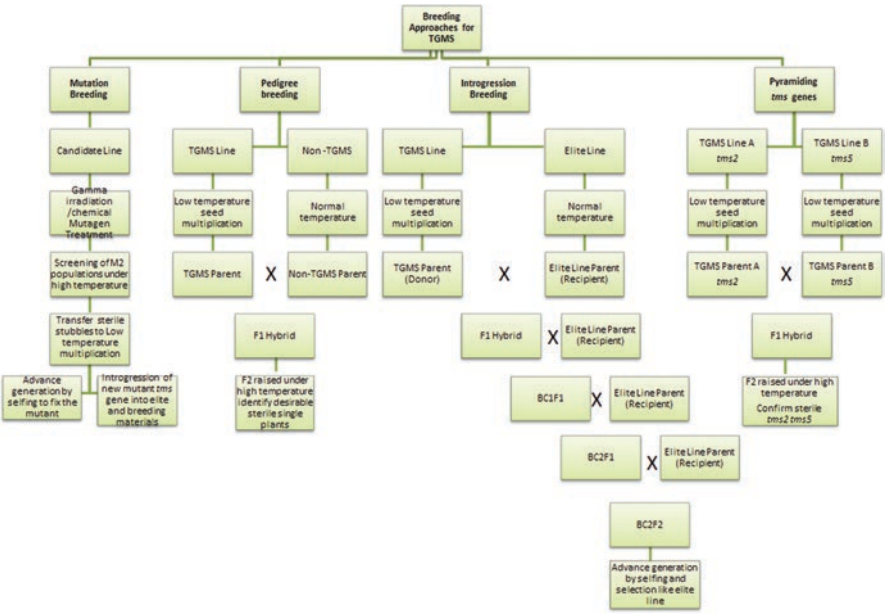

Fig. 4 Current breeding approaches for TGMS followed at IRRI

(high) temperatures (>26 °C). The restrictive high temperatures resulted in 89 differentially accumulated proteins (DAPs) in the anthers as compared to permissive low-temperature conditions. Out of the 89 DAPs, 46 had increased abundance and 43 had decreased abundance, which are distributed in most of the subcellular compartments of anther cells. Most have catalytic and binding molecular functions. Moreover, the gene ontology analysis for biological processes done by Wang et al. (2019) indicated that high-temperature induction caused the fertility-sterility conversion. This mainly adversely affects the metabolism of protein, carbohydrate, and energy and decreases the abundance of vital proteins closely related to defense and stress. This further impedes the growth and development of the pollen and weakens the overall defense and stress ability of Annong S-1.

Li et al. (2020) carried out RNA-Seq on rice TGMS lines at the microspore mother cell and meiosis stages under sterile and fertile conditions that revealed 1070 differentially expressed genes found to be enriched in protein folding, protein binding, regulation of transcription, transcription factor activity, and metabolic-related processes. They showed that hub genes (such as UbL40s) were predicted to interact with proteolysis-related genes and DNA-directed RNA polymerase subunit, and heat shock proteins (HSPs) interacted with kinases to play significant roles in regulating fertility alteration. Their study suggested that, besides UbL40s, DNA-directed RNA polymerase subunit, kinases, and HSPs might be involved in TGMS fertility alteration and could be applied for TGMS breeding (Li et al. 2020). Despite several of these in-depth studies, the TGMS trait mechanism still needs to be unraveled entirely for its immediate exploitation by breeders.

6 Breeding of TGMS and Pollen Parental Lines

Two-line breeding strategies for TGMS are currently carried out using four approaches: (a) the use of mutagenesis to induce new *tms* gene mutants from current materials, (b) conventional crossing and pedigree selection, (c) introgression of currently identified *tms* genes into elite lines, and (d) pyramiding known *tms* genes from different sources (Fig. 4). For each strategy, parental line selection remains the most crucial part to ensure hybrid vigor and address market segment requirements.

6.1 Different Available Approaches to Breed TGMS Lines

6.1.1 Mutation Breeding for the Identification of TGMS Mutants

Mutation breeding for the development of TGMS lines was first reported by Maruyama et al. (1991) for the development of Norin PL12 using gamma radiation. Furthermore, Ali et al. (1995) developed and characterized several TGMS lines using chemical and physical mutagens. Interestingly, Ali and Siddiq (1999) also identified a spontaneous mutant (JP38s) that showed a reverse TGMS trait, behaving as sterile at lower temperatures (<24 °C) and as fertile at higher temperatures (>30.5 °C). IRRI began a mutation breeding program using chemical mutagens in 2015 to discover new TGMS mutants, which are currently being characterized. The mutation populations in the M_2 generation need to be screened under high-temperature conditions to identify complete male sterility, and these are then stubbled and taken to low-temperature conditions to check for fertility reversions. Depending upon their seed settings in the stubbles, they are further generation advanced under low-temperature conditions to fix the TGMS mutants quickly. Upon fixation, these mutants are studied in different temperature regimes to characterize them physiologically (Ali et al. 1995, 2020 Unpublished).

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6.1.2 Pedigree Breeding

It is also essential to breed new materials through crossing TGMS parents with elite lines and selection in the F_2 generation for male-sterile single plants under high-temperature conditions. At IRRI, conventional crosses were made with the TGMS line A07 as a pollinator and elite breeding materials as the female parents (Ali et al. 2018). After the initial cross in the F_2 generation, the selected male-sterile single plants in high-temperature regimes are then stubbled and selfed seeds are produced under low-temperature conditions. These selected single plants are verified for the presence of the *tms5* gene across succeeding generations. Using this approach, a new TGMS line with the *tms5* gene will be developed (Ali et al. 2020 Unpublished).

6.1.3 Transfer from a Known TGMS Gene Source to Elite Lines

Another strategy for integrating TGMS in two-line hybrid rice is by introgression of *tms* genes. At IRRI, the TGMS line A07 is used as a donor for introgressing the *tms5* gene into elite breeding materials by two backcrosses and selecting the progenies in BC_2F_2 onward for the *tms5* gene. By using foreground markers and high-density background SNP markers, introgression of the *tms5* gene into elite materials is possible. However, it is essential to accurately characterize these materials upon fixation for their fertility-sterility alteration behavior.

6.1.4 Pyramiding TGMS Genes for Better Stability

Despite the independent successes in characterizing and isolating different TGMS genes in rice, only a few studies have dealt with the additive effect and pyramiding of different TGMS genes (Nas et al. 2005). Two- and three-gene pyramids constructed using the three TGMS donors, Norin PL 12 (*tms2*), SA2 (*tgms*), and DQ200047-21 (*tms5*), possessing the RM11 allele of Norin PL 12, RM257 allele of SA2, and RM174 allele of DQ200047-21 were selected. As expected, all selected progenies were male-sterile in sterility-inducing conditions (Nas et al. 2005). The pyramids developed from this effort were designated as IR80775-46 (with *tms2* and *tms5*) and IR80775-21 (with *tms2*, *tgms,* and *tms5*). Pyramiding *tms* genes is useful to improve the stability of the TGMS line and to widen the sterility phase. Currently, at IRRI, efforts are ongoing to pyramid *tms2* and *tms5* genes to understand the mechanisms of the genes and to improve the stability of the TGMS trait. The current



	A07	IR 132941-B-1-9-AC 43	IR 75589-31-27-8-33-1 (TGMS)
Maturity (days)	100	120	117
Plant height (cm)	81.7	54.0-60.0	75.0
Tiller number	7-11	10-12	15-20
Panicle length (cm)	23.0	17.0-20.0	23.0
Flag-leaf length (cm)	45.8	35.0	21.5-30.0
Flag-leaf width (cm)	1.6	1.2	1.6
Value-added genes	*GS3*	*GS3+Wxg2 and Wxg3*	*GS3+Wx-e6, Bph18+Bph3*

Fig. 5 New IRRI stable TGMS lines with low critical sterility temperature point at 24 °C

Table 3 TGMS lines developed at IRRI (modified from Ali et al. 2018)

S. no.	TGMS line
1	A07
2	A32
3	A36
4	A37
5	IR75589-31-27-8-33-1 (TGMS)
6	IR68301-11-6-4-4-3-6-6 (TGMS)
7	IR73827-23-26-15-7 (TGMS)
8	IR73834-21-26-15-25-4 (TGMS)
9	IR75589-31-27-8-33 (TGMS)
10	IR77271-42-5-4-36 (TGMS)

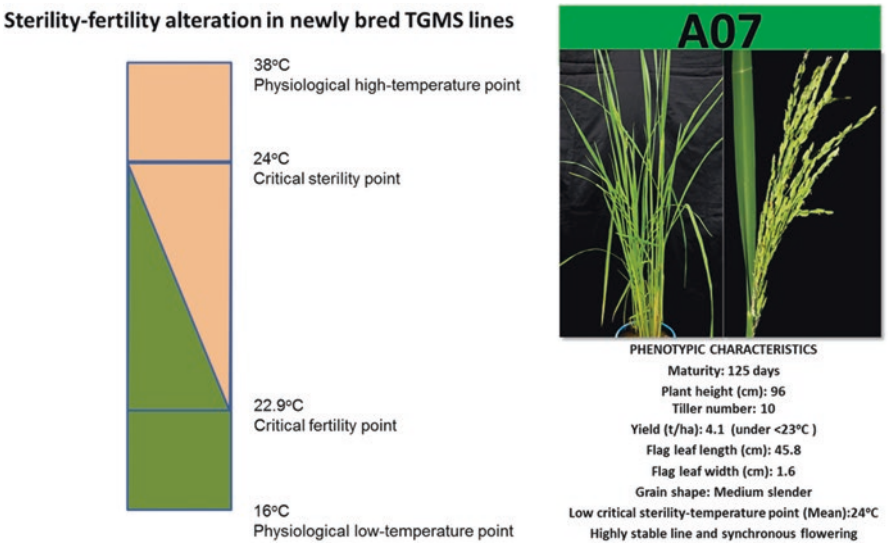

Fig. 6 New IRRI stable TGMS line with low critical sterility temperature point. (Source: Ali et al. 2018)

TGMS pyramiding studies at IRRI used line A07 as a pollinator parent (Ali et al. 2020 Unpublished).

6.2 Rapid Fixation of Segregating TGMS Lines

Conventionally, generation advancement is accomplished by growing the plants under natural low-temperature locations, for example, Lucban-Quezon (14.0805°N, 121.5427°E) and Tublay-Benguet (16.50805°N, 120.63524°E). This method remains the most popular as it is the most cost-efficient and requires the least technical work. This method, however, has its disadvantages as well. First, the environmental variables (temperature, humidity, and day length) at the location could cause genetic purities, mainly if fluctuations occurred during the plant's panicle initiation. Second, it requires labor-intensive cultural management of the field to prevent pests and diseases, especially under higher altitude locations in the tropics. Regardless of the fixation method, marker-assisted selection (MAS) is integral to the generation advancement of TGMS lines. MAS ensures the integrity of the *tms* gene and the genetic purity of the TGMS lines across generations.

Pedigree breeding and generation advancement of desirable TGMS segregants and mutants are challenging until the lines are correctly fixed. Recently, using new techniques of speed breeding under rapid generation advancement (RGA) facilities with specialized lighting, one can fix the segregating TGMS trait within 2–3 years, and this can be put to use to develop hybrid combinations. The use of the RGA method is a viable alternative to save on time and costs vis-à-vis field conditions. RGA hastens the fixation of new lines by advancing single seeds per line from a segregating population under controlled conditions (Collard et al. 2017). Instead of the usual dry and wet seasons, RGA allows several generations of advancement in a single season by growing the plants in trays instead of transplanting in the field to facilitate faster growth. Generations of TGMS breeding lines are advanced at low temperature (<22 °C) in plant growth facility (PGF) chambers. It is essential to maintain the critical temperature and humidity necessary to induce pollen fertility and self-seed setting in plants, thus requiring more labor costs, a PGF, and technical expertise.

To speed up the fixation of TGMS traits in the mutants and segregants, one can use a doubled-haploid (DH) approach. It is essential to identify the right segregants and mutants for fixation through the DH approach (Fig. 5). Many times, the DH TGMS lines, once fixed, may not be the ideal ones to match the market requirements. IRRI has previously developed four TGMS lines using DH technology: A07, A36, A32, and A37 (Ali et al. 2018) (Table 3). Among them, A07 has already been validated as highly stable and it has a low CSTP of 24 °C (Fig. 6).

Moreover, the *tms* gene present in this line (*tms5*) is the most extensively studied *tms* gene and it is used in different breeding programs as well (Wang et al. 2003; Nas et al. 2005; Yang et al. 2007; Kadirimangalam et al. 2019). Finally, DH technology offers the best potential among the three approaches. The use of DH technology

ensures the fastest method of fixing recombinant genotypes, encompassing six generations of population advancement typically required to fix the population in just a single season (Yao et al. 2018). Moreover, the use of DHs eliminates the presence of deleterious alleles and background noise, which are typically observed in a natural population.

6.3 Breeding Pollen Parents

Heterotic pool-based breeding of pollen parents, more diverse and distinct from the TGMS pool, is required. These materials need to be improved within the pool and avoid contamination from materials nearer the TGMS heterotic pool. Breeders need to select for the target traits that help in pollen dehiscence, staggering flowering characteristics, and heavy tillering to provide a continuous pollen supply. Furthermore, the pollen parents need to address market segment needs so that the hybrids developed fit well. Pedigree breeding, single seed descent with genomic selection, along with RGA approaches could help to speed up the pollen parental breeding process. Specific traits such as genes with resistance against major insect pests and diseases that address market segment needs could be incorporated through a marker-assisted backcross (MABC) breeding approach.

6.4 Two-Line indica/japonica *Hybrids*

The two-line system is ideal for exploiting *indica/japonica* hybrids as there is no barrier for the identification of pollen parents, which could be any parent other than the TGMS parent. The TGMS gene could preferably be in the *indica* parental background, and with the use of a wide-compatibility (WC) gene in any one of the parents, one could develop *indica/japonica* hybrids. Shukla and Pandey (2008) suggested brighter prospects of combining improved *japonica* and *tropical japonica* germplasm having WC genes with *indica* TGMS lines for the exploitation of intersubspecific heterosis. Recently, with the discovery of reliable WC gene-based markers, ones such as *S5* could be highly useful for selection to combine with *tms* gene-based markers. At IRRI, the *S5* gene from different sources is backcrossed into TGMS line A07. Hybrid rice seed production of intersubspecific hybrids may be challenging due to the varying timing of spikelet opening and pollination of the two subspecies, especially in tropical environments. So, we need to carefully identify parental lines from these two subspecies closer to each other.

7 Breeding Two-Line Hybrids

Two-line rice hybrids have higher heterosis than three-line rice hybrids as any nonTGMS line could be used as a pollen parent, thus creating more extensive opportunities. Unlike the three-line system, CMS requires only restorers with restorer fertility (*Rf*) genes to restore fertility in the F_1 hybrid. Thus, it is a much narrower range within which heterosis needs to be exploited. On the other hand, TGMS-based two-line hybrids open up more opportunities to use the intersubspecific hybrids (*indica/japonica*) as the *japonica* subspecies has a low frequency of restorer genes. At IRRI, all source nurseries are genotyped, and heterotic pools are formed based on the genetic distances. Heterotic pool-based breeding is being followed to identify the best combinations for the two-line hybrids. To improve the heterotic pools, we have to make crosses within the pool. There is a need to maintain different heterotic pools carefully and to avoid contamination from other heterotic pools. To develop new heterotic hybrids, we can attempt crosses between distant pools.

Fig. 7 IR134554H, a multiple-stress-tolerant two-line rice hybrid developed at IRRI

Table 4 Performance of hybrids over the best inbreds and hybrids at IRRI-South Asia Hub, Hyderabad, in WS 2018, and at ISARC-Varanasi in kharif 2019

Designation	IRRI-South Asia Hub, Hyderabad (WS2018)									ISARC Varanasi (kharif 2019)		
								Yield advantage (%) over check			Yield advantage (%) over check	
	Days to flowering	Plant height (cm)	Panicle length (cm)	Productive tillers (no.)	Total spikelets per panicle	Spikelet fertility (%)	Total grain yield (kg/ha)	US337 (comm. hybrid)	MTU1010 (best variety)	Total grain yield (kg/ha)	Arize 6444Gold (hybrid check)	Sava127 Pro (hybrid check)
IR134554 H	101	92	21	12	158	80	7767.0	10.0	21.0	7956.7	6.4	12.9
IR81958H (Mestiso 77)	92	94	22	12	161	78	7638.0	8.0	19.0	7987.0	6.8	13.4
IR90872 H	95	88	22	18	173	79	7532.0	7.0	17.0	7606.0	1.7	8.0
IR81265H (Mestiso 61)	92	84	23	16	199	84	7164.0	2.0	11.0	7704.7	3.1	9.4
IR82391H (Mestiso 68)	95	88	21	13	163	78	6885.0	−2.0	7.0	7790.7	4.2	10.6
IR121020 H	87	78	20	13	153	79	6218.0	−12.0	−3.0	7040.7	−5.8	−0.1
IR106638 H	94	80	22	14	201	82	6153.0	−13.0	−4.0	7746.3	3.6	10.0
IR106616 H	90	85	22	12	221	83	6032.0	−14.0	−6.0	7676.0	2.7	9.0
IR81255H (Mestiso 89)	91	83	21	16	199	81	5947.0	−16.0	−8.0	7818.3	4.6	11.0
IR82386H (Mestiso 71)	93	82	22	13	168	68	5477.0	−22.0	−15.0	7927.0	6.0	12.5
DRR DHAN 44	83	81	21	13	157	76	3398.0	–	–	–	–	–
US 312	102	87	21	12	148	71	6881.0	−2.0	7.0	–	–	–
US 382	106	95	20	11	169	77	6756.0	−4.0	5.0	–	–	–
US 337	94	101	23	11	187	73	7051.0	0.0	10.0	–	–	–
MTU 1010	89	93	21	16	196	80	6436.0	−9.0	0.0	–	–	–

Designation	IRRI-South Asia Hub, Hyderabad (WS2018)									ISARC Varanasi (kharif 2019)		
								Yield advantage (%) over check			Yield advantage (%) over check	
	Days to flowering	Plant height (cm)	Panicle length (cm)	Productive tillers (no.)	Total spikelets per panicle	Spikelet fertility (%)	Total grain yield (kg/ha)	US337 (comm. hybrid)	MTU1010 (best variety)	Total grain yield (kg/ha)	Arize 6444Gold (hybrid check)	Sava127 Pro (hybrid check)
Arize 6129 Gold (hybrid check)	–	–	–	–	–	–	–	–	–	7249.3	−3.0	2.9
VNR 2355 Plus (hybrid check)	–	–	–	–	–	–	–	–	–	6636.0	−11.2	−5.8
Super Moti (hybrid check)	–	–	–	–	–	–	–	–	–	6617.3	−11.5	−6.1
BPT 5204 (inbred check)	–	–	–	–	–	–	–	–	–	6499.3	−13.1	−7.8
Sarju 52 (inbred check)	–	–	–	–	–	–	–	–	–	5559.3	−25.6	−21.1
MTU 7029 (inbred check)	–	–	–	–	–	–	–	–	–	6958.0	−6.9	−1.2
Arize 6444 Gold (hybrid check)	–	–	–	–	–	–	–	–	–	7476.7	0.0	6.1
Sava 127 pro (hybrid check)	–	–	–	–	–	–	–	–	–	7045.3	−5.8	0.0

7.1 Combining Ability Nurseries

The general combining ability (GCA) of an inbred is its average performance across a series of hybrid combinations, and it is primarily due to the additive effects of genes. The GCA effects of the parental lines help in the identification of suitable parental lines (Chandirakala et al. 2012). The most promising TGMS lines developed in high combining ability backgrounds could be used to further identify and validate their general combining ability. For this, a line × tester design could be used to identify high GCA of lines. This also allows identifying combinations with high specific combining ability that could be immediately exploited. A combining ability nursery needs to be regularly created to identify TGMS lines with high GCA and pollen parents from the breeding pipelines. Chen et al. (2010) stressed the importance of identifying PTGMS with high combining ability as this is the basis of robust heterotic hybrid rice varieties. Cao and Zhao (2014) showed that successful hybrids are directly determined by the combining ability of the sterile line, and sterile lines with high GCA have higher chances to produce heterotic combinations. In situations with poor GCA of TGMS lines, it is good to have pollen parents with high GCA to develop heterotic hybrids. Shukla and Pandey (2008), with a broad set of line × tester crosses, found that the parents with good GCA did not always produce the best hybrid combinations due to a lack of higher-order additive interaction, and they suggested evaluating the specific combinations. They found TGMS line 365-8S to be the best general combiner for all six traits: grain yield, panicle length, grain number per panicle, earliness in flowering, panicle number per plant, and 1000-grain weight.

7.2 Breeding Trials

Once hybrid combinations are identified, small-scale seed production either by hand crosses or in field conditions should be sufficient to carry out an observation yield trial (OYT). An OYT evaluation of the F_1s under best management conditions would allow the identification of good performing hybrids, and these should be forwarded to an advanced yield trial (AYT) in a larger plot size with proper replications and the best market checks. Simultaneously, the AYT is screened for resistances to insect pests and diseases. The highly performing hybrids should be identified and sent for grain quality evaluation. Based on all the data, the best hybrids need to be produced in large quantities and also evaluated for their hybrid seed reproducibility for ensuring their success when screened in multienvironment trials (METs). The best candidate hybrids tested under METs lay the foundation for the identification of the best hybrids for a given target location and market segment. IRRI conducted two demonstration trials in India to evaluate the performance of some newly developed two-line hybrids. One hybrid (IR134554H) performed exceedingly well at both Hyderabad and Varanasi (Table 4, Fig. 7).

Table 5 Market needs segment per region for hybrid rice

Countries	Product concept	Duration (days)	Potential hybrid area (m ha)	Core target geography	Yield advantage over best hybrid (%)	Yield advantage over best OPV (%)	Check variety	Plant height (cm)	Key defensive traits	Key agronomic traits	Grain quality	Producibility benchmark (yield/ha)	Key gaps in the present products
India	Mid-early segment	110–125	8	PUN, HAR, CG, CU, JH, MP, GJ, AS, UP, WB, KAR, W, C, Assam	5–8	15–20	MTU1010 and US 312 (inbred)	110–120	Blast, neck blast,* BLB, BPH, false smut,* stem borer,* drought tolerant	High vigor, >85% spikelet fertility, non-lodging, cold tolerant (seedling), MTU 1010 & IET 4786 grain type, fine grain	HRR: 55–65% MRR: >70% AC: 18–24% LS	>3.0 t/ha, staggering <15 days	Lack of stress-tolerant materials, producibility, lodging,* false smut*
Bangladesh	MS grain segment (boro)	125–140	4	Sylhet, Dhaka, Chittagong, Rajshahi, Ranpur, Khulna, Barisal Divisions	10	20 (>9.0 t/ha)	BRRI dhan 81(inbred) BRRI dhan 5 (hybrid)	90–100	BLB, SB, BPH	High vigor, spikelet fertility, cold tolerant, Jeerasail grain type (>85%)	MS with high amylose (>24%), milling (>65%)	>3.0 t/ha, staggering <15 days	Lack of stress-tolerant materials (salinity, drought, cold, diseases, insects
	Mid-early segment (T. aman)	115–120	2		10	20 (>7.0 t/ha)	BRRI dhan 49 (inbred) BRRI Hybrid 6 (hybrid)	100–110	*False smut,* BLB*	High vigor, spikelet fertility, early, drought tolerant, BRRI dhan49 grain type (>85%)	Slender grain with high amylose (>24%), milling outturn (>65%)	>3.0 t/ha, staggering <10 days	Lack of stress-tolerant materials (salinity, drought, cold, submergence, stagnation at 15–30-cm depths, diseases, insects)

(continued)

Table 5 (continued)

Countries	Product concept	Duration (days)	Potential hybrid area (m ha)	Core target geography	Yield advantage over best hybrid (%)	Yield advantage over best OPV (%)	Check variety	Plant height (cm)	Key defensive traits	Key agronomic traits	Grain quality	Producibility benchmark (yield/ha)	Key gaps in the present products
Philippines	Mid-early segment	95–115	2	Region 1, Region 3, Region 4A/4B	5	15–20	NSIC Rc 222 (inbred) Mestiso 3 (hybrid)	≥100	BLB, BPH, blast, stem borer,* RTV, non-lodging	Stability, more tillers, uniform grain maturity, non-lodging, non-shattering	AC 17–24%, >55% HRR, >65% MRR, LS, less chalk, slight aroma	≥2.5 t/ha	Susceptible to SB, poor standability, yield coupled with GQ, BLB, BPH, low HRR, susceptibility to fungal diseases
Indonesia	Mid-late segment	120–130	2	Bali, Central Java, East Java, Aceh, South Sulawesi, Lampung	≥10	≥20	Ciherang (inbred) Hipa 18, Hipa 20 (hybrid)	90–115	Blast, neck blast,* BLB, BPH, false smut,* stem borer,* drought tolerant, RTV, BPH, RRSV/ RGSV	More productive tillers, more filled grains, lodging tol., wide adaptability, threshability	AC 18–22%, low-med GT, soft GC, >50% HRR, >65% MRR, <10% chalkiness, good palatability, transluscent, S-LS	>2.5 t/ha, staggering <10 days, high OCR (>50%)	RSV-BPH, BLB, blast, drought
Vietnam	Medium segment	> 110–130	2046.1 t/ha	North	1–5	>20	Nhị Ưu 838, Thien uu, BC 15 (inbreds) TH3–3 (hybrid)	110–120	BLB, BPH	Yield	Intermediate AC, high HRR, low chalk	>2.5 t/ha	LS grain hybrid with BLB, BPH, SB traits
	Early segment	85–105	1.4 mt (early/LS)	South & Central	1–3	<20		110–120	BLB, BPH	Very early	Intermediate AC, high HRR, low chalk	>2.5 t/ha	LS grain hybrid with BLB & BPH traits

7.3 Insect Pest and Disease Resistance

Two-line rice hybrid yield potential could be fully realized by incorporating resistance to major diseases and insect pests (bacterial leaf blight (BLB), blast (BL), false smut (FS), sheath blight (SHB), tungro, green leafhopper (GLH), brown planthopper (BPH), stem borer, leaf folder, and gall midge). Most insect pest and disease resistances are governed by major genes and could be easily introgressed into parental lines depending on market segment requirements. Two-line breeding offers better opportunities to convert the TGMS parent to acquire disease and insect pest resistance as compared to a CMS/maintainer parent, which is more cumbersome and requires more time (Ali et al. 2018). In this regard, IRRI has developed a global product concept addressing different market needs, which could be useful, require fewer resources, and result in higher impact. Researchers at Huazhong Agricultural University (HAU) introgressed *Xa*7, *Xa21*, and *Xa23* genes into C815S, a popular TGMS parental line, to develop five BLB-resistant cultivars: Hua1005S, Hua1002S, Hua 1009S, Hua 1006S, and Hua1001S (Jiang et al. 2015). Two-line hybrids with *Xa23* showed a resistance reaction to seven *Xanthomonas oryzae* pv. *oryzae* (Xoo) strains. Hua1006S was the most promising TGMS parent among them with a higher degree of resistance based on *Xa23* besides better plant type and grain quality features (Jiang et al. 2015). Currently, at IRRI, introgression of BLB and blast resistance genes into elite TGMS and pollen parental lines is carried out through marker-assisted selection.

7.4 Grain Quality Considerations Addressing Market Needs

A wider array of heterotic two-line rice hybrids opens up better options for developing customized grain quality that caters to market needs (Table 5). IRRI's two-line rice hybrid Mestiso 61 with good grain quality matched the market needs of the Philippines. It was successfully licensed to SL Agritech Company in the Philippines with limited exclusivity for a 6-year period. However, it is still available for license to the private seed industry for other countries under the Hybrid Rice Development Consortium. This hybrid gave an average yield of 6.7 t/ha during the dry season and 6.4 t/ha during the wet season across the Philippines. The yield potential of this hybrid was nearly 10 t/ha, with 55% head rice recovery and amylose content of 20.5%, ideally fitting Philippine market needs. We developed a strategy to breed and customize grain quality as per market requirements (Allahgholipour et al. 2006; Pang et al. 2016). In this approach, breeders identify good-quality lines that will cater to the varied interests of consumers across rice-consuming countries by screening the breeding materials for eating and cooking quality (ECQ) and keeping the popularly preferred good-quality varieties as controls in the study. Furthermore, work is ongoing to identify advanced rice breeding lines/cultivars with similar apparent amylose content (AAC), gelatinization temperature (GT), and rapid vis-

cosity analysis (RVA), properties like those of the popular high-quality rice varieties, through simple cluster analysis. A two-line hybrid from China, Pei-Liang-you 1108, has relatively good ECQ, and through our study, we identified seven lines in the HC21 cluster clade with similar AAC, GT, and RVA and hence with comparable ECQ. Likewise, another two-line hybrid with good ECQ, Jin-ke-you651, allowed us to identify 11 hybrid lines within the HC18 cluster clade that had similar AAC, GT, and ECQ (Pang et al. 2016).

It is essential to develop rice hybrids with better ECQ that address market needs, paving the way for the expansion and adoption of rice hybrids in Asia and Africa. Higher hybrid rice yields have no value if they do not translate into higher percentage head rice recovery (>55%), leading to increased farmers' income.

8 Seed Production Challenges

Two-line hybrid rice technology largely depends on the identification of TGMS lines that need to be multiplied under low-temperature conditions, and hybrid rice seed production requires a minimum of 2 weeks of stable high temperature to reach the sterile phase. To achieve these two different aspects of seed production carefully, we have different approaches to identify appropriate locations based on agrometeorological data. However, this needs validation before large-scale seed production.

Key Challenges

- Addressing market requirements for different target places varies: for example, long-duration hybrids for the Indian market segment may require a longer duration of TGMS and pollen parents.
- Identification and exploitation of hybrid rice seed production and TGMS self-seed multiplication sites.
- Development of usable and stable TGMS parental lines matching market segment requirements.
- The relative heterosis of two-line rice hybrids needs to be superior to that of the existing best three-line hybrids in the market.
- Two-line hybrid rice technology should assure lower seed costs on account of better hybrid seed reproducibility rates of 3 t/ha and higher self-seed multiplication rates (>4.5 t/ha), making this seed feasible for use by farmers.

8.1 Identification of Ideal Locations for Self-Seed Multiplication of TGMS and Hybrid Rice Seed Production

TGMS-based two-line hybrid rice technology mainly depends on the identification of suitable areas for both self–seed multiplication and hybrid rice seed production (Table 6). Earlier, a systematic analysis of 50 years of agrometeorological data

helped in the identification of appropriate sites in India (Siddiq and Ali 1999). Interestingly, the authors identified places located in India between 500 and 700 m above sea level from May to September for both hybrid seed and self-seed multiplication of the TGMS lines. Furthermore, through experimental validation, these places were confirmed as suitable for hybrid rice seed production, TGMS seed multiplication, and locations ideal for both operations (Siddiq and Ali 1999).

Critical considerations for the choice of place could be the hills, coastal plains, or interior plains, keeping within the physiological sterility limits of <40 °C to >16 °C. The Two-line Hybrid Rice Research Station was established under Tamil Nadu Agricultural University in the Nilgiris hills at 1200 m above sea level in a place known as Gudalur as early as 1995 in India (Soundararaj et al. 2002). Male-sterile TGMS selections at high temperatures at Trichy were made and immediately sent as stubbles to Gudalur to allow their self-seed multiplication and generation advancement. The most suitable time for matching the temperature conducive to self-fertility was from June to November. Shuttle breeding helped to identify 15 highly stable TGMS lines with better stigma exsertion of 40–66%, and many are in the pipeline. Nearly 800 ha of paddy lands are available for commercial self-seed multiplication of promising TGMS lines (Soundararaj et al. 2002). In the Philippines, Lucban, Nueva Vizcaya, and Benguet are all identified as highly suitable for self-seed multiplication of TGMS lines. In Nueva Vizcaya, the mean temperature from the beginning of October to the end of February in the next year is less than 22 °C, making it a suitable place to reproduce TGMS line seed. The mean temperature at Lucban from January to February was <23 °C, and so all the TGMS lines possessing a CFTP of <23 °C could be multiplied at Lucban. The TGMS lines should be completely male sterile to ensure the safety of hybrid seed production. Interestingly, we observed that the mean temperature at IRRI, Los Baños, was higher than 25 °C almost all year. So, the CSTP of fertility-sterility alteration of TGMS lines in the Philippines could be set at >24 °C for ensuring completely safe hybrid seed production, especially from April to June.

Pollen of A07 was partially fertile to completely sterile at Lucban as observed from 5 May to 17 June and completely sterile (with no pollen type) at Los Baños. A07 possesses a lower CFTP to turn completely fertile at <24 °C. A07 seeds produced in Nueva Vizcaya are possible where lower temperature prevails as compared to Lucban (Ali et al. 2020 Unpublished). Recently, with GIS technologies, IRRI has successfully identified a suitable choice of sites for hybrid seed production and TGMS self-seed multiplication based on 20 years of agrometeorological data. The potential GIS maps for the Philippines, identifying the places suitable for self-seed multiplication and hybrid rice seed production, are shown in Fig. 8. A map with a 0.08° spatial resolution and limited climatic data from 2010 to 2018 was used to avoid results affected by climate change trends. The following assumptions were used for locations selected based on temperature meeting a stable criterion for 28 days minimum each year, especially for hybrid seed production: (a) average daily temperature of >28 °C and ≤36 °C and (b) a minimum temperature of >24 °C. Likewise, for TGMS self-seed multiplication, a criterion of average temperature of <24 °C and Tmin >16 °C was used.

Table 6 Ideal locations for two-line hybrid seed production and TGMS self-seed multiplication (modified from Ali et al. 2018)

Seed production operation	Ideal places
Hybrid seed production	India: Aduthurai, Trichy, Killikulum, Madurai, Karnal, Delhi Philippines: Los Banos
TGMS seed production	India: Aduthurai, Gudalur, Samalkota, Karnal; Philippines: Lucban, Benguet, Nueva Vizcaya
Hybrid seed production & TGMS seed multiplication	India: Aduthurai and Samalkota

9 Wide-Scale Adoption and Use of Two-Line Hybrid Rice Technology

To achieve wide-scale adoption of two-line hybrid rice technology, we need ideal TGMS lines that should possess a higher combining ability, outcrossing rate, and market-desirable grain quality features along with insect and disease resistance (Fig. 6). During the sterile induction phase, the plants must be 100% male sterile with more than 99.5% pollen sterility and must behave stably under well-defined fertility-sterility alteration conditions. Higher seed setting above 45% in the self-seed multiplication phase is essential. Ideal TGMS lines should have lower CSTP (24 °C) and lower CFTP (22 °C). However, researchers are still attempting to lower the CSTP to 23 °C (mean temperature), which will render the TGMS lines highly stable, especially during the sterile phase, and make them highly suitable for hybrid rice seed production. The frequency of heterotic hybrids is much higher for two-line hybrids than for three-line hybrids as any nonTGMS parent could be used as a pollen parent, thereby increasing hybrid breeding efficiency. Furthermore, as there is no need for restorer genes in the male parents of two-line hybrids, this is highly ideal for developing *indica/japonica* hybrids as most *japonica* lines do not possess restorer genes. Since there is no need for a maintainer line for seed multiplication, this makes seed production much simpler and highly cost-effective. Two-line hybrids have obvious superiority over three-line hybrids for rice grain yield, quality, and insect pest and disease resistance (Chen et al. 2010). In this regard, the best two-line hybrids should address market segment requirements with a 30–35% yield advantage over market check inbreds and with higher seed reproducibility rates (>3 t/ha).

Two-line hybrid rice technology is feasible for tropical conditions for which the temperature regimes are highly suitable for its exploitation. TGMS parental lines with lower CSTP of 23 °C are highly essential for the success of this technology. At IRRI, we are trying to reach 22 °C for CSTP, which is even more stable and would ensure the wide-scale adoption of two-line hybrid rice technology. In this regard, the Two-Line Hybrid Rice Study Group involving key hybrid rice seed companies agreed to join hands in 2019 primarily to test-verify and validate potential TGMS lines, pollen parents, and F_1 hybrids in the target geographies. The study group will be able to jointly confirm the strength of two-line hybrid rice technology, especially

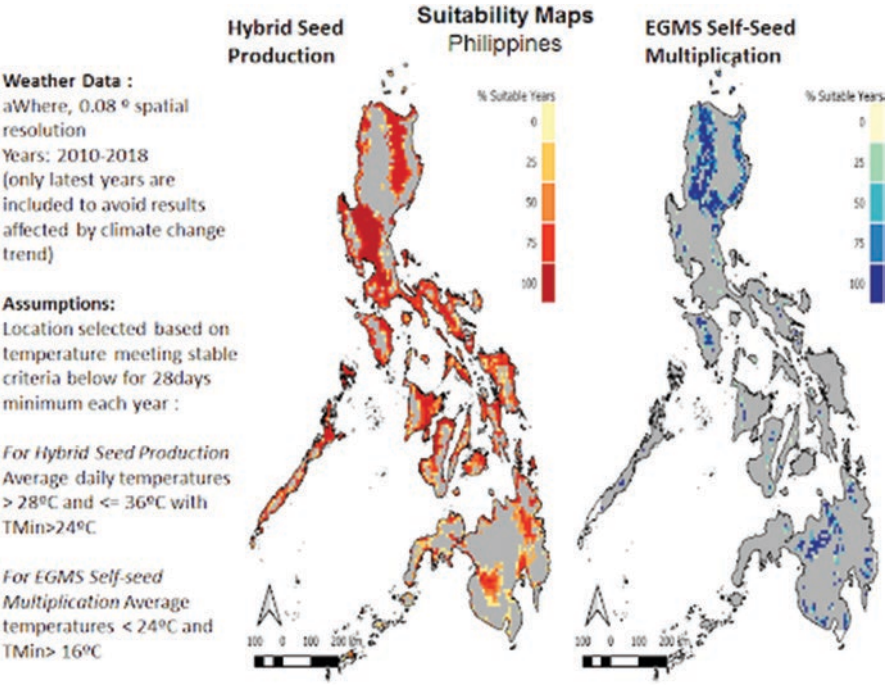

Fig. 8 Suitability maps for hybrid rice seed production and self-seed multiplication of TGMS lines for the Philippines developed by the IRRI GIS team

for its feasibility in South Asia. IRRI will continue to invest in this crucial technology for bringing the benefits of two-line rice hybrids to the rice farmers in South Asia. The accomplishment of this study group would ensure a lower cost of hybrid seeds, higher heterosis of two-line hybrids, and potential combinations meeting the market needs of the target regions. The success of two-line hybrid rice technology in tropical Asia would shift the attention of hybrid rice development in China toward South Asia, thus triggering widespread adoption of two-line rice hybrids.

10 Future Directions and Conclusions

The recent discovery of genome editing tools has opened up more opportunities to correct the genes of interest, including the *tms* gene, and to make them more stable and with precise expression. However, in many countries, genome editing is still under the genetically modified (GM) domain, including the Philippines. Li et al. (2019) introduced specific mutations into the *TMS5, Pi21*, and *Xa13* genes in Pinzhan intermediate breeding material using the CRISPR/Cas9 multiplex genome editing system. They demonstrated multiplex gene editing by finding transgene-free

homozygous triple *tms5/pi21/xa13* mutants obtained in the T_1 generation that displayed characteristics of TGMS with improved resistance to rice blast and bacterial leaf blight. However, recent publications on editing the *TMS5* gene and also achieving multiplex gene editing have increased our confidence to improve TGMS lines (Barman et al. 2019; Li et al. 2019; Zhou et al. 2016).

Wang and Deng (2018) described the development and implementation of the "third-generation" hybrid rice breeding system that is based on a transgenic approach to propagate and use stable recessive nuclear male-sterile lines. Using this approach, the male-sterile lines and hybrid rice produced using such a system are nontransgenic and hold great promise to boost the production of hybrid rice and other crops (Wang and Deng 2018).

To conclude, two-line hybrid rice technology primarily concentrates on the identification of proper TGMS parental lines with a lower CSTP (23 °C) and matching market segment requirements. The hybrids developed out of these TGMS parental lines should also meet market needs by achieving consumer and farmer acceptance that includes duration, grain shape, grain quality, and insect pest and disease resistance. Furthermore, these top-performing hybrids should have a high hybrid rice seed reproducibility of >3 t/ha to allow the private sector to adopt them. Also, hybrid rice seed costs would become relatively cheaper and enable farmers to invest in the purchase of seeds. Two-line rice hybrids have several advantages over three-line rice hybrids, and they could be easily upscaled once they match market needs. The Two-line Study Group was formed in 2019 at IRRI to understand the fundamental challenges for the wide-scale adoption of two-line hybrid rice technology and validate the research efforts by IRRI to meet these challenges and make the technology feasible. The study group is in the process of testing and verifying IRRI TGMS materials in the target regions. Recent advances in the field of GIS and the precise identification of suitable locations for hybrid rice seed production and TGMS self-seed multiplication, especially in the tropical countries in Asia and Africa, have given us the confidence to scale up two-line hybrid rice technology.

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