การพัฒนาพันธุ์ข้าวลูกผสมโดยใช้ระบบการผลิตข้าวลูกผสมแบบสองสายพันธุ์

Development of Hybrid Rice Varieties Using the Two Line Hybrid System

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บทคัดย่อ

การพัฒนาพันธุ์ข้าวลูกผสมเป็นอีกเทคโนโลยีที่เข้ามาช่วยเพิ่มปริมาณผลผลิต การศึกษาครั้งนี้มุ่งเน้นพัฒนาพันธุ์ข้าวลูกผสมแบบสองทาง โดยคัดเลือกสายพันธุ์แม่จากลักษณะทรงต้น การติดเมล็ด และความเป็นหมันในช่วงอุณหภูมิวิกฤต (Thermogenic male sterile) ที่อุณหภูมิสูงสุด/ต่ำสุด (35/26 ^{O}C) ซึ่งถูกควบคุมด้วยยีน *tms2* จำนวน 2 สายพันธุ์คือ PFT1 และ PFT2 คัดเลือกสายพันธุ์พ่อจำนวน 30 สายพันธุ์ จากจำนวน 205 สายพันธุ์ที่ได้จาก 3 แหล่งพันธุกรรม โดยมีสายพันธุ์ PM134 และ PM115 ให้ผลผลิตสูงถึง 961.5 และ 934.9 กิโลกรัมต่อไร่ ตามลำดับ จากนั้นนำสายพันธุ์พ่อแม่ที่ได้มาผลิตสายพันธุ์ข้าวลูกผสมจำนวน 60 คู่ผสม โดยเป็นการสร้างคู่ผสมจากการผสมด้วยมือ แล้วนำลูกผสมชั่วที่ 1 ที่ได้มาทดสอบผลผลิตโดยเปรียบเทียบกับสายพันธุ์พ่อแม่และพันธุ์มาตรฐานที่ให้ผลผลิตสูงในปัจจุบันจำนวน 10 พันธุ์ ผลการศึกษาพบว่าข้าวลูกผสมส่วนใหญ่ให้ผลผลิตสูงกว่าสายพันธุ์พ่อแม่และพันธุ์มาตรฐาน โดยเฉพาะข้าวลูกผสม 10 คู่ผสม ได้แก่ PF1/PM149, PF1/PM053, PF2/PM117, PF1/PM147, PF2/PM121, PF1/PM003, PF2/PM018, PF2/PM149, PF1/PM092 และ PF2/PM064 ให้ผลผลิต 2,595.84, 2,488.51, 2,488.77, 2,257.34, 2,235.26, 2,168.38, 2,152.06, 1,999.23, 1,994.18 และ 1.992.77 กิโลกรัม/ไร่ ตามลำดับ ซึ่งให้ผลผลิตสูงเมื่อเทียบกับข้าวพันธุ์ชัยนาท 1 ที่เป็นพันธุ์ข้าวมาตรฐาน คือ 1,222.40 กิโลกรัมต่อไร่ ดังนั้นการดำเนินการในขั้นต่อไปคือการทดสอบการผลิตเมล็ดพันธุ์ข้าวลูกผสมจากคู่ผสมที่ให้ผลผลิตสูงเหล่านี้ในแปลงผลิตเพื่อพัฒนาสู่ระบบการผลิตเมล็ดพันธุ์ที่เหมาะสมต่อไป

คำสำคัญ : ข้าวลูกผสมระบบสองสายพันธุ์ ยีน *tms2* เพศผู้เป็นหมันเนื่องจากอุณหภูมิ ความดีเด่นเหนือพ่อแม่

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ABSTRACT

This research aim to develop hybrid rice varieties using the two line hybrid system. For development of 2 thermo sensitive male sterile (TGMS) line, Norin PL12 was used as donor source of the *tms2* gene to develop female parents. Phenotypic selections of male sterility critical temperature, seed set and plant type were consequence applied for female parents. Two lines, PF1 and PF2 demonstrated a complete male sterility at mean maximum/minimum temperature was 35/26 ^{O}C were selected and used as female parents. Male parents were selected 30 line from 205 improved varieties. The criteria of selection are based upon the potential yield and other important agronomic characteristics. The results showed that the PM134 and PM115 yielded 961.5 and 934.9 kg/rai respectively. Evaluation of heterosis, combining ability and potential yield are critical step in the hybrid breeding program. Heterosis and combining ability were studied by grain yield and other yield component traits using F_1 crossed by hand pollination crosses. The 60 F_1 progenies from 2 female recipients and 30 male parents the high-yield donors were selected. Ten interesting F_1 hybrids had significantly higher yield than the best check variety; CNT1. The average yield of PF1/PM149, PF1/PM053, PF2/PM117, PF1/PM147, PF2/PM121, PF1/PM003, PF2/PM018, PF2/PM149, PF1/PM092 and PF2/PM064 were 2,595.84, 2,488.51, 2,488.77, 2,257.34, 2,235.26, 2,168.38, 2,152.06, 1,999.23, 1,994.18 and 1.992.77 kg/rai. The average yield of hybrids were higher than CNT1 (1222.40 Kg/rai)

Keywords : Two-line Hybrid Rice, *tms2*, Thermo sensitive male sterile, heterosis, combining ability

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INTRODUCTION

Nowadays, Thailand produces rice for both local consumption and export worldwide. Despite housing 63 million of inhabitants, it is able to yield up to 23 million tons of rice grain while approximately 12-13 million tons are consumed within country (Shull, 1980). Regarding to an increase of world's inhabitants along with 2 percent rising in demand for rice, it is proposed that Thailand (in the next 20 years) has to plant rice at least 30 million tons in order to maintain world's rice exporter leader. However, average yields across country are just over 381 kilograms per rai which is lower than other opponents. Hence, hybrid rice benefits heterosis by expecting higher yield than pure line varieties or varieties derived by conventional breeding programme for 20-30 percent (Yuan, 1997). Hybrid rice technology is one of breeding approaches to increase national rice yield in Thailand. Development of hybrid rice is necessary to increase average yield by combining the use of heterosis and male sterility in rice germplasm. (Yuan et al 2003). This project aims to develop hybrid rice varieties using the two line hybrid system.

RESEARCH METHODOLOGY

Selection of TGMS lines

The *tms2* gene derived from Norin PL12 was introgressed into Kao Dawk Mali 105 (KDML105) by Marker assisted selection until BC_3F_3 generation (Lopez et al.2003). Plant type selection was applied to select lines which were non-photoperiod sensitivity, short plant height, good yield components, cooking quality and completely sterility under critical temperature. Before flowering period, the pollen and spikelet fertilities were recorded. The pollen grains were stained with 1% IKI strain. The panicles that emerged were bagged before anthesis to prevented cross pollination and the number of well filled grains were counted at the time of maturity. The number of pollen fertility expression and well-filled grains were expressed as percentage.

Selectionof male parents

Six main source of rice germplasm were used in this study (Table1). Thai germplasm consisted of 3 groups, Rice gene discovery unit (RGDU), Phisanulok rice center (PSL) and Rice department (RD), based on the institutes where rice varieties are developed. IRRI source consisted of 3 groups, IRRI improved varieties, IRRI new plant type (NPT) and Philippine upland varieties (PUL). A total of 205 rice varieties and 5 standard cultivars including SPR1, CNT1, PTT1, IR64 and IR57514 were evaluated for yield, yield components, blast disease and bacterial blight test.

Table 1 Six main sources of rice germplasm were used in this study.

No.	Group	source	No. of Varieties
1	IRRI	International Rice Research Institute	80
2	Philippine up land	International Rice Research Institute	17
3	IRRI New plant type	International Rice Research Institute	33
4	KU	Kasetsart University, Khampangsaen	15
5	PSL	Phisanulok Rice Research Center	43
6	RD	Rice department	17
			205

Combining ability

Thirty high-yield rice varieties were used as male parent to evaluate combining ability. Top crossing with two female parents were done at RGDU, Kasetsart University Kamphaeng Saen Kampus in 2008. F_1 progenies from sixty crossing were evaluated yield and its components including tiller number per hill, number of grains per panicle, 1000 grain weight and total grain yield compared to their

parents and standard varieties. The experimental design was augmented with three blocks and each containing 20 F_1crosses, 2 female parents, 20 male parents and 5 standard varieties. Plot size was 0.75x1 m^2 with a spacing of 0.25 m x 0.25 m.

RESULTS

Selection of TGMS lines

Out of 154 TGMS lines and two elite lines (PF1 and PF2) that showed a complete male sterility at 35/26 ^{O}C were selected and used as female parents. Typical characteristics of these lines were shown on Table 2 and 3.

Table 2 Typical characteristics of 2 TGMS lines; PF1 and PF2

Variety	Bagging Panicle			Strain with 1%IKI				Temp (C)	
	No.of filled grain	No.of grain	% Fertile	No.of sterile pollen	No.of fertile pollen	pollen shape	% sterile	Maximum Temp.	Minimum Temp.
PF1	114	0	0	15	0	DP[1/]	100	35	26
PF2	105	0	0	18	0	EP[2/]	100	35	26

[1/]DP = distorted pollen grain, [2/]EP = empty pollen grain

Table 3 Typical characteristics of 2 TGMS lines; PF1 and PF2

Variety	Day to 50% flowering	Plant height (cm.)	Tiller/hill	Panicle/hill	Panicle length (cm)
PF1	105	83.2	11	9	21
PF2	91	112.1	10	8	24.1

Selection of male parent

The rice varieties from Pitsanulok Rice Research Center showed a good performance in plant types. Most of rice varieties from PUL were photo-sensitivity and showed tall plant height that was unflavored characteristics for being a male parent in hybrid rice production.

PM134, an improved variety from Phisanulok Rice Research Center, produced the highest yield (961.5 kg/rai). PM115, new released variety, was ranked second with the average yield of 934.9 kg/rai (Table4). Yield of other varieties was below 900 kg/rai. Rice varieties from PSL group showed the

highest average yield (674.2 kg/rai) and the rice varieties from PUL group had the lowest average yield (265.7 kg/rai). (Table 5) All group varieties had a potential grain yield excepted the PSL. The varieties provided the highest yield was from Pitsanulok Rice Research center.

Table 4 Potential yield and other important characteristics of male parents lines and check varieties.

Entry	Variety group	Variety	Plant height (cm)	No. of Panicle /hill	Estimated yield/ไai (kg)	Reaction of Blast disease	Reaction of BLB disease
1	PSL	PM134	102.1	11.3	961.5	R	R
2	RD	PM115	106.3	11.3	934.9	R	R
3	KU	PM124	119.7	10.2	877.0	R	S
4	PSL	PM147	108.7	12.5	866.1	R	R
5	PSL	PM143	99.1	13.1	839.3	R	MR
6	IRRI	PM002	99.3	13.4	827.6	MR	S
7	PSL	PM135	104.5	12.1	824.0	R	R
8	PSL	PM132	105.1	12.9	805.4	R	R
9	PSL	PM149	109.7	14.1	801.2	R	R
10	PSL	PM142	99.7	12.5	799.5	R	R
		CNT1	114.8	14.2	571.3	R	S
		IR57514	116.2	11.9	592.9	R	S
		IR64	91.6	15.7	563.5	R	MR
		PTT1	109.9	17.4	589.3	R	R
		SPR1	118.9	14.3	664.9	R	R
	LSD .05		4.1	8.5	136.6		

R = Resistance MR = Intermediate S = Susceptible

BLB = **Bacterial leaf blight**

Table 5 Potential yield and other important characters of rice varieties from different sources.

Group	Source	Amount	Plant height (cm)	No. of Panicle /hill	Estimated yield/Rai (kg)
1	International Rice Research Institute	81	103.70 (±10.24)	14.40 (±3.52)	565.40 (±132.77)
2	International Rice Research Institute	17	106.20 (±16.45)	11.70 (±4.15)	265.70 (±186.43)
3	International Rice Research Institute	33	102.10 (±13.59)	12.80 (±3.76)	480.60 (±203.56)
4	Kasetsart University, KhampangSaen	15	112.80 (±16.22)	11.50 (±3.44)	498.60 (±249.88)
5	Phisanulok Rice Research Center	43	105.80 (±5.60)	12.60 (±1.46)	674.20 (±114.75)
6	Rice department	16	111.20 (±20.59)	12.00 (±2.59)	584.30 (±137.36)
	Total	205	105.30 (±12.24)	13.20 (±3.29)	550.70 (±185.98)

F_1 hybrid yield trail

Sixty two-line hybrids from crossing between 2 TGMS recipients; PF1 and PF2 and high-yield donors 30 lines that selected from the previous work. Promise ranges of seed qualities, high yield and high productivity, especially the varieties from PSL. The results showed that the average of panicle number per plant, number of grains per panicle, 1000 grain weight and grain yield of the best standard variety; CNT1 were 14, 139, 27.8 and 1222.40, respectively. The 10 selected F_1hybrids showed yield and its components higher than CNT1 (Table 6). Interestingly, these ten F_1 crosses of PF1/PM149, PF1/PM053, PF2/PM117, PF1/PM147, PF2/PM121, PF1/PM003, PF2/PM018, PF2/PM149, PF1/PM092 and PF2/PM064 produced 2,595.84, 2,488.51, 2,488.77, 2,257.34, 2,235.26, 2,168.38, 2,152.06, 1,999.23, 1,994.18 and 1.992.77 kg/rai of grain yield, respectively while CNT1 yielded 1222.40 kg/rai (Table 6).

Table 6 Yield and yield components of 10 F_1 promising crosses, female parent (PF1, PF2) and best standard check varieties (CNT1).

No.	Cross	Tiller/hill	Grains/panicle	1000 Grains weight	Grain yield/rai
1	PF1/PM149	27	223	33.60	2595.84
2	PF1/PM053	26	171	35.40	2488.51
3	PF2/PM117	26	105	33.80	2448.77
4	PF1/PM147	26	179	34.30	2257.34
5	PF2/PM121	18	148	30.70	2235.26
6	PF1/PM003	33	183	28.10	2168.38
7	PF1/PM018	36	184	36.70	2152.06
8	PF2/PM149	24	166	35.20	1999.23
9	PF2/PM092	28	133	33.40	1994.18
10	PF1/PM064	29	170	32.90	1992.77
	PF1	9	72	24.40	404.77
	PF2	8	70	24.60	352.67
	CNT1	14	139	27.79	1222.40
	LSD .05	6.69	14.96	0.43	15.93

DISCUSSION

We also found that the PF1 line has a high combining ability and gave the high yield when crossing with any R line. While the PF2 line gave the very good yield when crossing with some R line. The results indicated the possibility of obtaining more heterosis hybrids only in specific cross combinations. (Bal Joshi, 2001) In some interesting crosses, the number of panicle per hill showed poor potential of restorer (R) lines. Any way, the other factors had effect on yield such spikelet number panicle,spikelet fertility percentage and 1000-grain weight (Virmani and Edwards, 1983). With appropriate choice of parental lines it appears possible to develop F1 rice hybrid possessing distinct yield superiority over the best check varieties parent lines. Yield components should be considered to increase the yield through selections (Yuan et al., 1994) The combination of PF1/PM149, PF12/PM053 and PF2/PM117 had higher than the best check variety suggested that these hybrids of crosses could developed for commercial hybrid rice production in the further research. Therefore, the initially steps of hybrid seed production is being now to get essential data on the way to identify suitable system and conditions for Thailand.

CONCLUSION

PF1 line demonstrates an extravagant general-crossing ability, while PF2 proposes excellent specific-crossing ability. However, the generated varieties are now facing a problem in percentage of grain seed for F_1 seed production. In addition, PF1/M149 is found out to be the suitable variety to be employed as hybrid rice due to high yield (2595 kg/rai), well adaptability and good performance. Test of seed production proposes more than 250 kg/rai in all experiments with plantation ratio between donor and recipient is 2:10. Nonetheless, this hybrid is in the process of evaluating in large scale production and will be release as new hybrid rice.

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