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ภาคผนวกที่ ๑

การวิเคราะห์หาความแปรปรวนระหว่างแม่พันธุ์และพ่อพันธุ์เพศผู้ปกติโดยวิธี Female × Male Analysis ตามวิธีของ Kempthorne (1957) โดยที่

$$\text{C.F. (crosses)} = \frac{(\text{ผลรวมของลูกผสมของแม่พันธุ์และพ่อพันธุ์เพศผู้ปกติ})^{๒}}{\text{จำนวนซ้ำ} \times \text{จำนวนแม่พันธุ์} \times \text{จำนวนพ่อพันธุ์เพศผู้ปกติ}}$$

$$\text{S.S. (total)} = \text{ผลรวม } (\text{ลูกผสมของแม่พันธุ์และพ่อพันธุ์เพศผู้ปกติ})^{๒} - \text{C.F. (crosses)}$$

$$\text{S.S. (block)} = \frac{\text{ผลรวม } (\text{ผลรวมของลูกผสมภายในบล็อคหรือซ้ำเดียวกัน})^{๒}}{\text{จำนวนแม่พันธุ์} \times \text{จำนวนพ่อพันธุ์เพศผู้ปกติ}} - \text{C.F. (crosses)}$$

$$\text{S.S. (female)} = \frac{\text{ผลรวม } (\text{ผลรวมของพ่อพันธุ์เพศผู้ปกติที่ผสมกับแม่พันธุ์เดียวกัน})^{๒}}{\text{จำนวนซ้ำ} \times \text{จำนวนพ่อพันธุ์เพศผู้ปกติ}} - \text{C.F. (crosses)}$$

$$\text{S.S. (male)} = \frac{\text{ผลรวม } (\text{ผลรวมของแม่พันธุ์ที่ผสมกับพ่อพันธุ์เพศผู้ปกติเดียวกัน})^{๒}}{\text{จำนวนซ้ำ} \times \text{จำนวนแม่พันธุ์}} - \text{C.F. (crosses)}$$

$$\text{S.S. (crosses)} = \frac{(\text{ผลรวมของลูกผสมของแม่พันธุ์และพ่อพันธุ์เพศผู้ปกติ})^{๒}}{\text{จำนวนซ้ำ}}$$

$$\text{S.S. (female} \times \text{male)} = \text{S.S. (crosses)} - \text{S.S. (female)} - \text{S.S. (male)}$$

$$\text{S.S. (error)} = \text{S.S. (total)} - \text{S.S. (block)} - \text{S.S. (female)} - \text{S.S. (male)} - \text{S.S. (female} \times \text{male)}$$

ตารางภาคผนวกที่ ๑ การวิเคราะห์ความแปรปรวนโดยวิธี Female × Male Analysis

source of variance	Df	S.S.	M.S.
Block	r–1	S.S. (block)	Block
Female	f–1	S.S. (female)	Female
Male	m–1	S.S. (male)	Male
Female × Male	(f–1) × (m–1)	S.S. (female × male)	Female × Male
Error		S.S. (error)	Error
Total	rfm–1		Total

r = จำนวนซ้ำ

f = จำนวนแม่พันธุ์

m = จำนวนพ่อพันธุ์เพศผู้ปกติ

การประเมินค่าความสามารถในการรวมตัวทั่วไป (general combining ability, gca)

แม่พันธุ์ (female)

$$g_i = \frac{x_{\cdot i \cdot}}{mr} - \frac{x_{\cdots}}{fmr}$$

g_i	=	ค่าความสามารถในการรวมตัวทั่วไปของแม่พันธุ์ i
$x_{\cdot i \cdot}$	=	ผลรวมค่าเฉลี่ยทั้งหมดของลูกผสมเดี่ยวที่เกิดจากแม่พันธุ์ i
$x_{\cdots}$	=	ผลรวมค่าเฉลี่ยลูกผสมเดี่ยวทั้งหมด lt คู่
f	=	จำนวนแม่พันธุ์
m	=	จำนวนพ่อพันธุ์เพศผู้ปกติ
r	=	จำนวนซ้ำ

พ่อพันธุ์เพศผู้ปกติ (male)

$$g_j = \frac{x_{.j.}}{fr} - \frac{x_{...}}{fmr}$$

g_j = ค่าความสามารถในการรวมตัวทั่วไปของพ่อพันธุ์เพศผู้ปกติ j

$x_{.j.}$ = ผลรวมค่าเฉลี่ยทั้งหมดของลูกผสมเดี่ยวที่เกิดจากพ่อพันธุ์เพศผู้ปกติ j

$x_{...}$ = ผลรวมค่าเฉลี่ยลูกผสมเดี่ยวทั้งหมด lt คู่

f = จำนวนแม่พันธุ์

m = จำนวนพ่อพันธุ์เพศผู้ปกติ

r = จำนวนซ้ำ

การประเมินค่าความสามารถในการรวมตัวเฉพาะ (specific combining ability, sca)

$$s_{ij} = \frac{x_{.ij.}}{r} - \frac{x_{.i.}}{mr} - \frac{x_{.j.}}{fr} + \frac{x_{...}}{fmr}$$

s_{ij} = ค่าความสามารถในการรวมตัวเฉพาะของลูกผสมเดี่ยว ij

$x_{.ij.}$ = ค่าเฉลี่ยของลูกผสมเดี่ยว ij

$x_{.i.}$ = ผลรวมค่าเฉลี่ยทั้งหมดของลูกผสมเดี่ยวที่เกิดจากแม่พันธุ์ i

$x_{.j.}$ = ผลรวมค่าเฉลี่ยทั้งหมดของลูกผสมเดี่ยวที่เกิดจากพ่อพันธุ์เพศผู้ปกติ j

$x_{...}$ = ผลรวมค่าเฉลี่ยลูกผสมเดี่ยวทั้งหมด lt คู่

f = จำนวนแม่พันธุ์

m = จำนวนพ่อพันธุ์เพศผู้ปกติ

r = จำนวนซ้ำ

คำนวณความคลาดเคลื่อนมาตรฐานจากสูตร

$$\text{S.E. (gca for female)} = \sqrt{\frac{Me}{rm}}$$

$$\text{S.E. (gi–gj) female} = \sqrt{\frac{2.\ Me}{rm}}$$

$$\text{S.E. (gca for male)} = \sqrt{\frac{Me}{rf}}$$

$$\text{S.E. (gi–gj) male} = \sqrt{\frac{2.\ Me}{rf}}$$

$$\text{S.E. (sca effect)} = \sqrt{\frac{Me}{r}}$$

$$\text{S.E. (Sij–Skl)} = \sqrt{\frac{2.\ Me}{r}}$$

หาค่าต่ำสุดเพื่อแสดงความแตกต่างทางสถิติ

LSD_{α} ของความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่าความสามารถในการรวมตัวทั่วไปของแม่พันธุ์

= t_{α} S.E. (gca for female)

LSD_{α} ของการเปรียบเทียบค่าความสามารถในการรวมตัวทั่วไปของแม่พันธุ์

= t_{α} S.E. (gi–gj) female

LSD_{α} ของความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่าความสามารถในการรวมตัวทั่วไปของพ่อพันธุ์เพศผู้ปกติ

= t_{α} S.E. (gca for male)

LSD_{α} ของการเปรียบเทียบค่าความสามารถในการรวมตัวทั่วไปของพ่อพันธุ์เพศผู้ปกติ

= t_{α} S.E. (gi–gj) male

LSD_{α} ของความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่าความสามารถในการรวมตัวเฉพาะ

= t_{α} S.E. (sca effect)

LSD_{α} ของการเปรียบเทียบค่าความสามารถในการรวมตัวเฉพาะ = t_{α} S.E. (Sij–Skl)

ภาคผนวกที่ ๒

เปรียบเทียบพันธุ์พริกหนุ่มเขียวลูกผสมชั่วที่๑ ๙ พันธุ์กับพ่อพันธุ์ ๓ พันธุ์ แม่พันธุ์ ๓ พันธุ์และพันธุ์มาตรฐาน ๓ พันธุ์ ในฤดูหนาว ๒๕๕๓

ตารางภาคผนวกที่ ๒ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

SOV.	d.f.	Mean Square					
		ความสูงต้น (ซม.)	ความกว้างทรงพุ่ม (ซม.)	นน.ผล/ต้น (กก./ต้น)	ผลผลิต (กก./ไร่)	จน. ผล/ต้น	นน.ผล (ก.)
Replication	2	32.7467	88.3558	0.0118	488316.8334	19.5616	0.9369
Treatment	17	238.8496**	64.7060	0.0960**	3927890.4049**	149.6236**	387.8264**
Error	34	38.3560	49.2929	0.0072	298694.2657	8.6940	6.2865
C.V. (%)		9.11	9.29	12.34	12.44	11.15	7.45

ตารางภาคผนวกที่ ๓ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

SOV	d.f.	Mean Square					
		ความกว้างผล (ซม.)	ความยาวผล (ซม.)	ความหนาของเนื้อผล(มม.)	L*	Chroma	Hue angle
Replication	2	0.0012	1.1990	0.00002	5.7036	10.8983	1.4704
Treatment	17	0.7105**	15.7266**	0.00312**	60.3898**	36.0007**	20.2636**
Error	34	0.0162	0.4930	0.00019	3.5440	5.3614	2.0199
C.V. (%)		4.82	4.09	5.88	3.65	5.12	1.12

ตารางภาคผนวกที่ ๔ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

SOV	d.f.	Mean Square		
		คลอโรฟิลล์เอ (มก./๑๐๐กรัมน้ำหนักผลสด)	คลอโรฟิลล์บี (มก./๑๐๐กรัมน้ำหนักผลสด)	คลอโรฟิลล์ทั้งหมด (มก./๑๐๐กรัมน้ำหนักผลสด)
Replication	2	1.852E-06	1.852E-06	7.407E-06
Treatment	17	1.313E-04**	1.483E-04**	3.734E-04**
Error	34	7.734E-06	1.852E-06	7.407E-06
C.V. (%)		27.34	20.65	14.91

ตารางภาคผนวกที่๕ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

SOV	d.f.	Mean Square			
		ปริมาณของแข็งที่ละลายน้ำได้(%)	ความชื้น (%)	วิตามินซี (มก./๑๐๐ กรัมน้ำหนักผลสด)	แคพไซซิน (Scoville unit)
Replication	2	0.2585	0.0422	0.2800	31938.8889
Treatment	17	2.0288**	14.5665**	2.4474**	6412764.7059**
Error	34	0.0803	0.6333	0.2379	24868.3007
C.V. (%)		5.38	0.92	8.56	8.89

ตารางภาคผนวกที่ ๖ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

Source of Variance	df.	Mean Square		
		ความสูงต้น (ซม.)	ความกว้างทรงพุ่ม (ซม.)	นน.ผล/ต้น (กก./ต้น)
Replications	2	35.55	80.04	0.010
Treatments	14	215.98**	75.48	0.108**
Parents	5	310.07**	57.71	0.056**
Parents vs. Crosses	1	0.20	44.86	0.228**
Crosses	8	184.15**	90.41	0.125**
Lines	2	302.38	61.40	0.135
Testers	2	210.16	30.60	0.072
Lines x Testers	4	112.02*	134.83*	0.147**
Error	28	39.57	48.75	0.008
%CV		9.51	9.25	13.32

*, ** significant difference at P<0.05 and P<0.01 levels, respectively.

ตารางภาคผนวกที่ ๗ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

Source of Variance	df.	Mean Square					
		ผลผลิต (กก./ไร่)	จน. ผล /ต้น	นน.ผล (ก.)	ความกว้างผล (ซม.)	ความยาวผล (ซม.)	ความหนาของเนื้อผล (มม.)[1]
Replications	2	409651.4	22.597	1.31	0.000	1.110	0.000
Treatments	14	4421335.0**	124.087**	439.23**	0.665**	18.425**	0.010**
Parents	5	2294860.4**	44.744**	610.85**	0.997**	19.679**	0.016**
Parents vs. Crosses	1	9359011.7**	0.001	13.11	0.092*	39.721**	0.002
Crosses	8	5133172.0**	189.188**	385.24**	0.530**	14.978**	0.008**
Lines	2	5542065.0	365.410	485.44	0.868	1.719	0.013
Testers	2	2969892.8	43.416	431.27	0.504	10.836	0.003
Lines x Testers	4	6010365.0**	173.963**	312.12**	0.373**	23.679**	0.008**
Error	28	328790.2	7.938	7.26	0.019	0.559	0.001
%CV		13.34	11.26	7.72	5.02	4.39	8.38

[1] Transformed data by log(x)+1.

*, ** significant difference at P<0.05 and P<0.01 levels, respectively

ตารางภาคผนวกที่ ๘ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

Source of Variance	df.	Mean Square					
		ความยาวของก้านผล	L*	Chroma	Hue angle	ปริมาณของแข็งที่ละลายน้ำได้(%)	ความชื้น (%)
Replications	2	0.02	8.80	11.30	2.22	0.15	0.09
Treatments	14	0.97**	53.47**	29.92**	19.73**	2.11**	17.19**
Parents	5	1.35**	41.68**	22.51**	18.99**	2.91**	18.45**
Parents vs. Crosses	1	0.23	14.79*	10.80	3.43	1.16**	3.25*
Crosses	8	0.83**	65.67**	36.95**	22.23**	1.72**	18.15**
Lines	2	1.44	68.84	47.35	15.43	4.05	8.07
Testers	2	0.82	99.62	40.48	27.01	0.11	37.44
Lines x Testers	4	0.53**	47.11**	29.98**	23.23**	1.36**	13.54**
Error	28	0.09	3.43	5.82	2.21	0.09	0.67
%CV		5.85	3.56	5.31	1.17	5.65	0.94

*, ** significant difference at P<0.05 and P<0.01 levels, respectively.

ตารางภาคผนวกที่ ๙ ความแปรปรวนของของลักษณะทางพืชสวนและสมบัติทางเคมีของของพริกหนุ่มเขียว ในฤดูหนาว ๒๕๕๓

Source of Variance	df.	Mean Square				
		วิตามินซี (มก./๑๐๐ กรัม น้ำหนักผลสด)	คลอโรฟิลล์เอ (มก./๑๐๐กรัม น้ำหนักผลสด)	คลอโรฟิลล์บี (มก./๑๐๐กรัม น้ำหนักผลสด)	คลอโรฟิลล์รวม (มก./๑๐๐กรัม น้ำหนักผลสด)	แคพไซซิน (Scoville unit)
Replications	2	0.32	0.004	0.003	0.002	26246.67
Treatments	14	2.67**	0.528**	0.786**	0.616**	7493985.71**
Parents	5	3.28**	0.961**	1.037**	0.983**	5516400.00**
Parents vs. Crosses	1	0.22	0.914**	2.581**	1.610**	16333.33
Crosses	8	2.60**	0.209**	0.405**	0.263**	9664683.33**
Lines	2	8.75**	0.129	0.588	0.239	11864633.33
Testers	2	0.55	0.051	0.088	0.053	7238233.33
Lines x Testers	4	0.55	0.327**	0.471**	0.380**	9777933.33**
Error	28	0.24	0.002	0.002	0.002	22989.52
%CV		8.59	2.47	2.64	2.16	8.53

[1] Transformed data by log(x)+4.

*, ** significant difference at P<0.05 and P<0.01 levels, respectively.

ภาคผนวกที่ ๓

เสนอผลงานที่ International Conference on Solanaceae Resistance Breeding Technologies, Genetics and Genomics February 17 –19, 2011, Le Meridien Hotel, Chiang Mai, Thailand เรื่อง Cytoplasmic male sterile and sterility maintainer cultivars of *Capsicum annuum* L. วันที่ ๑๗ กุมภาพันธ์ ๒๕๕๔

Cytoplasmic Male Sterile and Sterility Maintainer Cultivars of *Capsicum annuum* L.

Maneechat Nikornpun[1]/ and Krisda Sukwiwat[2]

ABSTRACT

Two male sterile cultivars, A1 and A2, were crossed with the C1 cultivar, selfed and backrossed for two generations. The first and second backcross progenies showed higher capsaicin content than their F1 hybrids. The differences were statistically significant. The second backcross generation progenies were selfed and crossed with several male fertile cultivars, designated cultivars, C2, C3, and C4, to produce hybrids. The hybrids yielded as well as some checks and had variation of capsaicin contents. One good female parent was selected from the A1 crossed with C1-3. It was selfed and crossed with 3 good maintainer cultivars, B1, B2 and B3. The progenies were backcrossed with respective maintainer parents for 3 generations. These backcross lines could serve as female parents for hybrid production. Second backcross generation lines of three crosses of male sterile lines and the sterility maintainer lines were distributed to two seed companies in Thailand in 2010. Seeds of the third backcross generations of sterile chilies and male sterility maintainers are available from the Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

Keywords: chili, F1 hybrid, cytoplasmic male sterility and pungency.

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INTRODUCTION

Local and open-pollinated cultivars of chilies are widely used by farmers in Thailand. In the northern part of Thailand, a particular type of hot chili is widely grown for a special type of chili paste. Cultivars of hot chilies used for this type of paste are different from local cultivars of hot chilies in other parts of the country. Many open-pollinated and some F1 hybrid cultivars are available. However, most farmers prefer to produce their own chili seeds rather than purchasing them from seed stores. They are reluctant to purchase the F1 hybrid seeds because of the high price. The use of male sterility in chilies would decrease the cost of production of the F1 hybrid seeds (Berke, 1999). Emasculation of male flowers would no longer be needed. However, sterile cultivars of chilies do not have high pungency and other characteristics that are required for the hot chili cultivars Wang *et al.* (2004).

Male sterility in chilies was reported by Martin and Grawford in 1951 and Peterson in 1958 and subsequently by Basset (1986), Novak and Beltach (1973), Shifriss and Pilowsky (1993), Shifriss (1997), Gulvas *et al.*(2006) and Wang *et al.*(2006). Both genic male sterility and cytoplasmic-genic male sterility have been used in the breeding and seed production of hybrid chilies (Zou *et al.,* 2001, Fan and Liu, 2002 and Yazawa *et al.,* 2002). This has proven to be an effective way of producing F1 hybrid chilies. Cytoplasmic-genic male sterility has been used more than genic male sterility. Improvement in male sterile lines for the stability of pollen sterility is needed. Identification of heterosis, general combining ability and specific combining ability is also needed to develop superior hybrids (Meshram, 1986, Ahmed *et al.* 1999, Ahmed and Panday, 2002, Linganagouda *et al.,* 2003, Zou *et al.,* 2007, Gelata *et al.,* 2004, Gelata *et al.,* 2004, Prasad *et al.,* 2003, Singh and Claudhary, 2005 and Kanthaswarry *et al.,* 2003).

MATERIALS AND METHODS

Selection and evaluation of locally grown cultivars.

Twelve cultivars of hot chilies were collected from the local areas around Chiang Mai University and Kasetsart University and designated cultivar C2 to C13. They were selfed for three generations before being grown during the rainy season at Chiang Mai University in a randomized complete block design with three replications. Soil type of the area is sandy loam , average day temperature was 32.6±0.7 oC, average night temperature was 27.2±0.9 o C , relative humidity was 78.5 % and light intensity was 432.8 W/m2. Twenty plants were used in each treatment. They were grown in the field. Fertilizer was applied at the rate 200 kg. of 15N-15P-15 K/ha. Insecticides such as imidacloprid, fipronil sulfur and methomyl were used at recommended rates once a week. Thirty days old seedlings were planted in a double row bed, at spacing of 50 x 50 cm. with a plot size of 9 m2. High yielding cultivars with good fruit quality and good plant type were selected for test crosses.

Development of germplasm: Improvement of male sterile lines for yield and capsaicin levels.

Cytoplasmic male sterile – hot chilies were developed from the two F1 hybrid, hot chilies described by Milerue (2000), These hybrids, A1 x C1 and A2 x C1, are high yielding but do not have high pungency. They were produced from cytoplasmic male sterile lines, A1 and A2, crossed with restorer line,C1, so they are heterozygous (*Rfrf)* for restorer genes.

Therefore, they segregate for sterility upon selfing. After selfing, fertile plants either *SRfRf, SRfrf* and male sterile plants with *Srfrf* were obtained. We selfed for three generations and then selected male sterile plants for backcrossing with the C1 cultivar (Figure 1)

Three male sterile female lines, A2 x C1-1, A1 x C1-2 and A1 x C1-3 were selected as the most desirable. Progenies from the first and second backcross generations of these three lines were tested for capsaicin content in comparison with the F1 hybrids in a randomized complete block design with three replications. Number of plants in each treatment, plot size and field management were the same as mentioned in the earlier experiment. Male sterile plants with desirable characteristics were selected from the second backcross generation of A2 x C1-1, A1 x

C1-2 and A1 x C1-3. They were crossed with the fertile plants within the same population and the best male sterile progenies were selected for 2 to 3 generations. These were then crossed with 3 good maintainer lines, B1, B2 and B3 to produce hybrids which were backcrossed with the maintainer lines for ๓ generations.

Crossing and progeny tests.

Backcross 2 progenies of A1 x C1-2, A1 x C1-3, and A2 x C1-1, were selected for high yields and good horticultural characteristics. They were crossed with the three local cultivars, C2, C3, C4, and evaluated for capsaicin contents and heterosis compared to the single cross hybrids, A1xC1 and A2xC1. The nine F1 hybrids produced were tested and compared with the three local cultivars, C2, C3 and C4, and with three commercial cultivars, S1, S2 and S3. A randomized complete block design with three replications was used. Number of plants in each treatment, plot size, spacing and field management were the same as mentioned in the earlier experiment. Fruit yield, fruit length and width, and capsaicin content were recorded. The capsaicin content was measured as mentioned using spectrophotometry at 750 nm (Anan *et al.*,1996). Heterosis (%) was calculated as Average of F1 hybrid – Average of better parent divided by Average of better parent x 100.

Remark : Abbreviations of cultivars; A1-Ky 1-1, A2-CF, C1 Bang-Chang, C1-1-Bang-Chang 10, C1-2-Bang-Chang 14, C1-3- Bang-Chang 16, C2-337, C3-437, C4-137, C5-233, C6-135, C7-1121, C8-634, C9-234, C10- 935, C11-737, C12-1201 and C13-4310, S1-Jom-Tong 2, S2-NhumKhiew, S3- San-Patong, B1- CA 1445, B2- CA1449 and B3-CA1450.

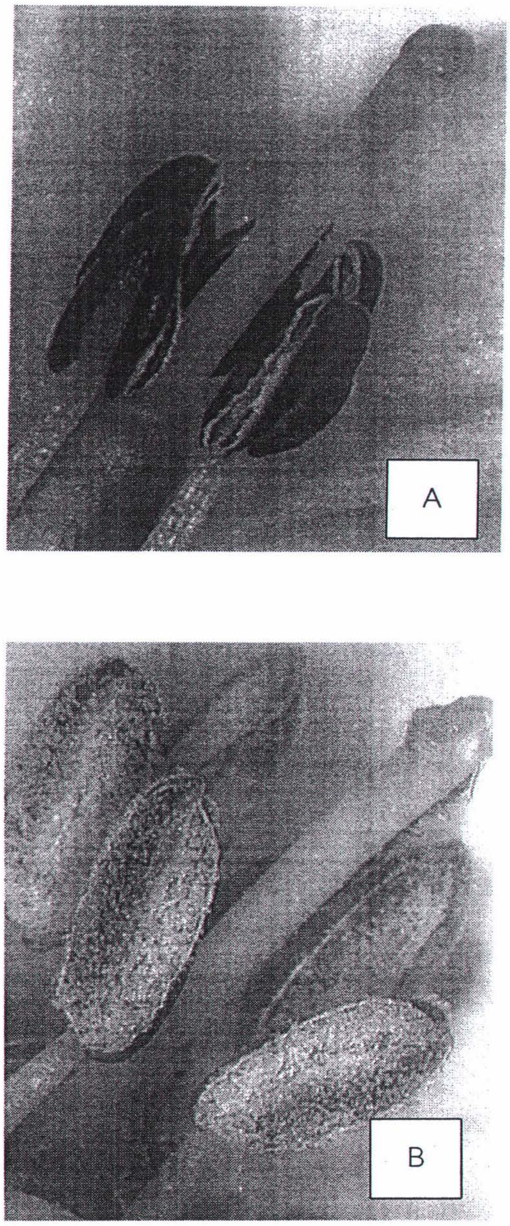

Figure 1 Sterile flowers (A) and fertile flowers (B) of chilies.

RESULTS

Selection and evaluation of locally grown cultivars.

The twelve cultivars of hot chilies that were collected showed fruit yield ranging from 9.1 to 19.4 t/ha (Table 1). The differences in yields were statistically significant. The three highest yielding cultivars were cultivars C2, C3 and C4 which showed 19.4, 15.4 and 12.7 t/ha, respectively. They had good horticultural characteristics in terms of plant and fruit type such as compact bush plants, male fertility, preferred fruit color at the mature stage, fruit pendency and heavy fruit set.

Development of male sterile and maintainer lines.

The F1 hybrids, A2 x C1 and A1 xC1, (Milerue, 2000) were grown to compare with their first and second backcross progenies. The capsaicin content of these was tested and the data is presented in Table 2.

The F1 hybrid , A2 x C1, showed a capsaicin content of 7,493 Scoville units. The first and second backcross progenies of A2 x C1-1, showed a capsaicin content of 8,063 and 9,147 Scoville units, respectively. The differences were statistically significant. Capsaicin levels increased markedly when the female line A2 x C1-1, was backcrossed with the C1 cultivar.

The percentage of heterosis for capsaicin contents of the first and second backcross generations over the C1 cultivar was 7.6% and 13.44%, respectively. The F1 hybrid, A1 xC1, showed a capsaicin content of 1,313 Scoville units. The first generation backcross progenies of A1 xC1-2 and A1 x C1-3, showed a capsaicin contents of 5,290 and 4,703 Scoville units, respectively. The percentage of heterosis over the C1 for progenies A1 xC1-2 and A1 x C1-3, was 302.82% and 258.18%, respectively. The second backcross generation progenies of, A1 x C1-2 and A1 x C1-3, had a capsaicin content of 7,220 and 10110 Scoville units, respectively. The percentage of heterosis for bc2 progenies, A1 x C1-2 and A1 x C1-3 was 36.48% and 114.96%, respectively. The differences in capsaicin content between the F1 hybrid, A1 x C1, and the first and second backcross progenies were statistically significant. One good female line was selected from A1 x C1-3. Since it lacked fertility restorers and was male sterile, it was crossed with 3 good maintainer cultivars, B1, B2 and B3. Theprogenies were backcrossed with respective maintainer parents for 3 generations. Three second backcross generations of male sterile lines, (A1 x C1-3) x B1, (A1 x C1-3) x B2 and (A1 x C1-3) x B3 , and the sterility maintainer lines, B1, B2 and B3 (Figure 2), were distributed to two seed companies in Thailand in 2010.

Crossing and progeny tests.

The nine F1 hybrid progenies between male sterile lines and local cultivars showed fruit yields ranging from 20 to 38.4 t/ha (Table 3). The yields were significantly different. The highest yielding hybrids were (A2 x C1-1) x C4, (A1 x C1-2) x C4, (A1 x C1-2) x C3 and (A2 x C1-1) x C2, which showed 38.4, 36.1, 35.2 and 34.4 t/ha, respectively. These yields were not significantly different from those of the parental, C4, and the commercial cultivar S1. However, they were significantly higher than those of the parental cultivars, C2 and C3, and commercial cultivars, S2 and S3. Among these high yielding cultivars, (A2 x C1-1) x C2, showed the best heterosis at 38.66%. F1 hybrids, (A2 x C1-1) x C4 and (A1 x C1-2) x C4, showed low heterosis, 5.2% and 0%, respectively.

The capsaicin contents of the F1 hybrids showed a range from 1,020 to 7,520 Scoville units (Table 3). Two high yielding F1 hybrids, (A2 x C1-1) x C4 and (A1 x C1-2) x C4, showed the lowest capsaicin levels, 1,470 and 1,020 Scoville units, respectively. These levels were significantly lower than those of their parental cultivar, C4, which showed 9,310 Scoville units and those of all commercial cultivars. Two other high yielding F1 hybrids, (A1 x C1-2) x C3 and (A2 x C1-1) x C2, showed capsaicin levels of 5,580 and 4,880 Scoville units, respectively. These levels were significantly lower than one of the parental cultivar, C3, but were more or less the same levels as those of the commercial cultivar, S1.

Table 10 Fruit yield of local cultivars of chilies tested in rainy season.

Local cultivar	Yield (t/ha)
C2[x]	19.4a[y]
C3	15.4b
C4	12.7c
C5	12.0c
C6	11.3c
C7	11.1c
C8	10.6d
C9	10.5d
C10	9.4e
C11	9.6e
C12	9.4e
C13	9.1e
LSD0.05	0.9
CV (%)	8.18

[x] Abbreviations of cultivars ; C2-337, C3-437, C4-137, C5-233, C6-135, C7-1121, C8-634, C9-234, C10-935, C11-737, C12-1201 and C13-4310.

[y] Means followed by the same letters indicate no differences at $P < 0.05$ by Least significant difference.

Table 11 Capsaicin content and percentage of heterosis of cms chili lines selected from F1 hybrids between cms sources and high capsaicin chili and bc1 and bc2 generations to the high capsaicin parent.

Germplasm	Capsaicin (Scoville unit)	Heterosis (%)
F1 hybrids		
A2 x C1[x]	7,493d[y]/	-
A1 x C1	1,313f	-
BC1		
A2 x C1-1	8,063c	7.6
A1 x C1-2	5,290e	302.89
A1 x C1-3	4,703e	258.18
BC2		
A1 x C1-3	10,110c	114.96
A2 x C1-1	9,147b	13.44
A1 x C1-2	7,220d	36.48
LSD 0.05	627	-
CV(%)	1697	-

[x]Abbreviations of cultivars ; A1-Ky1-1, A2-CF, C1- Bang-Chang, C1-1- Bang-Chang 10, C1-2- Bang-Chang 14 and C1-3-Bang-Chang 16.

[y]Means followed by the same letters indicate no differences at $P < 0.05$ by Least significant difference. BC = backcross

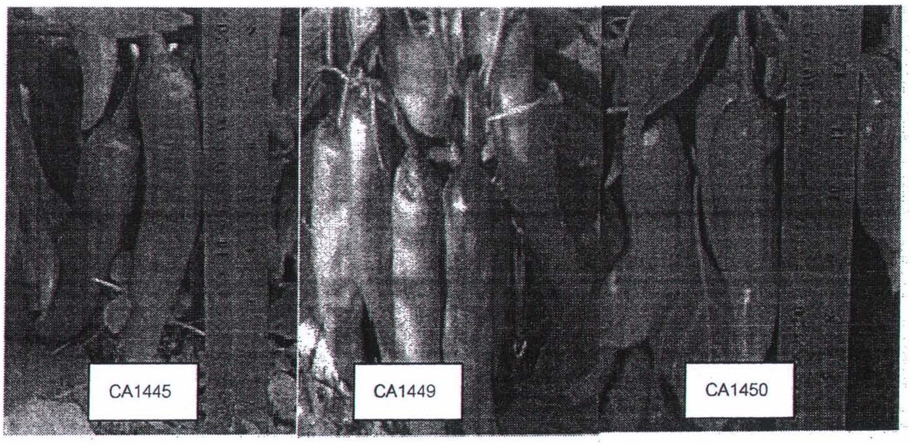

Figure 2 Characteristic fruit of maintainer lines useful for cms hybrid production in chilies

Table 12 Fruit yield and horticultural characteristics of hybrids between cms lines and local cultivars of chilies

Cultivar t/ha	Yield (cm)	Fruit length (cm)	Fruit diameter (cm)	Yield/pt (kg)	% Heterosis	Capsaisin (Scoville unit)
Local cultivars						
C4	33.8a1/	14.21d	1.61b	0.901a		9,310a
C3	27.6c	12.72e	1.37cd	0.692b		8,290a
C2	19.6d	17.77b	1.68b	0.637b		1,050d
Hybrids with cms lines						
(A2xC1–1)xC4	38.4a	15.34e	1.84a	0.947a	5.2	1,470d
(A1xC1–2)xC4	36.1a	15.37e	1.97a	0.901a	0	1,020d
(A1xC1–2)xC3	35.2a	15.43e	1.65b	0.872a	25.97	5,580b
(A2xC1–1)xC2	34.4a	19.93a	1.42c	0.883a	38.66	4,880b
(A1xC1–2)xC2	30.9b	19.37a	1.40c	0.760b	19.3	3,720c
(A1xC1–3)xC2	30.8b	15.67c	1.59b	0.810b	–10	7,520a
(A1xC1–3)xC3	25.0c	13.69d	1.43c	0.699b	1.04	5,480b
(A1xC1–3)xC2	24.1c	18.92a	1.37cd	0.697b	9.47	4,460b
(A2xC1–2)xC3	20.0d	14.10d	1.23de	0.513c	–25.78	5,030b

Table 12(Cont.) Fruit yield and horticultural characteristics of hybrids between cms lines and local cultivars of chilies.

Cultivar	Yield (t/ha)	Fruit length (cm)	Fruit diameter (cm)	Yield/pt (kg)	% Heterosis	Capsaicin (Scoville unit)
Commercial cultivars						
S1[x]	33.5a[y]	15.34e	1.97a	0.775b	-	4,050b
S2	20.1d	11.68e	1.11e	0.539c	-	3,900c
S3	18.1d	18.11b	1.56b	0.475c	-	4,430b
LSD0.05	2.65	1.47	0.14	0.24	-	2,227
C.V.(%)	5.72	10.6	19.10		-	28.52

[x]Abbreviations of cultivars ; A1–Ky1–1, A2–CF, C1–1– Bang–Chang 10, C1–2– Bang–Chang 14, C1–3– Bang Chang 16,C2– 337, C3–437, C4–137, S1– Jom Tong 2, S2– Nhum Khiew and S3– San Patong.

[y] Means followed by the same letters indicate no differences at $P \leq 0.05$ by Least significant difference.

DISCUSSION

We have identified usable sources of cytoplasmic male sterility in hot chilies. The sources came from crosses of A1 and A2 cultivars onto C1. The hybrids produced had low capsaicin content and were not acceptable to farmers. However, backcrosses of the hybrids to the C1 parent increased the capsaicin content. After two backcrosses to C1, suitable female lines with high capsaicin were developed. However, the best of these lines were male sterile and needed maintainer lines. Instead of developing isogenic maintainer lines, which is a long process, we used unrelated maintainer lines to develop sterile F1 hybrids.

We collected and evaluated 12 hot chili cultivars from Northern Thailand and chose the top three cultivars for yield and horticultural characteristics. We crossed these three local cultivars with the best three cms lines to produce 9 hybrids. These hybrids were compared for yield and capsaicin content with the three local cultivars and three commercial cultivars used as checks. Hybrid combinations that had yields and capsaicin contents equal to the best commercial and local cultivars were identified. These male sterile lines of the third backcross generations of sterile chilies, (A1 x C1-3) x B1, (A1 x C1-3) x B2 and (A1 x C1-3) x B3, along with three maintainer lines, B1, B2 and B3 are made available to all plant breeders from the Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, The Faculty of Agriculture, can be contacted at maneechat.n@gmail.com. The Chiang Mai University requires a material transfer agreement from users to pay part of the production cost to support the institution.

CONCLUSIONS

Twelve local cultivars of hot chilies were selfed for three generations. Three high yielding cultivars with good fruit quality and good plant type were selected. The two F1 hybrid, hot chilies from Milerue (2000), that are high yielding but do not have high pungency were selfed for three generations and then male sterile plants were selected for backcrossing with the C1 cultivar for ๒ generations. Progenies from the first and second backcross generations were tested for capsaicin content in comparison with the F1 hybrids. Capsaicin levels increased markedly when the female lines were backcrossed with the C1 cultivar. One good female line was selected from A1 x C1-3. Since it lacked fertility restorers and was male sterile, it was crossed with 3 good maintainer cultivars, B1, B2 and B3. The progenies were backcrossed with respective maintainer parents for 3 generations. Three second backcross generation of male sterile lines and the sterility maintainer lines were distributed to two seed companies in Thailand.

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Combining Abilities of Maintainers of Chilies (*Capsicum annuum* L.)

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ABSTRACT

The male sterile chili cultivar, A, is a good source of male sterility but lacks a good maintainer line to produce adequate seed for hybrid production. Two F1 hybrids which showed high percentages of male sterility were selected from crosses between the male sterile cultivar, A, and two maintainer cultivars. The F1 hybrids from A crossed to the maintainer lines were crossed with five restorer lines to produce ten fertile line x tester hybrids. The hybrids were significantly different in yields when tested by using a randomized complete block design with three replications. The hybrid, (AxB1) x C2, was the highest yielding among all the hybrids. However, it was not significantly different from the male parents and commercial checks. The specific combining ability of the hybrid, (AxB1) x C2, was good for three characteristics. In addition, it showed a positive and significant heterobeltiosis for number of fruit per plant. The general combining ability of the female parent, (AxB1), was good for three characteristics. The male parent, C2 was good for four characteristics. The line x tester, (AxB1) x C2, was the best combination identified in this study. These lines could be used to produce seed of competitive three way hybrids very economically and this could foster the development of additional hybrid chili cultivars.

Keywords: General combining ability, specific combining ability, maintainer, and restorer lines.

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INTRODUCTION

Hybrid production using cytoplasmic male sterility in chilies is dependent on the presence of cytoplasmic male sterility and nuclear restorer genes (Martin and Grawford, 1951, Peterson, 1953, Novak and Beltach, 1973, Basset, 1986, Shifriss and Pilovsky, 1993 Shifriss, 1997, Gulvas, *et. al.* 2006 and Wang, *et. al.* 2006). The development of a good source of male sterility and improvement in an isogenic maintainer line is a time and labor consuming process. Selection of other existing maintainer cultivars which have different genetic backgrounds from the sterile line should require less time than development of isogenic maintainers. Using different sources of sterility maintaining lines to cross on the CMS line, A, would result in sterile F1 hybrids. Crossing these sterile F1 hybrids with fertility restorer lines which have different genetic backgrounds from the sterile line would produce fertile three way hybrids which would be cheap to produce. Selection of three lines with good combining ability would result in productive three way hybrids. Towards this end, heterobeltiosis, general combining ability and the specific combining ability of various sterile and fertile parents for use in producing three way hybrids were identified. These studies aimed to develop a system for cheap three way hybrid production using the A male sterile line.

MATERIALS AND METHODS

Selection of a sterile cultivar, seed production of line x tester hybrids and field testing.

These two sterile hybrids were crossed with different plants of five restorer cultivars, C1, C2, C3, C4 and C5 in a testcross method (Kempthorne, 1957) and Singh and Chaudhary, 1979). Ten crosses of male sterile hybrids, A x B1 and A x B2, by restorer lines successfully set fruit. Seed were extracted from the fruit and dried. The ten hybrids were compared with the parents and commercial cultivars as checks. A randomized block design with three replications was used. Ten plants were used in each treatment. They were planted in a double row bed, at a spacing of 50 x 50 cm. with a plot size of 4.5 m๒. The field management was conducted

according to the local conventional culture practice. Horticultural characteristics were evaluated at harvesting time. Heterobeltiosis over the better parent for some horticultural characteristics; yield, fruit number per plant, fruit weight and fruit length were calculated (Chen *et.*

al., 2003). General and specific combining abilities of two female parents, A x B1 and A x B2, and all male parents were calculated for yield, fruit weight, fruit number per plant, fruit weight, fruit length and fruit width were evaluated (Kempthorne, 1957) and Singh and Chaudhary, 1979). Analysis of combining ability was carried out by using R software.

Remark : Abbreviations of cultivars ; A–KY16, B1–PEPAC35, B2– PEPAC37, C1–CA1445–7–8, C2–CA1447–2–9, C3– CA1448–5–13–8, C4–CA1449–5–6 and C5–CA1451–1–10.

RESULTS

Field testing of line x tester hybrids.

Fruit yield.

The fruit yield of all line x tester hybrids, parents, maintainers, and commercial F1 hybrid checks showed a range from 357 to 7,388 kg/ha, which was a significant difference (Table 1). The line x tester hybrid, (AxB1) x C2, was the highest yielding among the line x tester hybrids. The second highest yielding cultivar was the line x tester hybrid, (AxB1) x C3. However, the yields of both were not significantly different from most of the commercial checks. Only these two hybrids could be used commercially among the ten hybrids when yield and fruit characteristics were considered.

Heterobeltiosis.

Heterobeltiosis of the line x tester hybrids compared with the better parents (% Hp) showed that there were two hybrids, (AxB1) x C2 and (AxB1) x C3, which showed significant heterobeltiosis of some characteristics. Heterobeltiosis of 80.95% was observed in the number of fruit per plant for hybrid, (AxB1) x C2.

Heterobeltiosis of 21.83% was observed in fruit length for (AxB1) x C3 (Table 1). Both characteristics were major contributing factors for the highest yields of these two hybrids among all hybrids tested.

Combining ability.

A male sterile cultivar, single cross A x B1 was the only cross which showed significant differences in general combining abilities for yield, fruit length and fruit weight per plant. Analysis of variance of horticultural characteristics is shown in Table 2. The general combining abilities of this single cross were 734.84 1.25 and 55.67, respectively (Table 3).This sterile hybrid was used as female parent to development a line x tester hybrids. It has good potential to be used with other restorer cultivars as well. Some male parents, C2 and C3, Cultivar, C2 showed positive general combining abilities in yield, fruit weight, fruit weight per plant and fruit length at1,146.95, 3.14, 109.62 and 1.56, respectively.

Cultivar, C3 showed positive general combining abilities in yield, number of fruit per plant, fruit weight per plant, and fruit length at 1,417.97, 23.85, 107.42 and 1.39, respectively. The line x tester hybrid, (AxB1) x C2 ,was the only cross which showed positive and significant difference in some specific combining abilities (Table 3). This hybrid showed significant and positive combining abilities in yield, fruit weight per plant and number of fruit per plant at 2,313.81, 175.79 and 14.85, respectively.

Table 13 Horticultural characteristics of line x tester hybrids and their male parents of *Capsicum annuum* L.

Cultivar	Yield (kg/ha)	%HP	Fruit number per plant	%Hpw	Fruit weight (g)	%HP	Fruit length (cm)	%HP
line x tester hybrids								
(A x B1) x C1[x]	2,179def	23.11	16efghij	-16.79**	13.3def	59.28	9.03efghi	-10.24
(A x B1) x C2	6,576a[y]	11.27	38abcd	80.95**	14.55def	-46.21**	13.23abcd	-5.63
(A x B1) x C3	4,270bc	178.07	45ab	164.71**	8.59ghi	-17.64	11.72bcde	21.83*
(A x B1) x C4	671ef	-80.88*	5ghij	-73.68**	8.25ghi	-59.97**	7.86fghij	-23.84
(A x B1) x C5	379f	-91.35	3hij	-85.71**	8.2ghi	-63.92**	9.38efgh	-19.35
(A x B2) x C1	641cde	-63.78	5ghij	-73.68**	8.25ghi	-1.20	5.94ij	-40.95*
(A x B2) x C2	479f	-91.90**	2ij	-90.48**	11.92fgh	-55.93**	7.88fghij	-43.79**
(A x B2) x C3	2,726cde	77.53	39abcd	129.41**	7.36hi	-29.43	9.06efghi	-5.82
(A x B2) x C4	1,982def	-46.54	21defghij	10.53	10.35fghi	-49.78**	7.75ghij	-24.90*
(A x B2) x C5	899ef	-79.47	7ghij	-66.67**	10.06fghi	-55.74*	8.12fghij	-30.18

w% Hp heterobeltiosis

[x]Abbreviations of cultivars; A–KY16, B1–PEPAC35, B2–PEPAC37, C1–CA1445–7–8, C2–CA1447–2–9, C3–CA1448–5–13–8, C4, CA1449–5–6, C5–CA1451–1–10, D–Chakrapat , E–Jomthong2, F–YoksiamyMeans followed by the same letter indicate no difference at $P \leq 0.05$ by Duncan's multiple range test. z*, ** significant difference from zero at $P \leq 0.05$ and 0.01 levels, respectively.

Table 13 (cont.) Horticultural characteristics of line x tester hybrids and their male parents of *Capsicum annuum* L.

Cultivar amount per plant	Yield (kg/ha)	%HP	Fruit weight (g)	Fruit (cm)	%HP	Fruit length	%HP
Male parents							
C1	1,770 def		19efghij	8.35ghi		10.06	
C2	5,909.4 ab		21defghij	27.05a		14.07	
C3	1,536 def		17efghij	10.43fghi		9.62	
C4	3,511 cd		19efghij	20.61 bc		10.32	
C5	4,378 bc		21defghij	22.73 ab		11.63	
Commercial cultivars							
D	6,017 ab		24cdefg	20.14bc		15.23	
E	7,388 a		42abc	16.97cde		13.64	
F	5,746 ab		33bcde	17.89cd		14.17	
LSD0.05	2,110.40		17	3.9		2.18	
CV(%)	40.17		48.77	17.27		12.54	

Table 14 Analysis of variance of horticultural characteristics of chilies.

Source of Variance				Mean Square			
Yield (kg/ha)	d.f.	Fruit weight(g)	No. of fruit/plant	Fruit weight /plant (g/pt)	Fruit length (cm)		Fruit width (cm)
Replications	2	278036.88	0.85	2.88	1595.74	0.12	0.01
Crosses	9	12118147.97**	17.86*	843.54**	69549.16**	13.23**	0.11**
Lines	1	16199591.89**	7.32	365.05	92973.61**	46.61**	0.03
Testers	4	10797945.00**	24.54**	1243.59**	61972.54**	12.19*	0.19**
Line xTester	4	12417989.96**	13.81	563.12*	71269.66**	5.92	0.04
Error	18	1608319.70	5.22	147.06	9230.62	2.69	0.02

*,** significant difference at $p \leq 0.05$ and $p \leq 0.01$ levels, respectively.

Table 15 General combining ability (GCA value) and specific combining ability (SCA value) of characteristics in female, male parents and line x tester hybrids

	Cultivar	Yield (kg/ha)	Fruit(no./pt)	Fruit weight (g)	Fruit Weight (g/pt)	Fruit length (cm)	Fruit width (cm)
GCA							
Lines	A x B1[x]	734.84*[y]	3.49	0.49	55.67*	1.25**	-0.03
	A x B2	-734.84*	-3.49	-0.49	55.67*	-1.25**	0.03
Tester	C1	-670.19	7.59	0.69	-50.77	-1.51*	0.22**
	C2	1,446.95**	1.99	3.15**	109.62**	1.56*	0.07
	C3	1,417.98**	23.85**	-2.11*	107.42**	1.39*	-0.27**
	C4	753.35	5.09	-0.78	-57.07	-1.19*	0.00
	C5	-1,441.39**	-13.16**	-0.95	-109.20**	-0.25	-0.01
SCA							
line x tester hybrids	(A x B1) x C1	34.04	1.75		2.58		
	(A x B1) x C2	2,313.81**	14.85*		175.29**		
	(A x B1) x C3	37.18	-0.22		2.82		
	(A x B1) x C4	-1,390.28	-11.16		-105.32		
	(A x B1) x C5	994.74	-5.22		-75.36		
	(A x B1) x C1	-34.04	-1.75		-2.58		

x Abbreviations of cultivars; A–KY16, B3–PEPAC35, B2–PEPAC37, C1–CA1445–7–8, C2–CA1447–2–9, C3–CA1448–5–13–8, C4–CA1449–5–6 and C5– CA1451–1–10

y *, ** significant difference from zero at $P \leq 0.05$ and 0.01 levels, respectively.

Table 15 (cont.) General combining ability (GCA value) and specific combining ability (SCA value) of characteristics in female, male parents and line x tester hybrids.

	Cultivar	Yield (kg/ha)	Fruit number per plant	Fruit weight (g/pt)
SCA				
line x tester hybrids				
	(A x B2) x C2	-2,313.81**	-14.85*	-175.29**
	(A x B2) x C3	-37.18	0.22	-2.82
	(A x B2) x C4	1,390.28	11.16	105.32
	(A x B2) x C5	994.74	5.22ns	75.36

DISCUSSION

F1 hybrids which were used as female parents for three way hybrids, showed variability in the ratios of male sterile to fertile plants. Such variability might be caused by the environment. Lee *et al.* (2008) reported that restoration of pollen fertility in cytoplasmic male sterility is governed by fertility restoration genes, some modifier genes, and by the environment. This was supported by Wang *et al.* (2004) who showed that the improvement in female chili for perfect male sterility requires a controlled environment. The line x tester hybrid, (AxB1) x C2 , gave the highest yield of all the hybrids and significant specific combining ability The yield was significantly different from other hybrids, but it was not significantly different from the male parents and the commercial checks. This hybrid showed heterobeltiosis for the number of fruit per plant compared with the male parents. Results were supported by Meshram and Mukewar (1986), Ahmed *et al.* (1999), Pandey *et al.* (2003), Ahmed and Pandey (2002), Gelata *et al.* (๒๐๐๘), Prasad *et al.* (2003), Singh and Chaudhary (2008), Kanthaswamy *et al.* (2003), Linganagouda *et al.* (2003), Meshram and Mukewar (1986), Sousa *et al.* (2003), in that the F1 hybrid showed heterosis in fruit yield, number of days to flower, plant height, the number of branches, fruit length, and the number of fruit per plant. Analysis of combining abilities showed that the female parent, AxB1, showed good general combining abilities for fruit yield, fruit weight per plant and fruit length. The restorer, C2 showed good general combining abilities for fruit yield, fruit weight, fruit weight per plant and fruit length. Therefore, the line x tester hybrid, (AxB1) x C2 , showed good specific combining abilities for fruit yield, the amount of fruit per plant and fruit weight per plant. Results were supported by Ahmed *et al.* (1999), Ahmed and Pandey (2002), linganagouda *et al.* (2003) and Zou *et al.* (2007). The male maintainer, B1, is suitable for use in increasing seed of the sterile line A. The restorer, C2 is suitable as a male parent for producing a line x tester hybrid with A x B1. Development of a hybrid should serve as an incentive for a new hybrid development due to the low cost of seed production. The development of additional restorer cultivars should prove to be an advantage in additional three way hybrid production. A few hybrid chillies are available commercially in Thailand. The hybrid seeds are expensive. The Thai seed industry needs new and different sources of male sterile cultivars, maintainers and restorer cultivars. These studies have shown that the maintainer, B1, can be used successfully with the male sterile,

A. Cultivars, C2 and C3, are good restorers for the cross, AxB1. One can request A, B1, C2 and C3 from Chiang Mai University for his seed business. The suggested line x tester crossed hybrid would be the way to reduce the cost of hybrid seeds.

CONCLUSIONS

The male sterile chili cultivar, A, is a good source of male sterility but lacks a good maintainer line to produce adequate seed for hybrid production. The line, B1, combines well with A to produce adequate amounts of male sterile F1 hybrid seeds. In order to develop an economical system for hybrid chili seed production, five accessions of chilies were evaluated for their ability to restore fertility to the A x B1, single cross, and their good combining abilities for various yield and quality attributes. The male sterile single cross, A x B1, showed significant differences in general combining abilities for yield, fruit length and fruit weight per plant. The line x tester hybrids, (AxB1) x C2 and (AxB1) x C3, showed significant hererobeltiosis for the number of fruit per plant and fruit length, respectively. The restorer, C2 showed positive general combining abilities in yield, fruit weight, fruit weight per plant and fruit length. The line x tester hybrid, (AxB1) x C2 was the highest yielding among the line x tester hybrids tested, indicating that it was the best combination. The second highest yielding was the line x tester, (AxB1) x C3. However, the yields of both were not significantly different from most of the commercial cultivars. These lines could be used to produce seed of competitive line x tester hybrids very economically and this could foster the development of additional hybrid chili cultivars.

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Maintainers of Chilies (*Capsicum annuum* L.)

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ABSTRACT

Thirteen accessions of chilies were evaluated by crossing them onto a male sterile source, five accessions were determined to be maintainer cultivars. Three accessions were determined to be homozygous dominant for restorer genes. Five accessions were determined to be heterozygous in restorer genes. Vine, inflorescence and fruit physico-chemical qualities and vine growth habit of the field grown chilies were evaluate without an experimental design.

Keywords: Fertility restorer, maintainer, heterozygous and homozygous dominant.

INTRODUCTION

Cytoplasmic male sterility of chile cultivars was evaluated for their prospective genotypes. Identification of male sterility maintainers of chilies was the main task because of limitation of current maintainers. Seed companies have few sources of chili maintainers. Searching for high fertility restorer and stable maintainer lines are major tasks for plant breeders since chili hybrids need those lines for efficient and low cost seed production. Horticultural characteristics and fruit physico-chemical qualities of the cultivars are important as well.

The Faculty of Agriculture, Chiang Mai University announces the release of open-pollinated cultivars of chilies (*Capsicum annuum* L.) that are both fertility restorer and male sterility maintainer cultivars.

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Cytoplasmic-male sterility in chilies (*Capsicum* spp.) which is dependent upon a sterile cytoplasm and a nuclear non-restorer gene (*rfrf*) was first documented by Peterson (1958). Today, several seed companies use the genic mechanism *rfrf* on a large scale for producing chili hybrids (*Capsicum annuum* L.) -whereas, the cytoplasmic genic source is used mainly for breeding pungent (*SRfrf*) chili hybrids (Shifriss, 1997). Cytoplasmic male-sterility is maternally inherited through the cytoplasm and the abortion of pollen is caused by mutation in mitochondrial genomes (Laser and Lersten, 1972). Restoration of pollen fertility is controlled by restorer genes (*Rf*) (Peterson, 1958). Dominant restorer alleles have been found in several hot chili genotypes (Peterson, 1958 and Woong, 1990). Wang *et al.* (2004) identified a major Quantitative Trait Loci (QTL) which was mapped on chromosome P6 for fertility restoration in chilies (*Capsicum annuum* L.), accounting for 20- 69% of the phenotypic variation. Four additional minor QTL were also detected on chromosomes P5, P2 and linkage groups PY3 and PY1, accounting for 7-17% of phenotypic variation in chilies. Most of the alleles for fertility restoration increasing fertility originate from the restorer parent, except for two alleles at minor QTLs. Phenotypic analysis and genetic dissection indicated that breeding chili for complete sterility of female lines and high hybrid fertility requires complex combinations of alleles from both parents and a strict control of environment (Wang *et al.*, 2004). An effective method for identifying restorer genes such as a CAPS marker have been characterized (Lee *et al.*, 2007 and Kim *et al.*, 2005). Conventional breeding with fertility scoring of pollen was used to identify genotypes of chili accessions in Thailand (Nikornpun *et al.*, 2009).

MATERIALS AND METHODS

Chili cultivars, A, B, C, D, E, and F were obtained from Kasetsart University, Kampangsan. The exact source of origin is unknown as they were collected about 15 years ago. Some of them were locally collected and some of them were collected from other countries. They have been kept in a cold storage and have never

been released. They were self-pollinated for 4 generations with concomitant single plant selection for vine. growth, high fruit set, fruit shape, fruit size, fruit color, genotypes for restorers and maintainers. Superior genotypes were identified and selfed in each generation.

Description and performance of the selected lines.

Thirteen accessions of *Capsicum annuum* L. were grown at Chiang Mai University during the winter of 2006-7. Soil type of the area is sandy loam , average day temperature was 31.2±0.7oC, average night temperature was 16.0±0.9 oC , relative humidity was 67.8±2.4 % and light intensity was 331.3 W/m2. Thirty days old seedlings were transplanted into 8x12 inch black plastic bags containing rice hulls and sandy soil mixed in 1:1 ratio, additionally, 0.5 kg. of cow manual and 10 g. of fertilizer 15N-15P-15K were mixed into the mixed soil in each bag. They were grown in a greenhouse. There was no experimental design. Liquid fertilizer contained 150 g. of 15N-0P-0K, 65 g. of 13N-0P-42K, 75 g. of 0N-52P-34K and 5 g. of trace element which consisted of Mg 9.0%, Fe 4.0%, Mn 4.0%, Cu 1.5%, Co 0.05%, Zn 1.5% , B 0.5% and Mo 0.1% were mixed in 100 liters of water. Insecticide such as imidacloprid, fipronil sulfur and methomyl were used at recommended rates once a week. The 13 accessions were crossed onto 4 cytoplasmic male sterile chilies obtained from The Asian Vegetable Research and Development Center, Taiwan, who transferred these lines to Kasetsart University, Kampangsan. The lines were G1, G2, G3 and G4. The hybrids produced were G1xA1, G1xC1, G1xE1, G2xA3, G2xC2, G3xB2,,G4xA2, G4xB1, G4xB3, G4xD1, G4xF1, G4xF2 and G4xF3.

About 50 days after anthesis when the fruits were at red ripe stage, seeds were harvested and dried. The F1 hybrids were grown for evaluation of the viability of their pollen. Thirty plants were grown for each F1 hybrid using the same place and same cultural practices as mentioned above. Pollen from open-flowers was stained with 1% acetocarmine to score for pollen fertility (Rai *et al.* 2001, Pakozdi *et al.* 2002, and Yoon *et al.* 2006). Based on the red stained color and morphology of pollen indicated the viability of pollen was used to classify different genotypes. They were classified as follows:

a. If all plants for a cross possesses non-viable pollen , then the male parent for that F1 hybrid was designated as sterile maintainer genes reside in the nucleus (*rfrf*), and normal cytoplasm (*N*).

b. If all F1 plant for a cross possesses viable pollen, then that male parent was designated as restorer genes reside in the nucleus as *RfRf* genotype and the cytoplasm is either normal (*N*) or sterile (*S*).

c. If some F1 plants for a cross possesses viable pollen and some non-viable pollen, then the male parent was designated as heterozygous for restorer genes in the nucleus (*Rfrf*), and the cytoplasm was conserved either normal (*N*) or sterile (*S*).

Vine, inflorescence and fruit characteristics and vine growth habit of the field grown chilies were evaluate without an experimental design (IPGRI *et al.*, 1995). A completely randomized design with three replications and 20 plants in each treatment was carried out in pot-grown plants in the greenhouse and fruit moisture, total soluble solid, chlorophyll, vitamin C, and capsaicin content of the lines were evaluated by methods proposed by Whitham *et al.* (1971) and Ranganna (1986). Laser Fruit color was measured by using a chromameter.

RESULTS

Description and performance of the selected lines.

Of the 13 accessions of chilies evaluated, five accessions, A1, B1, C1, E1, and F1, were determined to be maintainer cultivars with genotypes of *N rfrf* (Table 1 and Fig. 1). Three accessions, A3, B3 and F3, were determined to be homozygous dominant in restorer genes with genotypes of *N/SRfRf*. Five accessions, A2, B2, C2, D1 and F2 were determined to be heterozygous in restorer genes with genotypes of *N/SRfrf* . These have already been distributed to ten seed companies in Thailand during 2008-2011. The maintainer and restorer lines were used to improve the F1 hybrid development program of the companies. Unavailability of maintainer and restorer lines encouraged the seed companies to sign an agreement contract with Chiang Mai University. The agreement requires for some percentages (10%) from whole sale seed production cost to return to the University. Horticultural characteristics of the maintainer cultivars were recorded (Table 2). Fruit physico- chemical qualities, including chlorophyll, vitamin C, capsaicin content and fruit color were recorded (Table 3). Abbreviations of cultivars, A-CA1445, A1-CA1445-5-12, A2-CA1445-3-10, A3-CA1445-7-8, B-CA1447, B1- CA1447-3-13, B2-CA1447-3-2, B3-CA1447-2-9, C-CA1448, C1-CA1448-1-3, C2-CA1448-5-13-8, D-CA 1449, D1- CA 1449-5-6, E-CA1450, E1- CA1450-7, F-CA1451, F1-CA1451-4-8, F2-CA1451-1-9, F3-CA1451-1-10, G1- Seungchon [CMS] / 6 * TitParis, G2- Seungchon [CMS] / 6 * Saegochu / 5 * PBC 385, G3- Seungchon [CMS] / 7 * Arunalu and G4- Seungchon [CMS] / 6 * Kunja.

Table 16 Prospective genotypes of chili cultivars[x].

Cultivar	Genotype	Remark
A1[y]	*Nrfrf* [z]	Maintainer
A2	*N/SRfrf*	Restorer
A3	*N/SRfRf*	Restorer
B1	*Nrfrf*	Maintainer
B2	*N/SRfrf*	Restorer
B3	*N/SRfRf*	Restorer
C1	*Nrfrf*	Maintainer
C2	*N/SRfrf*	Restorer
D1	*N/SRfrf*	Restorer
E1	*Nrfrf*	Maintainer
F1	*Nrfrf*	Maintainer
F2	*N/SRfrf*	Restorer
F3	*N/SRfRf*	Restorer

[x] Non experimental design.

[y] Abbreviation of cultivars; A1-CA1445-5-12, A2-CA1445-3-10, A3-CA1445-7-8, B1- CA1447-3-13,B2- CA1447-3-2, B3-CA1447-2-9, C1-CA1448-1-3, C2-CA1448-5-13-8, D1-CA 1449-5-6, , E1- CA1450- 7, F1CA1451-4-8, F2-CA1451-1-9, F3-CA1451-1-10 (the cultivars came from lines A-CA1445, B- CA1447, C- CA1448, D- CA 1449, E-CA1450, and F-CA1451 cultivars crossed onto the male sterile lines and showed different genotypes of male sterility).

[z] *Nrfrf* – normal cytoplasm and homozygous recessive of restorer genes which designated as maintainer, *N/SRfrf*– normal or sterile cytoplasm and heterozygous of restorer genes which designated as restorer and *N/SRfRf*– normal or sterile cytoplasm and homozygous dominant of restorer genes which designated as restorer.

Table 17 Vine, inflorescence and fruit characteristics and vine growth habit of chili maintainers[x].

Cultivar / IPGRI descriptor	A1	B1	C1
1. Inflorescence and fruit[z]			
1.1 Male sterility	0(absent)	0(absent)	0(absent)
1.2 Fruit color in immature stage	3[y](green)	3(green)	3(green)
1.3 Fruit color in mature stage	9(dark red)	8(red)	8(red)
1.4 Fruit shape	1(elongate)	1(elongate)	1(elongate)
1.5 Fruit shape at pedicel attachment	4(cordate)	5(lobate)	2(obtuse)
1.6 Neck at base of fruit	0(absent)	0(absent)	0(absent)
1.7 Fruit shape at blossom end	1(pointed)	1(pointed)	1(pointed)
1.8 Fruit blossom end appendage	0(absent)	0(absent)	1(present)
1.9 Fruit cross-sectional corrugation	3(slightly corrugate)	3(slightly corrugate)	3(slightly corrugate)
1.10 Fruit surface	2(semi wrinkled)	2(semi wrinkled)	2(semi wrinkled

[x] Non experimental design.

[y] Numbers infront of parentheses are designated for the characteristics in the parentheses by IPGRI , AVRDC and CATIE (1995).

[z] Inflorescence–a flower cluster, appendage–external organ, corrugate–contract into grooves and ridges, semi–wrinkled–not smooth.

Table 17 (cont.) Vine, inflorescence and fruit characteristics and vine growth habit of chili maintainers.

Cultivar / IPGRI descriptor	A1	B1	C1
Height (cm)	46.00+5.66	45.33+5.13	54.50+0.71
Width of canopy (cm)	71.50+6.36	63.67+4.04	74.00+1.41
Leaf length (cm)	7.00+0.00	5.67+0.21	5.60+0.14
Leaf width (cm)	3.90+0.28	2.57+0.36	2.60+0.14
No. of days to first flowers	40	40	40
No. of days to 50% flowering	72	72	71
Fruit length (cm)	22.18+0.46	14.98+2.78	10.74+0.01
Fruit width (cm)	3.09+0.03	2.96+0.56	2.42+0.01
Fruit diameter (cm)	3.09+0.03	2.96+0.56	2.42+0.01
Cortex thickness (mm)	3.17+0.04	3.49+0.52	2.05+0.07
Weight of fruit (g)	47.10+3.96	45.44+3.51	17.21+0.01
Weight of fruit/plant (kg)	1.73+0.31	1.05+0.07	0.05+0.00
No. of fruit/plant	36.50+3.54	24.00+1.41	29.50+0.71
No. of fruit/kg	21.31+1.79	22.08+1.70	58.11+0.05
No. of seeds/fruit	133.00+19.80	117.00+61.30	80.50+0.71
Weight of 100 seeds (g)	0.59+0.02	0.44+0.02	0.48+0.03

Table 17 (cont.) Vine, inflorescence and fruit characteristics and vine growth habit

Cultivar IPGRI descriptor	D1	E1	F1
1. Inflorescence and fruit	0(absent)	0(absent)	0(absent)
1.1 Male sterility			
1.2 Fruit color in immature stage	3(green)	3(green)	3(green)
1.3 Fruit color in mature stage	8(red)	8(red)	8(red)
1.4 Fruit shape	1(elongate)	1(elongate)	1(elongate)
1.5 Fruit shape at pedicel attachment	5(lobate)	2(obtuse)	5(lobate)
1.6 Neck at base of fruit	0(absent)	0(absent)	0(absent)
1.7 Fruit shape at blossom end	1(pointed)	1(pointed)	1(pointed)
1.8 Fruit blossom end appendage	0(absent)	0(absent)	0(absent)
1.9 Fruit cross-sectional corrugation	3(slightly corrugate)	3(slightly corrugate)	3(slightly corrugate)
1.10 Fruit surface	1(smooth)	1(smooth)	1(smooth)

Table 17 (cont.) Vine, inflorescence and fruit characteristics and vine growth habit of chili maintainers.

Cultivar / IPGRI descriptor	D1	E1	F1
Horticultural characteristics	56.67+2.89	57.33+2.52	52.33+2.52
Height (cm)			
Width of canopy (cm)	81.00+4.58	69.00+1.73	85.00+4.80
Leaf length (cm)	4.73+0.25	6.33+0.29	5.03+0.06
Leaf width (cm)	2.37+0.12	3.27+0.25	2.40+0.10
No. of days to first flowers	40	43	43
No. of days to 50% flowering	72	72	70
Fruit length (cm)	12.88+1.79	14.01+2.34	12.05+0.62
Fruit width (cm)	2.72+0.24	3.01+0.16	2.74+0.15
Fruit diameter (cm)	2.72+0.24	3.01+0.16	2.74+0.15
Cortex thickness (mm)	2.75+0.22	3.33+0.10	2.97+0.20
Weight of fruit (g)	30.43+3.09	30.24+4.94	26.60+1.80
Weight of fruit/plant (kg)	0.62+0.01	0.62+0.01	0.85+0.01
No. of fruit/plant	22.50+0.71	45.00+2.65	23.00+3.61
No. of fruit/kg	33.04+3.35	33.63+5.16	40.80+2.99
No. of seeds/fruit	87.67+44.12	86.33+41.50	70.67+15.57
Weight of 100 seeds (g)	0.35+0.05	0.38+0.03	0.61+0.04

Table 18 Fruit yields, fruit color and fruit chemistry of chilies.

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Cultivar	L*[z]	Chroma	Hue angle (°)	Fruit weight/pt (kg)	Moisture (%)	TSS (%)	Vitamin C (mg/100 g fw)	Capsaicin (Scoville unit)
S[y]1	55.18a[x]	39.65a	109.80e	0.1940ab	78.71b	6.43a	133.98c	6,340a
S2	37.07c	21.13c	125.94a	0.1552b	81.38b	5.73ab	117.30e	6,710a
A	38.17c	25.84c	122.62bc	0.1800ab	86.86a	5.27bc	114.74e	4,830b
B	38.74c	26.56c	123.52ab	0.2184a	89.04a	4.37d	147.44b	4,740b
C	45.49b	33.40b	120.06cd	0.1524b	77.83b	5.87ab	143.59b	4,620b
D	43.85b	32.24b	118.96d	0.1593b	86.24a	5.23bcd	158.98a	6,600a
E	38.01c	25.95c	124.24ab	0.2272a	87.92a	5.40b	122.43de	4,230b
F	38.23c	24.62c	123.54ab	0.2212a	88.01a	4.50cd	126.92cd	4,710b
LSD0.05	4.66	5.46	2.73	0.0588	4.78	0.88	9.12	1,452.33
C.V. (%)	-	-	-	-	3.26	9.53	3.96	15.68

Randomized complete block design. ,[x] Mean followed by the same letters indicate no difference at P≤ 0.05 by Least significant difference. [y] Abbreviation of cultivars; A-CA1445, B-CA1447, C-CA1448, D-CA 1449, E-CA1450, F-CA1451, S1- control cultivar Pijit. 27-1-2-1 and S2- control cultivar CA 1038. [z] L*-Lightness factor, Chroma-Color saturation, Hue angle(o)-Angle of color saturatio , Moisture (%) , TSS (%)-Total soluble solids, Vitamin C (mg/100 g fw) and capsaicin (Scoville unit)

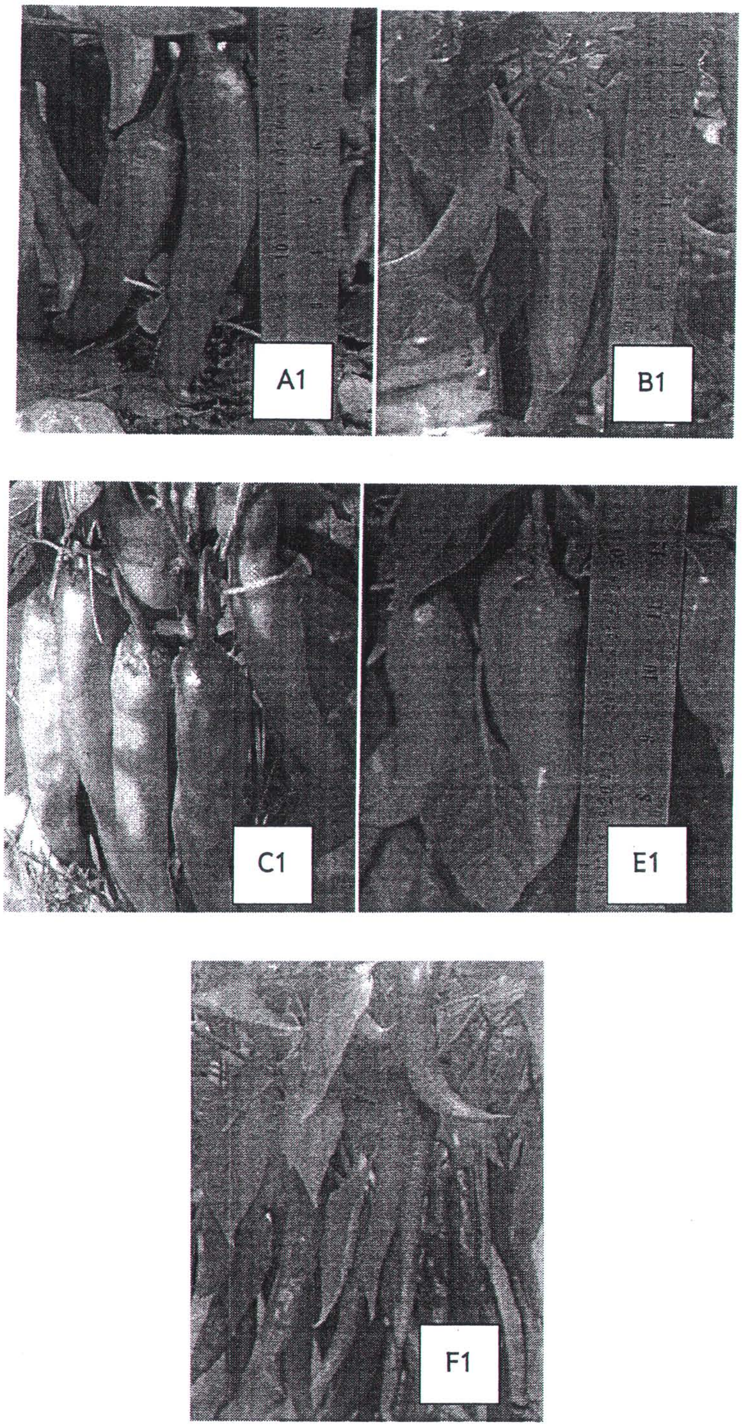

Figure 1 Heavy fruit set of chili maintainer cultivars which are open–pollinated cultivars. Abbreviations of cultivars, A1–CA1445–5–12, B1–CA1447–3–13,C1–CA1448–1–3, E1– CA1450–7 and F1– CA1451–4–8.

DISCUSSION

The fertility scoring method has been used successfully in indicating fertile pollen (Rai *et al.* 2001, Pakozdi *et al.* 2002, and Yoon *et al.* 2006). Results from this experiment also showed that unknown male sterility in chilies could be determined by the fertility scoring method. However, the method is a time and labor consuming method. It requires two growing seasons. Kim *et al.* (2005) and Lee *et al.* (2008) have suggested a CAPS marker closely linked to the fertility restoration of cytoplasmic male sterility. It would require much less time and labor than the previous method.

CONCLUSIONS

The determined male accessions of chilies showed interesting groups of genotypes; normal male fertile chilies (*N/SRfRf*), maintainer chilies (*N rfrf*), and male fertile chilies which had heterozygous in restorer genes of chilies (*N/SRfrf*). Some lines such cultivars, A1, B1, C1, E1 and F1 showed interesting horticultural characteristics and they were maintainer cultivars. They could be used in cultivar development of F1 hybrids. The uses of cytoplasmic male sterility in F1 hybrid chilies were reported by Shifriss, 1997, Rai *et al.* 2001 and Pakozdi *et al.* 2002. Seeds of these maintainer and restorer cultivars are available on request. Cultivars, maintainer and restorer genotypes of A1, A2, A3, B1, B2, B3, C1, C2, D1, E1, F1, F2 and F3 are available at the Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, e-mail: maneechat.n@gmail.com

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ภาคผนวกที่ ๔

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