Genetic Resources for Fertility Restoration Lines and Maintainers of *Capsicum annuum* L.

ABSTRACT

Male fertility reactions of one hundred and forty-one accessions of chilies were classified. Three groups were found. Some accessions maintained male sterility and were determined to carry a non-sterile cytoplasm and to lack fertility restoration genes or *N rfrf* genotype. Some accessions segregated for the ability to restore male sterile cytoplasm and were determined to be heterozygous in restorer genes with genotype *N/SRfrf*. Some accessions restored fertility of CMS and had the genotype *N/SRfRf*. A few maintainers with good horticultural characteristics were selected. They were selfed and selected for a few generations and then their progeny were evaluated. There were differences in the genetic stability of cytoplasmic male-sterility among the selected lines. Some lines were good maintainers, but a few lines were discarded. The stable maintainers were distributed to 10 seed companies and the government of China. Some F1 hybrid chilies produced using these lines have been commercially sold both in China and Thailand.

Fruit physio-chemical qualities of maintainer accessions, restorers and heterozygous accessions were also recorded. The level of capsaicin of the accessions varied from 3,250 to 8,850 Scoville units. The level of vitamin C showed a range of 4.43 to 103.16 mg./100g.fw. Horticultural characteristics of the accessions were recorded and the fruit physio-chemical qualities of the accessions were reported.

Keywords: Restorers, heterozygous accessions and maintainers.

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INTRODUCTION

Cytoplasmic male-sterility (CMS) in chilies has previously been documented by Peterson (1958). Today, several seed companies use the genic mechanism *rfrf* on a large scale for producing chili hybrids (*Capsicum annuu*m L.), whereas, the cytoplasmic source is used mainly for breeding pungent (*SRfrf*) chili hybrids (Shifriss, 1997). CMS is maternally inherited (Budar *et al*.2001). Male sterility caused chromosome rearrangements and chromosome breakage leading to aberrations that could damage the genetic system responsible for the regular initiation, running and termination of late meiotic stages. The microspore degeneration starts either after the tetrad stage when the callose fails to dissolve and the microspores are not released from the sporads, or, in some cases, at various phases of the microgameto-genesis process (Vesselina *et al*. 2010). Male sterility is caused by suppression of mitochondrial genes that cause pollen abortion. Restoration of pollen fertility is controlled by nuclear restorer genes (*Rf*) (Schnable and Wise, 1998). Dominant restorer alleles (*Rf*) have been found in several hot pepper genotypes (Peterson, 1958 and Woong, 1990). Mankare and Korekar (2017) identified a few restorers and maintainers for CMS *C. annuum* lines by examining pollen fertility. An effective method for identifying restorer genes such as a CAPS marker or other markers were further suggested (Kim *et al*., 2005, Gulyas *et al*., 2006 and Lee *et al*., 2008). 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.) which accounted for 20-69% of the phenotypic

variation. Four additional minor QTL which accounted for 7-17% of phenotypic variation in chilies were also detected on chromosomes P5, P2 and linkage groups PY3 and PY1.

However, conventional breeding with fertility scoring of pollen was used to identify genotypes of chili accessions in this study. Searching for high fertility restorers and stable maintainer lines are major tasks for plant breeders but they are needed for efficient and low cost seed production of chili hybrids (Yang *et al.,* 2008). Conventional breeding with fertility scoring of pollen was used to identify genotypes of chili accessions in Thailand (Nikornpun *et al.,* 2009).

Figure 1. A commercial F1 hybrid variety from our germplasm in Chinese markets.

The objectives of these studies were to aid in producing food for the World by using cytoplasmic male sterility of chilies. Since 2009, after releasing male sterile lines, maintainer lines and restorers line of chili (Nikornpun *et al.,* 2009), Chiang Mai University has distributed the chili germplasm to 10 private sector organizations and Horticultural Research Institute at the Yunnan Academy of Agricultural Science, North Suburban, Kunming, Yunnan, China. Material transfer agreements were signed between 2009 and 2018. The Horticultural Research Institute at the Yunnan Academy of Agricultural Science has successfully produced and registered 2 outstanding F1 hybrid chilies commercially (Figure 1). About 200-400 kg. of hybrid chili seeds are produced yearly in China, calculated from the royalty fees which have been submitted to Chiang Mai University since 2017. About half of the royalty fees are donated to crippled patients, medical students, agriculture students, engineering students and horses in China. Known You seed company in Thailand is currently commercializing hybrid chilies in Asian countries.

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MATERIALS AND METHODS

One hundred and forty-one accessions of *Capsicum annuum* L. were obtained from Kasetsart University, Kamphangsaen. The exact source of origin is unknown as they were collected about 15 years ago. Some of them were from land races and some were collected from other countries. They have been kept in cold storage at Kasetsart University, Kamphangsaen and have never been released. They were grown at Chiang Mai University in six experiments with random planting during the winters of 2006-10. The accessions were crossed onto six cytoplasmic male sterile chilies obtained from The Asian Vegetable Research and Development Center, Taiwan. The lines were Seungchon [CMS] / 6 , TitParis, Seungchon [CMS] / 6 , Saegochu / 5 PBC 385, Seungchon [CMS] / 7 , Arunalu, Seungchon [CMS] / 6 , Kunja, Suwon [CMS] / 7 , PBC 292, Seungchon [CMS] / 6 , Tumpang and our own line KY16. They were evaluated for their male sterility levels by using pollen viability. Some good stable maintainer lines were observed and selfed seeds were grown and crossed onto the male sterile lines and the F1 seeds were grown and evaluated. When mature seeds were harvested and dried, the F_1 hybrids were grown for evaluation of the viability of the pollen. Thirty plants were grown for each F_1 hybrid in the same place and under 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). Pollen viability was rated based on the red stained color and the morphology of the pollen. The accessions were classified as follows:

a. If all F_1 plants from a cross possessed non-viable pollen, then the male parent for that F_1 hybrid was designated as having maintainer genes in the nucleus (*rfrf*), and normal cytoplasm (N).

b. If all F_1 plants from a cross possessed viable pollen, then that male parent was designated as a restorer, with restorer genes in the nucleus (*RfRf*), and the cytoplasm was either normal (N) or sterile (S).

c. If some F_1 plants from a cross had viable pollen and some non-viable pollen, then the male parent was designated as heterozygous for sterility controlling genes in the nucleus *(Rfrf),* and the cytoplasm was either normal (N) or sterile (S).

The evaluations were done on sandy-loam soil and the average daytime temperature was 31.2±0.7 ^{o}C, the average nighttime temperature was 16.0±0.9 ^{o}C, relative humidity was 67.8±2.4 % and light intensity was 331.3W/m^2. Thirty day old seedlings were transplanted into the field, along with 2 tons of cow manure and 31.25 kg. of fertilizer 15N-15P-15K/ha was mixed into the soil. Liquid fertilizer which contained 300 g. of 15N-15P-15K, 130 g. of 13N-0P-46K and 150 g. of 0N-52P-34K, 5 g. of trace elements which contained 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% was mixed in with 200 liters of water. Insecticides such as imidacloprid, fipronil sulfur and methomyl were used at recommended rates once a week. Plants were planted in a double row beds, 50 x 50 cm. with a plot size of 4.5 m^2.

Vine, inflorescence and fruit characteristics and vine growth habit of some of the field grown chilies were also evaluate (IPGRI *et al*., 1995). Moreover, about 50 days after anthesis, when the fruits were at the red ripe stage, fruit physio-chemical qualities, including chlorophyll, vitamin C, capsaicin content and fruit color were also evaluated (Whitham *et. al.*, 1971), Anan *et al*., 1996 and Ranganna. 1986).

Results

The results from the 6 experiments identified 3 groups of cytoplasmic male sterility of chili accessions. The first group of chilies, was heterozygous for restorer genes with genotypes N/S *Rfrf*.(Table 1). Some of these had small anthers without pollen and some had non-viable pollen (Fig. 2A and 2C). Some had normal size anthers with stained pollens (Fig. 2B and 2D). The second group was determined to be homozygous for restorer genes with genotypes of N/S *RfRf*. All of these had normal size anthers with stained pollens. The third group was determined to be homozygous with genotypes of N *rfrf* . All of these showed small anthers without pollen or little non-viable pollen.

A few maintainers with good horticultural characteristic were selected from the first experiment (Figure 2). They were self-pollinated and selected for stability of male sterility (*rfrf*) for a few generations. The offspring were grown in the following experiments and were tested for sterility reaction (.(Table 1 and 2). The maintainers showed differential stabilities:

a. Accession CA1286-1 showed full sterility N *rfrf* in the first experiment. When the sib lines of CA1286 were selfed and selected for 2 generations; CA1286-4-2-6

showed some fertile (*Rfrf*) and some sterile (*rfrf*) but CA1286-4-9-5 was fully sterie (*rfrf*). Accession CA1286-1 was also fully sterile (*rfrf*). Accessions CA1286-1 and CA1286-4-9-5 could be used as maintainers.

b. Accessions CA1303-1, CA1303-6 and CA1303-8 were tested 8 times. When CA1303-6 and CA1303-8 were selfed for 2 generations, most of the offspring were fully sterile (*rfrf*) except for 2 lines that were fertile (*Rfrf* and *RfRf*). Accessions CA1303-6 and CA1303-8 could be used as maintainers with a requirement for genotype testing because sterility is not stable, and fertility is occasionally restored.

c. Five lines of accession CA1445-1 was tested, 3 were sterile (*rfrf*) and 2 were fertile (*Rfrf*). Three sibbed lines from accession CA1445-2 was teste, 1 was sterile (*rfrf*) and 2were fertile (*Rfrf*). Two lines from accession CA1445-5 was tested, and both were sterile (*rfrf*). Three accession CA1445-7 was tested, and 1 segregated for *Rf* genes (*Rfrf*). and 2 were fully fertile (*RfRf*). Two lines from accession CA1445-8 was tested, and both were sterile (*rfrf*).. CA1445 is not suitable as a maintainer because of the instability of sterility *rfrf*.

d. The accession CA1447-1 segregated for *Rfrf* genes when the sibbed lines were selfed and selected for 2 and 3 generations. The offspring; CA1447-3-2-1-5, CA1447-4-12-9 and CA1447-812-9 segregated into 2 fully testoring (*RfRf*) and 1 segregating for *Rfrf* genes. This accession should be used as a restorer.

e. Accession CA1448 showed fully fertiile progeny (*RfRf*). When it was selfed and progeny selected for 2-3 generations, the offspring segregated with 4 *Rfrf* and 1 *rfrf*. This accession is not suitable to be a maintainer but it could be a restorer.

f. Progeny of accession CA1449-1 were fully fertiile (*RfRf*). When sib lines were selfed and selected for a few generations; 6 offspring of CA1449-2 segregated for restorer genes (*Rfrf*). CA1449-2 is not suitable to be a maintainer. While 3 lines of CA1449-3 were fully sterile *rfrf*. Therefore, CA1449-3 could be further selected to be a maintainer. Additionally, CA1449-5 and its progeny; CA1449-5-8, CA1449-5-8-3, CA1449-5-11 and CA1449-5-13 were fully sterile (*rfrf*). Therefore both accessions; CA1449-3 and CA1449-5 could be used as a maintainer.

g. Two lines from accession CA1450-1were tested, and they segregated for *Rfrf* genes. When the sibbed lines; were selfed and selected for a few generations; all 5 offspring; CA1450-2, CA1450-3, CA1450-5, CA1450-6 and CA1450-7-10 were fertile (*Rfrf* and *RfRf*). These accessions are not good as maintainers. However, CA1450-7 segregated into fully sterile (*rfrf*). When it was selfed and selected for 2-3 generations, CA1450-7-10-10 segregated into sterile *rfrf* and fertile (*Rfrf*) progeny. CA1450-7-1 and CA1450-7-1-72 segregated into sterile *rfrf* genes. CA1450-7-1 and CA1450-7-10-10 could be further selected to be maintainers.

Fruit physio-chemical qualities of some of maintainers, restorers and heterozygous accessions were recorded (Table 3-5).The level of capsaicin in the accessions showed a range of 3,250 to 8,850 Scoville units. The level of vitamin C

of the accessions showed a range of 4.43 to 103.16 mg./100g.fw. Horticultural characteristics were recorded according to IBPGR descriptors. Fruit physio-chemical qualities such as fruit color; L, Chroma, Hue, % moisture content, vitamin C, chlorophyll content and capsaicin were demonstrated. Seeds of the maintainers are available on request.

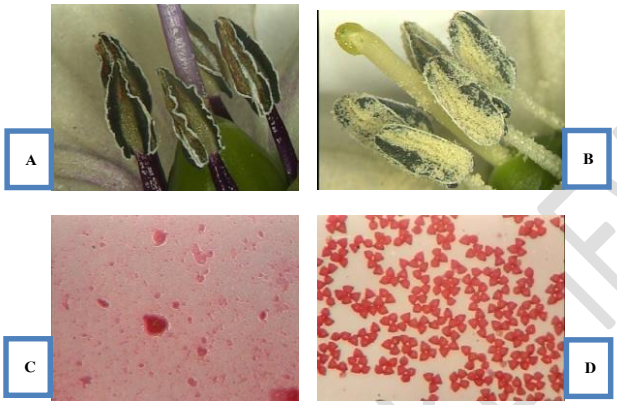

Figure 2. Chili flowers: (A) sterile anther, (B) fertile anther, (C) sterile pollen, (D) fertile pollen.

Table 1. Prospective genotypes of cytoplasmic male sterility of chili inbred lines. This table suggests there are a lot of maintainer lines.

Prospective genotypes	Accession
N *rfrf*	CA 005, CA 074, CA 254, CA 319-1, CA 365-1, CA 365-3, CA 443, CA 453,CA 457, CA 480, CA 489-1, CA 489-2, 598-4, CA 617, CA 636, CA 646, CA 715, CA 719, CA 720-3, CA 720-8, CA 778, CA 780, CA 780-1, CA 991, CA 1159-3, CA 1193-2, CA 1193-F-5, CA 1239, CA 1286-1, CA 1286-4-2-6, CA 1286-4-9-5, CA 1297, CA 1303-1, CA 1303-6, CA 1303-6-8-7, CA 1303-6-8-8, CA 1303-6-20-7, CA 1303-8-1-5, CA 1374, CA 1377, CA 1404, CA 1440-6, CA 1440-H, CA 1441-1, CA 1441-J-3-6, CA 1441-J-8-3-6, CA 1442, CA 1442-1, CA 1442-5,CA 1442-D, CA 1442-F-7,CA 1443-4, CA 1443-A, CA 1445-1, CA 1445-2, CA 1445-2-6-18-1, CA 1445-5-2, CA 1445-5-8, CA 1445-8, CA 1446, CA 1448-5-13-8-3, CA 1449-3-8-3, CA 1449-3-9-8, CA 1449-5, CA1449-5-8, CA 1449-5-8-3, CA 1449-5-11, CA 1449-5-13, CA 1450-7, CA 1450-7-1, CA 1450-7-10-10, CA 1450-7-72,
N/S *RfRf*	CA 006, CA 319-2, CA 458, CA 479, CA 493, CA 500, CA 516, CA 597, CA 598-1, CA 614, CA 652, CA 654, CA 683-3, CA 738, CA 1159-1, CA 1303-8-1-5, CA 1428-G-1, CA 1428-F-1, CA 1445-7-8, CA 1447-4-12-9, CA 1447-8-12-9, CA 1448, CA 1449-1, CA 1450-2-14-1-4, CA 1547-5, CA 1574-5
N/S *Rfrf*	CA 042, CA 633, CA 634, CA 639, CA 649, CA 650, CA 683, CA 776, CA 1274, CA 1286-4-2-6, CA 1303-6-8-7, CA 1321, CA 1395-2, CA 1395-A-8, CA 1401, CA 1403, CA 1442-5, CA 1443-5, CA 1444, CA 1444-2, CA 1445-1, CA 1445-2-4-10-5, CA 1445-7-8-32-2, CA 1447-1, CA 1447-3-2-1-5, CA 1448-1-3-10, CA 1448-2-5, CA 1448-5-13-9, CA 1449-2-4-1, CA 1449-2-4-7, CA 1449-2-5-10, CA 1449-3-13-8, CA 1450-1, CA 1450-2-14-1, CA 1450-3-6-9, CA 1450-5-17-2, CA 1450-6-9-1, CA 1450-7-10-1, CA 1451, CA 1451-5-11-11, CA 1533-2,

Table 2. Segregating genotypes of cytoplasmic male sterility of chili maintainer offspring.

No.	Variety	Segregaing genotypes of offspring
1	CA 1286	CA 1286-1 N *rfrf*, CA 1286-1 N *rfrf,* CA 1286-4-2-6 N/S *Rfrf,* CA 1286-4-2-6 N *rfrf*, CA 1286-4-9-5 N *rfrf*
2	CA 1303	CA 1303-1 N *rfrf*, CA 1303-6 N *rfrf,* CA 1303-6 N *rfrf*, CA 1303-6 N *rfrf*, CA 1303-6-8-7 N *rfrf,* CA 1303-6-8-7 N/S *Rfrf,* CA 1303-6-8-8 N *rfrf*, CA 1303-6-20-7 N *rfrf,* CA 1303-8-1-5 N/S *RfRf* , CA 1303-8-1-5 N *rfrf*
3	CA 1445	CA 1445-1 N *rfrf*, CA 1445-1 N *rfrf,* CA 1445-1 N/S *Rfrf,* CA 1445-1 N *rfrf*, CA 1445-1 N/S *Rfrf,* CA 1445-2 N *rfrf,* CA 1445-2-6-18-1, CA 1445-2-4-10-5 N/S *Rfrf,* CA 1445-5-2 N *rfrf,* CA 1445-5-8 N *rfrf*, CA 1445-7-8 N/S *RfRf* , CA 1445-7-8 N/S *RfRf,* CA 1445-7-8-32-2 N/S *Rfrf*, CA 1445-8 N *rfrf*
4	CA 1448	CA 1448 N/S *RfRf*, CA 1448-1-3-10 N/S *Rfrf,* CA 1448-2-5 N/S *Rfrf,* CA 1448-5-13-9 N/S *Rfrf*, CA 1448-5-13-9 N/S *Rfrf,* CA 1448-5-13-8-3 N *rfrf*
5	CA 1449	CA 1449-1 N/S *RfRf*, CA 1449-2-4-1 N/S *Rfrf,* CA 1449-2-4-1 N/S *Rfrf*, CA 1449-2-4-7 N/S *Rfrf,* CA 1449-2-4-7 N/S *Rfrf*, CA 1449-2-5-10 N/S *Rfrf* CA 1449-2-5-10 N/S *Rfrf*, CA 1449-3-8-3 N *rfrf,* CA 1449-3-9-8 N *rfrf*, CA 1449-3-9-8 N *rfrf,* CA 1449-3-13-8 N/S *Rfrf*, CA 1449-5 N *rfrf,* CA 1449-5-8 N *rfrf* CA 1449-5-8-3 N *rfrf*, CA 1449-5-11 N *rfrf,* CA 1449-5-13 N *rfrf*
6	CA 1450	CA 1450-1 N/S *Rfrf*, CA 1450-1 N/S *Rfrf,* CA 1450-2-14-1 N/S *Rfrf,* CA 1450-2-14-1 N/S *Rfrf*, CA 1450-2-14-1-4 N/S *RfRf,* CA 1450-3-6-9 N/S *Rfrf*, CA 1450-3-6-9 N/S *Rfrf,* CA 1450-5-17-2 N/S *Rfrf*, CA 1450-5-17-2 N/S *Rfrf,* CA 1450-6-9-1 N/S *Rfrf*, , CA 1450-6-9-1 N/S *Rfrf,* CA 1450-7 N *rfrf*, CA 1450-7-1 N *rfrf*, CA 1450-7-10-1 N/S *Rfrf,* CA 1450-7-10-1 N/S *Rfrf,* CA 1450-7-10-10 N *rfrf,* CA 1450-7-10-10 N *rfrf*, CA 1450-7-72 N *rfrf*

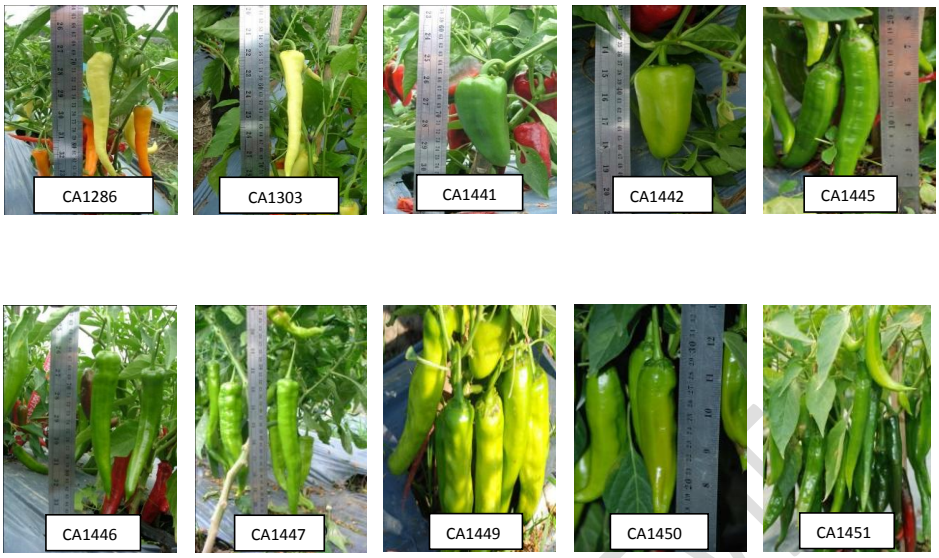

Figure 3. Characteristic of maintainer chilies: CA1286, CA1303, CA1441-J, CA1442, CA1445, CA1446, CA1447, CA1449, CA1450 and CA1451.

Table 3. Sources of maintainer chilies[x] (N*frf*)[y] with fruit physio-chemical qualities of some accessions.

		Fruit (cm.)								Chlorophyll			Capsaicin
									Vit. C	a	b		
No.	Accession	Length	Width	L[z]	Chroma[z]	Hue[oz]	%Moisture	TSS[z]	mg./100 g.fw.			Total	Scoville unit
1	CA005	9.57	2	61.66	44.63	113.8	89.83	6.53	5.7	0.04	0.02	0.06	5,660
2	CA042	8.43	1.95	52.71	43.33	116.48	88.45	7.6	12.03	0.07	0.03	0.1	7,730
3	CA254	7.22	2.16	67.34	41.96	112.22	91.18	5.83	4.43	0.03	0.01	0.04	6,300
4	CA319	8.48	1.5	32	18.25	130.6	88.58	7.6	15.19	0.25	0.12	0.37	8,850
5	CA617-D	9.14	1.54	47.12	40.63	120.26	87.75	8.4	67.72	0.1	0.05	0.15	4,320
6	CA720	7.16	1.94	43.4	39.29	120.36	85.99	9.87	103.16	0.11	0.07	0.18	5,020
7	CA780	7.52	2.98	49.66	44.16	116.62	90.79	6.17	34.18	0.06	0.04	0.1	4,580
8	CA1159	7.27	1.17	43.06	38.05	121.28	87.51	7.4	10.76	0.11	0.07	0.18	4,540
9	CA1159	7.27	1.17	43.06	38.05	121.28	87.51	7.4	10.76	0.11	0.07	0.18	4,540
10	CA1193	1.95	7.75	60.69	47.94	113.44	86.85	8.43	39.87	0.04	0.02	0.06	5,190
11	CA1193-F	6.93	2.37	58.26	49.02	112.44	87.71	8.07	3.87	0.03	0.02	0.05	6,840
12	CA1297	8.33	2.17	52.14	45.54	117.4	91.84	7.17	3.8	0.07	0.03	0.1	3,250
13	CA1303	11.22	1.94	64.84	41.44	111.72	90.04	5.23	23.23	0.01	0.01	0.02	4,330
14	CA1377-D-2	5.72	2.39	44.19	39.66	116.66	87.66	8.03	18.06	0.08	0.04	0.12	5,430
15	CA1441-1	7.98	4.06	55.79	46.31	114.4	93.81	5.17	29.75	0.06	0.03	0.09	3,320
16	CA1441-J-1-8	7.99	4.42	47.29	42.58	116.82	71.7	5.47	20.65	0.03	0.01	0.04	4,030
17	CA1441-J-3-1	7.99	4.42	47.29	42.58	116.82	71.7	5.47	20.65	0.03	0.01	0.04	4,030
18	CA1441-J-3-8	7.99	4.42	47.29	42.58	116.82	71.7	5.47	20.65	0.03	0.01	0.04	4,030
19	CA1442-1	8.29	4.62	51.34	42.17	114.76	79.98	5.17	21.94	0.03	0.02	0.05	8,120
20	CA1442-F-7	9.26	3.89	44.24	36.03	121.18	90.69	6.73	40	0.05	0.03	0.08	6,280
21	CA1443-4	10.11	2.92	45.07	39.87	120.08	90.21	5.67	14.84	0.08	0.04	0.12	4,800
22	CA1443-A	9.41	2.69	48.83	40.64	120.18	86.91	7.3	18.06	0.05	0.03	0.08	5,050
23	CA1445-4-1	11.18	2.76	42.38	32.73	123.36	93.64	5	12.03	0.1	0.05	0.14	3,875
24	CA1445-5-12	11.18	2.76	42.38	32.73	123.36	93.64	5	12.03	0.1	0.05	0.14	3,875
25	CA1447-3-13	12.12	2.27	46.42	43.31	119.9	91.2	5.43	46.54	0.02	0.01	0.03	5,480
26	CA1448-1-3	9	1.71	52.94	46.57	116.14	90.22	7.5	4.43	0.06	0.03	0.08	3,690
27	CA1449-3-9	12.75	2.16	51.06	43.08	117.54	91.91	5.27	29.11	0.09	0.04	0.13	4,610
28	CA1449-5-6	12.75	2.16	51.06	43.08	117.54	91.91	5.27	29.11	0.09	0.04	0.13	4,610
29	CA1450-4-8	11.12	2.46	43.45	36.04	122.52	90.99	5.17	6.45	0.11	0.06	0.17	4,700
30	CA1450-5-6	11.12	2.46	43.45	36.04	122.52	90.99	5.17	6.45	0.11	0.06	0.17	4,700
31	CA1450-7	11.12	2.46	43.45	36.04	122.52	90.99	5.17	6.45	0.11	0.06	0.17	4,700

[x] Random planting was used.

[y] N*rfrf* - normal cytoplasm and homozygous recessive of restorer genes which is designated as a maintainer.

[z] L*- the lightness factor, ranges from black (0) to white (100), Chroma- color saturation , ranges from grey or pale (0)to high color (60), Hue angle-the angle of color reflectance , ranges from 0 to 360 , TSS-total soluble solids, soluble solids content of a solution, referred to as the degrees Brix.

a- chlorophyll a.

b- chlorophyll b.

Table 4. Sources of fertility restorer of chilies[x] (N/S *RfRf*)[y] with fruit physio-chemical qualities of some accessions.

		Fruit (cm.)								Chlorophyll			Capsaicin
									Vit. C	a	b		
No.	Accession	Length	Width	L[z]	Chroma[z]	Hue[oz]	%Moisture	TSS[z]	mg./100 g.fw.			Total	Scoville unit
1	CA006-1	9.52	1.87	53.77	44.28	116.62	88.78	8.7	31.01	0.06	0.03	0.09	4,520
2	CA597-2	5.83	2.38	51.11	44.53	118.98	89.12	7.43	17.09	0.06	0.03	0.09	5,090
3	CA598-1	7.99	2.6	50.16	41.68	118.94	91.18	6.2	12.66	0.09	0.04	0.14	3,600
4	CA614-A-4	10.32	1.56	47.03	39.96	120.14	88.89	6.03	4.43	0.1	0.09	0.19	4,740
5	CA652-1	6.62	1.38	27.41	9.6	131.12	86.84	7.4	40.51	0.35	0.18	0.52	4,560
6	CA1297	8.33	2.17	52.14	45.54	117.4	91.84	7.17	3.8	0.07	0.03	0.1	3,250
7	CA1321-3	7.33	1.58	44.57	38.38	120.46	88.39	6.77	7.59	0.11	0.06	0.17	4,840
8	CA1428-F-1	8.86	2.49	39.77	32.93	121.72	87.81	6.63	57.86	0.03	0.01	0.04	7,280
9	CA1428-G-1	9.31	2.67	45.9	41.75	118.24	86.65	5.57	5.16	0.06	0.03	0.1	5,930
10	CA1451-1-10	10.6	1.96	46.26	39.27	121.02	93.26	5	4.43	0.11	0.06	0.17	5,120
11	CA1574-5	10.76	0.83	58.86	47.38	116.9	89.43	7.3	7.59	0.09	0.05	0.14	10,160

[x] Random planting was used.

[y] N/*SRfRf* - normal or sterile cytoplasm and homozygous dominant of restorer genes which is designated as a restorer.

[z] L*-the lightness factor, ranges from black (0) to white (100), Chroma- color saturation , ranges from grey or pale (0)to high color (60), Hue angle-the angle of color reflectance , ranges from 0 to 360 , TSS-total soluble solids, soluble solids content of a solution, referred to as the degrees Brix.

Table 5 Sources of heterozygous chilies[x] (N/S *Rfrf*)[y] with fruit size and fruit chemistry of some accessions.

		Fruit (cm.)								Chlorophyll			Capsaicin
									Vit. C	a	b		
No.	Accession	Length	Width	L[z]	Chroma[z]	Hue[oz]	%Moisture	TSS[z]	mg./100 g.fw.			Total	Scoville unit
1	CA042-5	8.43	1.95	52.71	43.33	116.48	88.45	7.6	12.03	0.07	0.03	0.1	7,730
2	CA614-A-4	10.32	1.56	47.03	39.96	120.14	88.89	6.03	4.43	0.1	0.09	0.19	4,740
3	CA683-1	8.5	2.12	48.54	43.21	118.64	88.81	7.17	72.15	0.09	0.05	0.14	5,120
4	CA1159-3	7.27	1.17	43.06	38.05	121.28	87.51	7.4	10.76	0.11	0.07	0.18	4,540
5	CA1286-4-2	10.39	2.06	63.36	38.95	111.76	91.39	5.57	91.14	0.02	0.01	0.03	3,760
6	CA1321-3	7.33	1.58	44.57	38.38	120.46	88.39	6.77	7.59	0.11	0.06	0.17	4,840
7	CA1403-7	6.98	1.06	57.37	52.84	116.06	87.31	8.9	5.06	0.05	0.03	0.08	9,410
8	CA1445-3-10	11.18	2.76	42.38	32.73	123.36	93.64	5	12.03	0.1	0.05	0.14	2,920
9	CA1447-3-2	12.12	2.27	46.42	43.31	119.9	91.2	5.43	46.54	0.02	0.01	0.03	6,230
10	CA1451-1-9	10.6	1.96	46.26	39.27	121.02	93.26	5	4.43	0.11	0.06	0.17	5,120

[x] Random planting was used.

[y] N/S*Rfrf*- normal or sterile cytoplasm and heterozygous of restorer genes which is designated as a restorer**.**

[z] L*-the lightness factor, ranges from black (0) to white (100), Chroma- color saturation , ranges from grey or pale (0)to high color (60), Hue angle-the angle of color reflectance , ranges from 0 to 360 , TSS-total soluble solids, soluble solids content of a solution, referred to as the degrees Brix.

Discussion

The use of male sterility in hybrid seed production is of great importance as it eliminates the need for mechanical emasculation. Cytoplasmic male sterility increasingly has been used in the F1 hybrid seed production of hot peppers (*C. annuum*) because it can reduce production costs by as much as 47% (Yang *et al.,* 2008) . Today, several international seed companies are using the genic male sterility system, msms, on a large scale for producing non-pungent hybrids, while cytoplasmic male sterility is used mainly for breeding pungent (S) *Rf rf* hybrids (Shifriss, 1997). The Faculty of Agriculture, Chiang Mai University, has released open-pollinated lines of chilies (*Capsicum annuum* L.) that are either restorers or male sterility maintainers. Ten seed companies and the Horticultural Research Institute, Yunnan Academy of Agricultural Science in China signed material transfer agreements with Chiang Mai University to use the chili cultivars between 2007 and 2009. They used the cultivars in their breeding programs to develop new F1 hybrid chilies. Two commercial F1 hybrids were developed for the Chinese market. The varieties were officially registered in China. Seeds of one variety, CT117, are available for commercial markets. At present, out of the 10 contracts with seed companies, only 3 companies have so far developed on-going programs of F1 hybrid development. A few hybrid chili varieties are available in the Thailand market this year. Fifty percent (50%) of the royalty fee goes to the Department of Rehabilitation Medicine, Faculty of Medicine, Chiang Mai University for medical students and patients; as well as agriculture students, engineering students and working horses in China.

The fertility-scoring method has been used successfully in indentifying fertile pollens (Rai *et al*. 2001, Pakozdi *et al*. 2002, and Yoon *et al*., 2006). Furthermore, results from this experiment showed that chili lines could be identified as maintainer or restorer lines by fertility scoring.

However, the method is time and labor consuming. It requires two growing seasons. Kim *et al.* (2005) and Lee *et al.* (2008) have suggested utilizing a CAPS marker that is closely linked to the fertility restoration genes. This method would require much less time and labor than the conventional method but has had limited success thus far.

Cytoplasmic male sterility (CMS) is an economically important trait for hybrid seed production. Disruption of meiosis in the pollen mother cells of cytoplasmic male sterile chili lines (S*rfrf*) causes male sterility. Chromosome rearrangements and chromosome breakage leading to aberrations can disrupt genetic system responsible for the regular meiosis. This could disrupt callose dissolving in the CMS plants (Vesselina *et al.,* 2010). Additionally, The CMS-associated protein Orf456 has been identified as a candidate gene for mediating male sterility in chilies (Kim *et al*., 2007). It has been found that the reduction of activity in ATP synthase in mitochondria also causes cytoplasmic male sterility (Li *et al*., 2013). There are nuclear restorers of fertility, *Rf* genes, that are distinct from genetic male sterility genes (Gulyas *et al*. (2006). The *Rf* genes have no expression unless sterile cytoplasm is present. *Rf* genes are required to restore fertility in the S cytoplasm that causes male sterility. Plants with the normal or N cytoplasm are fertile and those with the CMS cytoplasm with nuclear restorer genes are fertile, while the plants with the male sterile or S cytoplasm with no restorer genes are male sterile. The restoration of CMS in chili is controlled by a major nuclear gene termed restorer-of-fertility (*Rf*), along with several modifiers and some environmental factors (Wang *et al.*, 2004 and Huang *et al.,*2015) .

Conclusions

One hundred and forty one accessions of chilies segregated into 3 groups of prospective genotypes; maintainer lines with genotypes of *N rfrf* , heterozygous lines with genotypes of *N/SRfrf* and fertility restores with homozygous dominant restorer genes *N/SRfRf*. A few maintainers with good horticultural characteristic have been selected. There were differences in the stabilities of the cytoplasmic male-sterility among selected lines; and some lines were good maintainers, while a few lines were unstable and should be discarded. The accessions were distributed to 10 seed companies and the government of China and some F1 hybrid chilies have been commercially sold in both China and Thailand**.**

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