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Genetic Evaluation and Physico-chemical Properties of Chillies (*Capsicum annuum* L.)

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Abstract

Cytoplasmic male sterility of chilli cultivars was evaluated for their prospective genotypes. Fifteen pairs of F_1 hybrids were evaluated for their male pollen viabilities in three natures. Hybrids which showed no pollen occurred in 9 varieties whose male parents should have the prospective genotypes of N *rfrf* or B line. Hybrids with fertile pollens in some plants and sterile ones on others appeared in 4 varieties whose male parents should have prospective genotypes of N/S *Rfrf*. Hybrids showing fertile pollens were found in 2 varieties whose male parents should have the male sterility controlling genes in the nucleus as N/S *RfRf* or C line. Horticultural characteristics of six accessions of chillies were recorded. Chilli fruits of the accessions were analyzed for the physico-chemical properties in terms of moisture content, total soluble solids, vitamin C, capsaicin content and skin color. Accessions CA1445, CA1449 and CA1450 were found to have suitable characteristics as maintainer lines.

Keywords: *Capsicum*, chilli, male sterility, capsaicin, maintainer, physico-chemical properties

1. Introduction

Male sterility trait in pepper was first found in *Capsicum frutescens* (Martin & Crawford, 1951). Subsequently, Peterson (1958) found male sterility caused by interaction between cytoplasm (S-type) and the genes in the nucleus (*rf*) in *Capsicum annuum*. Shifriss (1997) studied and segregated various cases of natural sterile male pollens and concluded that male sterility was controlled by both the genes in the nucleus and the interaction between cytoplasm and the genes in the nucleus. Hybrid vegetable seeds generally produce high yield, good quality and consistent outputs (Khanobdee, 1992). However, producing hybrid seeds using hand emasculation of male pollen method is costly. The alternative method becomes the assurance of male sterility trait in hybrid seeds (Maneechat, 1999). Duvick (1959) exploited CMS for production of hybrid seeds with outstanding hybrid features to reduce cost associated with hand emasculation of male pollens of the parental lines, while assuring the hybrid seeds will be free from self-pollination action contamination (Mulyantoro et al., 2009; Wang et al., 2006). The farmers using hybrid seed chilli will be assured of consistent and higher output, especially from expressive heterosis in chilli hybrid seeds those results from heterozygosity (Bosland & Votava, 2000; Kordus, 1991).

2. Materials and Methods

2.1 Genetics Identification of Chilli by Pollen Viability

Sixteen accessions of *Capsicum annuum* L. were grown at Chiang Mai University during the winter of 2010-11. Average day temperature was 31.2±0.7 ^oC, average night temperature was 16.0±0.9 ^oC, relative humidity was 67.8±2.4 % and light intensity was 331.3 W/m^2. Thirty days old seedlings were transplanted into 8×12 inch black plastic bags containing rice hulls and sandy soil mixed in 1:1 ratio, additionally, 0.5 kg of cow manure and 10 g of fertilizer 15N-15P-15K were mixed into the mixed soil in each bag. They were grown in a greenhouse. Liquid fertilizer containing 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. Insecticides such as imidacloprid, fipronil sulfur and methomyl were used at recommended rates once a week. The 16 accessions were crossed onto 3 cytoplasmic male sterile chillies obtained from the Asian Vegetable Research and Development Center, Taiwan, who transferred these lines to

Kasetsart University, Kampangsan. About 50 days after anthesis when the fruits were at red ripe stage, seeds were harvested and dried. The F_1 hybrids were grown for evaluation of the viability of their pollens. A completely randomized design was used. Thirty plants were grown for each F_1 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; Yoon et al., 2006). The red stained color and morphology of pollen indicating the viability of pollen were basis for classifying different genotypes. They were classified as follows:

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

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

c. If some F_1 plants of a cross possessed 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).

2.2 Determination of the Horticultural Characteristics and Physico-chemical Properties of Chilli

Long green chilli varieties : CA683, CA1445, CA1447, CA1448, CA 1449 and CA1450 were selected from the germplasm and compared with three control varieties. 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 × 50 cm with a plot size of 4.5 m^2 and using the same cultural practices as mentioned above. Horticultural characteristics were also recorded (IPGRI, AVRDC, & CATIE, 1995). The green mature fruit was analyzed for the physico-chemical properties; total soluble solids, moisture content, vitamin C (Ranganna, 1986). The capsaicin content was measured as mentioned using spectrophotometry at 750 nm (Anan et al., 1996). The fruit color was measured using a chromometer.

3. Results

Fifteen F_1 hybrids were evaluated. They were classified into three groups (Table 1) according to their pollen viability (Figure 1). Nine F_1 hybrids showed no pollen fertility. This group consisted of : PEPAC 32-84 × CA1286-1, PEPAC 32-80 × CA1286-4, PEPAC 32-92 × CA1303-6, PEPAC 32-94 × CA1303-8, PEPAC 32 -103 × CA1441-J-8, PEPAC 38-25 × CA1442-5, PEPAC 38-11 × CA1445-1, PEPAC 32-37 × CA1449-3-9 and PEPAC 36-17 × CA1450-7. Accordingly, the male parental accessions: CA1286-1, CA1286-4, CA1303-6, CA1303-8, CA1441-J-8, CA1442-5, CA1445-1, CA1449-3-9 and CA1450-7 should have prospective genotypes of N *rfrf*. Four F_1 hybrids showed no pollen or sterile pollen on some plants and fertile pollens on other plants in the same hybrid. This group consisted of: PEPAC 38-18 × CA1303-2, PEPAC 32-110 × CA1443-5, PEPAC 32-23 × CA1448-1-3 and PEPAC 32-7 × CA1448-5-13. Accordingly, the male parental accessions: CA1303-2, CA1443-5, CA1448-1-3 and CA1448-5-13 should have prospective genotypes of N/S *Rfrf*. Two F_1 hybrids showed fertile pollens. This group consisted of: PEPAC 38-7 × CA683-3 and PEPAC 32-18 × CA1447-4-12. Accordingly, the male parental accessions: CA683-3 and CA1447-4-12 should have prospective genotypes of N/S *RfRf*.

Horticultural characteristics (IPGRI, AVRDC, & CATIE, 1995) were recorded for 6 selected varieties: CA683, CA1445, CA1447, CA1448, CA1449 and CA1450 and three control varieties: Jakkrapat, JomThong 2 and YokSiam as presented in Table 2. Plant and fruit characteristics of some varieties as shown in figure 2-4. The fruit of the hot chilli varieties CA 1447, CA1448 and CA1450 had a higher moisture content than those of other varieties and all control varieties (Table 3). The results indicated among the varieties tested, the moisture content, total soluble solids (TSS), vitamin C, capsaicin content and fruit color values differed at statistically significant levels. Total soluble solid values range between 4.30-6.93% with varieties CA1447, CA1448 and CA1450 having the highest values when compared with the others. The vitamin C content was expressed on the fresh weight basis of the chillies. The varieties CA1447, CA1448, CA1450, JomThong 2 and YokSiam had the highest vitamin C content. The group of varieties CA683 and CA1449 had the highest capsaicin when compared with other varieties. Canopy widths of all varieties show no statistical difference (Table 4).

Table 1. Prospective genotype of the F_1 hybrid and the male parents of chilli

F_1 hybrid	Prospective genotype	
	F_1 hybrid	Male parent
PEPAC 32-84 × CA1286-1	S *rfrf*	N *rfrf*
PEPAC 32-80 × CA1286-4	S *rfrf*	N *rfrf*
PEPAC 32-92 × CA1303-6	S *rfrf*	N *rfrf*
PEPAC 32-94 × CA1303-8	S *rfrf*	N *rfrf*
PEPAC 32-103 × CA1441-J-8	S *rfrf*	N *rfrf*
PEPAC 38-25 × CA1442-5	S *rfrf*	N *rfrf*
PEPAC 38-11 × CA1445-1	S *rfrf*	N *rfrf*
PEPAC 32-37 × CA1449-3-9	S *rfrf*	N *rfrf*
PEPAC 36-17 × CA1450-7	S *rfrf*	N *rfrf*
PEPAC 38 -18 × CA1303-2	S *Rfrf* : S *rfrf*	N/S *Rfrf*
PEPAC 32-110 × CA1443-5	S *Rfrf* : S *rfrf*	N/S *Rfrf*
PEPAC 32-23 × CA1448-1-3	S *Rfrf* : S *rfrf*	N/S *Rfrf*
PEPAC 32-7 × CA1448-5-13	S *Rfrf* : S *rfrf*	N/S *Rfrf*
PEPAC 38-7 × CA683-3	S *Rfrf*	N/S *RfRf*
PEPAC 32-18 × CA1447-4-12	S *Rfrf*	N/S *RfRf*

Note: N *rfrf*-normal cytoplasm and homozygous recessive of restorer genes which designated as maintainer, N/S *Rfrf*- normal or sterile cytoplasm and heterozygous of restorer genes which designated as restorer and N/S *Rfrf*-normal or sterile cytoplasm and homozygous dominant of restorer genes which designated as restorer.

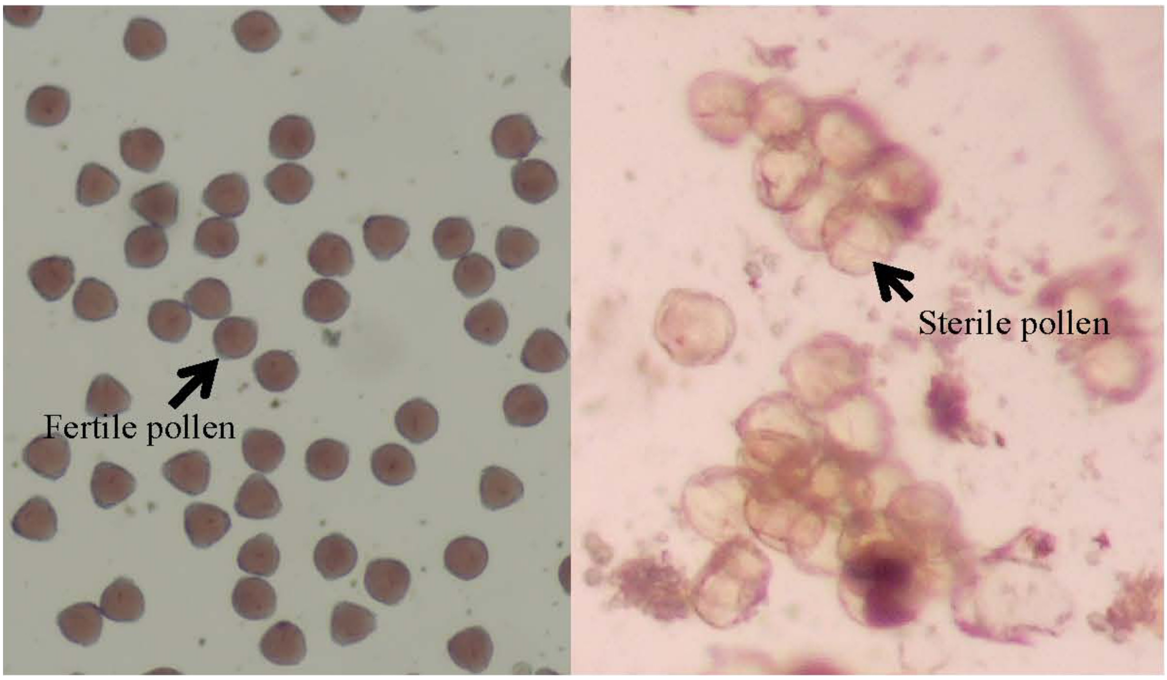

Figure 1. Fertile and sterile pollen of chilli accessions

Table 2. Vine, inflorescence and fruit characteristics and vine growth habit of chillies

Descriptors for *Capsicum* (1995)	CA683	CA1445	CA1447
1. Plant descriptors			
1.1 Stem color	1(Green)	1(Green)	1(Green)
1.2 Nodal anthocyanin	5(Purple)	5(Purple)	5(Purple)
1.3 Stem shape	1(Cylindrical)	1(Cylindrical)	1(Cylindrical)
1.4 Stem pubescence	3(Sparse)	3(Sparse)	3(Sparse)
1.5 Plant growth habit	7(Erect)	7(Erect)	5(Compact)
1.6 Branching habit	3(Sparse)	5(Intermediate)	3(Sparse)
1.7 Leaf density	7(Dense)	5(Intermediate)	5(Intermediate)
1.8 Leaf color	3(Green)	3(Green)	3(Green)
1.9 Leaf shape	3(Lanceolate)	3(Lanceolate)	3(Lanceolate)
1.10 Lamina margin	1(Entire)	1(Entire)	1(Entire)
1.11 Leaf pubescence	3(Sparse)	3(Sparse)	3(Sparse)
1.12 Plant height (cm)	80.89±6.74	69.33±2.40	62.11±5.40
1.13 Plant canopy width (cm)	72.22±7.19	79.78±5.09	76.11±7.24
1.14 Stem length (cm)	27.11±2.27	23.78±1.02	24.89±0.19
1.15 Mature leaf length (cm)	20.52±3.62	14.83±1.56	18.26±1.18
1.16 Mature leaf width (cm)	5.36±0.95	5.40±0.40	6.87±0.59
2. Inflorescence descriptors			
2.1 Days to flowering	72	71	69
2.2 Flower position	3(Pendant)	3(Pendant)	3(Pendant)
2.3 Corolla color	1(White)	1(White)	1(White)
2.4 Corolla spot color	no	no	no
2.5 Corolla shape	1(Rotate)	1(Rotate)	1(Rotate)
2.6 Anther color	5(Purple)	5(Purple)	5(Purple)
2.7 Filament color	1(White)	1(White)	6(Purple)
2.8 Male sterility	0(Absent)	0(Absent)	0(Absent)
2.9 Calyx pigmentation	0(Absent)	0(Absent)	0(Absent)
2.10 Calyx margin	2(Intermediate)	2(Intermediate)	2(Intermediate)
2.11 Calyx annular constriction	0(Absent)	1(Present)	1(Present)
3. Fruit descriptors			
3.1 Anthocyanin spots or strips	0(Absent)	0(Absent)	0(Absent)
3.2 Fruit color at intermediate stage	3(Green)	3(Green)	3(Green)
3.3 Fruit set	7(High)	7(High)	5(Intermediate)
3.4 Fruit color at mature stage	8(Red)	8(Red)	8(Red)
3.5 Fruit shape	1(Elongate)	1(Elongate)	1(Elongate)
3.6 Fruit shape at pedicel attachment	2(Obtuse)	3(Truncate)	3(Truncate)
3.7 Neck at base of fruit	0(Absent)	1(Present)	1(Present)
3.8 Fruit shape at blossom end	1(Pointed)	1(Pointed)	1(Pointed)
3.9 Fruit blossom end appendage	0(Absent)	0(Absent)	0(Absent)
3.10 Fruit cross-sectional corrugation	3(Slightly corrugated)	3(Slightly corrugated)	5(Intermediate)
3.11 Fruit surface	1(Smooth)	2(Semiwrinkled)	2(Semiwrinkled)
3.12 Placenta length	3(>1/2 fruit length)	3(>1/2 fruit length)	3(>1/2 fruit length)
3.13 Number of locules	2-3	2-3	2-3
3.14 Fruit length (cm)	14.20±0.30	13.65±0.49	18.92±0.28
3.15 Fruit width (cm)	2.00±0.08	2.12±0.06	3.16±0.10
3.16 Fruit pedicel length (cm)	5.92±0.04	4.01±0.24	5.40±0.10
3.17 Fruit wall thickness (mm)	0.193±0.01	0.207±0.01	0.263±0.01
3.18 Fruit weight (g)	16.533±2.70	17.933±0.51	47.500±3.35
4. Seed descriptors			
4.1 Seed color	1(Straw)	1(Straw)	1(Straw)
4.2 Seed surface	1(Smooth)	1(Smooth)	1(Smooth)
4.3 1000-seed weight (g)	6.662	4.763	6.089
4.4 Number of seed per fruit	3(>50)	3(>50)	3(>50)

Table 2. Vine, inflorescence and fruit characteristics and vine growth habit of chillies (continue)

Descriptors for *Capsicum* (1995)	CA1448	CA1449	CA1450
1. Plant descriptors			
1.1 Stem color	1(Green)	1(Green)	1(Green)
1.2 Nodal anthocyanin	5(Purple)	1(Green)	1(Green)
1.3 Stem shape	1(Cylindrical)	1(Cylindrical)	1(Cylindrical)
1.4 Stem pubescence	3(Sparse)	3(Sparse)	3(Sparse)
1.5 Plant growth habit	5(Compact)	5(Compact)	3(Prostrate)
1.6 Branching habit	5(Intermediate)	3(Sparse)	5(Intermediate)
1.7 Leaf density	5(Intermediate)	5(Intermediate)	5(Intermediate)
1.8 Leaf color	3(Green)	3(Green)	3(Green)
1.9 Leaf shape	3(Lanceolate)	3(Lanceolate)	3(Lanceolate)
1.10 Lamina margin	1(Entire)	1(Entire)	1(Entire)
1.11 Leaf pubescence	3(Sparse)	3(Sparse)	3(Sparse)
1.12 Plant height (cm)	58.56±2.22	72.45±8.28	52.89±1.02
1.13 Plant canopy width (cm)	66.89±1.02	74.33±8.45	76.22±7.07
1.14 Stem length (cm)	23.11±1.35	19.33±1.86	21.33±1.45
1.15 Mature leaf length (cm)	16.97±1.52	17.48±1.49	13.86±3.50
1.16 Mature leaf width (cm)	6.21±0.66	6.48±1.10	5.48±1.13
2. Inflorescence descriptors			
2.1 Days to flowering	69	72	69
2.2 Flower position	3(Pendant)	3(Pendant)	3(Pendant)
2.3 Corolla color	1(White)	1(White)	1(White)
2.4 Corolla spot color	no	no	no
2.5 Corolla shape	1(Rotate)	1(Rotate)	1(Rotate)
2.6 Anther color	5(Purple)	5(Purple)	5(Purple)
2.7 Filament color	1(White)	1(White)	6(Purple)
2.8 Male sterility	0(Absent)	0(Absent)	0(Absent)
2.9 Calyx pigmentation	0(Absent)	0(Absent)	0(Absent)
2.10 Calyx margin	2(Intermediate)	2(Intermediate)	2(Intermediate)
2.11 Calyx annular constriction	1(Present)	1(Present)	1(Present)
3. Fruit descriptors			
3.1 Anthocyanin spots or strips	0(Absent)	0(Absent)	0(Absent)
3.2 Fruit color at intermediate stage	3(Green)	3(Green)	3(Green)
3.3 Fruit set	5(Intermediate)	7(High)	5(Intermediate)
3.4 Fruit color at mature stage	8(Red)	8(Red)	8(Red)
3.5 Fruit shape	1(Elongate)	1(Elongate)	1(Elongate)
3.6 Fruit shape at pedicel attachment	Obtuse	Truncate	Truncate
3.7 Neck at base of fruit	0(Absent)	1(Present)	1(Present)
3.8 Fruit shape at blossom end	Pointed	Blunt	Pointed
3.9 Fruit blossom end appendage	0(Absent)	0(Absent)	0(Absent)
3.10 Fruit cross-sectional corrugation	3(Slightly corrugated)	5(Intermediate)	3(Slightly corrugated)
3.11 Fruit surface	2(Semiwrinkled)	2(Semiwrinkled)	2(Semiwrinkled)
3.12 Placenta length	3(>1/2 fruit length)	3(>1/2 fruit length)	3(>1/2 fruit length)
3.13 Number of locules	2-3	2-3	2-3
3.14 Fruit length (cm)	19.26±0.67	13.86±0.84	15.38±0.71
3.15 Fruit width (cm)	3.20±0.10	3.05±0.15	3.26±0.16
3.16 Fruit pedicel length (cm)	4.99±0.20	4.60±0.12	5.34±0.64
3.17 Fruit wall thickness (mm)	0.280±0.02	0.247±0.01	0.287±0.02
3.18 Fruit weight (g)	46.767±2.10	32.233±1.58	44.333±0.25
4. Seed descriptors			
4.1 Seed color	Straw	Straw	Straw
4.2 Seed surface	Smooth	Smooth	Smooth
4.3 1000-seed weight (g)	5.033	7.224	5.477
4.4 Number of seed per fruit	>50	>50	>50

Table 3. Analysis of physico-chemical properties of chillies

Variety	Moisture (%)	TSS (%)	Vitamin C (mg/100 g fw.)	Capsaicin (Scoville unit)
CA683	84.803 e [1]	4.533 d	3.846 c	3530.0 a
CA1445	83.497 f	6.133 b	5.128 b	260.0 f
CA1447	89.367 a	6.933 a	6.410 a	1700.0 d
CA1448	89.017 ab	6.233 b	6.410 a	190.0 f
CA1449	86.583 d	5.167 c	5.128 b	2750.0 b
CA1450	88.953 ab	4.567 d	6.410 a	2370.0 c
Jakkrapat (control 1)	87.897 bc	4.733 cd	5.128 b	2610.0 bc
JomThong 2 (control 2)	87.477 cd	4.833 cd	6.410 a	960.0 e
YokSiam (control 3)	87.577 cd	4.300 d	6.410 a	1700.0 d

[1] Means within column with different letters differ significantly at $P \leq 0.05$ according to DMRT.

Table 3. Analysis of physico-chemical properties of chillies (continue)

Variety	L*	Chroma	Hue angle (°)
CA683	48.047 e [1]	42.750 c	130.41 a
CA1445	53.827 bc	49.480 ab	125.99 b
CA1447	53.537 c	48.263 ab	125.49 b
CA1448	51.683 cd	46.297 b	126.40 b
CA1449	58.820 a	46.437 b	122.65 c
CA1450	49.987 de	42.927 c	127.13 b
Jakkrapat (control 1)	47.750 e	42.153 c	129.60 a
JomThong 2 (control 2)	44.620 f	40.303 c	130.70 a
YokSiam (control 3)	56.830 ab	50.713 a	126.09 b

[1] Means within column with different letters differ significantly at $P \leq 0.05$ according to DMRT.

Table 4. Canopy height and width of chillies

Variety	Height (cm)	Width (cm)
CA683	80.89 a [1]	72.22 ns
CA1445	69.33 bc	79.78
CA1447	62.11 cd	76.11
CA1448	58.56 d	66.89
CA1449	72.45 ab	74.33
CA1450	52.89 d	76.22
Jakkrapat (control 1)	76.00 ab	73.67
JomThong 2 (control 2)	81.78 a	78.78
YokSiam (control 3)	74.22 ab	76.22

[1] Means within column with different letters differ significantly at $P \leq 0.05$ according to DMRT.

Figure 2. Plant canopy and fruit characteristics of chillies

(a) Variety CA683 (b) Variety CA1445 and (c) Variety CA1450.

4. Discussion and Conclusion

Male sterility is very critical for plant breeding improvement program especially for the production of hybrid seeds of chillies (Min et al., 2009) as this trait can help save time and labor, hence the production cost, as well as enhance the purity of hybrid seeds (Mulyantoro et al., 2009; Yang et al., 2008). Male sterility can be distinguished into three groups : normal male fertile chilli (N/S *RfRf*), male sterile chilli; (N *rfrf*) and male fertile chilli which has heterozygosis in the genotype of the fertility restoration (N/S *Rfrf*) using fertility scoring for the evaluation of male sterility (Rai et al., 2001; Pakozdi et al., 2002; Yoon et al., 2006). Normal male fertile chilli (N/S *RfRf*) can be used as C line in breeding hybrid chillies as the parent has the homozygous male sterility controlling gene in the nucleus. This occurred in two varieties CA683 which has high capsaicin content and CA1447 which contain high moisture, total soluble solids and vitamin C and they can be utilized for breeding hybrids with the desirable characteristics. Nine varieties with pollen fertility were obtained. The CA1450 has high moisture content, total soluble solids and vitamin C and CA1449 contains high capsaicin and they should be exploited as maintainer or B line in breeding hybrids with these characteristics. The chilli varieties with heterozygous male sterility controlling gene may be used as C line in future breed improvement whose accession CA1448 has high content of moisture, total soluble solids and, vitamin C. The distribution of the F_1 hybrids in the three male pollen viability groups is supported by the research results of Shifriss (1997) which enable an understanding of the genetic mechanism in fertility restoration and the use of male sterility trait in hybrid development (Wang et al., 2006). It is also found that the fertility scoring method is robust in genetic evaluation for the present purpose (Pakozdi et al., 2002).

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