Kasetsart J. (Nat. Sci.) 37 : 5 - 13 (2003)

Development of Male and Female Parents of F_1 Hybrid in Chinese Cabbage

Ekapote Payakhapaab, Tragool Tunsuwan, Chokchai Chimonkon, Dumdern Karadee and Maneechat Nikonpun

ABSTRACT

Male and female parents were improved to produce good hybrid progenies of Chinese cabbage, a highly self-incompatible vegetable crop. Hybrid seed production using bees was also studied. Inbred seeds of 4 Chinese cabbage lines were produced using bud pollination which is a conventional technique for inbred lines. Results showed that line 40-9 gave the highest seed weight of 0.165 g/plant. Using seed set analysis technique to check self-incompatibility, lines 23-3-4, 27, 27-3-7, and 40-9 were found to be self-incompatible among the 9 Chinese cabbage lines evaluated. However, when fluorescent microscope technique was used, the self-incompatible lines were 23-3-4, 27, 27-3-7, 40-9, and 142. Four inbred lines with self-incompatibility were selected to produce seeds. When line 40-9 was used as female parent in reciprocal crosses by bee pollination, the progenies gave the highest seed yield, an indication of maternal effect. Crosses 40-9 x 142-5, 40-9 x 27-3-7 and 40-9 x 23-3-4 gave high seed weight of 4.8, 3.9 and 2.7 kg/rai, respectively. Comparison of 11 Chinese cabbage hybrids with 3 commercial varieties showed that hybrid 142-5 x 40-9 gave the highest head yield with 6,170 kg/rai, 36.3% higher than commercial varieties. Other crosses such as hybrids 23 x 27, 23 x 142, 27-3-7 x 23-".-4, 27-3-7 x 142-5 and 40-9 x 23-3-4 had good horticultural characteristics. These results indicate that improvement of parental lines is necessary to enhance good F_1 hybrid.

Key words : Chiness cabbage, hybrid

INTRODUCTION

Chinese cabbage is not only a popular vegetable throughout the country but also an economical crop for export. Chinese cabbage grown in Thailand are both open-pollinated and F_1 hybrid varieties. They are all imported from abroad so seed price is very high. Popular Chinese cabbage grown by farmers are hybrid varieties from Japan, Taiwan and Korea.

The Vegetable Seed Production Thailand project was supported by the International Development Research Centre, Canada through the Faculty of Agriculture, Chiang Mai University. The project aimed to develop varieties of some Brassica crops including Chinese cabbage and their seed production. Chinese cabbage varieties were collected from local markets and other countries. Good F_1 hybrid varieties were developed from these varieties (Tunsuwan *et al*., 1997). Seed production of open-pollinated Chinese cabbage was tested in few locations under highland condition and the results were satisfactory. Chimonkon *et al*. (1997) developed self-incompatibility

Department of Horticulture, Faculty of Agriculture, Chiang Mai University Chiang Mai 50200, Thailand.

Received date : 2/09/02 Accepted date : 02/12/02

characteristic of Chinese cabbage for F_1 hybrid seed production. This character can reduce labor cost because emasculation of female parent is not needed. Therefore, the price of F_1 hybrid seeds is reduced. Very often, the hybrid seeds can be collected from both parents.

Self-incompatibility is a special characteristic of some Brassica crops which promotes cross-pollination and prevents self-pollination of these crops. This characteristic is controlled by multiple allelic genes at a single locus. Pollen tube germination is inhibited by stigma if they carry the same self-incompatible genes (Nasrallah and Nasrallah, 1993; Nasrallah *et al*., 1994; Isogai *et al*., 1987). The is a sporophytic reaction in which 2n chromosome of S locus of both parents controls the reaction (Gaude *et al*., 1993; Pastuglia *et al*., 1997 and Nasrallah and Nasrallah, 1993). Chinese cabbage also carries self-incompatible genes of S locus. It is classified into a sporophytic reaction (Opena *et al*., 1988). The self-incompatible genes were used to develop F_1 hybrid Chinese cabbage for high yield and good quality. Additionally, bee pollination was studied for F_1 hybrid seed production.

MATERIALS AND METHODS

1. Hand pollination of an unopened flower bud for inbred seed production

The seeds of Chinese cabbage Inbred lines 23-3-4, 27-3-7, 40-9 and 142-5 were germinated for two days and then vernalized at 4-5°C for 15 days before they were planted in the field in winter of 1999 at Chiang Mai University. The inflorescence was covered with paper bag before blooming. When some flowers of the inflorescence started to open, the paper bag was removed. The young unopened flowers were forced to open by forceps. The pollen from opened flowers of the same inflorescence was used to pollinate the young flowers. All other opened flowers were removed. The inflorescence was covered with the same paper bag. The seeds were collected at maturity.

2. Testing for self-incompatibility levels

Two methods of testing for self-incompatibility levels of Chinese cabbage were used: seed set analysis and fluorescent microscope. In seed set analysis method, unopened and opened flowers of the same inflorescence were pollinated with pollen from the same plant (Shinohara, 1981 ; Opena *et al*., 1988). Ten plants with healthy inflorescence from each inbred lines were selected. About 3-4 healthy inflorescences per plant were covered with paper bags. The open flowers were removed before bagging. Two to three days after bagging, both unopened and opened flowers were emasculated and pollinated with pollen from the same inflorescence of the same plant. The unopened and the opened flowers were marked with string and the inflorescence was covered with the same paper bag. When the seeds matured, the number of pods that set and number of seeds per pod were counted.

Fluorescent microscope method was used to observe pollen tubes in styles of female flowers (Kho and Baer, 1968). Unopened and opened flowers were taken from a plant and pollinated with pollen from the same plant. The opened flowers were emasculated and cross-pollinated with pollen from different varieties.

Three types of flowers were collected: the self-pollinated of the unopened flower, the self-pollinated of the opened flower, and the cross-pollinated of the opened flower. The flowers were put on a slide in a petri dish containing potassium dichromate (K_2CrO_4) solution underneath the slide. The potassium dichromate kept the atmosphere in the petri dish at 98%. The flowers were left in the petri dish for 24 hrs. The styles of the flowers were dissected and placed in 1 N sodium hydroxide. The sample was boiled at 60°C for 30 minutes. The styles were washed with distilled water, stained with aniline blue solution (0.2% diluted in 2% potassium phosphate), and kept for 24 hrs. in a

refrigerator. Then they were squashed with glycerin on a slide and observed under fluorescent microscope. The number of pollen tubes in each style was counted.

3. F_1 hybrid seed production by bees

Four inbred lines of Chinese cabbage, 23-3-4, 27-3-7, 40-9 and 142-5, were vernalized at 4-5°C for 15 days. They were planted in a field at a distance of 30 cm between plants and 50 cm between rows. Six seedlings were planted for each line with 3 plants per row. Plot size was 2 x 2 m with two plots per crossing. Each plot was planted with 2 inbred lines, side by side. Twelve possible crosses were made including reciprocals. A bee hive was placed in each cross making sure that the number of bees in each net was enough for pollination. A salan net was used to cover the plots to protect against insects and to keep honey bees inside the net. The mature seeds were harvested when pods dried.

4. Varietal evalution of F_1 hybrid Chinese cabbage and control varieties

Randomized complete block design with 3 replications was used for this experiment. The F_1 hybrid varieties were tested against 3 control varieties such as Chang, Bomb 159 and Tapa 23. Each treatment was planted in a 2 x 2 meters plot. Eight plants were planted per plot at a spacing of 40 x 50 cm. Guard rows were planted around each replication.

RESULTS

1. Hand pollination of an unopened flower bud for inbred seeds production

Inbred lines of Chinese cabbage were different in seed set (Table 1). Line 40-9 gave the highest seed weight of 0.165 g/plant, followed by 27-3-7 and 27 with 0.153 and 0.123 g/plant, respectively. It was observed that unopened flowers in the middle of an inflorescence produced the highest seed number while unopened flowers at the top and bottom of the inflorescence produced few seeds or did not set seed at all. It was also observed that the flowers which gave good seed set were big but the yellow color of petals did not yet show up.

2. Testing for self-incompatibility levels

Testing for self-incompatibility levels by seed set analysis method

The self-incompatibility levels of the plant is indicated by the number of seeds set in the opened flowers. Some inbred lines such as lines 27-3-7 and 40-9 showed strong self-incompatibility. When the range of 0-25% of seed setting was used to indicate strong self-incompatibility levels, the strongest self-incompatible line was 27-3-7 which showed 22.01 % seed set. It was followed by 40-9 and 23-3-4 with 24.72 and 29.24 % seed set, respectively. When 25.5% of setting was used to indicate weak self-incompatibility, the weak self-incompatible

Table 1 Seed weight from self pollinated Chinese cabbage.

Line	Seed weight/plant (g)
40-9	0.165
27-3-7	0.153
27	0.123
23	0.076
142-5	0.047
23-3-4	0.032

Table 2 Levels of self-incompatibility of Chinese cabbage lines, tested by seed set analysis.

Line	Number of pod		Number of seed		Seed set[1] (%)	Conclusion[2]
	Unopened flower	Opened flower	Unopened flower	Opened flower		
23	15	15	210	80	38.10	WSI
23-3-4	15	15	236	69	29.24	WSI
27	15	15	193	96	49.74	WSI
27-3-7	15	15	231	51	22.01	SI
40-9	15	15	267	66	24.72	SI
142	15	15	253	88	34.78	WSI
142-5	15	15	245	79	32.24	WSI

1 0-25%-self-incompatibility, 26-50% weak self-incompatibility, 51-75% weak self compatibility and 76-100% self-compatibility.

2 SI = self-incompatibility
WSI = week self-incompatibility

lines were 23, 27, 142, and 142-5 (Table 2 and Figure 1).

Testing of self-incompatibility levels by fluorescent microscope technique

Chinese cabbage inbred lines were tested for their self-incompatibility levels by fluorescent microscope technique. Pollen tubes in styles of unopened and opened flowers were observed using fluorescent microscope after they were self-pollinated. The inbred lines showed weak self-incompatibility, weak and levels (Table 3). The weak self-incompatible lines, 23-3-4, 27, 27-3-7, 40-9, and 142, showed low percentage of pollen tubes in styles. Inbred lines 23, 40, and 142-5 showed some pollen tubes in their styles. They were classified in both weak self-incompatible and weak self-compatible groups. The self-compatible line was 23-3-1 which showed 80% of pollen tubes in opened flowers when self-pollinated (Figure 2).

Comparing the two methods of evaluating self-incompatibility levels, the results were somewhat different. Seed set analysis method showed strong self-incompatibility levels in lines 27-3-7, 40-9, and 23-3-4. However, the fluorescent microscope technique showed weak self-incompatibility levels in lines 23-3-4, 27, 27-3-7, 40-9, and 142. Some pollen tubes were found in these lines under the fluorescent microscope. This method of evaluation might be more sensitive than the seed set analysis method. The pollen tubes which were found in these lines might not be able to convey to seed setting.

3. Bee pollination for F_1 hybrid seed production

Four Chinese cabbage inbred lines, 23-3-4, 27-3-7, 40-9, and 142-5, were reciprocally crossed with the use of bees. Seed yield of the crosses is shown in Table 4. Seed weight ranged from 0.03 to 4.8 kg/rai. Cross 40-9 x 142-5 gave the highest seed yield, 4.8 kg/rai. It was followed by crosses 40-9 x 27-3-7 and 40-9 x 23-3-4 which yielded 3.9 and 2.9 kg/rai, respectively. Seed yield as such was rather low for seed production. There might be some problems in bee pollination. Probably, the size of the salan net (2.5 x 2.5 m) may be too small for bee activities. The bees tend to stay in the hive or are held on the salan. Maternal effects were detected in line 40-9 as a mother plant compared to the other mother lines (Table 4).

Table 3 Levels of self-incompatibility of Chinese cabbage lines, tested by fluorescent microscope technique.

Line	Control		Self pollinated		Cross pollinated		Conclusion
	Unopened flower (%)	Opened flower (%)	Unopened flower (%)	Opened flower (%)	Unopened flower (%)	Opened flower (%)	
23	-	-	80[1]	50	80	100	WSI[2],WSC
23-3-1	-	-	100	80	100	80	SC
23-3-4	-	-	100	30	80	100	WSI
27	-	-	50	30	30	100	WSI
27-3-7	-	-	80	30	100	100	WSI
40	-	-	80	50	100	50	WSI,WSC
40-9	-	-	100	30	80	100	WSI
142	-	-	30	30	100	80	WSI
142-5	-	-	80	50	100	100	WSI,WSC

[1] Number of pollen tube in style in percentage, 0-25%-self-incompatibility, 26-50% weak self-incompatibility, 51-75% weak self-compatibility and 76-100% self-compatibility.

[2] WSI = weak self-incompatibility
SC = self-compatibility
WSC = weak self-compatibility

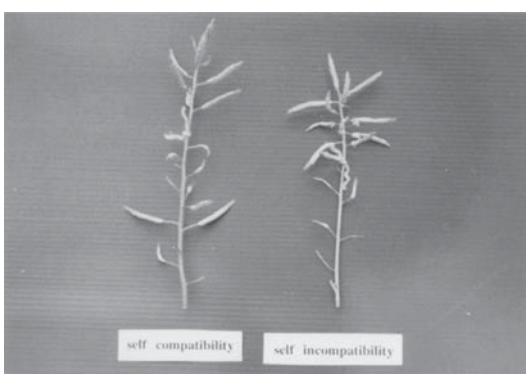

Figure 1 Self-compatible and self-incompatible inflorescence of Chinese cabbage.

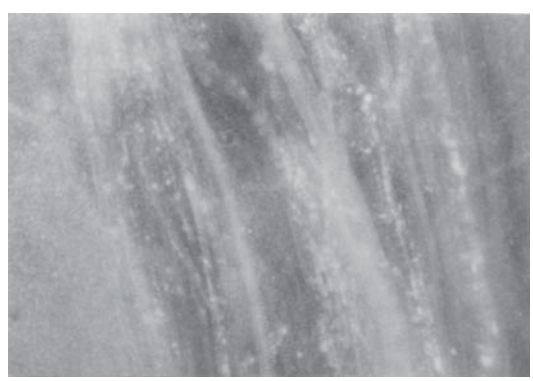

Figure 2 Pollen tube in a style of female in Chinese cabbage flower.

4. Varietal evaluation of F_1 hybrid Chinese cabbage and control varieties

Evaluation of 11 F_1 hybrid varieties of Chinese cabbage obtained from the crosses against 3 control varieties showed that most F_1 hybrid varieties gave higher fresh head yield than the control ones (Table 5).

Head fresh weight of F_1 hybrid varieties ranged from 3,665 to 6,170 kg/rai. Cross 142-5 x 40-9 gave the highest head fresh weight of 6,170 kg/rai followed by 23 x 142 and 27-3-7 x 142-5 which yielded 5,551 and 5,150 kg/rai, respectively, These yields were significantly different from all control varieties which gave head fresh yield ranging from 3,625 to 4,527 kg/rai. Considering head weight before trimming, the F_1 hybrid

Table 4 Seed weight of F1 hybrid Chinese cabbage.

Cross	Seed weight (kg/rai)	Cross	Seed weight (kg/rai)
40-9 x 142-5	4.826	27-3-7 x 23-3-4	0.197
40-9 x 27-3-7	3.941	142-5 x 27-3-7	0.144
40-9 x 23-3-4	2.714	23-3-4 x 40-9	0.128
142-5 x 40-9	1.642	23-3-4 x 142-5	0.085
23-3-4 x 27-3-7	1.403	27-3-7 x 142-5	0.051
27-3-7 x 40-9	0.773	142-5 x 23-3-4	0.030

Table 5 Fresh weight before and after trimming, trimming percentage, and solidity of Chinese cabbage head of F1 hybrid and control varieties.

Cross and variety	Head yield (Kg/rai)	Head weight before trimming	Head weight after trimming	Trimming (%)	Solidity of head (g/cm^3)	HSI
Cross						
142-5 x 40-9	6,170.0 a[3]	1,390.0 b	964.0 a	30.65	0.5719 a	1.228 e
23 x 142	5,551.0 b	1,329.0 b	867.3 b	34.74	0.5507 a	1.308 d
27-3-7 x 142-5	5,150.0 b	1,484.0 a	804.7 b	45.77	0.4566 c	1.311 d
27 x 23	4,911.0 c	1,248.0 b	767.3 c	38.52	0.4110 d	1.279 d
27-3-7 x 40-9	4,851.0 c	1,333.0 b	758.0 c	43.14	0.4916 b	1.356 c
40-9 x 142-5	4,736.0 c	1,181.0 c	740.0 c	37.34	0.5857 a	1.299 d
23 x 27	4,646.0 c	1,299.0 b	726.0 c	44.11	0.4499 c	1.432 b
40-9 x 27-3-7	4,414.0 d	1,107.0 c	689.7 d	37.70	0.4418 c	1.227 e
142 x 23	4,186.0 d	1,112.0 c	654.0 d	41.19	0.4511 c	1.289 d
40-9 x 23-3-4	3,785.0 e	974.0 d	591.3 e	39.29	0.5020 b	1.569 a
27-3-7 x 23-3-4	3,665.0 e	1,173.0 c	572.7 e	51.18	0.4243 d	1.513 a
Variety						
Chang	4,527.0 d	1,486.0 a	707.3 d	52.40	0.5269 a	1.404 b
Tapa 23	3,917.0 e	1,152.0 e	612.0 e	46.88	0.3997 d	1.343 c
Bomb 159	3,625.0 e	1,473.0 a	566.3 e	61.55	0.3931 d	1.427 b
C.V. (%)	7.65	6.38	7.65	-	6.58	3.35
$LSD_{.05}$	588.33	135.78	91.926	-	0.053	0.076

1. Solidity = $MHW/(0.524d_1^2d_2)$
   MHW = mean head weight
   d_1 = head width
   d_2 = head length
2. Head shape index; HSI = head width/head length
3. Means follow by the same letters indicate no differences at P = .05 by least significant difference.

varieties gave head weight ranging from 970 to 1,484 g (Table 5). The range was more or less similar to fresh head weight of the control varieties which ranged from 1,152 to 1,486 g. However, when the outer leaves were trimmed off, most of the F_1 hybrid varieties showed higher head weight than the control (Table 5, Figure 3 and 4). Head weight of the F_1 hybrid varieties after trimming ranged from 572.7 to 964 g/head, while the control varieties showed a range of 566.3 to 707.3 g/head. The head weights of these varieties were significantly lower than most of the F_1 hybrid varieties.

The control varieties showed higher percentage of trimming than most (BE SPECIFIC) of the F_1 hybrid varieties (Table 5). Variety Bomb 159 gave the highest trimming percentage of 61.55%. A good control variety such as Chang showed 52.40%. It was followed by variety Tapa 23 with 46.88%. The F_1 hybrid varieties showed a range of 30.65 to 51.18%. Therefore, the marketable yield of most of the F_1 hybrid varieties should be higher than the control. Head solidity of the control variety, Chang, was not significantly different from high yielding F_1 hybrid varieties, 142-5 x 40-9 and 23 x 142. However, varieties Bomb 159 and Tapa 23 were less solid than all of the F_1 hybrid varieties. High solidity of Chinese cabbage is more perferable than low solidity.

Another important characteristic of Chinese cabbage is head length. Long head is more preferable than short one, therefore, high head shape index is preferred.

Cylindrical head shape was observed in the F_1 hybrids 40-9 x 23-3-4 and 27-3-7 x 23-3-4 with head shape index of 1.569 and 1.513, respectively, which were significantly different from the control varieties. However, there were many F_1 hybrid

Figure 3 Head of F1 hybrid Chinese cabbage 142-5 x 40-9 (50) and control varieties (1-Chang, 2-Bomb 159 and 3-Tepa 23).

Figure 4 Head of F_1 hybrid Chinese cabbage 27-3-7 x 23-3-4 (44) and control varieties (1-Chang, 2-Bomb 159 and 3-Tapa 23).

varieties which their head shape index ranged from 1.227 to 1.311. Their heads were rather round which is undesirable in the market.

Other horticultural characteristics of most of F_1 hybrid varieties were more or less the same (Table 6). They had very tight heads, oval shape, thick petioles, pale and light green color of outer leaves, and yellow color of inner leaves.

The F_1 hybrid varieties had certain levels of disease resistance. Even though the hybrids were not screened for disease resistance, soft rot is a common disease for Chinese cabbage, The disease was not observed on the F_1 hybrid and the control varieties.

DISCUSSION

Improvement of F_1 hybrid varieties of Chinese cabbage requires good male and female parental lines with high levels of self-incompatibility. Comparison ot self-incompatibility test by using seed set analysis and fluorescent microscope technique showed that the former method was better than the later. Results showed that lines 23, 23-3-4, 27-3-4, 40-9, and 142-5 were self-incompatible and may have good potential for F_1 hybrid seed production. Among the lines tested for seed production using bud pollination technique, line 40-9 gave the highest seed weight of 0.165g/plant.

Production of F_1 hybrid seeds of the crosses showed that self-incompatibility could be utilized. Results showed that there was maternal effect. When line 40-9 was used as a female parent, the crosses gave the highest seed weight.

Varietal evaluation of F_1 hybrid Chinese cabbage showed that F_1 hybrid 142-5 x 40-9 gave higher head weight than standard varieties. However, the head shape was rather round. There were other good potential F_1 hybrids such as 23 x 27, 23 x 142, 27-3-7 x 23-3-4, 27-3-7 x 142-5 and 40-9 x 23-3-4.

CONCLUSION

Inbred lines 23, 23-3-4, 27-3-7, 40-9 and 142-5 were suitable to be used as parental lines due to the high levels of self-incompatibility which is

Table 6 Horticultural characteristics of F_1 hybrid Chinese cabbage.

Cross	Cross-section of petiole	Solidity of head	Thickness of petiole	Head shape	Color of outer leaves	Color of inner leaves
23 x 27	Flat	Firm	Thick	Obovate	Pale green	Yellow
23 x 142	Flat	Firm	Semi	Obovate	Light green	Yellow
27 x 23	Flat	Firm	Semi	Obovate	Pale green	Yellow
27-3-7 x 23-3-4	Flat	Firm	Thick	Obovate	Pale green	Yellow
27-3-7 x 40-9	Semiround	intermediate	Thick	Obovate	Light green	Yellow
27-3-7 x 142-5	Flat	Firm	Thick	Obovate	Pale green	Yellow
40-9 x 23-3-4	Flat	Firm	Thick	Ovate	Light green	Yellow
40-9 x 27-3-7	Flat	Firm	Thick	Obovate	Pale green	Yellow
40-9 x 142-5	Flat	Firm	Thick	Obovate	Pale green	Yellow
142 x 23	Flat	Firm	Thick	Obovate	Pale green	Yellow
142-5 x 27-3-7	Flat	Firm	Thick	Obovate	Light green	Yellow

good for F_1 hybrid seed production. All the lines gave high seed yield when used as a female parent. Among these lines, 40-9 showed maternal effect. It also gave high seed yield when inbred seeds were produced by self-pollination of the unopened flowers.

Bee pollination of F_1 hybrid seed production was not satisfied (SATISFACTORY?) because seed yield was quite low. Production of F_1 hybrid seed by bee pollination is useful due to low cost in inbred and hybrid seed production. However, whether this technique is expropriated (APPROPRIATE?) or not needs to be evaluated further.

Most F_1 hybrid varieties gave high head yield but their heads were rather round which is not desirable. Good potential F_1 hybrid varieties were 142-5 x 40.9, 23 x 27, and 23 x 142.

LITERATURE CITED

Briggs, F.N. and P.F. Knowles. 1967. **Introduction to Plant Breeding**. Reinhold Publishing Corporation: A Subsidiary of Chapman-Reinhold, INC., 426 p.

Chimonkon, C., M. Nikornpun and T. Tunsuwan. 1997. Varietal trial of Chinese cabbge,pp. 120-126. *In* **National Vegetable Meeting**, 15th, Bangkok,

Gaude, T., A. Friry, P.Heizmann, C. Mariac, M. Rougier, I. Fobis and C. Dumas. 1993. Expression of a self incompatibility gene in a self compatible line of *B.oleracea*. **Plant Cell** 5 : 75-86.

Isogai, A., S. Takayama, C. Tsukamoto, Y. Ueda, H. Shozawa, K. Hinata, K. Okazaki and A. Suzuki. 1987. S-locus-specific glycoproteins associated with self-incompatibility in *Brassica campestris*. **Plant Cell Physiol**. 28(7) :1279-1291.

Kho, Y.O. and J. Baer. 1968. Observing pollen tubes by means of fluorescence. **Euphytica** 17 : 298-302.

Nasrallah, J.B. and M.E. Nasrallah. 1993. Pollen-stigma signaling in the sporophytic self incompatibility response. **Plant Cell** 5 : 1325-1335.

Nasrallah, J.B., J.C. Stein, M.K. Kandasamy and M.E.Nasrallah. 1994. Signaling the arrest of pollen tube development in self-incompatible plants. **Science**. 266 : 1505-1508.

Opena, P.T., C.G. Kuo and J.Y.Yoon. 1988. **Breeding and Seed Produciton of Chinese Cabbage in the Tropic and Subtropics**. Technical Bulletin No.17. AVRDC. Taiwan. P. 92.

Pastuglia, M., V. Ruffio-Chable, V.Delorme, T.Gaude, C. Dumas and J.M. Cock. 1997. A function S locus anther gene is not required for the self incompatibility response in *B.oleracea*. **Plant Cell** 9 : 2065-2076.

Shinohara, S. 1981. **Principles of Vegetable Seed Produciton**. Tsukuba International Agriculture Training Center, Textbook V.C. No.26. 225 p.

Tunsuwan, T., C. chaimongkul and M. Nikornpun. 1997. Hybrid Chinese cabbage seed production project, pp. 105-119. *In* **National Vegetable Meeting**, 15th, Bangkok.