African Journal of Agricultural Research Vol. 4 (5), pp. 522-529, May 2009
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X © 2009 Academic Journals

Full Length Research Paper

Directional transfer of the genic multiple allele inherited male sterile line in Chinese cabbage

Li Cheng-yu, Feng Hui*, Wei Peng, Wang Li-li, Zhang Jun, Yang Hao-ning and Jiang Nan

Department of Horticulture, Shenyang Agricultural University, Shengyang 110161 Liaoning, China.

Accepted 27 April, 2009

A "directional transfer program" was designed and carried out according to the genotype on the male sterility locus of the target line, in order to improve the utilization of the multiple-allele inherited male sterile gene in Chinese cabbage and solve the problems in the transfer process of this gene. In the program, recurrent backcrossing was employed to transfer botanical traits, while the genotype was identified through test cross. In addition, we began to develop the male sterile line after the transfer of botanical traits was finished. Following the procedure, the male sterile gene was successfully transferred from "3A", a white leafstalk inbred line in Chinese cabbage, to "206", the target green leafstalk inbred line in cylindrical ecotype. The "GMS104", a male sterile line with similar botanical traits to "206" with 100% male sterility and 100% male sterile plants, was bred. With "GMS104" as the female parent, an excellent hybridized combination "GMS104×B4" was finally obtained and it passed the variety certification and registration of minor crops in Liaoning, China. Based on the results, it was certified that male sterility and the other botanical and economic traits could be transferred to a target line simultaneously following the "directional transfer program" designed in our study.

Key words: Chinese cabbage, genetic male sterile line, transfer.

INTRODUCTION

In higher plants, pollen maturation is normally followed by its release by dehiscence of the anthers (Goldberg et al., 1993). This normal process of pollen formation and release may be hampered, resulting in male sterility. This phenomenon has been, for a long time, recognized as an important trait for hybrid seed production as self-pollination of male sterile plants is prevented. Over decades, much advance has been achieved for understanding the mechanism of plant male sterility and applying male sterility in the practical production (Hockett et al., 1968; Gill et al., 1970; Brim and Stuber, 1973; Jan et al., 1977; Reddy et al., 1978; Yaegaki et al., 2003; Long et al., 2008).

Chinese cabbage (*Brassica rapa* L.) is an example of a typically allogamous plant with bisexual flowers, which has obvious heterosis. The pattern of hybrid seed production is very important for the utilization of its heterosis. Presently, the F_1 hybrid seeds are mainly obtained from the application of self-incompatibility in practice. However, disadvantages were found in the utilization of self-incompatible lines such as costly maintaining of parental materials, fast degeneration of vigor after several selfing generations and low purity of hybrid seed (Niu et al., 1980). The male sterile line is an economical and stable way to improve the current situation of cross breeding of Chinese cabbage. Therefore, worldwide breeders paid much attention on the breeding and utilization of male sterile lines (Zhang et al., 1990; Ke et al., 1992; Michael, 2004). The already obtained male sterile materials in Chinese cabbage could be classified into genic male sterility (GMS) and cytoplasmic male sterility (CMS) (Van Der Meer, 1987). Most CMS in Chinese cabbage originnated from Ogu and Pol CMS (Zhang et al., 2006). However, the male sterile line in Chinese cabbage with Ogu CMS could not be utilized in a large scale in practice, because it was often characterized by such disadvantages as leaf etiolation, nectary degeneration and slow growth etc (Ren et al., 1992; Sun et al., 2000). The male sterile line in Chinese cabbage bred based on Polima CMS has been employed in the hybrid seed production. But the thermal-sensitive character limited its perfomance

*Corresponding author. E-mail: fenghuiaaa@263.net
Tel.: 86-13898899863. Fax: 86-024-88487143.

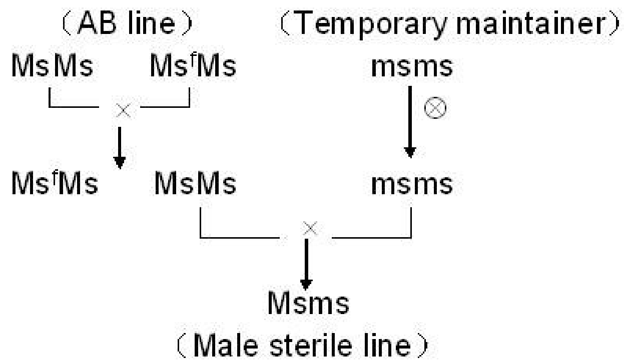

Figure 1. Genetic model for multiple allele inherited male sterile line in Chinese cabbage.

in the large scale production (Zhang et al., 2001). The multiple allele inherited male sterile material in Chinese cabbage discovered by Feng et al. (1995, 1996) was characterized by its stable and complete sterility performance, with 100% male sterile plants and without any negative cytoplasmic effects etc, which has become the focus of many breeders. A series of researches have been carried out on the breeding of male sterile lines, based on the "genetic hypothesis of genic multiple allele male sterile gene in Chinese cabbage" (Wen et al., 2001; Xu et al., 2003). However, most male sterile lines obtained in these researches could not be utilized in practice, because of the low evenness degree of hybrid seeds generated by these male sterile lines. In the genetic model of these male sterile materials, the male sterile line was the result of the cross between the male sterile plant of a male sterile "AB" line and the temporary maintainer. If significant differences existed in the genetic background between the "AB" line and the temporary maintainer, the male sterile line generated from the cross between these 2 materials could be considered as the F_1 seed. And with such male sterile line as the female parent, the hybridized seed is actually a 3-way crossing seed. Thus, a new way for the breeding and utilization of the male sterile materials is crucial. In the present study, with the male sterile material with 100% male sterile plants as the male sterility source, we designed a "directional transfer program". Therefore the problem in the transfer of such materials was successfully solved through the breeding of a new male sterile line, which is inherently stable, with similar botanical traits to the target line.

MATERIALS AND METHODS

Materials

Male sterile source and test-cross material: "3A", a white leafstalk genic male sterile line in Chinese cabbage in cylindrical ecotype. Target line: "206", a green leafstalk inbred line in cylindrical ecotype. Test-cross line: "AB01", a male sterile "AB" line in Chinese cabbage.

Methods

The study was performed in the research base of Shenyang agricultural university during the 6 years between 2002 and 2008. Conventional crossing, backcrossing, test crossing and selfing were employed to transfer the male sterility and the other traits. Botanical traits were investigated through a randomized block design with 3 repeats and 9 m^2 block area.

Formula for the sample size "n": $n \geq \ln(0.01)/\ln(1 - p)$, P: probability of occurrence for target individuals

RESULTS

Genetic characteristics of the genic multiple-allele inherited male sterility in Chinese cabbage

In the "genetic hypothesis of genic multiple-allele male sterile gene in Chinese cabbage", the model accounts for a single locus with three alleles, "Ms" allele for male sterility, "ms" allele for fertility and "Ms^f" for fertility restoration. The dominant-recessive relationship of these alleles was $Ms^f > Ms > ms$. Based on the genetic model for obtaining a male sterile line with 100% male sterile plants, the temporary maintainer is employed to cross with the homozygous sterile plant in the male sterile "AB" line (Figure 1). Genotypes of male sterile plants and fertile plants in an "AB" line were MsMs and Ms^fMs respectively. And the "AB" line could be maintained by sib crossing between the male sterile plant and fertile plant ($MsMs \times Ms^fMs \rightarrow 1/2\ MsMs,\ 1/2\ Ms^fMs$);

Genotyping of "206"

The special inheritance model of this locus results in totally 6 genotypes, of which 3 conventionally share an identical fertile phenotype, including Ms^fMs^f, msms and Ms^fms. These 3 genotypes could be distinguished by the test cross with a male sterile line (Msms) (Figure 2).

99 F_1 plants, which were totally fertile, were obtained from the cross between "3A" as the female parent and the target line "206". It indicated that the genotype of "206" is Ms^fMs^f.

Breeding of the male sterile line

Genetic model

The genetic model is presented in Figure 3 with "3A" (Msms) as the male sterility source and "206" (Ms^fMs^f) as the target line.

Result of the breeding

Following the model in Figure 3, the plants with 2 genotypes, including Ms^fMs and Ms^fms, existed in the F_1 population and shared an identical fertile phenotype. For genotyping each plant in the population, 7 plants were selected randomly and selfed. The identification result of

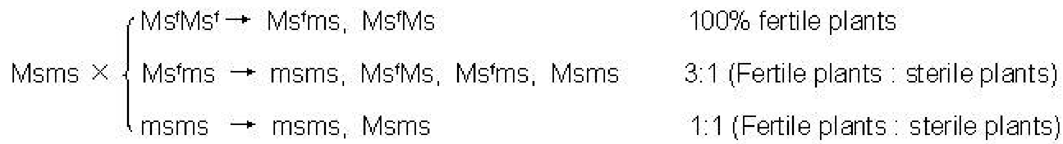

Figure 2. Genotyping of the target line "206".

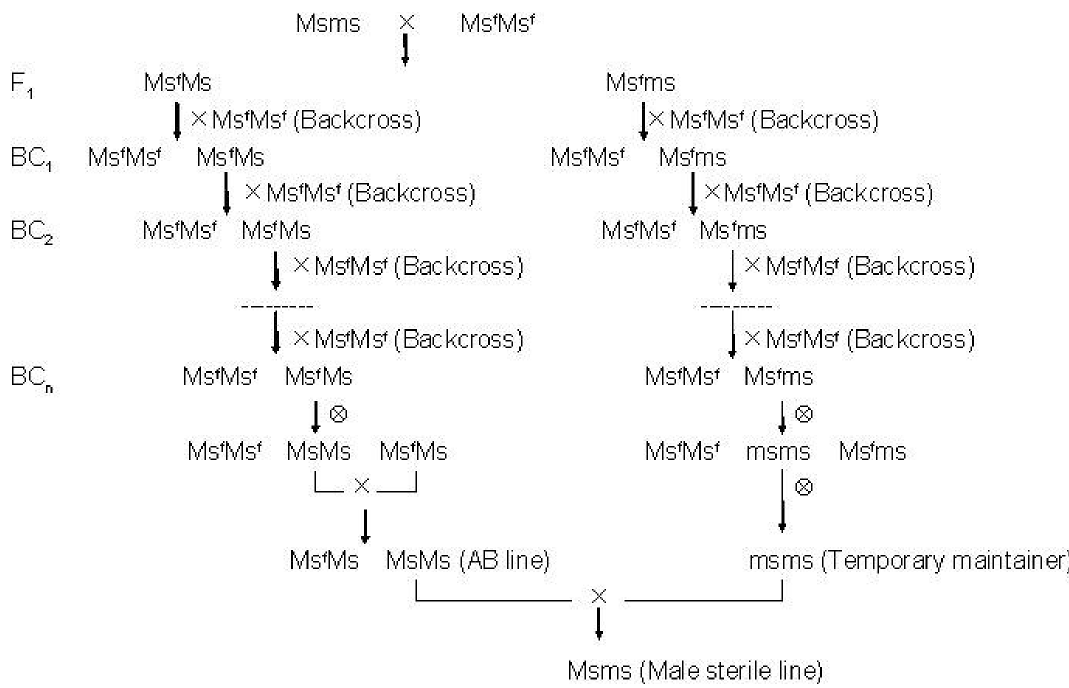

Figure 3. Genetic model for directional transfer of the multiple allele inherited male sterile line in Chinese cabbage

sterility or fertility was presented in Table 1. Simultaneously, these 7 F_1 plants were backcrossed with "206" to construct BC_1 population.

The genotype of the specific F_1 plant could be identified according to the segregated ratio for fertility/sterility in the corresponding F_2 population. One BC_1 population that resulted from the backcross with the F_1 plants with 2 genotypes, including Ms^fMs and Ms^fms, was selected respectively. From each selected BC_1 population, 7 plants were crossed with the male sterile plant (MsMs) in the "AB" line "AB01" to identify the genotype of them. The backcross progenies of plants identified as Ms^fMs and Ms^fms were selected, in which the "AB" line (MsMs, Ms^fMs) and the "temporary maintainer" (msms) in the BC_1 generation could be developed, while those of the Ms^fMs^f plants were eliminated. Following this procedure, the "AB" line and the "temporary maintainer" of BC_2, BC_3 and BC_4 could be developed, too. "GMS101", "GMS102", "GMS103" and "GMS104", the male sterile line bred respectively in the BC_1, BC_2, BC_3 and BC_4 generations, were obtained from the cross between the male sterile plant (MsMs) of the "AB" line in each generation and its corresponding "temporary maintainer" (msms).

In Table 2, we present the genotyping results of selected individuals in each backcross generation. A ratio of 1:1 for fertility and sterility was observed in all backcross populations. It indicates that the genotype of tested plants is Ms^fMs or Ms^fms. Thus, they were considered as target plants.

In Table 3, we present the segregated ratio for fertility and sterility of the selfing progenies of plants identified as Ms^fMs and Ms^fms in each backcross generation.

There were totally 3 genotypes, including Ms^fMs^f, Ms^fMs and MsMs, found in the selfing progenies of Ms^fMs, which resulted in the fertile and sterile plants segregated in a ratio of 3:1. Sib crossing was made between 5 fertile plants (Ms^fMs^f or Ms^fMs) and the male sterile plant (MsMs), respectively. The new "AB" line (Ms^fMs, MsMs) was obtained, in case that the sterility of progenies segregated for 1:1.

Table 1. Segregation ratio for fertility and male sterility in F_2 generation between male sterile line "3A" and fertile line "206".

Code of plant	Fertile plants: sterile plants	Theoretical ratio ($X^2_{0.05,1}$ = 3.84)	Genotype of F_1 plant	Genotypes of F_2 population
(3A×206)-1⊗	23:9	3:1(0.042)	Ms^fMs	Ms^fMs^f,Ms^fMs,MsMs
(3A×206)-2⊗	24:7	3:1(0.011)	Ms^fMs	Ms^fMs^f,Ms^fMs,MsMs
(3A×206)-3⊗	33:0	All fertile	Ms^fms	Ms^fMs^f,Ms^fms,msms
(3A×206)-4⊗	30:0	All fertile	Ms^fms	Ms^fMs^f,Ms^fms,msms
(3A×206)-5⊗	26:5	3:1(0.871)	Ms^fMs	Ms^fMs^f,Ms^fMs,MsMs
(3A×206)-6⊗	22:7	3:1(0.011)	Ms^fMs	Ms^fMs^f,Ms^fMs,MsMs
(3A×206)-7⊗	23:8	3:1(0.011)	Ms^fMs	Ms^fMs^f,Ms^fMs,MsMs

Table 2. Genotype identification of selected plants in each backcross generation during the transfer of male sterile line in Chinese cabbage.

Combinations	Genotype of tested plant	Fertile plants : sterile plants	Theoretical ratio ($X^2_{0.05,1}$=3.841)
AB01×((3A×206) -1×206)-1	Ms^fMs	31:28	1:1(0.068)
AB01×(((3A×206) -1×206)-1×206)-3	Ms^fMs	27:29	1:1(0.018)
AB01×((((3A×206)-1×206)-1×206)-3×206)-2	Ms^fMs	34:32	1:1(0.015)
AB01×(((((3A×206)-1×206)-1×206)-3×206)-2×206)-5	Ms^fMs	29:33	1:1(0.145)
AB01×((3A×206) -1×206)-2	Ms^fms	33:36	1:1(0.058)
AB01×(((3A×206) -1×206)-2×206)-4	Ms^fms	23:22	1:1(0.000)
AB01×((((3A×206) -1×206)-2×206)-4×206)-5	Ms^fms	29:28	1:1(0.000)
AB01×(((((3A×206)-1×206)-2×206)-4×206)-5×206)-3	Ms^fms	25:22	1:1(0.085)

Table 3. Segregation ratio for fertility and male sterility in the selfing progenies of plants with the genotypes of MsfMs and Msfms in each backcross generation during the transfer of male sterile line in Chinese cabbage.

Combinations	Genotype of the plant	Fertile plants : sterile plants	Theoretical ratio ($X^2_{0.05,1}$ = 3.841)
((3A×206) -1×206)-1⊗	Ms^fMs	26: 7	3:1(0.091)
(((3A×206) -1×206)-1×206)-3⊗	Ms^fMs	38:11	3:1 (0.061)
((((3A×206)-1×206)-1×206)-3×206)-2 ⊗	Ms^fMs	29:7	3:1(0.333)
(((((3A×206)-1×206)-1×206)-3×206)-2×206)-5⊗	Ms^fMs	31:13	3:1(0.273)
((3A×206)-3×206)-2⊗	Ms^fms	33:0	All fertile
(((3A×206)-3×206)-2×206)-4⊗	Ms^fms	29:0	All fertile
((((3A×206)-3×206)-2×206)-4×206)-5⊗	Ms^fms	36:0	All fertile
(((((3A×206)-3×206)-2×206)-4×206)-5×206)-3⊗	Ms^fms	31:0	All fertile

On the other hand, 3 genotypes, including Ms^fMs^f, Ms^fms and msms, also exited in the selfing progenies of the Ms^fms plant, all of which resulted in fertility. 16 fertile plants were selected and crossed with the male sterile plant (MsMs) in the selfing progenies of the Ms^fMs plant. The "temporary maintainer" was obtained, in case that the progenies were all sterile.

In Table 4, the segregated ratio of 1:1 could be found in the progenies from the sib crossing between fertile plants and sterile plants in each generation. It indicated that they were stably inherited male sterility "AB" line.

In Table 5, the phenotyping results for fertility and sterility were presented for the progenies from the cross between the male sterile plant in the "AB" line and "temporary maintainer".

The male sterile lines with 100% male sterility and male sterile plants could be bred, based on the "AB" lines obtained in the BC_1, BC_2, BC_3 and BC_4 generations and their corresponding "temporary maintainers". And these male sterile lines were nominated as GMS101, GMS102,

Table 4. Test-cross result of the “AB line” in each backcross generation during the transfer of genic male sterile line in Chinese cabbage.

Combinations	Fertile plants : sterile plants	Theoretical ratio ($X^2_{0.05, 1}$ = 3.841)
((3A×206) -1×206)-1-1×((3A×206) -1×206)-1-2	15:17	1:1(0.031)
(((3A×206) -1×206)-1×206)-3-5×(((3A×206) -1×206)-1×206)-3-3	39:36	1:1(0.053)
((((3A×206)-1×206)-1×206)-3×206)-2-3×((((3A×206)-1×206)-1×206)-3×206)-2-1	22:19	1:1(0.098)
(((((3A×206)-1×206)-1×206)-3×206)-2×206)-5-2×(((((3A×206)-1×206)-1×206)-3×206)-2×206)-5-6	22:25	1:1(0.085)

Table 5. Test-cross result of the temporary maintainer in each backcross generation during the transfer of genic male sterile line in Chinese cabbage.

Male sterile lines	Combinations	Fertile plants : sterile plants
GMS101	((3A×206) -1×206)-1-1×((3 A×206)-3×206)-2-5	0:47
GMS102	(((3A×206) -1×206)-1×206)-3-5×(((3 A×206)-3×206)-2×206)-4-2	0:52
GMS103	((((3A×206)-1×206)-1×206)-3×206)-2-3×((((3 A×206)-3×206)-2×206)-4×206)-5-3	0:45
GMS104	(((((3A×206)-1×206)-1×206)-3×206)-2×206)-5-2×(((((3A×206)-3×206)-2×206)-4×206)-5×206)-3-9	0:48

Table 6. Observation on botanical traits of the parent “206” and male sterile lines transferred in 4 backcross generations.

Traits	GMS101	GMS102	GMS103	GMS104	206
plant height (cm)	46.76	45.69	44.89	44.22	44.17
plant width (cm)	61.26	61.03	60.53	59.78	59.64
leaf head height (cm)	38.98	38.67	38.11	37.42	37.26
leaf head diameter (cm)	12.73	12.59	12.04	12.29	12.17
leaf head weight (kg)	2.69	2.52	2.34	2.30	2.24

Note: Data in the table is the average value of 60 plants.

GMS103 and GMS104, respectively.

Effect evaluation of directional transfer

Observation on botanical traits of the male sterile line and the target line

The optimal number of the backcross generation was determined according to the similarity between “206” and 4 male sterile lines including “GMS101”, “GMS102”, “GMS103” and “GMS104” (Table 6).

It could be found in Table 6 that, with the increase of the backcross generation number, the progenies were approaching to the recurrent parent “206” in botanical traits. GMS104, the male sterile line bred in BC_4 generation, is almost the same as “206” in botanical traits, which ultimately reached the objective of the directional transfer.

Observation of evenness degree on hybridized combinations with the male sterile line as the female parent.

In order to compare the similarity of genetic back-ground between newly bred male sterile “AB” lines and their corresponding temporary maintainers and also evaluate the value of these male sterile lines in practice, we observed the botanical traits of hybridized combinations and calculated the variation coefficients of major botanical traits. We obtained 4 hybridized combinations employing GMS101, GMS102, GMS103 and GMS105 as the female parents and an excellent inbred line B1 as the male parent. With the “Jinlv 75” as the CK, the botanical traits were observed and the variation coefficients of major botanical traits were calculated (Figure 4).

As it was showed in Figure 4, with the increase of backcross generation number, the variation coefficients of major botanical traits of each combination declined gradually. The variation coefficients of “GMS104×B1”, the combination obtained in BC_4 generation, were significantly lower than the CK, which indicated that the evenness degree of hybrids produced with the male sterile line bred in BC_4 generation is very high.

Breeding and utilization of hybridized combinations

With the newly bred GMS104 as the female parent, 5

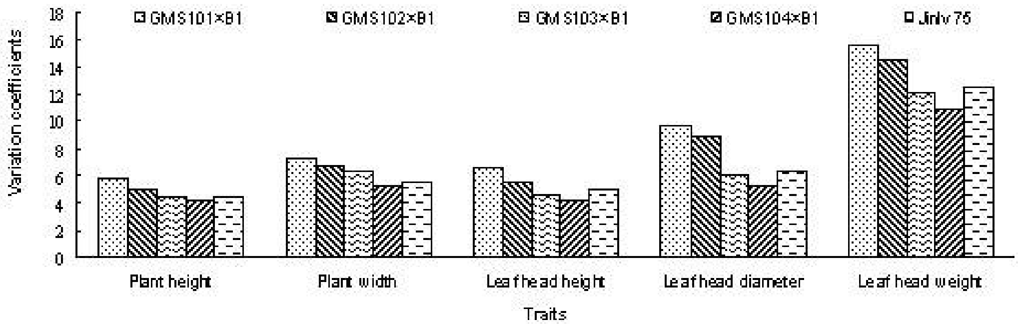

Figure 4. Variation coefficients of major botanical traits of hybridized combinations made with male sterile lines in four backcross generations in Chinese cabbage.

Table 7. Yield of hybridized combinations of Chinese cabbage in comparative experiment plot (unit: kg).

Combination	Repeats I	Repeats II	Repeats III	Average value
GMS104 × B4	117.88	122.82	119.01	119.90 a
Jinlv75	108.72	96.60	115.43	106.92 b
GMS104 × B2	109.20	102.24	105.77	105.74 b
GMS104 × B5	101.75	97.06	108.57	102.46 b
GMS104 × B3	94.32	103.31	102.01	99.88 b
GMS104 × B1	96.84	100.02	95.04	97.30 b

Note: Area of plot is 10 square meters.

combinations were generated by crossing GMS104 with 5 inbred lines including B1, B2, B3, B4 and B5. Comparative experiment was performed, employing "Jinlv 75" as the CK (Table 7).

According to the analysis of variance on the data listed in Table 7, the variance among varieties reached a significant level ($F = 14.33 > F0.01 = 5.99$). New multiple range method was employed to make a multiple comparison. The result indicated that the yield of "GMS104×B4" is significantly higher than that of the CK and the other combinations. In 1997, the combination passed the variety certification and registration of minor crops in Liaoning, China. It was nominated as "Shennong super No.9" and has been cultivated in a large area in China.

DISCUSSION

Selection of male sterile source and the target line

The cross-based transfer of the male sterile gene is an efficient way to breed male sterile lines in many kinds of crops. The objective of the directional transfer in the present study is to develop a new male sterile line which is similar to the target line in botanical traits. Thus, the selection of target lines is very important. A target line with a high combining ability, disease resistance and quality is the precondition for developing an excellent male sterile line. The major inbred lines widely used in the current production should be selected first.

According to the "Genetic hypothesis of genic multiple-allele male sterile gene in Chinese cabbage", the male sterility is controlled by 3 alleles on the same locus. In order to include all the three alleles in the transfer system, the genotype of the male sterility source material and the target line should be complemented.

Genotyping of fertile lines

As it was described in the "genetic hypothesis of genic multiple-allele male sterile gene in Chinese cabbage", 4 genotypes, including Ms^fMs^f, Ms^fMs, Ms^fms and msms, existed in the fertile plants. Generally, the genotype of the target lines could not be heterozygous Ms^fMs, because their selfed progenies segregated for a 3:1 ratio for fertility and sterility. The genotype of the target line could be identified by the test cross with a male sterile plant (MsMs) in an "AB" line and the genic male sterile line (Msms). If the male sterile line (Msms) is employed in the test cross, the segregated ratio in the test cross progenies of Ms^fMs^f, Ms^fms and msms should be all fertile, 3:1 and 1:1, respectively. However, a large population is necessary to distinguish between 3:1 and 1:1. If we use male sterile plant (MsMs) in the test cross, the segregated ratio in the test cross progenies is all fertile, 1:1 and all sterile, which is much easier to distinguish.

Advantages of directional transfer

The objective of the directional transfer in the present study is to develop a new male sterile line which is similar to the target material in botanical traits. The transfer of both the male sterility and the other traits were focused on. The "AB" line and its "temporary maintainer" could be considered as sib lines which were similar to the target line in most traits after recurrent backcrossing. As a result of the similar genetic background, the inheritance of traits of the male sterile line developed with this system is very stable. And the combinations bred with such male sterile line as the female parent are excellent in evenness degree. Based on this model, the genic male sterile gene could be transferred into the target inbred lines in Chinese cabbage and a new male sterile line which is similar to the target line in most traits could be developed, which improves the utilization of such male sterile lines.

Confirm of generation number for backcross

Directional transfer of the genic male sterile line in Chinese cabbage is performed by recurrently back-crossing male sterile source with the target lines. The number of backcross generation is very important for the directional transfer effect. If the number is too small, the effect of "directional" could not be reached. While if it is too big, serious degeneration might occur. Thus, during the directional transfer process, the number of backcross generation should be determined according to the similarity degree of botanical traits between the backcross progenies and the recurrent parent. In the present study, GMS104, the male sterile line bred in BC_4 generation, is almost the same to "206" in botanical traits. As a result, backcross for 4 generations is a proper choice for the directional transfer of genic male sterile lines. However, this number should increase, in case that significant differences exit between the target line and the male sterility source and none unexpected traits were found after the backcross for many generations.

Application of molecular marker technique

Molecular marker technique has been widely used in genetic mapping, map-based cloning, genetic diversity analysis, variety protection and breeding selection. For male sterility in *Brassica* crops, various molecular markers have been identified linked to the male sterile genes and fertility restorer genes (Hansen et al., 1997; Jean et al., 1997; Miao et al., 2000; Cao et al., 2005; Zhang et al., 2008; Wang et al., 2000; Hong et al., 2006; Wang et al., 2000a, b, 2005). In the directional transfer program, it requires more time, labor and space to select plants with desired genotypes, because test cross must be involved in each generation. Application of molecular markers tightly linked to the Ms allele provides a potential for effi-cient and earlier selection of the desired plants. Presently, the Ms-specific molecular markers are being developed in our lab.

Conclusion

1.) A "directional transfer program" was designed for the genic multiple-allele inherited male sterile gene, which solves the problems in the breeding and utilization of the male sterile line.

Based on the "Hypothesis for genic multiple male sterile gene in Chinese cabbage", we designed a "directional transfer program", aiming at developing a new male sterile line which is similar to the target material in botanical traits. Directional transfer gives attention on the transfer of both the male sterility and the other traits. The newly bred male sterile line is inherited stably, which guarantees a high evenness degree of hybrid seed.

2.) Following the directional transfer program, "GMS104", a genic male sterile line in Chinese cabbage, was bred, which is similar to the target inbred line "206" in both botanical and economic traits.

After recurrent backcrossing for 4 generations, a new male sterile line named "GMS104" with 100% male sterility and 100% male sterile plants was bred based on the directional transfer program, employing "3A", a white leafstalk male sterile line in Chinese cabbage in cylindrical ecotype, as the male sterility source and "206", a green leafstalk inbred line in cylindrical ecotype, as the target line. It verified that male sterility and the other traits could be transferred simultaneously following the "directional transfer program" and provided a theoretical and practical basis for solving the problems in the breeding and utilization of the genic male sterility in Chinese cabbage.

ACKNOWLEDGEMENT

This research was supported by a grant from the national natural science fund (30671414) and national "863" plan program (2006AA10Z170) of PR China.

REFERENCES

Brim CA, Stuber CW (1973). Application of genetic male sterility to recurrent selection schemes in soybeans. Crop Sci. 13: 528-530.

Cao JS, Yu XL, Ye WZ, Lu G, Xiang X (2005). Functional analysis of a novel male fertility *CYP86MF* gene in Chinese cabbage (*Brassica compestris* L. ssp. *chinensis* makino). Plant Cell Rep. 24: 715-723.

Feng H, Wei YT, Zhang SN (1995). Inheritance of and utilization model for genic male sterility in Chinese cabbage (*Brassica pekinensis* Rupr.). Acta Horticulturae 402:133-140.

Feng H, Wei YT, Ji SJ, Jin G, Jin JS, Dong WJ (1996). Multiple allele model for genic male sterility in Chinese cabbage. Acta Horticulturae, 467: 133-142.

Gill BS, Anand SC (1970). Genetic male sterility for hybrid seed production in wheat. Crop Sci. 10: 385-386.

Goldberg RB, Beals TP, Sanders PM (1993). Anther development: basic principles and practical applications. Plant Cell 5: 1217-1229.

Hansen M, Hallden C, Nilsson N, Sall T (1997). Marker-assisted selection of restored male-fertile *Brassica napus* plants using a set of dominant RAPD markers. Mol. Breed 3: 449-456.

Hockett EA, Eslick RF (1968). Genetic male sterility in Barley. I. Nonallelic genes. Crop Sci. 8:218-220.

Hong DF, Wan LL, Liu PW, Yang GS, He QB (2006). AFLP and SCAR markers linked to the suppressor gene (*Rf*) of a dominant genetic male sterility in rapeseed (*Brassica napus* L.). Euphytica 151: 401-409.

Jan CC, Qualset CO (1977). Genetic male sterility in wheat: inheritance. Crop Sci. 17: 791-794

Jean M, Brown GG, Landry BS (1997). Genetic mapping of nuclear fertility restorer genes for the '*Polima*' cytoplasmic male sterility in canola (*Brassica napus* L.) using DNA markers. Theor. Appl. Genet. 95: 321–328.

Ke GL, Zhao ZY, Song YZ, Zhang LG, Zhao LM (1992). Breeding of

alloplasmic male sterile line cms3411-7 in Chinese cabbage (*Brassica campestrls* L. ssp.*pekinensis* (lour) olsson) and its application. Acta Horticulturae Sinica, 19: 333-340.
Long YM, Zhao LF, Niu BX, Su J, Wu H, Chen YL, Zhang QY, Guo JX, Zhuang CX, Mei MT, Xia JX, Wang L, Wu HB, Liu YG (2008). Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. Proc. Natl. Acad. Sci. 105: 18871-18876.
Miao Y, Dreyer F, Cai DG, Jung C (2000). Molecular markers for genic male sterility in Chinese cabbage. Eupytica. 132: 227-234.
Michael JH (2004). The use of cytoplasmic male sterility for hybrid seed production. Molecular Biology and Biotechnology of Plant Organelles. Netherlands: Springer pp. 623-634.
Niu XK, Wu FY, Zhong HH, Li XS (1980). The selection and utilization of Chinese cabbage (*B.pekineensis* Rupr.) of male sterile AB line. Acta Horticulturae Sinica, 2: 25-32.
Reddy VS, Green JM, Bisen SS (1978). Genetic male sterility in Pigeon Pea. Crop Sci. 18: 362-364.
Ren CW, Cao SC (1992). A survey of some physiological characteristics related to the yellowing defect in cytoplasmic male sterile *Brassica campestris* ssp.*chinensis* with r-cms cytoplasm. Acta Horticulturae Sinica 19: 275 - 276.
Sun RF, Fang ZY, Zhang SJ, Li F, Niu XK, Wu FY (2000). Biochemical analysis of cold-tolerant Ogura CMS Chinese cabbage lines. Acta Horticulturae Sinica, 27: 187-192.
Van Der Meer QP (1987). Chromosomal monogenic dominant male sterility in Chinese Cabbage (*Brassica rapa* subsp. *pekinensis* (Lour.) Hanelt). Euphytica, 36: 927-931.
Wang JX, Yang GS, Fu TD, Meng JL (2000). Development of PCR-based markers linked to the fertility restorer gene for the polima cytoplasmic male sterility in rapeseed (*Brassica napus* L.). Acta Genetica Sinica, 27: 1012-1017.
Wang XW, Fang ZY, Run PT, Liu YM, Yang LM, Zhuang M (2000a). A SCAR marker applicable in marker assisted selection of a dominant male sterility gene in cabbage. Acta. Horticulturae Sinica. 27:143-144.
Wang XW, Fang ZY, Huang SW, Run PT, Liu YM, Yang LM, Zhuang M (2000b). An extended random primer amplified region (ERPAR) marker linked to a dominant male sterility gene in cabbage (*Brassica oleracea* var. *capitata*). Euphytica 112:267-273.
Wang YG, Feng H, Lin GR, Xu SF, Yang KY, Zhang NJ (2005). The Transfer of genetic male sterile lines in *Brassica campestris* L. ssp. *chinesis* (L.) Makino. Acta Horticulturae Sinica 32: 628-631.
Wen FY, Song LJ, Wang YL, Liu XH, Zhao B, Qiu YX (2001). Breeding of nuclear male sterile line in oingmaye type of Chinese cabbage. Acta Horticulturae Sinica 28: 133-138.
Xu M, Wei YT, Bai MY, Zhu T, Ren XB, Dai XY (2003). Introgression of dominant male sterile gene of Chinese cabbage into Purple-caitai. Acta. Horticulturae Sinica 30: 98-100.
Yaegaki H, Miyake M, Haji T, Yamaguchi M (2003). Inheritance of male sterility in Japanese apricot (*Prunus mume*) Hort. Sci. 38: 1323-1458.
Zhang LG, Hao DF, Ke GL (2001). Characteristics of sterility in CMS 3411-7 of Chinese cabbage sensitive to low temperature. Acta. Horticulturae Sinica 28: 415-420.
Zhang DS, Zhang FL, Wang YJ, Fang ZY, Yu YJ, Zhao XY, Xu JB (2006). Molecular identification of cytoplasmic male sterile line CMS96 in chinese cabbage. Mol. Plant Breed. 4: 545-552.
Zhang SF, Song ZH, Zhao XY (1990). Breeding of interactive genic male sterile line in Chinese cabbage (*Brassica pekinensis* rupr.) and utilization model. Acta. Horticulturae Sinica 17: 117-125.
Zhang SJ, Li F, Han HP, Zhang SF, Niu XG, Sun RF (2008). Molecular marker linked to a dominant genetic male sterile gene in Chinese cabbage. [*Brassica rapa* L. ssp *pekinensis* (Lour.) Olsson]. Scientia Agricultura Sinica 41: 2379-2385.