# A promising way to produce *B. napus* hybrid seeds by self-incompatibility pollination system

**Chaozhi Ma, Changbin Gao, Jianfeng Zhang, Fupeng Li, Xia Wang, Ying Liu, Tingdong Fu**
National Key Laboratory of Crop Genetic Improvement, National Center of Rapeseed Improvement in Wuhan, Huazhong Agricultural University, Wuhan 430070, P.R. China, ✉ E-mail: yuanbeauty@mail.hzau.edu.cn

## ABSTRACT

Hybrid breeding is one of the most efficient ways to improve seed yield of *B. napus* and self-incompatibility (SI) is one alternative for hybrid breeding. Self-incompatible *B. napus* line '271' was developed by introgressing an *S* haplotype of *B. rapa* into a *B. napus* line through interspecific hybridization, and has been improved to double low (low erucic acid; low glucosinolates) self-incompatible line S-1300. The SI of '271' and S-1300 is usually recessive to self-compatibility (SC) of natural *B. napus*, but dominant in some genetic background. '271' and S-1300 can be successfully used in *B. napus* hybrid breeding by producing three-way hybrids. However, breeding elite maintainers is difficult. In this report, S-1300 and its 44 restorers were used to study the possibility of two-way hybrid seed production. When spraying 3-5 % salt solution with hand or bees pollination, S-1300 produced 7.03 and 5.24 seeds per silique, respectively, which was about half of that in natural condition, showing that salt solution can overcome the self-incompatibility of S-1300, and that S-1300 can be amplified by salt-spraying with bee- or hand- pollination. The two-way hybrids had high positive mid-parent heterosis with an average of 28.91%. The hybrid with highest seed yield had very high positive mid-parent heterosis of 115%. Its seed yield was 15% over the check, zhongyouza2. Elite hybrid is expected to come out by selection or improvement of SI lines and restorers. Two-way hybrid of our SI system is one promising way for *B. napus* hybrid seed production.

**Keywords**: *Brassica napus* - Self-incompatibility – Heterosis - Hybrid

## INTRODUCTION

*Brassica napus* is among the world's most important oilseed crops for both edible oil consumption and biodiesel. China is the world's largest producer of rapeseed (85% of which is *B. napus*). Its growth areas and total production has increased to be one-third of globes', mainly due to the wide application of hybrids. The yield per unit of hybrid varieties is usually 10-15% more than that of OP varieties (Zhou and Fu, 2007). Hybrid breeding is one of the most efficient ways to improve seed yield of *B. napus*.

Self-incompatibility (SI) is a mechanism to prevent inbreeding and promoting outbreeding. It has been widely used for hybrid seed production in vegetables *B. rapa* (AA, n=10) and *B. oleracea* (CC, n=9). Although *B. napus* (AACC, n=19), from two self-incompatible species *B. rapa* and *B. oleracea*, is naturally self-compatible, self-incompatibility can be introduced into *B. napus* by introgressing from *B. oleracea* and *B. rapa* (Goring et al. 1992) or by the resynthesis of *B. napus* from *B. oleracea* and *B. rapa* (Gowers 1989; Rahman 2005).

We developed one self-incompatible *B. napus* line '271' by introgressing an *S* haplotype of *B. rapa* called Xishuibai into a *B. napus* line through interspecific hybridization (Fu and Liu 1975), and improved '271' to one double-low (low erucic acid, low glucosinolates) self-incompatible line called S-1300 (Ma et al, 1998). The SI of '271' and S-1300 is usually recessive to self-compatibility (SC) of natural *B. napus*, but dominant in some genetic background. When crossing with '271' or S-1300, the natural *B. napus* lines with recessive SC can produce self-incompatible $F_1$s, and therefore are referred to as maintainers; the natural *B. napus* lines with dominant SC can give rise to self-compatible $F_1$s, and so are referred to as restorers (Fu 1981; Ma et al, 1998). And so by producing three-way hybrids, '271' and S-1300 can be successfully used in *B. napus* hybrid breeding (Fu 1981). However, difficulty in propagating self-incompatible lines on a large scale limits the utilization of self-incompatibility in *Brassica napus*.

We report here one promising method to produce hybrid seed by SI lines and its restorers.

## MATERIALS AND METHODS

One *B. napus* self-incompatible line, S-1300, and 44 *B. napus* self-compatible lines, which are restorers of S-1300, were used in this study. They are Chinese semi-winter type and conserved in Huazhong Agricultural University, Wuhan, China. S-1300 was crossed as a female with restorers to obtain $F_1$ hybrids.

S-1300 was sowed in field at the autumn of 2003. When flowering in the spring of 2004, self-compatibility index (SCI) was investigated and 3-5% salt solution was sprayed to plants of S-1300. The experiments were repeated in 2004. $F_1$ plants were planted at the end of September in 2008. Plots were arranged in a randomized complete block design with three replications. The area of each plot is eight $m^2$. Plot seed yield was measured. Heterosis (%) over mid-parent (MPH) was evaluated using the formula: MPH= ($F_1$-MP)/MP×100; Heterosis (%) over Check (CKH) was evaluated using the formula: CKH= ($F_1$-Check)/ Check×100

$F_1$, MP and Check stand for hybrid mean, mid-parent mean and check mean, respectively. When three to five flowers were present on the major inflorescence of each plant, the top buds were cut off to inhibit indefinite flowering. The major inflorescence and two or three branches were then bagged. The bags were slapped gently every two days to assure enough self-pollination. The bags were removed approximately two weeks later in order to allow the seeds to develop in a more natural environment. After the seedpods matured, the seeds and flowers produced from each bag were counted, respectively, and the seed set per number of flower or silique was calculated.

## RESULTS

### Amplification of S-1300 by spraying salt-solution

The self-incompatible line S-1300 had few seed sets with an average of 0.08 and 0.88 seeds per silique when selfing by bag and selfing in isolation, respectively (Table 1). It could produce almost normal seeds after salt-spraying with selfing by hand and salt-spraying with bee pollination. The average seed set is 7.03 and 5.24 seeds per silique, respectively, which is about half of that in natural condition. The results showed that salt solution can overcome the self-incompatibility of S-1300; S-1300 can be amplified by salt-spraying with bee- or hand-pollination.

Table 1 Seed set of S-1300 in different treatments

| treatment | Silique per plant | Seed per plant | Seed per silique |
|---|---|---|---|
| Selfing by bag | 36.45 | 3.03 | 0.08 |
| Selfing in isolation | 27 | 2.82 | 0.88 |
| Salt-spraying and hand-pollination | 147.03 | 1033.22 | 7.03 |
| Salt-spraying and bee-pollination | 242.11 | 1268 | 5.24 |
| S-1300×Restorer | 280.35 | 3610.91 | 12.88 |
| In natural condition | 325.3 | 3806.0 | 11.7 |

### Seed production of two-way hybrid

When crossing with restorers, S-1300 can get as many seed set as in natural condition (Table 1). Figure 1 show the visualization of seed set of S-1300 in different conditions.

a b c d

Fig. 1 Visualization of seed set of self-incompatible line S-1300 in different conditions a: selfing in isolation; b-selfing and bud-pollinating; c: propagation by salt solution; d: hybrid seed production

**Seed yield heterosis of two-way hybrids**

Of the 44 two-way hybrids, 42 hybrids have positive mid-parent heterosis. The range is from -3.45% to 115.66%, with an average of 28.91%. This indicated that two-way hybrids have high mid-parent heterosis. Elite hybrid can be bred by selection or improvement of SI lines and restorers.

Heterosis over check (zhongyouza2) was evaluated. Most of the hybrids have negative heterosis. The average heterosis is -14.31%, which is much low than zero. However, there are six hybrids with positive heterosis over check. The maximum one is 15.24%.

Table 2 Heterosis over mid-parent and over check of two-way hybrid' seed yield

| | MPH (%) | CKH (%) |
|---|---|---|
| Max | 115.66 | 15.24 |
| Min | -3.45 | -37.95 |
| Average | 28.91 | -14.31 |
| No of combination over zero | 42 | 6 |
| No of combination under zero | 2 | 38 |

## DISCUSSION

*B. rapa* and *B. oleracea* are naturally self-incompatible. Self-incompatibility is widely used in their hybrid seed production. For they are majorly used as vegetables, expensive methods such as $CO_2$ gas treatment can be used to amplify self-incompatible lines; self-compatible hybrids are not necessary. On the contrast, *B. napus* is naturally self-compatible and is utilized mostly for oilseeds. The dominant self-incompatible lines can't be used in *B. napus* because of their inability to develop self-compatible hybrids. The recessive self-incompatible lines can produce self-compatible hybrids, but can not be used in practice due to the difficulty of their propagation on large scale.

Our SI system is usually recessive to self-compatibility (SC) of natural *B. napus*, but dominant in some genetic background. *B. napus* lines with recessive SC can produce self-incompatible $F_1$s, and therefore can amplify SI lines and are referred to as maintainers. Three-way hybrids by our SI system have successfully bred. However, backcross of self-incompatible $F_1$ plants with maintainer results in the segregation of *S* phenotypes, which brings out difficulty of breeding elite maintainers.

Our results showed that salt-spraying with bee-pollination can be used to amplify self-incompatible line S-1300. In this case, only SI lines and theirs restorer, other than maintainers, are enough for hybrid seed production. Furthermore, the two-way hybrids have high positive mid-parent heterosis with an average of 28.91% in our research. The hybrid with highest seed

yield has very high positive mid-parent heterosis of 115%. Its seed yield 15% over the check, zhongyouza2.

Elite hybrid is expected to come out by selection or improvement of SI lines and restorers, though two-way hybrid is not registered yet. Most cultivated B. napus accessions are capable of restoring the SI of our SI system, and so breeding restorers is becoming easier than cytoplasm male sterile system. However, an easy method to identifying self-incompatibility phenotype is to be developed in order to simplify the two-way hybrid breeding program.

## ACKNOWLEDGEMENTS

This research was financed by funds from the National Key Basic Research Special Foundation of China (2007CB109000) and supported by the Industry Technology System of Rapeseed in China (NYCYTX-00501).

## REFERENCES

Zhou Y.M. and T.D. Fu, 2007: Genetic improvement of rapeseed in China. Proceedings of the 12th international rapeseed congress, volume I: 210-212.

Goring D.R., P. Banks, W.D. Beversdorf and S.J. Rothstein,1992: Use of the polymerase chain reaction to isolate an *S*-locus glycoprotein cDNA introgressed from *B. campestris* into *B. napus* ssp. *oleifera*. Mol. Gen. Genet. 234: 185-192

Gowers S., 1989: Self-incompatibility interactions in *Brassica napus*. Euphytica 42: 99-103

Rahman M.H., 2005: Resynthesis of *Brassica napus* L. for self-incompatibility: self-incompatibility reaction, inheritance and breeding potential. Plant Breeding 124: 13-19

Fu T.D. and H.L. Liu, 1975: Preliminary report on breeding of self-incompatible lines of *Brassica napus*. Oil Crops of China 4: 77-85 (in Chinese)

Fu, T.D., 1981: Breeding of maintainer and restorer of self-incompatible lines of *Brassica napus*. Cruciferae Newsletter 6: 40-42

Ma C.Z., T.D. Fu, G.S. Yang, J.X. Tu, X.N. Yang and F. Dan, 1998: Breeding for self-incompatibility lines with double-zero on *Brassica napus* L. Journal of Huazhong Agricultural University 17(3):211-213(in Chinese)

Ma C.Z., Y.F. Jiang, F. Dan, B. Dan and T.D. Fu, 2003: Breeding for maintainer of self-incompatible lines and its potential in *Brassica napus* L. Journal of Huazhong Agricultural University 22:13-17 (in Chinese)