Breeding of an environment sensitive male sterile line 373S in *Brassica napus* L.

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Abstract

The authors found a chimerically male sterile rapeseed (*Brassica napus* L.) in the open-pollinated family from a spontaneous male sterile population. An environment-sensitive male-sterile line '373S' was bred from this chimerically male sterile plant after three-generation selfing. The correlations between the daily male fertility and atmospheric temperature, relative humidity, and daylength from 1 to 10 days prior to blooming were analyzed. Temperature 4 to 7 days prior to blooming and humidity 6 days prior to blooming significantly correlated with the male fertility. '373S' will become male fertile when temperature is below 11°C but complete male-sterile when temperature is over 15°C. No significant correlation between the male fertility and day-length was found. Therefore, '373S' was considered as a thermo-sensitive male sterile line. Owing to the thermo-sensitive characters, '373S' can multiply itself by sowing one-week early in winter rapeseed area and meanwhile, produce two-line hybrid in spring rapeseed areas or in winter rapeseed area by sowing 2-3 week late.

Key words: *Brassica napus* L., two-line hybrid, thermo-sensitive male sterility

The exploitation of plant heterosis is an effective approach to provide more food production than conventional breeding method. Male sterility is the most important approach for the utility of the heterosis because commercial hybrid can be produced on a large scale based on these systems. Difficulties in breeding elite male sterile lines and producing commercial hybrid seed will hamper the development of hybrid crop.

Recently, the development of two-line hybrid rice, rapeseed, and wheat is very quickly in Asian, especially in China. Normally, a two-line hybrid was based on environment (thermo and/or photoperiod) sensitive male sterility (EMS). The male fertility is regulated by temperature and / or daylength, normally, the pollens are completely sterile when the plant is grown under warmer and long-day conditions, and fertile pollens are produced when the plant is grown under cooler and short-day conditions. Thus, EMS can be planted in stripe as the female parent to produce hybrid seeds under long day conditions, and can be selfed under short-day conditions. Therefore, in "two-line" system, the maintainer line of male sterility in "three-line" hybrids was not required, which greatly reduce the costs in labor, time, and resources in hybrid production. Second, almost all normal varieties can be used as restore lines, this save much time for breeding restore lines. A further advantage is that employ EGMS system can avoid some potential adverse effects of male-sterile cytoplasm, for instance, Texas CMS in maize is susceptible to southern corn leaf blight and Ougra CMS in *Brassica* cause chlorosis on plants under cold condition.

In China, rapeseed was cultivated from subtropical to cold zone and both spring and winter type was used, so it is easy to find different regions either for the multiply of EMS or for hybrid producing. EMS was considered as an important approach for hybrid rapeseed breeding. Up to now, one thermo-sensitive genic male sterile (TMS) line 'Xiang 91S' (Xi et al 199) and a photoperiod-thermo sensitive genic male sterile (PTMS) line 'H90S' (Wang et al) have been identified and their sterility was controlled by interaction between 2-4 pairs of recessieve gene and 1-3 pairs of thermo-sesitive gene, and three pairs of recessieve gene, respectively. 'Xiang 91S' has been improved to be double low lines and a hybrid "Xiangzayou 5" (Wu et al., 2005) based on it had been registered. Li et al (2002) and Su et al (1999) also reported two EMS lines 'K121S' and 'Zunai S' in *Brassica juncea.*

Some time environment sensitive cytoplasmic male sterility (ECMS) was also included in EMS. Yang et al (1), Liu et al (1998), and Dong et al (2004) found some ECMS types in *B.napus*. Some hybrid such as "Liangyou 586" (based on ECMS) (Liu et al., 2000) and "Huayouza 12"(based on ECMS from Polima CMS), had been registered recently. However, these system only simplify the multiplication of female parent by substituting self-pollination for cross-pollination and did not resolve the problem of CMS. Notwithstanding, discovery and identification of new type of EMS will help researchers grasp more knowledge about male sterility and reveal the interaction mechanism between microspore development and ecological factors. We found and breeded three EGMS lines '373S', 'Hy 50 S', and 'CZ803S' in *Brassica napus* and their quality were in improvement now. In the present paper, the breeding and response to temperature, daylength, and humidity of the environment-sensitive male-sterile line '373S' was investigated.

Material and Method

Material

In the spring of 2001, a spontaneous male sterile population was found at Yangling (Latitude 108°E, Longitude 34°,15′N)

when the authors are making field collection. The seeds of five plants in this population was taken back and sowed in experimental field. However, only one plant in a family of them coding 02-373 showed about 10 male sterile flowers at the initiation of blooming but then became male fertility and all the other plants was male fertile. The author assumed that the fertility of this plant might be influenced by ecological factors such as atmospheric temperature and daylength. So this plant was bagged to make self-pollinating. Three male fertile plants and twenty-nine sterile plants were obtained in next generation. However, the male sterility of these twenty-nine sterile plants was not stable. They become 100% male fertile at the initiation of blooming but 100% male sterile at the latter stage of blooming. This phenomenon corresponded well to the regulation of local climate change, i.e. temperature rising and day length prolong in late March. An environment-sensitive male-sterile line named '373S' was bred from this chimerically male sterile plant after three-generation successive selfing.

Method

The correlation between the change of male fertility and the atmospheric temperature, relative humidity, and daylength from 1-10 days prior to flowering was analyzed. To daily exam the change of male fertility, male fertility index (MFI) was record as 0, 1, 2, 3, 4, 5, and 6 for a single flower with zero to six stamens with elongated filament and normal anther that can shed pollen. MFI for individual plant was the average of MFI for all fresh flowers on the day, and MFI for a male sterile line was the average of MFI for ten random-selected individuals.

Results

The fertility of '373S' fluctuated in the early spring due to the drastic change of climate at Yangling. '373S' became 100% male fertile at the initiation of blooming (late March to early April) but 100% male sterile at the latter stage of blooming (from late April). In the transition stage from male fertile to sterile, the number of anther that could shed pollen in a flower would decrease gradually.

The correlations between the daily male fertility and atmospheric temperature, relative humidity, and day-length from 1 to 10 days prior to blooming were analyzed. The results showed that temperature 4 to 7 days prior to blooming and humidity 6 days prior to blooming significantly and negatively correlated with the male fertility (Table 1). '373S' will become male fertile when temperature is below 11°C but complete male-sterile when temperature is over 15°C (data not showed). Relative humidity 6 days prior to blooming also significantly correlated with the male fertility. However, the sensitivity to humidity was not validated because the increase of humidity was always accompanied by the fall of temperature in the spring. No significant correlation between the male fertility and day-length was found. Therefore, '373S' was considered as a thermo-sensitive male sterile line.

Table 1 The correlation coefficient between male-fertility index (MFI) and the temperature, atmospheric humidity, and day length before blooming

Ecological factor	Days prior to blooming									
	1	2	3	4	5	6	7	8	9	10
Daily average temperature	-0.103	-0.249	-0.372	-0.523^{b}	-0.620^{b}	-0.683^{b}	-0.535^{b}	-0.115	0.331	0.448
Daily maximum temperature	-0.180	-0.207	-0.247	-0.427^{a}	-0.610^{b}	-0.689^{b}	-0.526^{b}	-0.086	0.353	0.436
Daily minimum temperature	-0.318	-0.524^{b}	-0.557^{b}	-0.504^{b}	-0.420^{a}	-0.485^{b}	-0.452^{a}	-0.097	0.272	0.384
Atmospheric humidity	0.142	-0.129	-0.267	-0.128	0.160	0.375^{a}	0.219	-0.098	-0.096	0.025
Day length	-0.005	0.078	0.053	0.020	-0.017	-0.063	-0.117	-0.179	-0.254	-0.345

Note: *a* and *b* meant significant at 95% and 99% probability level, respectively.

Discussion

The inheritance analysis results showed that the male sterility of '373S' may be controlled by 2 pair of recessive nuclear gene (unpublished). Owing to the thermo-sensitive characters, 373S can multiply itself by sowing one-week early in winter rapeseed area and meanwhile, produce two-line hybrid in spring rapeseed areas or in winter rapeseed area by sowing 2-3 week late. The advantages of this method are: (i) male sterile line is multiplied by selfing, and does not need a maintainer as CMS line;(ii) any other genotype can be used as parent, this ensure the flexibility of choice parent; (iii) some negatives associated with CMS can be avoided.

However, the production of hybrid on larger scale in these systems maybe limited by the instability of atmosphic temprature before flowering since it could render the fertility. So it needs to select the EMS line with lower critical temperature in the breeding and improvement of '373S'.

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