Development and studies of a thermosensitive genic male sterility breeding system in rapeseed mustard *Brassica juncea*

LI Shikai[1], HE Jiangming[1], LIU Xuyun[2], ZHANG Xishun[2], LI Weifen[1]

[1]*Horticulture Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650205, China*
[2]*Industrial Crop Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650205, China*
Email: lishikai@sina.com

Abstract

A temperature (photoperiod) sensitive male sterile (MS) line *K121S* of rapeseed mustard (*Brassica juncea* L.) was developed from Yunnan mustard in 1999. The MS exhibited stable sterile with 100% sterility in summer sowing but fertile with 70% self-seed rate in autumn sowing at Kunming natural situation. Fertility alteration indicated that the expression of fertility was strongly affected by temperature but photoperiod; higher temperature can cause male sterility and lower temperature male fertility. The daily average temperature (DAT) of 3-5d before blooming influenced significantly fertility, it needed a critical temperature of over 10.8 ° C from fertility to sterility. Genetic study suggested *K121S* belongs to a thermosensitive genic male sterility (TGMS) system with fertility controlled by two pair recessive duplicated sterile genes (ms_1 and ms_2) which interact with a pair of recessive inhibitor gene with sensitivity to temperature (*i*). The inhibitor gene is active and the MS genes can be suppressed to cause the male fertile when DAT is below the critical point, but it's inactive and the MS genes can be expressed normally when DAT is above the critical value. So besides the TGMS (TypeIII) *K121S* ($ms_1ms_1ms_2ms_2ii$), in this system there are another two types GMS line, (TypeI)*116A* ($ms_1ms_1ms_2ms_2II$) is not sensitve to temperature and can be kept in 1:1 ratio by sister-cross of sterile plant with fertile plant with genotype ($MS_1ms_1ms_2ms_2II$) or ($ms_1ms_1MS_2ms_2II$), (TypeII)*120A*($ms_1ms_1ms_2ms_2Ii$) is also not temperature sensitive but just can be maintained by TypeIII under autumn sowing in Kunming situation. A full sterile population with insensitive to temperature can be obtained by TypeI crossed to TypeIII in lower temperature situation and can be used to produce hybrid in any condition. Therefore, TGMS two-lines method and GMS three-lines method can all be used to produce hybrids based on the new system in *Brassica juncea* L.

Key words: *Brassica juncea*, Thermosensitive genic male sterility (TGMS), Fertility alteration, Inheritance

1 Discovery and development of the new sterile system

Rapeseed mustard (*Brassica juncea* L.) including spring type and winter type is an important oilseed crop in western China, most winter type is grown in upland of Yunnan, Guizhou and Sichuan, where climate is low rainfall and drought during the period of growth. Cytoplasmic male sterility (CMS) was studied and hybrids was developed to increase the crop yield in Yunnan from 1986 (Shi *et al.*, 1990; 1991), two outstanding hybrids with low erucic content based on "*OuxinA*" and "*YimenA*" CMS was registered and utilized commercially (Qiu *et al.*, 1998; Dong *et al.*, 1999). However, the application of CMS three lines hybrids was limited by lower seeds production because of insufficient pollination and low seed setting. Moreover, it needs long time to breed three lines resulted in a lower efficiency hybrid breeding.

In 1997, a fertile parent in a sister cross of genic male sterility (GMS) "*05S*" was found to show full sterile in summer sowing, the left seeds was sowed in September to check the flower fertility, it showed fertile or partial fertile at flowering in January 1998, self seeds was sowed in June, the flower fertility still showed full sterile at flowering in July of 1998, the ecotypic male sterility of this material was preliminary confirmed. By the method of propagation in autumn sowing and selection in summer sowing, an ecotypic male sterile line named "*K121S*" was developed in 1999(Li *et al.*, 2002). At the same time, a stable GMS line with 50% sterile plant named "*116A*" was also developed from "*05S*" by continual sister cross and its fertility was not influenced by environment. A genetical model of the system was set up and the temperature sensitive gene role was discussed (Li *et al.*, 2004; 2006). This finding will provide an alternative way for mustard hybrids breeding.

2 Fertility alteration research of "*k121s*"

*2.1 The fertility expression of "*K121S*" under the natural situation*

Different sowing date experiment was conducted continuously in two years from 2000 to 2002 to evaluate the fertility behavior of "*K121S*" in Kunming, China, Yunnan academy of agricultural sciences (YAAS). 13 sowing dates including 6 in autumn from Sep 5 to Dec 18 and 7 in summer from Apr 4 to Aug 27 were arranged in each year. The results showed "K121S" had a visible and stable fertility alteration characteristic, the self seeding rate could up to 70% under the autumn sowing condition from Sep 5 to Oct 16, but in summer sowing from Apr 4 to Jul 4 it was zero (fig.1 and fig.2).

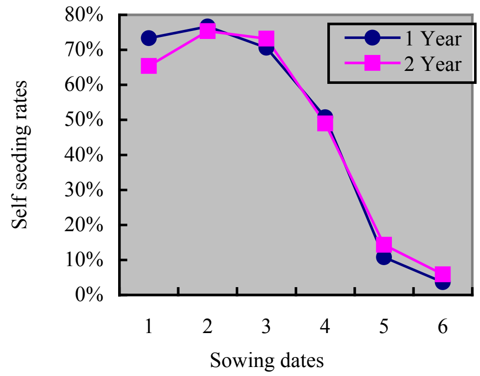

Fig.1 Fertility performance at autumn devided sowing in two years

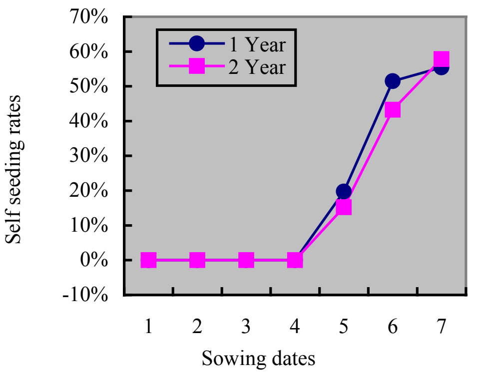

Fig.2 Fertility performance at summer devided sowing in two years

2.2 The influence factor analysis to fertility alteration

The temperature and photoperiod were considered as the candidate factors to influence the fertility alteration. Using the fertilities data of different date sowing, the correlations between self seeding rate with temperature and daylength conditions at seedling, bud and florescence stage was firstly analyzed, results showed that the fertility was significantly correlation with the daily average air temperature (DAT) in florescence, daylength in bud stage and florescence. However, the role of daylength in bud stage and florescence was excluded by applying stepwise regression analysis method (data not show). Results suggested the fertility alteration was mainly affected by the DAT- florescence, and the "*K121S*" was belonged to the temperature sensitive type.

The farther analysis to the relationship between self seeding rates and day-to-day DAT before flowering indicated the fertility was strongly affected by the DAT of three to five days before flowering (table 1), so that, three to five days before flowering was considered to be the sensitive stage to temperature.

2.3 The critical temperature of fertility alteration

A critical DAT of 10.8 °C was predicted by the regression model "Y=0.1346x-1.4561, R^2=0.8424" set up by sterile degree (Y) and the DAT of fertility alteration sensitive stage (x).

Table 1 the correlation coefficient between self seeding rates and the DAT of 0 to 20 day before flowering

Days before flowering	Correlation coefficient	Sig. (2-tailed)	Days before flowering	Correlation coefficient	Sig. (2-tailed)	Days before flowering	Correlation coefficient	Sig. (2-tailed)
20	0.5765	0.0309	13	0.7421	0.0024	6	0.5786	0.0302
19	0.4962	0.0712	12	0.6730	0.0083	5	0.8382	0.0002
18	0.5341	0.0491	11	0.5905	0.0262	4	0.8509	0.0001
17	0.5147	0.0596	10	0.7206	0.0036	3	0.8038	0.0005
16	0.2186	0.4527	9	0.6273	0.0163	2	0.7701	0.0013
15	0.4478	0.1083	8	0.4626	0.0958	1	0.7070	0.0047
14	0.7683	0.0013	7	0.5832	0.0286	0	0.5484	0.0423

3 Genetical analysis for the new system

3.1 The fertility of the test crosses F_1s

All F_1s including reverse crosses between "*K121S*" and "*OuxinA*" CMS maintainers and restorers were fertile whenever at summer sowing or autumn sowing situation in Kunming (data not showing). The results indicated the sterility of "*K121S*" was controlled by recessive nucleus gene, and was not matter with cytoplasm.

3.2 The fertility of the F_2s and BC1s populations

The fertility of F_2 and BC_1 progenies from the crosses between "*K121S*" and two fertile materials "*Kunyang Wulong*" and "*Wuding Laoshuiba*" at summer or autumn sowing situation in Kunming was evaluated and the result was listed in table 2. The fertility segregation of F_2 agreed with the proportion of 61:3 at autumn sowing but 15:1 at summer sowing; BC_1 accorded with the proportion of 7:1 at autumn sowing but 3:1 at summer sowing in Kunming situation. The results suggested the fertility inheritance was related to three pair interaction genes.

3.3 Fertility of the reciprocal crosses between "K121S" and "116A" and "120A"

Fertility performance of the reciprocal crosses between "*K121S*" and "*116A*" and "*120A*" was investigated and the result was listed in table 3. The cross between *K121S* and the fertile plant of *116A* always showed agree with the segregation proportion of one to one on fertile and sterile plant at all situations, but the reverse cross was full sterility any in summer or autumn sowing situation. Dissimilarly, the progeny of *K121S* crossed with the fertile plant of *120A* exhibited the fertility similar to *K121S* that was fertile at autumn sowing but full sterile at summer sowing, the reciprocal cross showed agree with

1:1segregation ratio between fertile and sterile plant at autumn sowing situation but full sterile at summer. The results suggested strongly that the three types MS line have the same site for fertility genes.

Table 2 the fertility of the F_2s and BC_1s populations at Kunming situation

Crosses	Generations	Seasons	Fertile	Sterile	E. Prop.	X_c^2	P
K121S×*Kunyang Wulong*	F_2	Autumn	591	32	61:3	0.26	0.61
		Summer	189	10	15:1	0.32	0.57
	BC_1	Autumn	301	30	7:1	3.27	0.07
		Summer	128	15	3:1	0.36	0.55
K121S×*Wuding Laoshuiba*	F_2	Autumn	677	26	61:3	1.48	0.22
		Summer	342	18	15:1	0.76	0.38
	BC_1	Autumn	279	35	7:1	0.41	0.52
		Summer	79	6	3:1	1.83	0.18
		Summer	264	50	3:1	3.06	0.08

Table 3 the fertility of reciprocal crosses between "*K121S*" and "*116A*" in different situation of Kunming

Crosses	Years	Fertility	
		Autumn	Summer
K121S×116A(Fertile plant)	2004	110F: 88S; X_c^2 (1:1)=2.23, p=0.14	57F: 62S; X_c^2 (1:1)=0.13, p=0.71
	2005	162F: 171S; X_c^2 (1:1)=0.19, p=0.66	83F: 89S; X_c^2 (1:1)=0.15, p=0.70
116A(Sterile plant)×*K121S*	2004	Full sterile	Full sterile
	2005	Full sterile	Full sterile
K121S×120A(Fertile plant)	2004	Fertile	Full sterile
	2005	Fertile	Full sterile
120A(Sterile plant)×*K121S*	2004	132F: 147S; X_c^2 (1:1)=0.70, p=0.40	Full sterile
	2005	116F: 129S; X_c^2 (1:1)=0.59, p=0.44	Full sterile

Note: F, Fertile; S, Sterile.

4 Conclusion

4.1 The inheritance and utilizing way of the new sterile system

Genetic researches suggested *K121S* belongs to a thermosensitive genic male sterility (TGMS) system which fertility controlled by two pair recessive duplicate sterile gene (ms_1 and ms_2) interact with a pair of recessive inhibitor gene with sensitivity to temperature (*i*), genotype is ($ms_1ms_1ms_2ms_2ii$). The inhibitor gene is active and the MS genes can be suppressed to cause the male fertile when DAT is below to the critical point, but it's inactive and the MS genes can be expressed normally when DAT above the critical value. Besides the TGMS *K121S* (TypeIII), in this system there are another two types GMS line which no sensitivity to temperature: TypeI- *116A* ($ms_1ms_1ms_2ms_2II$) can be kept by fertile plant with genotype ($MS_1ms_1ms_2ms_2II$) or ($ms_1ms_1MS_2ms_2II$) in 1:1 ratio through sister-cross, TypeII - *120A* ($ms_1ms_1ms_2ms_2Ii$) just can be maintained by TypeIII under autumn sowing in Kunming situation. A full sterile population with insensitive to temperature can be obtained by TypeI crossed to TypeIII in lower temperature situation and can be used to produce hybrid in any condition. Therefore, TGMS two lines method and GMS three lines method can all be used to produce hybrids based on the new system.

*4.2 The fertility stableness and safe utilization of the TGMS line "*K121S*"*

Research indicated that the fertility of "*K121S*" was strongly affected by DAT of 3-5 day before flowering, it's full fertile under the 10.8 °C, till above 18.2 °C it change to complete sterile. So that, hybrid production can be performed safely under the situation of flowering DAT is above 18.2 °C, the condition is easy to find in China, like as summer sowing in Kunming or spring sowing in Xining of Qinghai province, and so on.

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