

The Breeding and Utilization of the CMS Shaan 2A in *Brassica napus*

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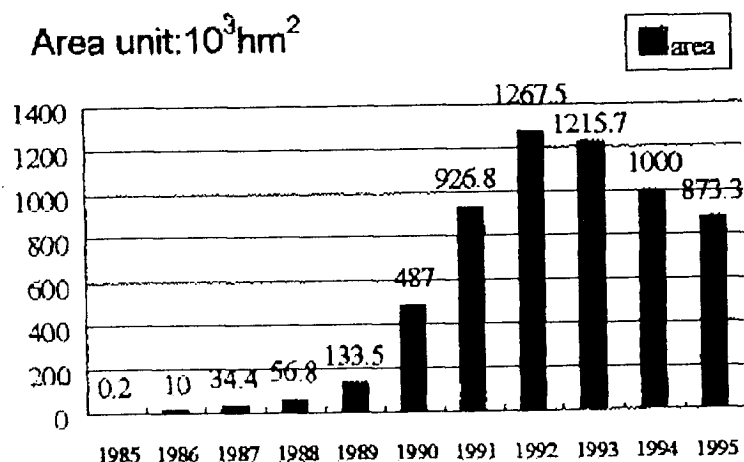
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The cytoplasm male-sterile line Shaan 2A was bred by Professor Li Dianrong in 1983, and the perfection of the triline was achieved in the same year. Professor Li was successful to use CMS Shaan 2A to cross with R-line Ken C1 to develop the hybrid «Za 37» rapeseed combinations, which was approved of identification and named as «Qinyou No. 2» by the Identification Committee of Crop New Varieties of Shaanxi Province in 1985.

«Qinyou No.2» was approved of identification by the experts organized by the Ministry of Agriculture and won the national technical patent in 1986. And in 1992 the extended areas

of «Qinyou No. 2» reached 1267,5 thousand ha (see figure 1), but later, the extended areas decreased a little for the hybrid seed production suffered from natural disasters to different extents so that the hybrid seeds did not meet the needs of hybrid rapeseed - «Qinyou No.2» production. It was estimated that in the fall of 1995, the sown areas of «Qinyou No.2» reached 1,400 thousand ha or so, accounting for 1/4 of winter rapeseed sown areas in China and widely spreading over 14 provinces and autonomous regions along the Yellow River, the Huaihe River and the Yangtze River valleys.

Fig. 1 : The sown areas with Qinyou No. 2 in the years 1985-1995



2. The floral organ's morphological feature of CMS Shaan 2A and pollen vigor

2.1. Morphological feature of floral organ.

By determination, the observational value of floral organ of maintenance line «Shaan 2B» and restoring line Ken C1 is similar. But floral organ of Shaan 2A occurred greatly in metamorphosis, mainly shown in the following aspects: firstly, the stamen height of Shaan 2A is only 42,88 % of Shaan 2B. Although there are no apparent differences in the pistil height of «Shaan 2A» and «Shaan 2B», the ratio of pistil height/stamen height of «Shaan 2A» itself reaches 2,23, whereas the ratio of pistil height/stamen height of Shaan 2B

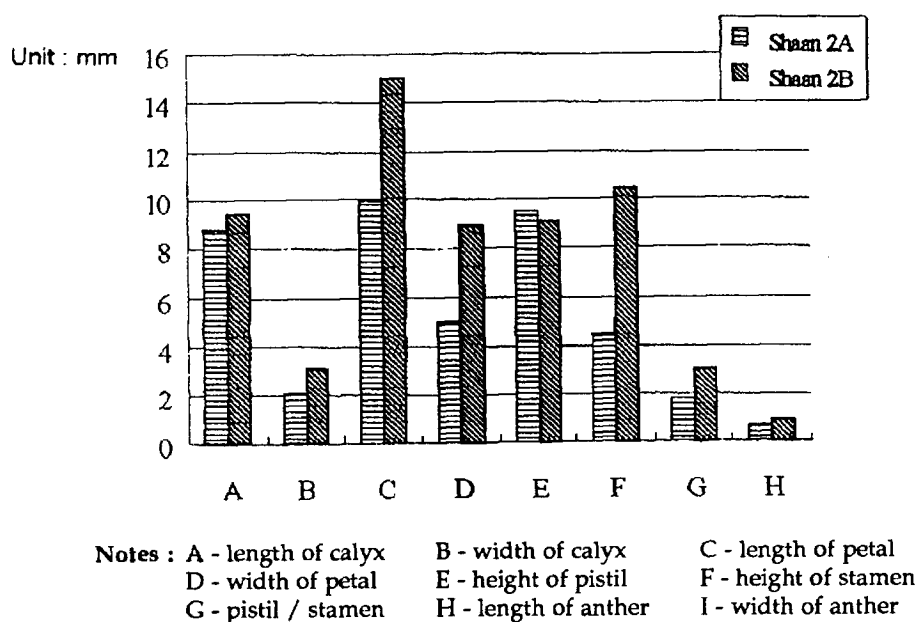
is only 0,87. Secondly, there is the apparent difference in the width of the petal and calyx and anthers length between sterility and fertility. The calyx width of «Shane 2A» is 68,4 % of «Shane 2B»; the petal width is 55,89 % of Shane 2B and the anther length is 60 % of «Shane 2B». But the metamorphosis variations in floral organ sterile line Shane 2A have completely restored normality in hybrid F1 generation (see Table 1). Floral organ of «Shaan 2A» and «Shaan 2B» are distinctly different in width of floral calyx, length and width of floral petal, height of stamen and length of anther. This is one of the main morphological features of CMS Shaan 2A (see Figure 3).

Table 1. The morphological comparison of floral organ between «Qinyou No. 2» and its triline (2)

Item	CMS Shaan 2A	Maintenance line Shaan 2B	Shaan2A/ Shaan 2B (%)	Restoring line Ken C ₁ (R)	Qinyou No.2 (Shan 2A × Ken C ₁)
Length of calyx	8.76 ± 0.81	9.43 ± 0.66	92.90	9.41 ± 0.20	9.98 ± 0.45
width of calyx	2.10 ± 0.18	3.07 ± 0.40	68.40	2.37 ± 0.18	2.40 ± 0.19
Length of petal	9.92 ± 1.01	15.00 ± 0.87	66.13	14.93 ± 0.62	15.74 ± 0.82
width of petal	4.98 ± 0.82	8.91 ± 0.98	55.89	8.49 ± 0.38	8.70 ± 0.58
height of pistil	9.51 ± 0.75	9.10 ± 0.88	104.51	8.99 ± 0.54	9.12 ± 0.45
height of stamen	4.49 ± 1.12	10.47 ± 0.62	42.88	10.56 ± 0.23	10.11 ± 0.46
pistil/stamen	2.23 ± 0.57	0.87 ± 0.08	256.32	0.85 ± 0.04	0.90 ± 0.07
length of anther	1.83 ± 0.42	3.05 ± 0.23	60.00	3.23 ± 0.11	3.19 ± 0.17
width of anther	0.67 ± 0.14	0.86 ± 0.13	77.91	1.18 ± 0.06	1.14 ± 0.10

unit: mm

Figure 3. The floral organ morphological comparison of CMS Shaan 2A and maintenance line Shaan 2B



2.2. Pollen's morphological feature and vigor

The shape of normal pollen of maintenance Line Shaan 2B is elliptic, whose long diameter is 31,0 + or - 2,3um or so, whose diameter of equator surface is 12,5 um, but the pollen of CMS Shaan 2A appears to be ball-like, whose diameter is 19,0 + or - 3,1 um in general, the smallest is only 7 um or so. The survival rate of Shaan 2A pollen is very low, while vigor-free pollen rate accounts for 96 % and the metamorphosis rate of pollen reaches as high as 97,5 %. The pollen survival rate of F1 generation obtained from Shaan 2A pollinated with the pollens from restoring line - Ken C1 reached as high as 99,8 %, whose metamorphosis rate was decreased to 3,6 %. Accordingly, it can be seen from this that the direct cause or reason of Shaan 2A sterility is its pollen abortion morphological metamorphosis or death; and that when the restoring line - Ken C1 is used to cross with Shaan 2A, the pollen morphology or shape and survival rate of Hybrid F1 generation can restore their normal fertile levels (see Table 2).

Table 2. The pollen's death rate and metamorphosis rate

Gene Type	Death rate %	Metamorphosis rate (%)
CMS Shaan 2A	96,00	97,50
Maintenance line Shaan 2B	3,4	1,5
Restoring line Ken C1	0,6	9,0
Qinyou No. 2 (Shaan 2A x Ken C1)	0,2	3,6

3. The Stability of CMS Shaan 2A.

After crossings and successive backcrossings, the sterile rate of Shaan 2A reached 100 % in 1983, but its sterile degree was 95,5 %-99,2 %. In the initial stage of blooming, there is 0,8 %-4,5 % flowers with traces of pollens (see Table 3).

The very few traces of pollens in CMS Shaan 2A were observed along the Yellow River and the Huaihe River valleys where the breeding area was located, but more traces of pollens were found along the Yangtze River valley, which just indicated that the behaviors of CMS Shaan 2A sterility were affected by the environment constraints. Since CMS Shaan 2A in the initial blooming stage was under the conditions of low temperature, the several early-blooming flowers on the top of the single plant showed the traces of pollens so that we called it as «low temperature sensitive type». In reproducing seeds along the Yangtze River valley, such measures as taking off the main axis, increasing the male parent row rates, promoting the male parent growth vigor and postponing the sowing date, etc. should be adopted to solve the problem of traces of pollens.

Table 3. Sterile rate and sterile degree of CMS Shaan 2A

Year month	Sterile rate			Sterile degree	
	Shaanxi	Hubei	Jiangsu	Shaanxi	Hubei
1983 3-4	100,0	100,0	100,0		
1983 3-4	100,0	98,57	100,0		95,50
1986 3-4	100,0	99,03	100,0	99,01	99,20
Average	100,00	99,20	100,00	99,01	97,35

Notes : The male sterile rate is averaged in accordance with the percentage so that the male sterile rate in Hubei is 99,2%. However, if the male sterile rates averaged in accordance with the weighted number of observed plants, the male sterile rate is 98,9 %. The sterile degree is average in accordance with the percentage so that the sterile degree in Hubei is 97,35 %, but if the sterile degree is averaged in accordance with the weighted number of observed flowers, the sterile degree is 98,14 %.

4. The sterile controlled gene of CMS Shaan 2A.

In 1983 when the combination of Shaan 2A x Ken C₁ was made and F₁ generation had its self- crossing, the separation rate between the fertile plants and sterile plants was 3.11:1.

In 1983 and 1985 when Shaan 2A was backcrossed with the hybrid F₁ generation as the male parent plants respectively, the

separation rate of next generation was 1:1. When X² test was used, its results showed that the difference was not extremely significant. It can be considered from this that as far as the nuclear gene of Shaan 2A is concerned, Shaan 2A is controlled by a pair of recessive sterile genes so that its restoring traits show the complete dominance. Shaan 2A genotype is S (rfrf) (See Table 4).

Table 4. Separation rate of F₂ generation and BC₁ in hybrid

Year	Cross combination	Generation	Fertile (a)	Sterile (b)	a:b	X ²
1983 - 1985	Shaan 2A x Ken C ₁ F ₁	F ₂	659	210	3.14:1	0,32
1983	Shaan 2A x (Shaan 2A x Ken C ₁)F ₁	BC ₁	116	115	1.01:1	0,00

5. The restoring source of CMS Shaan 2A

In the years of 1981-1986, some 138 parts of germplasm resources of rapeseeds obtained from both home and abroad were used to carry out testcrosses with Shaan 2A, of

which there were only 30 parts of germplasm resources which were able to restore the sterility of the CMS Shaan 2A with the results shown in Table 5.

Table 5. The restoring ability for Shaan 2A with different genotypes

germplasm origin	testing cross (parts) amount	number of restoring	number of non-restoring	% of restoring in tested number
Canada	6	0	6	0
Australia.....	5	0	5	0
Asia	10	0	10	0
Europe	24	1	23	4,17 %
China 1	22	4	18	18,18 %
China 2	/1	25	46	35,21 %
Total	138	30	108	21,74 %

Notes : «China 1» is the bred cultivar - «China 2» is the bred line.

The test results indicated that the rapeseed cultivars with high erucic acid contents as well as the cross bred cultivars and lines from Europe had a better restoring ability for Shaan 2A sterility, whereas those from Canada, Australia and Asia as well as those cultivars with Asia pedigrees bred or developed in China had no restoring ability for CMS

Shaan 2A. And at the same time, the cytoplasm of Ken C₁ belongs to the «S» type in accordance with the studies ; and also, the cell nucleus of Ken C₁ has a pair of dominant restoring fertile genes (RfRf), whose genotype is S (RfRf), which can restore the sterility of Shaan 2A completely.

6. The Utilization of CMS Shaan 2A

Based on the statistical results from 23 regional trial points in 3 years of 1984-1986 in Shaanxi Province, the yield of Qinyou No.2 - the hybrid rapeseed cultivar cross developed with CMS Shaan 2A and restoring line Ken C₁ was 2887,5-3163,5 kg/ha, with an average yield of 3044, 0 kg/ha, while the yield of rapeseed cultivar in the control was 2211.0-2629,5 kg/ha, with an average yield of 2397 kg/ha.

Accordingly, Qinyou No. 2 had a yield increase of 27.51 % higher than that in the control on an average so that the average rapeseed

increase was 647 kg/ha (See Table 6). In addition, the statistical results from 41 demonstration points in Shaanxi Province in 1985 showed that the average yield of Qinyou No. 2 was 3180 kg/ha, while that in the control was 2481 kg/ha so that Qinyou No. 2 has a yield increase of 28.20 % higher than that in the control. Again in 1986, the statistical results from 43 demonstration points in Shaanxi Province showed that the average yield of Qinyou No. 2 was 3130 kg/ha, while that in the control was 2052 kg/ha, so that Qinyou No. 2 had a yield increase of 52,5 % higher than that in the control.

Table 6. Regional test results of Shaanxi Province in the year of 1984-1986

Year	Number trial points	Place in yield trail	Yield of Qinyou No.2 (kg/ha)	Yield of the control (kg/ha)	% higher than that in the control
1984	6	1	3 081.0	2 211	39.35
1985	8	1	3 163.5	2 629.5	20.33
1986	9	1	2 887.5	2 350.5	22.85
Total average	23		3 044.0	2 397.0	27.51

Since Qinyou No. 2 is characterized with strong adverse resistance, wide adaptation, stable- and high yields, it has been approved of identification and extension in Shaanxi, Henan, Anhui, Hunan, Sichuan, Guizhou and Jiangsu provinces since 1985. In 1992, Qinyou No. 2 was again approved of identification as the «national level rapeseed cultivar» by the Chinese Crop New Variety Identification Committee. It can be estimated during the 9th Five-Year plan for national economic development (from the year of 1996 to the year of 2000) that Qinyou No. 2 will be the principal rapeseed cultivar in the winter-type rapeseed producing areas along the Yellow River, the Huaihe River and the Yangtze River valleys. As of 1978, Shaan 2A CMS germplasm resources have been transferred to some institutions of higher learning, research institutes and academies to carry out research on the transbreedings. At present, a batch of such fine quality hybrid rapeseed cultivars with single low and double lows has been successfully bred and entered the trials, demonstrations and extension as Huaza No. 2, Single Hybrid No. 1, Zayou No. 59, NKU-4 and Zayou No. 77, etc. The vegetable Research Lab of Agricultural Reclamation Scientific Research Center of Shaanxi Province has transferred the hybrid Chinese cabbage with Shaan 2A CMS cytoplasm which has entered the demonstration trial. At present, Shaan 2A CMS has been used in breeding and transbreeding

CMS lines and hybrid rapeseed cultivars characterized with strong heterosis vigor, high yielding and strong disease resistance in China, for instance, the yellow-seeded hybrid rapeseed cultivar bred has the oil contents improved by 1,5 - 4,5 % higher than the similar black hybrid rapeseed cultivars. However, the disadvantages of CMS Shaan 2A are some traces of pollens in the initial flower stage affected by the environment factor when sown in the fall along the Yangtze River valley with the warmer or higher temperatures in the longer winter. As far as the problem of traces of pollens is concerned, we are now probing into the possibility of utilization of using transbreeding line F1 generation to produce hybrids as well as GMS and CMS merged except that the method has been mentioned about in these areas for the hybrid seed production should be adopted to control the traces of pollens.

Reference Material

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