Studies on mechanism of trace pollen production of male sterile lines in *Brassica napus*

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Abstract

The mechanism of trace pollen production of male sterile lines in *Brassica napus* were studied. The results showed:(1) The flowers of 681A sterile line are smaller than its flowers of maintainer line and restorer line both, and with all yellow flower. The morphological character of flower organ in 681A can be divided six grades; (2)681A showed that fertile of pollen for low temperature condition and sterile of pollen for high temperature condition, and. .its critical temperature was about 10°C That means that sterile of pollen in over 10°C and fertile of pollen in below 10°C; (3) Bud size about 2mm was most sensitive stage in Low temperature; (4) The buds become smaller in high temperature treatment then that in low temperature treatment and the anthers length only 1 mm, triangle, milk white or yellow brown and no pollen, over 80 percent stigmas appear in ⩽2mm buds, but other size buds a few stigma appear. In addition, it can be observed a phenomena of double pistils or three pistils, but number of stamen still was six as nomal flower.

Key words: Male sterile line, heterosis, trace pollen, *Brassica napus*

It is reported that rapeseed F_1 hybrids can increase seed yield due to heterosis. But there is a sreious problem, that is, production of a little quantity of pollen called trace pollen by cytoplasmic male sterile(CMS) lines used as the female parents, which will result in seed yield decrease. Some people presumed it seems relate to trace pollen. Studies were undertaken in order to investigate causes of trace pollen production of a cytoplasmic male sterile line in *Brassica napus*.

1. Material and methods

1.1 Material

The CMS line 681A was used. 681A was obtained from the nature variation of XIANGYOU no.13 variety plant in the field, then the nature variation plant was passed through many generations hybridization and selection.

1.2 Methods

1.2.1 Field treatment

681A was planted on the field at Hunan Agricultural University. Six plants of 681A were observed. The number of buds with ⩾1mm size and the length of the largest buds of main inflorescence of six plants for every time at interval of 2 days since March 8, 2002 were recorded. In addition the ratio of stamen length to pistil length of blossom out according to order on main inflorescence every day were also recorded and every flower were fixed by F.A.A fixation solution since March 16,2002 until the end on April 6, 2002 In the meantime the air temperature was also recorded.

1.2.2. Artificial climate chamber treatment

And other 681A was treated in the September 30,2003 by artificial climate chamber with high temperature(20/17°C) and low temperature(10/7°C) respectively and the lights of both were 13 hours every day. The high temperature treatment was divided two steps: (1) when the main inflorescence occurrence first flower taking off difference sizes buds from main Inflorescences of three plants were observed under the microscope, and confirm the relation between bud size in length and stage of pollen development; (2) when that the largest bud is at 5 mm on the first branch, selection 4 branches retained the buds according to 4 grades with ⩽2mm, 2.1-3mm, 3.1-4mm, 4.1-5mm to continue treatment until the buds developed from 2 mm to 5 mm, then the flowers or buds were picked from the 4 branches respectively and under the microscope were observed. Others of plants were treatment with low temperature, it is same method as above .

2. Main results

2.1 Morphological character of flower organ in 681A

The flowers of 681A sterile line are smaller than its flowers of maintainer line and restorer line both, and with all yellow flower. The morphological character of flower organ in 681A can be divided six grades: (1) similitude normal flower, which have over 4 anthers with tittle pollens in six anthers, and stamen length to pistil length as ⩾ 1/2, which can be called No.4 grade: (2) there were tittle pollens in 2-3 anthers of six anthers and stamen length to pistil length as ⩽ 1/2,which can be called No.3 grade ;(3) there were tittle pollens in 1-2 anthers of six anthers and stamen length to pistil length as ⩽ 1/2, which can be called No. 2 grade; (4) there were trace pollens and stamen length to pistil length is also smaller than 1/2, which can be

called No. 1 grade . The variance of pollen number from No. 4 to No.1 grade were getting less and less. If no any pollen in the anther of stamen, it can be called No.0 grade . Normal flower it can be called No.5 grade .

2.2 Development of bud of 681 A

Table 1 showed the buds size 2mm developed to≥6mm buds about 10 days at start with flower of 681A.

Table 1 Development of buds of 681A

Observed Date (month/day)	3/8	3/10	3/12	3/14	3/16	3/18	3/20
Largest bud in length(mm)	2.0	2.8	3.8	4.5	5.2	6.1	First flower
>1mm No.bud	21	27	34	38	43	49	54

2.3 The relationship between tittle pollen and temperature in the natural condition

681A showed that fertile of pollen for low temperature condition and sterile of pollen for high temperature condition, and. .its critical temperature was about 10°C That means that sterile of pollen in over 10°C and fertile of pollen in below 10°C. Along with raise of temperature from 8.25±3.7 to 16.5±0.7 and the sterility of pollen was also raise, in other words the temperature was getting higher and higher, the tittle pollens were getting less and less, finally turn to complete male sterile. And this time the stage of development of 681A is at the end of flower stage.

2.4 The relationship between tittle pollen and control temperature by artificial climate chamber

2.4.1 Low temperature treatment(10°C/7°C)

The results as Table 2 showed that different size buds of first branch of three plants were treated with 10°C/7°C temperature in artificial climate chamber for 15days, when buds sizes developed to 3.1 mm-4mm in length have appeared tittle pollen, its flowers number were 3.5 percent of the observed flower number, and others when buds sizes from about 2mm developed to 4.1 mm-5mm in length have more flower with .tittle pollen, that went up by 84.1 percentage and the flower have opened from over 2mm buds size showed all no any pollens. It can be presumed that bud size about 2mm was most sensitive stage in Low temperature

Table 2. The relationship between tittle pollen and temperature in the natural condition (Sterile line: 681A)

Abloom date(month/day)	3/16-3/23	3/24-3/29	3/30-4/3	4/4 until harvest
Observed flower No.	113	237	88	100
trace pollen No.(%)	100	78.8-100	37.5-97.4	0
Grade of trace pollen	1-4	0-4	0-2	0
Temperature°C variation ($\bar{X} \pm s$)	8.25±3.7	8.40±1.6	11.5±1.3	16.5±0.7
The lowest temperature°C	1.5	6.5	8	15.5

Table 3 The results of buds in different size of first branch of three plants were treated with low temperature(10 **°C**/7 **°C**) for 15 days

Plant order	No.1	No.2	No.3	Total
Bud Size ⩽2mm				
No.of flower	8	6	10	24
No.of Trace Pollen flower	0	0	0	0
Percentage(%)	0	0	0	0
Bud size 2.1-3mm				
No.of flower	20	18	24	62
No.of Trace Pollen flower	0	0	0	0
Percentage(%)	0	0	0	0
Bud size 3.1-4mm				
No.of flower	32	26	28	86
No.of Trace Pollen flower	2	0	1	3
Percentage(%)	6.2	0	3.6	3.5
Bud size 4.1-5mm				
No.of flower	14	18	12	44
No.of tittle Pollen flower	6	15	12	37
Percentage(%)	71.4	83.3	100	84.1
Have opened flower				
No.of flower	2	3	4	9
No.of Trace Pollen flower	2	3	4	9
Percentage(%)	100	100	100	100

2.4.2 high temperature treatment(20°C/17°C)

The buds become smaller in high temperature treatment then that in low temperature treatment and the anthers length only 1 mm, triangle, milk white or yellow brown and no pollen, over 80 percent stigmas appear in ⩽2mm buds, but other size buds a few stigma appear. In addition, it can be observed a phenomena of double pistils or three pistils, but number of stamen still was six as nomal flower.

According to above results showed trace pollen produce of Male Sterile Lines 681A was relate with temperature variance .its critical temperature was about 10°C That means that sterile of pollen in over 10°C and fertile of pollen in below 10°C. Along with raise of temperature from 8.25±3.7 to 16.5±0.7 and the sterility of pollen was also raise, in other words the temperature was getting higher and higher, the tittle pollens were getting less and less, finally turn to complete male sterile. And this time the stage of development of 681A is at the end of flower stage.

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