STUDIES IN MALE STERILITY OF RAPESEED INDUCED BY CHEMICAL MALE GAMETOCIDE

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Abstract

Studies were made in male-sterility of rapeseed /Brassica napus L./ induced by male-gametocide. When applied at the bud stage, the percentage of male-sterilized flowers caused by male-gametocide MG_1, MG_2, MG_3 was 60% to 80%. Cytological studies observed mainly pollen-abortion in male sterile plants induced by MG_1, MG_2 and MG_3. Biochemical studies showed variations in content of free amino acid in male sterile anthers, banding patterns of peroxidase isoenzyme at bud and flowering stage. Heterosis in seed yield of produced hybrids by male-gametocide was significant. The seed yield of crosses Xianyou5 x Chuanyou9 was over the better parent 13.43% to 35.27%. This new technique has been applied.

Introduction

Chemical male-gametocide has been used for induction of male sterility and hybrid seeds production in crops. It is a new method of utilizing heterosis that has been of interest all over the world in recent thirty years, especially in rice, wheat, maize, cotton and vegetable. But a few reports for rapeseed /B.napus/ had been found. Since 1977 experiments with 30 chemicals were carried out and MG_1, MG_2, MG_3 were found to be the best chemicals in inducing male sterility in rapeseed /B.napus/. In this paper, the effect and mechanism of male sterility induced by MG_1, MG_2, MG_3 in rapeseed /B.napus/ are reported to guide chemical male-gametocide usage and provide basis for exploring new male-gametocide.

Materials and methods

Varieties of B.napus i.e. Xian-yon 5, Xian-nong-you 2, Sheng-Li and 83-64-1 /double low/ et.al. were used. MG_1, MG_2, MG_3 et al. used in this study were final products or their mixture. Different effective concentrations one time or several times of spraying at different stages and dosage were studied, and sterility of plants was investigated. Five main inflorescences were taken as a sample every 3 days after spraying, then fixed in Carnoy's fixing solution and kept in 70% alcohol solution. Pollen mother cell and microspores taken from the buds of the lowest part of inflorescence were observed with the use of microscope. The composition of free amino acids and respiratory intensity in anther were evaluated by thin-layer chromatography and W-s microrespiratory tester. In addition banding pattern of peroxidase isoenzyme in anther was analysed.

Results and discussion

1. Male sterility of rapeseed induced by MG_1, MG_2 and MG_3.

MG_1, MG_2 and MG_3 showed a rather efficient induction of male sterility in rapeseed based on repeated observations. Spraying at bud stage with suitable concentration resulted in 60-80% complete sterile /CS/ plants or more than 80%. There were still some semi-sterile /SS/ plants, bud-sealed /BS/ plants and dead /D/ plants as well as CS plants /Table 1/. The characteristics of these four types of plants, involving morphology and vigours of pollen and pistil could be summed up.

CS plant. Height of the plants was dwarfed. The growth was poor. The leaves were small. The most outstanding performance was the small flower, its size was only half of the normal flower. The petal was narrow. The pistil was a little shorter than normal. Otherwise, filament and anther of stamen were significantly shortened or seriously degenerated, and there were pollen grains or there were unregular pollen grains and 90% of these pollen grains had no vigour. No seeds were produced through self-pollination

by bagging.However,on average more than 8 seeds per siliqua were beared when cross pollination occured /Tab.2,Fig.1a/.

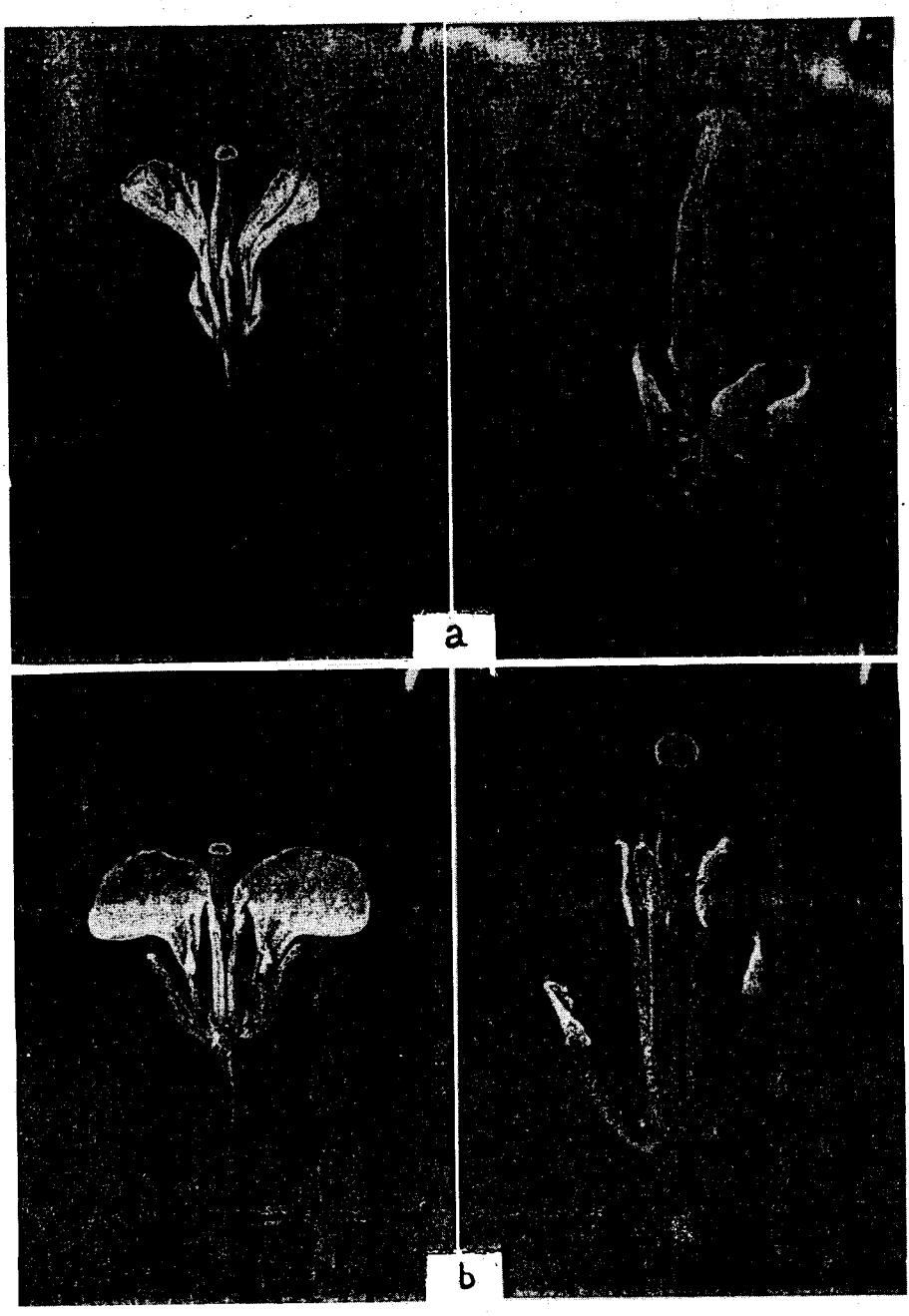
Table 1. Effects of chemicals on rapeseed /1985-86/.

Chemicals	Concentration %	No. of treated plants	% CSP	SSP	BSP	DP
MG_1	0.03	30	80.3	8.5	5	6.2
MG_2	1.5	30	81.1	3.9	0	15.0
MG_3	0.4	30	65.6	34.4	0	0

SS plant. Slightly dwarfed in height, almost normal growth, small leaves, slightly smaller flowers than in normal plants were observed. Stamen was slightly shorter than pistil. Anther possessed pollen grains which were small in size and unregular in shape. About 50% of pollen grains had no vigour. The rate of seed-producing by self pollination was low and became normal when cross pollination occured /Table 2, Fig. 1b/.

Table 2. The effect of MG_1 with effective concentration of 300ppm on the structure of flower, vigour of pollen and seed production in rapeseed /1978-79/.

		CS	SS	CK
Diameter of flower /mm/		13.7	19.2	24.1
Width of petal/mm/		5.7	8.1	12.1
Length of petal /mm/		14.0	13.9	15.4
Length of filament /mm/		6.2	7.5	9.2
Length of anther /mm/		1.30	2.05	2.22
Length of pistil /mm/		8.7	9.5	11.0
Size of pollen grain /um/		8.1±2.44	11.2±1.13	12.1±0.89
Vigour of pollen				
	No.of pollen observed	632	706	497
	No of dead pollen	573	316	19
	Ratio /dead/total/	90.7	43.2	3.9
Seed production	Self-pollination by bagging			
	No.of siliqua	0	0.19	0.38
	No.of seeds	0	1.04	4.56
	Cross-pollination			
	No.of siliqua	0.92	0.94	1.0
	No.of seeds	8.64	11.95	18.9


a
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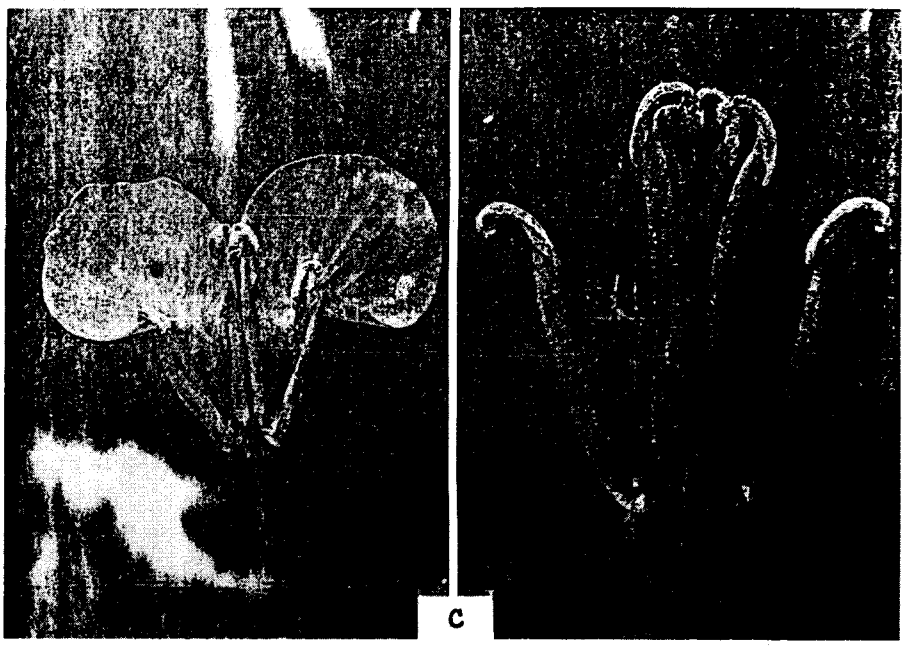

Fig.1 Morphology of male sterile flower induced by MG_1, and normal flower in rapeseed.

a. Complete sterile flower

b. Semi sterile flower

c. Normal flower

BS plant. The plant was dwarfed in height. Buds on main inflorescence or inflorescences on upper branches could not flower and seed withered, died and finally dropped in due time when they grew to some extent.
The process of withering, drying and dropping on the same inflorescence took place primarily at young buds, then extended outward. Some useless branches appeared at the lower part of the plant during the end of flowering.

D plant. The plants stopped growing after spraying. A lot of red, dead leaves appeared at the base of the main stem, then upper leaves became red and died. Finally the whole plant died.

It was considered that the appearing of these four types of plants was mainly associated with the phase of plant growth and dosage that individual plant received.

2. Primary studies on the mechanism of male sterility induced by MG_1.

/1/ Development of pollen mother cells and microspores, Based on observation of preparated anthers, which were sprayed at the stage of stamen, pistil or petal differentiation we found pollen mother cells glued together, as well as abnormal meiosis /microspores withered at tetrad phase or abnormal pollen grains formed/. When sprayed at meiosis the microspores of tetrad also withered or the tetraspores remained to be uninucleate and produced abnormal pollens. Generally, spraying a little later might cause pollen abortion and lose of vigour.

/2/ The composition of free amino acids in anther. The composition of free amino acids in anther of CS plant induced by the male gametocide was analyzed by thin-layer chromatography and was compared with that of both normal and genetic male sterile plants. Based on these observation, it was shown that the anthers of CS plant induced by the male gametocide had only three kinds of amino-acid i.e. Alanine, Aspartic acid and Asparagine and their content was very slight. These anthers were very similar to those of genetic male sterile plant, but compared with

those of normal plant they lacked six kinds of amino acid /Table 3/. The disappearance and decrease of free amino acid were the symbol of obstruction of protein metabolism in anther, which resulted in male sterility.

/3/ Variations on banding patterns of peroxidase isoenzyme in anthers.

The male steryle anthers lack one band at monokaryon stage, and no band at trikaryon stage /Fig.2/

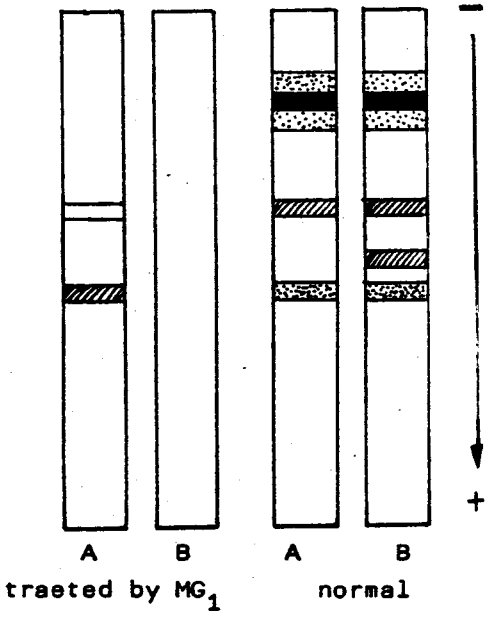

Fig.2 Variations on banding patterns of peroxidase isoenzyme in anthers
A-- monokaryon stage, B--trikaryon stage

/4/ Respiratory intensity in anthers.

The respiratory intensity of anthers in CS plants induced by male gemetocide was weaker than that of normal during both bud and flowering stage /Table 4/.

3. Heterosis in seed yield of hybrids produced by male gametocide.

Some studies have been done on male sterility of rapeseed induced by the male gametocide in recent years and

this method has been applied to hybrid seeds production. The hybrid seeds were produced in insolated plots with a row ratio of 2/♀/ : 2/♂/ or 3/♀/ : 2/♂/. More than 15 kg hybrid seeds per mu could be harvested. The hybrids produced by male gametocide showed great heterosis of vigorous growth, number of branches, number of siliqua and high seed yielding /increasing 10-20% or more than that of commercial cultivars/. The seed yield of crosses Xianyou 5 x Chuanyou 9 was 13.43% to 35.27% over the better parent. This new technique has been applied.

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Table 3. The composition of free amino acids in anthers of plants with male sterility induced by the male gametocide.

Materials	Phe	Val	2-amino-butyric acid	Ala	Glu	Asp	Asn	Gln	Lys or Arg
Male sterility of Xian you 5 induced by the male gametocide	-	-	-	++	-	++	+++	-	-
Normal Xianyon 5	+++	++	+	+++	+++	+++	+++	++	++
Xianai A/CMS/	-	-	-	++	-	++	+++	-	-

+++ a lot in content, ++ intermediate, + trail, - zero

Table 4. Respiratory intensity between normal plant and male sterile plant induced by male-gametocide /exchange of gas ml/g min/.

	Xianyou 5		Sheng Ii	
	bud stage	flowering stage	bud stage	flowering stage
Male sterility	4.1903	14.0193	2.2324	14.7143
Normal	4.6535	18.2919	6.2324	16.8888