Male sterility of *Brassica* induced by herbicide tribenuron-methyl

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Abstract

A sulfonylurea Herbicide tribenuron-methyl was used to induce male sterility in *B.carinata*, *B.rapa, B.juncea, and B.napus* at the rate of 0.5μg per plant. Application at the bolting-stage with ⩽2mm longest buds and reaped 10 days later resulted in 92%, 98%, 99% and 100% plants to be male sterile in *B.juncea, B.carinata, B.napus*, and *B.rapa,* respectively, as well as some slight phytotoxicity, i.e. reduction of plant height, space between floral buds and size of floral organs. The results suggested that tribenuron-methyl could be used as an efficient chemical hybridizing agent for *Brassica*.

Key words: *Brassica*; male sterility, chemical hybridizing agent, herbicide

Brassica is very important resource of vegetable, cooking oil, and condiment for human being. Significant heterosis for seed yield and other agronomic traits had been well documented in *Brassica*. The use of hybrids in agricultural crops is considered as an important technique to enhance plant production and value. The ability to prevent the selfing of the female parent, which would depress the seed purity, is very important in producing hybrid seed. Therefore, the exploitation of male sterility is the major approach to ensure outcrossing in the female parent and economically produce F_1 seed in large scale.

Using of some chemical substances called chemical hybridization agents (CHAs) or gametocides to induce male sterility in female parents provide a rapid, flexible and effective system for hybrid production. Theoretically, almost any normal inbred line may serve as a female parent and does not require establishing a CMS or NMS system. This will substantially reduce the time required for hybrid development. A good CHA should have the following characteristics: it should not cause significant environmental or health risk, and should be economic and easy to apply. The treated line should not in general be harmed by the CHA, and there should be flexibility in time of application to overcome adverse weather effects.

In rapeseed, much effort have been made to identify an effective and safe CHA chemicals but without much success. Researchers had developed various types of CHAs (Guan et al 1998, Guan and Stringam 1998, Singh and Chauhan 2004). However, to our knowledge, only zinc methyl arsenate was ever used in producing commercial hybrid in *B.napus* (Guan and Stringam 1998). Some commercial hybrids including 'Shuza No.2', 'Shuza No. 3', 'Shuza No.5', 'Yuhuang No.1', 'Yuhuang No.2', 'Xaingzayou No.1', 'Xaingzayou No.6', etc, have been developed using zinc methyl arsenate, and registered in China. This demonstrated the potential use of CHAs in hybrid breeding. However, the capability of zinc methyl arsenate inducing male sterility is too low. It resulted in about only 80% complete male sterility in *B. napus* (Guan and Stringam 1998) and much lower in *B.rapa* (Tian et al 1992) as well as serious phytotoxicity. Moreover, methyl arsenate could cause pollution to environment. Therefore, development of more effective CHA without environmental and health risk is still important to utilize the heterosis of rapeseed. In a previous experiment, the herbicide Express® (Du pont, USA), which contain 75% active ingredient tribenuron-methyl, and 25% inert ingredient, was found to be effective to induce male sterility on *B.napus* at the application rate from 0.5% to 2% of the labeled rate (15g/ha) for weed control (Yu et al 2006). Tribenuron-methyl, (methyl 2-[[[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) methylamino] carbonyl] amino] sulfonyl] benzoate), is a widely used sulfonylurea herbicide owning to its superior efficacy of weed control as well as low residue, and low toxicity to animals. The present paper reports its efficacy on inducing male sterility and phytotoxic effect on some *Brassica* subspecies.

Material and method

Plant materials

B.carinata cultivar 'SVP Sao Tome', *B.rapa.* cultivar 'Tobin', *B.juncea* cultivar 'Shaanbei Huangjie', *B.napus* cultivar 'Qinyou No.3' were used in the experiment. The seeds of *B.carinata* cultivar 'SVP Sao Tome' were kindly provided by Genebank of Research Institute of Crop Production, Prague, Czech Republic.

Field experiment

Field experiment was carried out at Northwest Agriculture and Forestry University, Yangling (Longitude 108°E, Latitude 34°.15'N), China. *B.rapa, B.juncea,* and *B.napus* were sowed in autumn of 2005 but *B.carinata* was sowed in the spring of 2006. Each treated plot had 70 plants that were sowed in five two-meter-long rows, with 50 cm spacing between rows and 13 cm between plants. Every two replications were separated by the contrast (treated only with water) served as male parents. The inflorescences and upper leaves in each plot were sprayed with 2 ml of 0.25 μg a.i./ml of tribenuron-methyl per plant at the bolting stage with ⩽2mm longest floral bud. The treatments were repeated again 10 days later. The lifetime and the percentage of complete /partial sterile plants in each plot were investigated. The pollen viability was estimated under a light

microscope by Aceio Carmine staining test. Ten plants were sampled randomly from these open plants, and the data on plant height, the size of twenty flowers per plant and seed set rate were collected to estimate the phytotoxicity. Seed set rate of 10 sampled plants (open-pollinated) was calculated with a formula: seed set rate percentage = (number of seeds per treated plant / number of seeds per contrast plant) ×100.

Results and analysis

The treatments with tribenuron-methyl were found to be quite effective in inducing pollen sterility in the four *Brassica* families. The treatments resulted in 92%, 98%, 99% and 100% plants to be male sterile in *B.juncea, B.carinata, B.napus*, and *B.rapa,* respectively. The male-sterile flowers were showed in Fig.1.

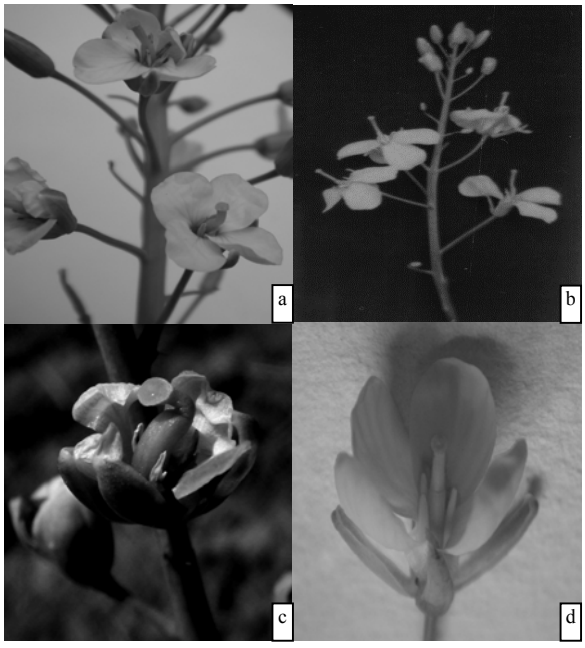

Fig. 1 Male sterile flowers induced in *B.napus* (a)*, B.juncea* (b)*, B.carinata* (c)*,* and *B. rapa* (d)

The development of vegetative and reproductive parts was suppressed due to the treatments (Table 1). The initiation of flowering of *B.carinata, B.rapa, B.napus,* and *B.juncea* were delayed 2 to 3 days. The height of treated plants decreased and the main inflorescences shortened compared to the control plants. The length of pistil, diameter of corolla was also reduced. However, the flowers opened widely, and the honey glands worked well, just like some nuclear male sterility. Thus these reduction in the size of flora organs were not serious as some inheritable male-sterility such as *Ogura* CMS and *Polima* CMS. Seed set on the plants under open pollination was 56.6%, 34.9%, 33.3%, and 49.2% for *B.carinata, B.rapa, B.juncea,* and *B.napus* compared to the corresponding control. The reduction in seed set was largely because there are not enough pollen for pollinating in male sterile plants but the reduction in the number of floral buds and ovules per ovary was also be considerable. Above all, phytotoxic effect was not serious at the test dosage of tribenuron-methyl because the seed-set is at the same level as some inheritable male-sterility such as *Ogura* CMS and *Polima* CMS in *B.rapa,* and *B.napus* under open-pollinated condition.

Table 1 Efficacy of inducing male sterility and the phytotoxicity (the differences subtracted from the corresponding contrast) of tribenuron-methyl on *Brassica*

plant	Complete sterile plants (%)	Partial sterile plants (%)	Fertile plants (%)	Pollen viability (%)	Plant height (cm)	Initiation of anthesis (day)	Diameter of corolla (mm)	Pistil length (mm)	Seed-set rate of open-pollination (%)
B.carinata	98	2	0	1.3	-5.8	+1	-1.5	-0.9	56.6
B.rapa	100	0	0	0.0	-6.1	+2	-2.0	-0.7	34.9
B.juncea	92	7	1	6.8	-15.7	+3	-1.9	-1.5	33.3
B.napus	99	1	0	0.0	-3.5	+2	-2.2	-1.1	49.2

Conclusion and discussion

Based on the obtained results, it could be concluded that the duplicate treatment with 0.5μg tribenuron-methyl appeared to be sufficient to induce over 92% plants in *B.napus, B.carinata, B.juncea,* and *B. rapa* to be male sterile under investigated conditions. If partial male-sterile plants were rogued from hybrid-producing field, it is possible to produce hybrid with high varietal purity.

Tribenuron-methyl is suggested to be a promising CHA for *B.rapa, B.napus*, B.carinata, *B.juncea* because of higher efficacy than methyl arsenate, the CHA ever being successfully used in China, very low cost, low residue and low toxicity to animals at the application rate.

B. carinata has several characteristics such as non-dehiscent siliques and tolerance to heat and drought, and resistance to blackleg disease and aphids and flea beetles. So it has been thought as a promising crop in Africa, Canada, and India. However, hybrid breeding for this crop is hampered by unavailability of male sterility system. High level of male sterility induced by tribenuron-methyl brings an opportunity for utility the heterosis of *B. carinata*.

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