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The Sulphonylurea Herbicide Monosulphuron Ester Sodium as a Special Male Gametocide in *Brassica napus* L.

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Abstract

Cheng Y.-F., Cui J.-M., Li Z.-J., Hu Z.-X., Xing Z.-N., Wang J., Zhao H.-X., Hu S.-W. (2015): The sulphonylurea herbicide monosulphuron ester sodium as a special male gametocide in *Brassica napus* L. Czech. J. Genet. Plant Breed., 51: 16–22.

The sulphonylurea herbicide monosulphuron ester sodium (MES) was first used to induce male sterility in rapeseed (*Brassica napus* L.). Nearly 100% male sterility was achieved after a single treatment with 10 ml of 0.05–0.10 μg/ml MES solution per plant to the cultivars Zhongshuang No.9 and Zhongshuang No.11 at the uninucleate stage of the longest buds. No adverse effects on main agronomic traits and female fertility were observed. In the subsequent experiment, 48 out of 49 different accessions showed male sterility after spraying with 10 ml of 0.10 μg/ml MES solution per plant. Seven out of these 48 accessions were male sterile for the entire flowering period. Seed number per plant of these MES-treated plants was not significantly reduced compared to the controls. The hybridity was 95.5% in the case of seeds harvested from the ZS09 inbred line if sprayed twice with 10 ml of 0.10 μg/ml MES solution per plant and pollinated by the SH11 male line. These results suggest that MES is an efficient chemical hybridising agent for *B. napus*.

Keywords: chemical hybridising agent; male sterility; MES; rapeseed

Commercial production of hybrids in bisexual plant species often depends on male sterility. Cytoplasmic male sterility (CMS) and nuclear male sterility (NMS) have been widely used to economically produce hybrid seeds on a large scale, but they are tedious and time consuming. In contrast, the chemical hybridising agent (CHA) approach is a rapid, flexible, and effective method (Guan 2012). The availability of safe and selective chemicals capable of inducing male sterility without causing any significant adverse effects on plant growth and development is a prerequisite for this approach.

In rapeseed, several chemicals are used to induce male sterility (Guan *et al*. 1981, 1998; Guan 1995, 2012; Guan & Stringam 1998; Zhang *et al*. 1999; Chen *et al*. 2002; Singh & Chauhan 2003; Yu *et al*. 2005, 2009; Yan *et al*. 2006; Liu *et al*. 2007; Jing *et al*. 2008; Yu & He 2014). To our knowledge, only methyl arsenates, tribenuron-methyl, and amidosulphuron have been used to produce commercial hybrid seeds on a large scale (Guan & Stringam 1998; Yu *et al*. 2006, 2009; Yu & He 2014). However, methyl arsenates are environmentally hazardous. Tribenuron-methyl (Yu *et al*. 2006; Zhang *et al*.

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2010) and amidosulphuron (Yu *et al*. 2009), developed by DuPont (Wilmington,USA) and Aventis (Paris, France), respectively, do not meet the requirement of breeding practice in China. Currently, several dozen commercial hybrids based on CHA-induced male sterility have been registered according to the Bulletin of the Chinese National Crop Variety Approval Committee (Guan 2012). Indeed, CHA-induced male sterility is increasingly becoming an important approach for exploiting rapeseed heterosis in China.

Monosulphuron ester sodium (MES) is a new sulfonylurea herbicide designed by Nankai University, China, in 2007 (Kou *et al*. 2006, 2013) and has no environmental or health risks (Wang *et al*. 2008). Now, MES is commercially available (Kou *et al*. 2013). In this report, we first tested MES as a rapeseed CHA. The objectives of the study were (1) to find a suitable MES concentration to induce male sterility, (2) to compare the male sterility-inducing ability among different accessions, (3) to test the efficiency of MES by using isolated hybrid seed production trials. Our results suggested MES as an efficient CHA for producing hybrid seeds in *B. napus*.

MATERIAL AND METHODS

Material. MES was obtained from Professor Zhengming Li, Nankai University, Tianjin, China. MES was prepared as 500 ml of 20 mg/l MES stock solution with 5 ml of dimethylformamide solvent and 3 µl of Tween-80 as an emulsifier. The control contained an equal concentration of dimethylformamide and Tween-80 without MES. Fifty-three rapeseed accessions (*B. napus*) were used in the experiment, including cvs. Zhongshuang No.9, Zhongshuang No.11, ZS09, SH11, and 49 others (Table 1). One hundred and twenty pairs of simple sequence repeat (SSR) primers (Piquemal *et al.* 2005; Bus *et al.* 2011; the Website of Brassica DB, http://ukcrop.net/perl/ace/search/BrassicaDB) were obtained from the Shanghai Shenggong Company Laboratory.

Design of field experiments. Field experiments were performed on the experimental farm of Northwest A&F University, Yangling (longitude 108°E, latitude 34°15'N), Shaanxi, China, during the crop seasons from autumn of 2008 to 2013. Conventional agronomic practices were followed. In 2008–2009, six treatments on two rapeseed cultivars, Zhongshuang No.9 and Zhongshuang No.11, i.e. 10 ml of 0.00, 0.01, 0.05, 0.10, 0.50, and 1.00 µg/ml of MES per plant, were arranged in completely randomised blocks with three replicates. A group of 100 plants was grown in each plot, which consisted of five 2-m long rows, with a distance of 500 mm between rows and 100 mm between plants. When the plants of these two accessions were at the bolting stage with the longest floral bud < 2 mm, i.e. the uninucleate stage of pollen development (Guan 1995), foliar spraying with one of the MES dosages using a hand sprayer was applied to each plot.

A split plot factorial experiment with three replicates was performed to test the effect of MES on the 49 accessions (Table 1) of different origins for two crop seasons from 2009 to 2011. Each plot contained 100 plants at the same density as in the previous experiment. Each accession was sprayed with 10 ml of 0.10 µg/ml of MES solution per plant at the uninucleate stage.

Observation of agronomic traits and estimation of pollen viability. Flowering time and the percentages of male sterile plants were recorded in each plot. Three or four inflorescences of 10 randomly sampled plants from each plot were bagged and manually self-pollinated to estimate the maximum selfed seed set of the female parents. At maturity, ten plants were also sampled randomly from the remaining open plants, and data on seed setting rate, plant height, siliques per plant, seed number per silique, and seed number per plant were collected. The seed setting rate of 10 sampled plants (selfed and open pollinated) was calculated using the formula: seed-set rate (%) = (number of seeds per treated plant/number of seeds per control plant) × 100. Seed number per plant was calculated using the formula: seed number per plant = siliques per plant × seed number per silique.

Pollen viability was estimated by acetocarmine staining (Yu *et al*. 2009).

Isolated seed production trial. An isolated seed production trial was carried out in 2011–2012. The plants of the female parent ZS09 and the male parent SH11 were grown in a plot consisting of 13 10-m long rows at the same density as in the previous experiment, with a 2:1 female and male ratio. Each ZS09 plant was sprayed with 10 ml of 0.10 µg/ml MES solution at the bolting stage, which was repeated 15 days later, and fertilised by honeybees and/or the wind. Seeds from the treated female plants were harvested at maturity.

PCR genotyping of hybrids. The hybridity of ZS09 × SH11 hybrid seeds obtained from the above described isolated seed production trial was tested using the SSR technique with 200 F_1 seedlings accord-

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Table 1. Performance of some important traits of 49 rapeseed accessions by foliar spraying with monosulphuron ester sodium (MES) at 0.1 μg/ml concentration

No.	Accessions	Sterile period (days)	Percentage of sterile plant (%)	Plant height (cm)	Siliques per plant	Seed No.	
						per silique	per plant
1	82089	7 (20)	100.0	96.1 (139.4)	256.8 (359.8)	10.3 (17.7)	5947.2 (6361.3)
2	C3	20 (23)	100.0	123.2 (156.2)	322.8 (475.8)	23.1 (23.2)	7443.9 (11019.6)
3	638	22 (22)	100.0	157.6 (166.8)	481.8 (464.8)	13.2 (12.7)	6350.2 (5894.4)
4	220	0 (20)	0.0	151.8 (147.6)	261.6 (359.8)	22.5 (23.0)	5891.3 (8830.4)
5	Qin7R	15 (22)	100.0	160.6 (155.0)	330.4 (287.0)	22.2 (24.7)	7341.4 (7095.4)
6	Huyou15-1	18 (24)	100.0	137.4 (152.6)	430.6 (425.0)	22.4 (24.8)	9654.3 (10532.0)
7	Huyou15-2	19 (24)	100.0	141.4 (155.6)	333.8 (337.6)	22.0 (24.9)	7350.0 (8393.0)
8	Ningyou18	15 (22)	100.0	156.6 (161.4)	338.8 (326.4)	20.4 (22.0)	6924.3 (7194.4)
9	ZA7	18 (20)	100.0	149.4 (169.8)	376.4 (393.0)	24.1 (21.9)	9056.3 (8614.9)
10	Huyou15-3	20 (23)	100.0	97.0 (132.2)	310.4 (434.8)	22.3 (23.2)	6934.0 (10105.0)
11	Z6C	21 (23)	100.0	143.4 (147.6)	288.6 (379.4)	20.7 (20.8)	5968.2 (7899.3)
12	D1526	20 (23)	100.0	133.2 (158.6)	312.4 (411.6)	21.8 (24.5)	6798.0 (10092.4)
13	D1560	20 (20)	100.0	90.6 (115.8)	371.4 (290.6)	21.7 (22.4)	8045.4 (6498.4)
14	QSC	15 (19)	100.0	140.4 (170.4)	356.8 (324.4)	24.7 (22.4)	8798.5 (7273.1)
15	Zheshuang72	21 (23)	100.0	142.6 (147.0)	324.8 (345.0)	24.3 (22.8)	7905.6 (7865.9)
16	Y6	13 (18)	100.0	140.2 (169.2)	268.6 (317.4)	23.0 (18.6)	6182.8 (5910.0)
17	Y7	13 (19)	100.0	165.6 (173.8)	354.8 (368.0)	21.5 (21.2)	7642.4 (7794.1)
18	C103	12 (20)	100.0	129.6 (155.0)	340.2 (344.2)	18.1 (17.2)	6151.0 (5906.0)
19	SH11	12 (19)	100.0	119.0 (177.0)	481.4 (364.2)	21.3 (18.8)	10234.7 (6832.4)
20	2000-5	20 (20)	100.0	105.2 (169.4)	357.6 (715.8)	20.2 (21.5)	7238.0 (5031.1)
21	9722	20 (20)	100.0	128.4 (161.6)	481.4 (297.6)	22.7 (25.7)	10927.6 (7648.0)
22	Gailiangzhong 2	20 (20)	100.0	105.2 (135.2)	357.6 (285.6)	22.9 (22.4)	8181.6 (6403.2)
23	D89	12 (20)	100.0	142.0 (160.8)	371.0 (311.8)	23.78 (20.7)	8784.9 (6466.7)
24	Zhongshuang No.7	15 (18)	100.0	131.2 (128.6)	393.4 (279.8)	27.2 (26.5)	10700.1 (7415.4)
25	Zhongshuang No.6	15 (20)	100.0	141.6 (129.2)	414.2 (325.2)	25.9 (22.0)	10744.4 (7161.4)
26	Zhongshuang No.5	16 (20)	100.0	126.4 (136.4)	258.4 (278.6)	24.3 (25.4)	6278.7 (7065.0)
27	Zhongshuang No.4	16 (19)	100.0	124.6 (163.0)	359.4 (329.2)	20.9 (22.6)	7526.4 (7453.3)
28	Zhongshuang No.2	20 (22)	100.0	158.4 (145.8)	326.2 (312.4)	26.6 (22.6)	8664.3 (7065.8)
29	Zhongshuang No.9-1	20 (20)	100.0	117.2 (116.4)	287.6 (243.2)	20.1 (24.1)	5774.8 (5850.7)
30	Zhongshuang No.9-2	20 (20)	100.0	120.8 (144.4)	396.2 (360.0)	21.0 (24.4)	8335.6 (8770.3)

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Table 1 to be continued

No.	Accessions	Sterile period (days)	Percentage of sterile plant (%)	Plant height (cm)	Siliques per plant	Seed No.	
						per silique	per plant
31	Zunyou No.1	15 (18)	100.0	127.4 (154.2)	385.6 (376.8)	24.5 (25.5)	9439.1 (9623.3)
32	Qingyou14	13 (22)	100.0	135.6 (138.6)	213.8 (311.2)	24.0 (22.8)	5123.4 (7095.0)
33	Zhongshuang No.10	12 (20)	100.0	123.8 (129.4)	260.4 (322.0)	23.5 (24.6)	6118.3 (7908.0)
34	Oaza	15 (18)	100.0	148.8 (126.8)	339.4 (211.0)	22.0 (23.1)	7453.1 (4866.2)
35	Soo-3	16 (18)	100.0	146.6 (140.0)	413.4 (358.8)	16.1 (20.7)	6647.4 (7420.4)
36	Odila	15 (18)	100.0	176.2 (164.0)	402.8 (414.2)	21.0 (22.8)	8458.9 (9443.8)
37	Slogan	15 (18)	100.0	130.6 (133.6)	393.0 (268.8)	22.6 (25.2)	8866.4 (6773.8)
38	Sonata	14 (19)	100.0	134.3 (137.2)	378.4 (283.6)	16.6 (25.4)	6274.4 (7192.4)
39	ZL-02-4	16 (19)	100.0	107.6 (141.2)	306.8 (353.4)	15.8 (23.7)	4835.0 (8382.6)
40	ZL-Var-55	14 (19)	100.0	128.6 (133.2)	352.2 (285.4)	16.0 (20.1)	5635.4 (5748.3)
41	KS3018	10 (20)	100.0	127.4 (139.2)	377.0 (411.0)	21.6 (25.1)	8157.9 (10324.3)
42	KS4114	15 (20)	100.0	126.6 (153.4)	432.6 (356.4)	16.3 (24.1)	7060.0 (8589.0)
43	KS4322	14 (20)	100.0	153.6 (155.2)	280.6 (334.0)	15.7 (24.5)	4411.1 (8176.3)
44	KS2185	14 (19)	100.0	119.8 (126.0)	411.2 (255.6)	23.8 (22.2)	9787.4 (5684.6)
45	KS4085	14 (19)	100.0	121.6 (158.8)	439.4 (346.4)	21.9 (23.3)	9613.9 (8071.2)
46	KS3302	12 (22)	100.0	152.6 (146.0)	481.6 (344.8)	16.6 (23.3)	7974.6 (8027.1)
47	9F47	15 (17)	100.0	137.6 (172.6)	541.2 (393.8)	18.2 (20.3)	8822.4 (7654.7)
48	Bronowski	16 (18)	100.0	119.0 (129.4)	223.8 (187.8)	27.4 (25.6)	4073.3 (3805.0)
49	SD138	14 (16)	100.0	110.4 (150.8)	296.2 (294.6)	24.3 (25.4)	8110.0 (7548.9)
Control[1]		19.9 ± 2.2		148.40 ± 15.68	344.03 ± 0.29	22.6 ± 2.6	7526.3 ± 1516.4
MES- treatment[1]		15.5 ± 4.0**		132.75 ± 18.63**	356.63 ± 71.90	21.1 ± 3.6**	7564.4 ± 1658.1

Data in parentheses indicate the control values, for sterile period, refers to the flowering period in controls; [1]data were expressed by mean ± SD; **meaning significantly different between the MES treatment and the control at 0.01 level

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ing to the protocol described by Song *et al*. (2011). The hybridity of the hybrid was calculated by the formula: hybridity% = (sum of individuals with typical hybrid SSR pattern/total sum of samples) × 100.

Statistics. To compare male sterility-inducing ability among different accessions, analysis of variance for the observed traits was performed using the split plot factorial design model with Design of Experiment, Processing of Data, Simulation Analysis DPS software (Tang & Feng 1997). Comparisons of the observed traits between the MES-treatment and the control were conducted using one-way ANOVA program of the SPSS 11.0 software (SPSS Inc., 2001).

RESULTS

Suitable MES concentration and its male sterility-inducing ability among different accessions. Two rapeseed accessions, Zhongshuang No.9 and Zhongshuang No.11, were used in a pilot experiment to determine the range of MES concentrations that induce male sterility. As a result, 0.05–0.10 μg/ml MES solution was a suitable concentration range that induced 99%–100% complete male sterility (Figure 1) in the two rapeseed accessions. Male sterility was maintained nearly to the end of the flowering period, without significant side-effects on seed yield (the data are not shown).

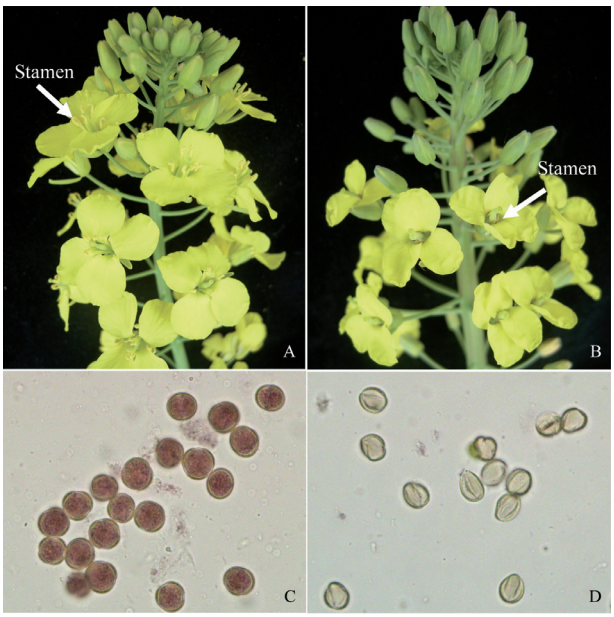

Figure 1. Comparison between the fertile (A, C) and sterile (B, D) flowers, stamens and pollen; Viable pollen was stained red with acetocarmine (C), but inviable pollen was not stained (D)

Forty-nine different rapeseed accessions were used to investigate the male sterility-inducing ability of 0.10 μg/ml MES solution in 2009–2011. Significant differences between the 49 rapeseed accessions were observed for the tested traits (the data are not shown). The results indicated that except for accession 220, male sterility could be induced in the remaining 48 accessions (Table 1). The sterile period of seven of these 48 accessions lasted the entire flowering period, although a significant difference in flowering period was observed between the MES-treated and control plants (Table 1). Among the four agronomic traits tested, only plant height and seed number per silique decreased significantly in the treated plants. Siliques per plant and seed number per plant were not significantly affected (Table 1). Spraying with 0.10 μg/ml MES solution once was inadequate to maintain male sterility for the different rapeseed accessions.

Isolated hybrid seed production trial. One pair of SSR primer, BRMS-042 (forward primer, GGATCAGTTATCTGCACCACAA, reverse primer, TCGGAATTGGATAAGAATTCAA), was screened out from 120 pairs of SSR primers which could amplify a polymorphic locus between the parent lines ZS09 and SH11. It amplified a 300bp band and a 200bp band in line SH11 and ZS09, respectively, and both bands in the ZS09 × SH11 hybrid. So this pair of primer was used to identify the hybridity of the hybrid seeds of ZS09 × SH11. The results of the hybrid production trial in 2012 showed that the average hybridity of seeds from the female line ZS09 treated twice with 10 ml of 0.10 μg/ml MES solution per plant and pollinated by a male parent SH11 was 95.5% according to the SSR analysis, which met the hybridity requirement in China (Ministry of Agriculture of P.R. China 2000).

On the basis of these results we concluded that twofold treatment with 1.0 μg MES solution per plant was sufficient to induce > 95% of male sterile plants and to produce hybrid seeds under the conditions investigated.

DISCUSSION

Establishing a highly effective low-pollution CHA approach is critical for using crop heterosis. Our group previously identified that two acetolactate synthase inhibitor herbicides, tribenuron-methyl and amidosulphuron, can induce complete male sterility in rapeseed when applied twice at a dosage

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of 30–100 mg/ha (Yu *et al*. 2006, 2009). In this investigation, we reported that MES, also an acetolactate synthase inhibitor herbicide, induced complete male sterility in 48 out of 49 rapeseed accessions when applied at a concentration < 1%. Such dosage is 100 times lower than the dosage that is required for herbicidal activity, without significant adverse effects on seed number per plant. However, only seven out of 48 accessions were male sterile for the entire flowering period with a one-time application of MES solution. Hence, twofold spraying of 10 ml of 0.10 µg/ml MES solution per plant (equivalent to 150 mg/ha) at the bolting stage and then after 15 days was required to maintain male sterility for the entire flowering period. As a result, seed purity reached 95.5% in the ZS09 × SH11 cross. Concerning the level of male sterility, time efficiency, dosage range, and yield parameters, the efficiency of MES as gametocide is comparable with two previously reported chemicals, tribenuron-methyl and amidosulphuron in rapeseed (Yu *et al.* 2006, 2009). MES is very cheap and does not pose any environmental hazard or health risk (Kou *et al*. 2006; Wang *et al*. 2008). Therefore, this study provides an alternative CHA for rapeseed hybrid breeding.

Moreover, the sulphonylurea herbicide MES proved to be an efficient CHA for *B. napus* at a concentration of approximately 1% of that needed for herbicidal activity.

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