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Outcrossability of *Brassica napus* L. and *B. rapa* L. in an Experimental Field

Makoto YAMAMORI[1]*

National Agriculture Research Center for Tohoku Region, National Agriculture and Food Research Organization (NARO) (Morioka, Iwate 020–0198, Japan)

Abstract

The outcrossing rate of oilseed rape (*Brassica napus*) between two cultivars was assessed in an experimental field. During flowering (from the end April to the end of May), the wind from the pollen donor (♂) to the seed parent (♀) prevailed. The crossing rate (%) of the seed parent decreased as the distance from the pollen donor parent increased; 4.1% at 0.25 m distance, 1.4% at 1 m, 0.4% at 5 m, 0.15% at 10 m, 0.09% at 30 m, and 0.01% at 60 m. In another experiment, the hybridization rate between 55 cultivars of *B. rapa* (♀) and *B. napus* (♂) cultivars was assessed by placing *rapa*-planted pots in a *napus* field. The rate of 55 *rapa* cultivars ranged from 2% to 50% in the field, which might show the *rapa* cultivar-differences of crossability between the two species.

Discipline: Plant breeding
Additional key words: hybridization, pollen dispersal, GM crop

Introduction

Oilseed rape (*Brassica napus* L., AACC genome) is a self-compatible plant and has an outcrossing trait (hybridization by pollens from other individuals). The outcrossing of oilseed rape is caused by both wind and insects. Since outcrossed seeds will produce progenies with segregated characteristics, outcrossing frequency is important in seed production by oilseed rape breeders or seed farms. Tasaka (1943)[11] examined the outcrossing of a Japanese cultivar in an experimental breeding field, and showed 19.5% hybridization at 1.8 m distant from the pollen parent, 8.5% at 5.4 m, etc. Interplant outcrossing rates reported by foreign researchers range from 12% to 55% (average 30%)[1]. However, since then, there has been no Japanese research on the outcrossing of oilseed rape.

The outcrossing of genetically modified (GM) crops, including oilseed rape, requires research because non-GM cultivars would be pollinated by GM cultivars when farmers cultivate them in the same area. GM oilseed rape has been cultivated in Canada, and Japan imports oilseed rape mainly from Canada (annually, about two million tons). While GM oilseed rape (*B. napus*) has not yet been cultivated in Japan, the outcrossing rate was examined in this study in order to expand knowledge about the outcrossability of *B. napus*. This paper describes the relationship between the outcrossing rate (%) of a seed parent (recipient) and the distance from a pollen parent (donor) in a field of a *napus* cultivar (Experiment 1). Another experiment (Experiment 2) shows spontaneous hybridization between *B. napus* cultivar Nanashikibu or Tohoku 96 (donor) and 55 *B. rapa* cultivars (recipient) in a field to elucidate the variation of hybridization frequencies among *rapa* cultivars.

Materials and methods

1. Experiment 1: Outcrossing rate of an oilseed rape cultivar (*B. napus*) at different distances from the pollen donor field

An oilseed cultivar, Kamikitanatane, which contains about 45% erucic acid in seed oil was used as the pollen parent (pollen donor, ♂), and Kirariboshi, which does not contain erucic acid, (0%) as the seed parent (pollen recipient, ♀). These cultivars were sown in September 2003, 2004, 2006, and 2007, and harvested in June of the following year. The field of Kamikitanatane (♂) was 30 ×

Present address:
[1] National Institute of Crop Science, NARO (Tsukuba, Ibaraki 305–8518, Japan)
*Corresponding author: e-mail yamamori@affrc.go.jp
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10 m and that of Kirariboshi (♀) was 30 × 60.5 m (Fig. 1). Both cultivars were drill-seeded at 50-cm intervals in 30-m-long rows and ca 400 g/10a. Seeds set in Kirariboshi were harvested from one or two plants at three sites (A, B, C), which were 0.25, 1, 5, 10, 30, and 60 m apart from the Kamikitanatane field. Three pot-planted Kirariboshi in the Kamikitanatane field were also harvested in order to examine the interplant crossing of Kirariboshi. The wind during the flowering of the cultivars was monitored, and the average wind direction and average wind speed at every hour were recorded.

The erucic acid content of the seeds was measured by gas chromatography. When a harvested seed of Kirariboshi contained erucic acid, it was judged to be a seed outcrossed by the pollen of Kamikitanatane.

2. Experiment 2: Crossing between *B. napus* cv. Nanashikibu or Tohoku 96 (♂) and 55 *B. rapa* cultivars (♀)

Fifty-five *B. rapa* cultivars were used (Table 1), of which 20 *B. rapa var. nippo-oleifera* were provided by the National Institute of Agrobiological Sciences, and the other *var. rapa*, *nipposinica*, *chinensis*, *pekinensis* and *perviridis* were purchased from seed companies. In 2006, a field (25 x 20 m) of *B. napus* cv. Nanashikibu in 60-cm interval rows was used as pollen parent (♂), and cv. Tohoku 96 drill-seeded in 50-cm interval rows (29 x 10 m) was used in 2007. In 2006, 29 *B. rapa* cultivars (♀) and in 2007, 26 *rapa* cultivars were seeded in a greenhouse. *B. rapa* cultivars were transplanted into 16 cm-diameter pots (mostly six pots and one plant per pot) at the beginning of April. *B. rapa* tended to start flowering earlier than *B. napus*. Therefore, after removing the open flowers of *B. rapa*, the pots were moved into the ♂ field at about the end of April. The pots were divided into two groups, e.g., 3+3 or 3+2 pots, and each group was randomly placed at 2-m intervals in rows (Fig. 2). The flowers of ♀ were set about the same height of the ♂ flowers to allow greater hybridization. At the end of flowering, the pots were moved into a glasshouse to avoid rain, and the seeds were harvested at maturity. One-hundred seeds were analyzed by flow cytometry (Ploidy analyzer, PA, Partec Co.), and crossed seeds (2n= 29) could be identified because they contained DNA amounts between *napus* (2n=38) and *rapa* (2n=20).

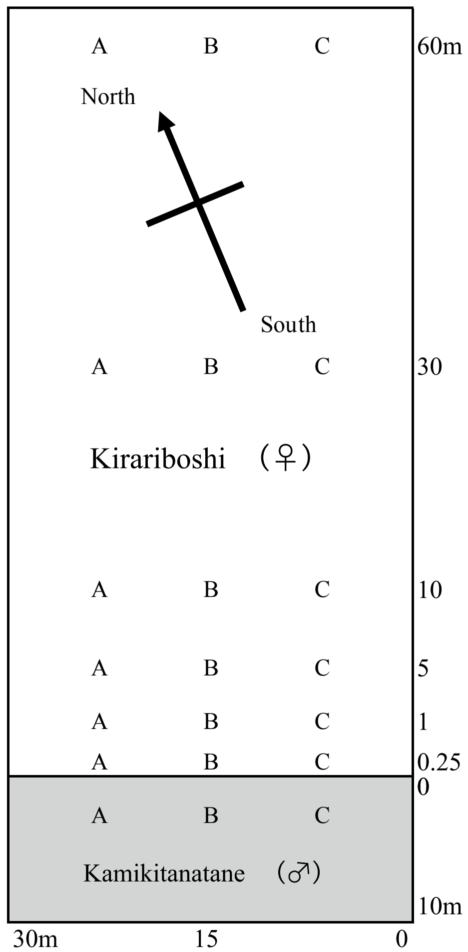

Fig. 1. Harvesting sites A, B, and C in the experimental field

Results and discussion

1. Experiment 1

The flowering durations of the two cultivars Kamikitanatane and Kirariboshi were almost the same, i.e., from late April or early May to the end of May or the beginning of June. The prevailing wind direction (Fig. 3) over the four years was south-southwest (SSW, 20.9%) and south (18.6%), and the average wind speed was 2.14 m/s for SSW and 1.82 m/s for south. Since the data suggest pollen flow by the wind is from Kamikitanatane (♂) to Kirariboshi (♀), the pollen parent and seed parent were suitably arranged in the experimental field. While insects, honeybees, etc., are pollinators for the fertilization of oilseed rape, the contribution of insects to outcrossing was not analyzed in this study. Three years of observation of insect fauna in the field[9] showed more than 75 species were caught around oilseed rape flowers, and the honeybee seemed to be important as a pollinator because it had a comparatively large body size and was a major flower-visiting insect. Other insects seemed not to be important pollinators because they were smaller, they did not move as much among the flowers, they seemed not to visit the flowers, or they were minor visitors, etc.[9] Another report[7] points out the importance of the honeybee and the bumblebee as a pollinator.

Seeds harvested from the three sites A, B and C were summed and the crossing rate was calculated (Table 2). The outcrossing of the pot-planted Kirariboshi in the Kamikitanatane field ranged from 9.4% to 19.0% (11.6% on average). The crossing rate of the Kirariboshi decreased as the distance from the Kamikitanatane field increased: 1.4% at 1-m distance, 0.4% at 5 m, 0.2 % at 10 m, and 0.09% at 30 m. The rate was 0.01% at 60 m (the most distant site in this experiment), which showed very low frequency of outcrossing. In this study, 60-m distance was the farthest from where the seeds were harvested. While other research[1] has reported the data for more than 1,000 m distance, the present results showed the outcrossing rate decreases as the distance from the donor field increases within the examined range (0.25 – 60 m).

Tasaka (1943)[11] examined the crossing rate of a *B. napus* cultivar Ohyoshu using the pot-planted seed parent (♀). The pots were placed at 1.8 – 108 m apart from a pollen parent field with an area of 80 a. The crossing rate was 19.5% at 1.8 m from the pollen field, 7.5% at 36 m, and 3.4% at 108 m, etc. The interplant crossing rate of the Ohyoshu in the four years of experiments was 26.8%, and the crossed seedlings/seedlings examined were 3,844/14,361. These values were greater than the values

Table 1. List of *B. rapa* cultivars used

Number in Fig. 4	Cultivar name	Variety	Number of plants examined*	Number in Fig. 4	Cultivar name	Variety	Number of plants examined*
1	Mie zairai	*nippo-oleifera*	3+2	29	Tokinashi kokabu	*rapa*	3+2
2	Shakushina	*nippo-oleifera*	3+0	30	Kanamachi kokabu	*rapa*	3+3
3	Miyagiwase	*nippo-oleifera*	2+2	31	Honbeni daimaru	*rapa*	3+3
4	Naniwashu	*nippo-oleifera*	3+2	32	Honbeni akamaru kabu	*rapa*	3+3
5	Akatsukawase	*nippo-oleifera*	3+2	33	Hida akakabu	*rapa*	3+3
6	Akihoshu	*nippo-oleifera*	3+3	34	Hinona	*rapa*	3+2
7	Wasena	*nippo-oleifera*	3+2	35	Tennoji kabu	*rapa*	3+1
8	Motoippon	*nippo-oleifera*	3+3	36	Waseharihari kyoumizuna	*nipposinica*	2+2
9	Iyohi kabu	*nippo-oleifera*	3+3	37	Nakatesirokukisensuji kyoumizuna	*nipposinica*	3+3
10	Wase akana	*nippo-oleifera*	3+2	38	Wase mibuna	*nipposinica*	3+3
11	Kuma zairai	*nippo-oleifera*	3+3	39	Nakate mibuna	*nipposinica*	3+2
12	Tottori zairai	*nippo-oleifera*	3+1	40	Wase harihari605 kyoumizuna	*nipposinica*	3+3
13	Houkidane	*nippo-oleifera*	3+3	41	Okuteshirokukisensuji kyoumizuna	*nipposinica*	3+3
14	Enuma zairai	*nippo-oleifera*	3+3	42	Hiroshimana	*chinensis*	3+1
15	Aoguki	*nippo-oleifera*	3+3	43	Chingensai	*chinensis*	2+2
16	Hiki zairai	*nippo-oleifera*	3+0	44	Osaka shirona	*chinensis*	4+2
17	Nagasaki zairai	*nippo-oleifera*	3+3	45	Taasai	*chinensis*	3+3
18	Yasu zairai	*nippo-oleifera*	3+0	46	Pakuchoi	*chinensis*	3+2
19	Saga zairai	*nippo-oleifera*	3+3	47	Kyoto 3gou	*pekinensis*	3+3
20	Hokkai	*nippo-oleifera*	3+3	48	Aichi hakusai	*pekinensis*	3+2
21	Wase imaichikabu	*rapa*	3+2	49	Kaga hakusai	*pekinensis*	3+3
22	Shougoin daimaru	*rapa*	3+2	50	Ogata santousai	*pekinensis*	3+2
23	Atsumi kabu	*rapa*	3+3	51	Maruba komatsuna	*perviridis*	2+1
24	Yurugi kabu	*rapa*	3+3	52	Uzuki	*perviridis*	3+3
25	Mogami jikabu	*rapa*	3+2	53	Shougatuna (Mochina)	*perviridis*	3+3
26	Yajima kabu	*rapa*	3+2	54	Shinkuroha komatuna	*perviridis*	3+3
27	Shinkintokichou	*rapa*	3+2	55	Fukidachina	-	3+2
28	Kiso murasakikabu	*rapa*	3+3				

* : Plants of a cultivar were divided into two groups, e.g. 3+3, 3+2 etc., and placed in the field.

in this study. The crossing rate will be affected by many factors[1]: e.g., the relative size of the pollen source and seed parent plot, environmental conditions (wind speed and direction, temperature, etc.), and the presence of insects. Two cultivars in this study were cultivated by drill seeding while Tasaka (1943)[11] placed the seed-parent (cv. Ohyoshu) pots away from the pollen field, resulting in a higher density of the seed-parent Kirariboshi. Therefore, the seed-parent Kirariboshi in this experiment may have been fertilized by its pollen more easily than the Ohyoshu in Tasaka's experiment, which resulted in a smaller outcrossing rate in this study. Furthermore, the results from interplant crossing suggest the cultivar-differences between Kirariboshi (11.6%) and Ohyoshu (26.8%). Beckie et al. (2003)[1] summarized the outcrossing frequencies of *B. napus*, in which some of the data are consistent with the present study.

For detecting outcrossed seeds, this study used gas chromatography (erucic acid), which examined 45,218 seeds over the four years of experiments. For detecting crossed seeds, other researchers used other markers, e.g., herbicide resistance. They examined many more samples, e.g., 100,000 seeds per plot[8], which would produce more exact results. Research[8] in Australia used commercial fields, and the farthest field examined was 4.5 km away from the pollen source field. Compared to these, the present study examined smaller seeds and the area of the

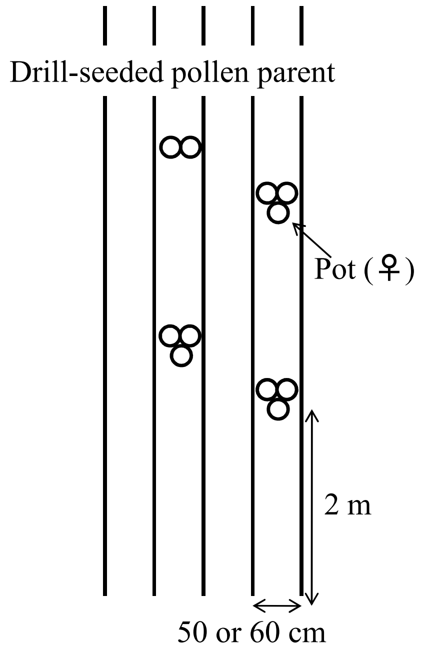

Fig. 2. Pot arrangement in the experimental field
Part of the field is shown.

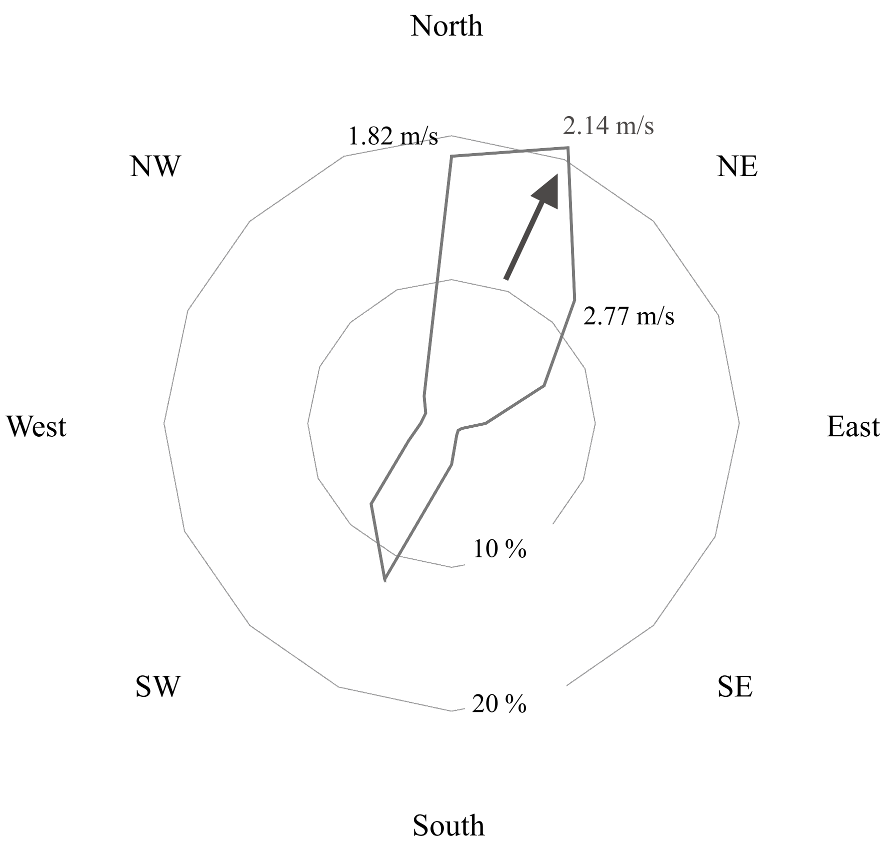

Fig. 3. Average wind direction and wind velocity over four years during flowering

experimental field was limited, and the results showed a sharp decline in the crossing rate as the distance from the pollen source increased.

Though GM oilseed rape is not cultivated in Japan, it is reported that seeds of imported GM oilseed rape happen to spill during transportation from a port and germinate on the roadside. This may become a pollen source for hybridization with non-GM oilseed rape (♀)[6]. Thus a comparatively small-sized study like this one will provide practical data for gene flow problems in Japan.

2. Experiment 2

Some of the harvested seeds from *B. rapa* (♀) precociously geminated (preharvest sprouting) in *rapa* (maternal) pods although the pots had been placed in the glasshouse. The extent of the preharvest sprouting varied among *rapa* cultivars as shown in Fig. 4. Hauser and Østergård (1999)[3] reported such precocious germination in F_1 seeds of *B. rapa* × *B. napus* but almost no precocious germination in *B. napus* × *B. rapa*. One-hundred seeds per cultivar, including geminated seeds, were analyzed by flow cytometry. In order that the outcrossing frequency reflects the precocious germination, the germinated seeds were examined roughly in proportion to the extent of the precocious germination, although the geminated seeds would not have survived in the field. The outcrossing of the 55 *rapa* cultivars ranged from 2% to 50% (average 22.8%) (Fig. 5). Analysis of variance did not show significant differences among the outcrossing rates of six varieties (P > 0.10). Although the current data are derived from a one-year experiment, the results suggest there is variation in the crossing rate among the 55 *rapa* cultivars. Since there are differences in the degree of self-incompatibility among the *rapa* cultivars[4], it may be a possible cause for the outcrossing variation among *rapa* cultivars in this study. In other words, less self-incompatibility of a cultivar may lead to less outcrossing. Syafaruddin et al. (2006)[10] showed that the number of pollen grains deposited on the pistil of seven *rapa* cultivars significantly varied under open-pollination conditions. The number of pollens from the *napus* vs *rapa* on the *rapa* pistil might differ among the rapa cultivars in the present study. This kind of factor might contribute to the present variation in the outcrossing rate. These points remain to be examined in future studies.

Jørgensen and Andersen (1994)[5] reported 93% and 56% hybrid seeds were produced in two experiments using *B. rapa* as the seed parent (♀). Since they planted single *rapa* plants in a *napus* field at intervals of 25 m, a *rapa* plant will be much less fertilized by pollens from

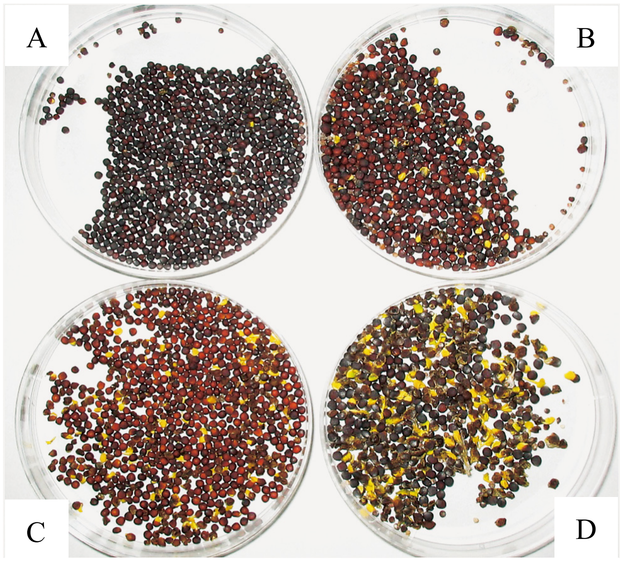

Fig. 4. Precocious germination of harvested seeds from *B. rapa*

The extent of germination increases A<B<C<D.

Table 2. Outcrossing rate (%) of Kirariboshi (♀) at diffent distances from Kamikitantane (♂)

Distance from ♂ (m)	Outcrossing rate in the year				Total		
	2004	2005	2007	2008	Number of seeds		Outcrossing rate (%)
					Examined	Outcrossed	
♂ field	-	18.97	9.42	10.42	2,068	240	11.61
0.25	2.96	4.44	5.15	3.92	5,840	239	4.09
1	1.09	1.64	-	-	3,260	44	1.35
5	0.35	0.53	-	-	3,228	14	0.43
10	0.22	0.07	-	-	3,300	5	0.15
30	0.09	0.10	0.17	0.00	12,586	11	0.09
60	0.00	0.02	0.00	0.00	14,936	1	0.01

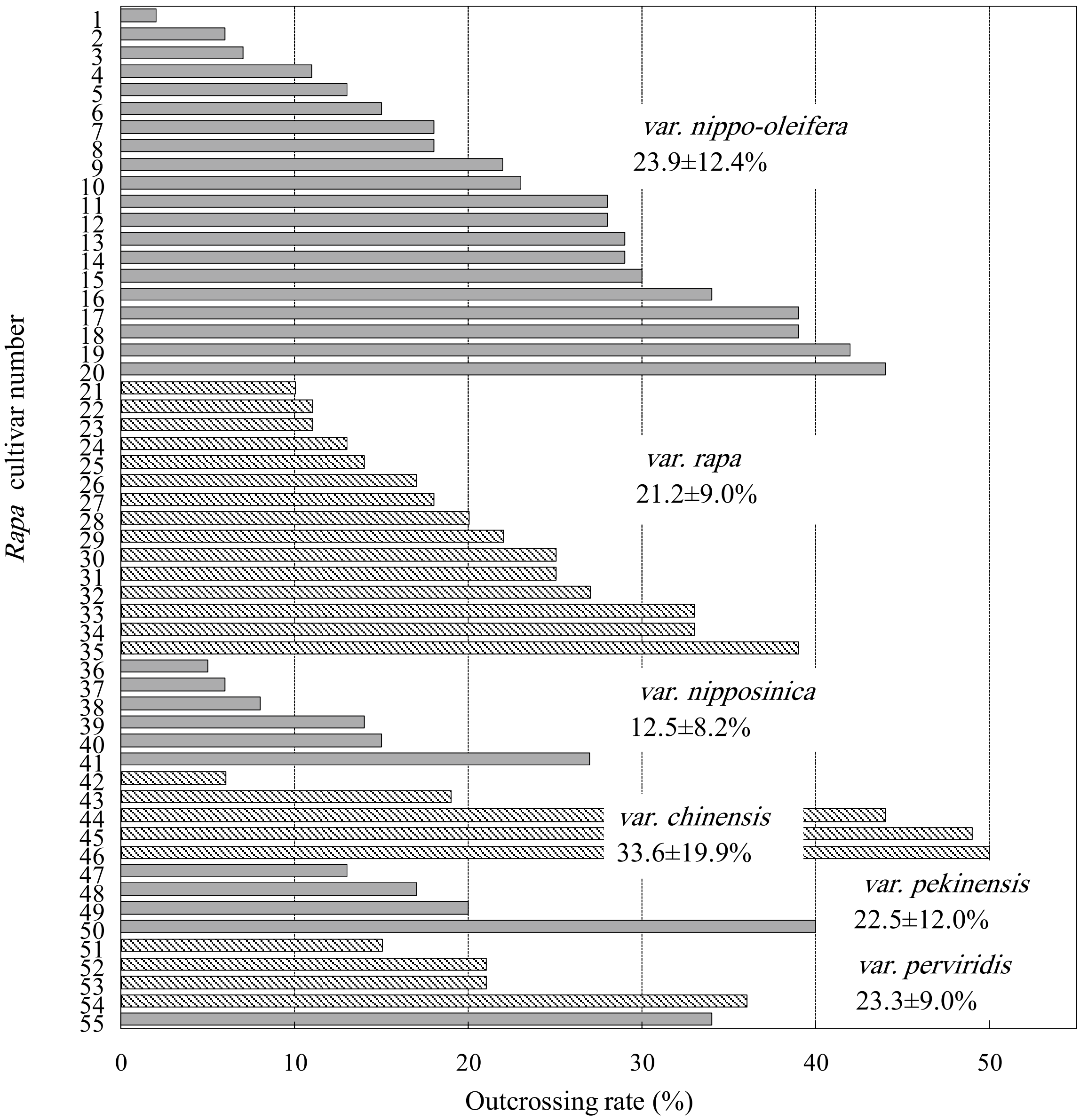

Fig. 5. Outcrossing (%) of 55 cultivars of *B. rapa*
Cultivar numbers correspond to those in Table 1.

other *rapa* plants compared to the present study. Therefore, their vales of hybrid seeds will be larger than the present results. Bing et al. (1996)[2] showed a much lower hybrid frequency when 50:50 seed mixture of *rapa* and *napus* was sown in a row, which was a higher density of *rapa* in a row than in this study. Warwick et al. (2003)[12] showed ca. 7% hybrid frequency in an experiment where *rapa* density was one plant per 1 x 1 m grid in a *napus* 5 x 5 m field. They also reported a 14% hybrid frequency in the *rapa* population collected near a commercial *napus* field. These results mean that the degree of outcrossing depends on the density and spatial distribution of *rapa* relative to *napus*[12].

Acknowledgments

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