

THE EVALUATION OF SELF-INCOMPATIBILITY AND CROSSABILITY IN CHOSEN *Brassica* SPECIES BASED ON THE OBSERVATION OF POLLEN TUBES GROWTH AND SEED SET

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Abstract. Most flowering species including *Brassica* genera have genetically controlled ability to out-crossing (gametophytic or sporophytic self-incompatibility – SI). In *Brassica*, self-incompatibility is sporophytically controlled by a single multiallelic locus, termed the *S* locus, with more than 100 haplotypes identified so far. From the breeding practice point of view, outcrossing is desired for obtaining acceptable yield level. So, the aim of the present study was to estimate the self-incompatibility and crossability in interspecific hybridization of five chosen *Brassica* species. The crossed species were: *B. napus* var. *oleifera* cv. Californium, *B. carinata*, *B. fruticulosa*, *B. rapa* ssp. *pekinensis* as well as two varieties of *B. oleracea* (var. *alboglabra* and var. *gongyloides*). The mode of pollination consist of diallel cross-pollination. The pollination was done in the glasshouse of Genetic and Plant Breeding Department of the Poznan University of Life Sciences. The crossability was evaluated on the base of the pollen germination index (PGI) and seed set in all tested cross combinations. The observations of pollen grains germination and pollen tubes penetration were made in pistils of female component of interspecific crosses. They showed unilateral incompatibility in the crosses between *B. rapa* ssp. *pekinensis* and *B. napus* var. *oleifera* cv. Californium (used as paternal form) as well as bilateral incompatibility in crosses between *B. napus* and *B. oleracea* var. *alboglabra*. Generally, better pollen germination and penetration of pollen tubes were observed in a cross combination where *B. napus* was used as a maternal parent. Both pre-zygotic and post-zygotic incompatibility barriers were found between analyzed species.

Key words: Brassicaceae, interspecific hybridization, pollen tubes, self-incompatibility, fluorescent microscopy

INTRODUCTION

Winter oilseed rape became very important crop in the world and in Poland during last two decades. Such a high increase of cultivation area is causing many problems e.g. with plant diseases. So, there is a great need for a wider variability of important traits in oilseed rape. In *Brassica*, interspecific hybridization is a potential and useful method for transferring valuable traits (e.g. seeds quality and resistance to some diseases) between species of commercial interest [Seyis *et al.* 2003]. However, interspecific hybridization between a tetraploid and a diploid *Brassica* species is difficult and failures occur at many stages starting from pollination incompatibility to pre/post-germination barriers. Most interspecific crosses do not produce mature seeds due to failure of endosperm development [Nishiyama *et al.* 1991]. Depending upon the barriers, specific techniques can be used to overcome the problems. Systematic investigations on the type of barriers and associated methods to overcome them can provide the basis for obtaining hybrids at higher frequencies and between desired genotypes. For example to overcome post-zygotic interspecific incompatibility in reciprocal crosses between *B. rapa* and *B. oleracea*. [Springer *et al.* 2007], ovule culture could be used. Similarly, the cross between *B. napus* and *B. oleracea* is normally unsuccessful, but the use of *in vitro* culture technique – embryo rescue, can produce hybrids [Weerakoon 2011, Tang 2012]. Such kinds of hybridization have been extensively studied by many researchers [Leflon *et al.* 2006, Sheikh *et al.* 2010, Bennett *et al.* 2012]. Most attempts on interspecific hybridization were based on conventional techniques of hand pollination. In these studies the frequency of hybrids was low. In addition hybrids were obtained at relatively higher frequency when *B. rapa* was used as a female parent indicating a strong incompatibility in the reverse cross [Ammitzboll *et al.* 2005]. Similar unidirectional incompatibility was also noticed when *B. napus* and *B. carinata* were synthesized and is frequently encountered in other interspecific crosses as well. The incompatibility barriers may occur at any stage from pollination to fertilization or at later stages of development to a fertile plant.

Many hermaphrodite flowering plants avoid self-fertilization through genetic systems of self-incompatibility (SI). SI allows a plant to recognize and to reject self or self-related pollen, thereby preserving its ovules for outcrossing. In the angiosperm plants exist two types of SI: sporophytic and gametophytic. In sporophytic self-incompatibility (SSI), the SI phenotype of the pollen is determined by maternal plant in which it was created. This form of SI was identified in the families: Brassicaceae, Asteraceae, Convolvulaceae, Betulaceae, Caryophyllaceae, Sterculiaceae and Polemoniaceae. In *Brassica* SI is sporophytically controlled by a single *S*-locus with multiple alleles. About half the species in the Brassicaceae family have self-incompatibility and the others are self-compatible [Franklin-Tong *et al.* 2010]. SI is important for high-quality seed production in *Brassica* vegetables, such as cabbage, turnip and Chinese cabbage. It is known, that the crossability study would give an idea on the cross compatibility relationship among the species, the direction of success of crossing and the crossability barriers of some combinations, if any.

The objective of this investigation was to check the crossability between *Brassica napus* and four another *Brassica* species and existing prezygotic barriers in hybridization of chosen *Brassica* species.

MATERIAL AND METHODS

Seeds of four species used in experiment i.e. *B. carinata* cv. Dodolla, *B. fruticulosa* (RCAT062227), *B. rapa* ssp. *pekinensis* (HRIGRU7569) and two species of *B. oleracea* var. *alboglabra* (HRIGRU9829) and var. *gongyloides* (HRIGRU005389) came from the Warwick HRI Genetic Resources Unit, while seeds of *Brassica napus* var. *oleifera* f. *biennis* cv. Californium, was from the resources of Genetics and Plant Breeding Department, Poznan University of Life Sciences (PULS). The reciprocal crosses was carried out in the glasshouse of Genetics and Plant Breeding Department during spring 2011. The plants were grown without controlled photoperiod and during flowering time (April) the temperature ranged from 24°C to 28°C. Each genotype was represented by 4 to 6 plants. The pollen of pollinators was placed on stigmas immediately after emasculation, which was done at the closed bud stage. From 40 to 174 flower buds in each cross combination were pollinated (Table 1).

Table 1. Pollen germination index (PGI) and effectiveness of self and wide hybridization of tested *Brassica* genotypes

Tabela 1. Index kiełkowania pyłku (PGI) i efektywność samo- i obcozapylenia u badanych genotypów *Brassica*

Cross combination Kombinacja krzyżowania	PGI after 48 h PGI po 48 h	No. of pollinated flowers Liczba zapylnych kwiatów	No. of seeds Liczba zawiązanych nasion	Seeds per pollination ^a Liczba nasion / zapylenie
<i>B. napus</i> – SP	2.6	48	224	4.7 ± 3.7 ^a
<i>B. rapa</i> ssp. <i>pekinensis</i> – SP	1.6	62	115	1.85 ± 2.1 ^a
<i>B. fruticulosa</i> – SP	1.1	42	17	0.4 ± 2.5 ^a
<i>B. carinata</i> – SP	2.3	56	168	3.0 ± 2.4 ^a
<i>B. oleracea</i> var. <i>alboglabra</i> – SP	0.8	71	0	0.0
<i>B. oleracea</i> var. <i>acephala</i> – SP	1.4	60	0	0.0
<i>B. napus</i> x <i>B. fruticulosa</i>	2.6	85	51	0.6 ± 1.7 ^a
<i>B. fruticulosa</i> x <i>B. napus</i>	2.7	105	0	0.0
<i>B. napus</i> x <i>B. carinata</i>	2.6	121	416	3.4 ± 3.2 ^a
<i>B. carinata</i> x <i>B. napus</i>	2.1	100	156	1.5 ± 2.5 ^a
<i>B. napus</i> x <i>B. oleracea</i> var. <i>alboglabra</i>	1.7	78	2	0.02 ± 0.8 ^a
<i>B. oleracea</i> var. <i>alboglabra</i> x <i>B. napus</i>	1.1	40	0	0.0
<i>B. napus</i> x <i>B. oleracea</i> var. <i>gongyloides</i>	2.1	82	16	0.19 ± 1.4 ^a
<i>B. oleracea</i> var. <i>gongyloides</i> x <i>B. napus</i>	2.4	174	0	0.0
<i>B. napus</i> x <i>B. rapa</i> ssp. <i>pekinensis</i>	2.8	83	204	2.4 ± 1.6 ^a
<i>B. rapa</i> ssp. <i>pekinensis</i> x <i>B. napus</i>	0.8	57	14	0.24 ± 2.0 ^a

^a the number of obtained seeds per pollinated flower and the standard deviation – liczba zawiązanych nasion na zapylny kwiat i odchylenie standardowe

Pollinated pistils were divided into two parts. Half of them were allocated for the microscopic examinations. For this purpose pistils were collected 48 h after pollination and next they were fixed and stained with aniline blue [Antkowiak *et al.* 2006, Tedder 2011]. The rest of pistils left on the plants to seeds formation. Observations of pollen grain germination and pollen tube (PT) growth were conducted after self (SP) and cross-pollination (CP) of analyzed *Brassica* species. The assessment was made by applying fluorescent microscopy technique. Ten pistils were analyzed in each combination. Intensity of pollen grains germination and pollen tubes penetration was expressed by the

six degree scale [Niemann *et al.* 2007], where 0 was the absence of PT, 1-4 – intermediate number of PT and 5 – the largest number of PT. Self-incompatibility (SI), self-compatibility (SC) or crossability (CC – cross-compatibility, CI – cross-incompatibility) were evaluated on the basis of the pollen germination index (PGI) according to Kaneko *et al.* [2009]:

$$\text{PGI} = (b + 2c + 3d + 4e)/(a + b + c + d + e),$$

- a – number of pistils with pollen grains,
- b – number of pistils in which pollen grains do not germinate,
- c – number of pistils in which pollen grains germinate on stigmas,
- d – number of pistils in which pollen tubes enter the style tissue,
- e – number of pistils in which pollen tubes penetrate close to or enter the ovules.

In the case of PGI equal or higher than 2 it was concluded that there was compatibility.

Effectiveness of wide hybridization of *B. napus* with other *Brassica* species was expressed by the number of seeds obtained per pollinated flower (Table 1).

RESULTS

1. The crossability evaluation based on the observation of pollen tubes growth in particular cross combinations.

In three of five tested *Brassica* species i.e. *B. rapa* ssp. *pekinensis*, *B. fruticulosa*, *B. oleracea* the germination of pollen grains after their self-pollination was very weak. In this combinations there was lack of germination or the pollen tubes were visible only on the stigmas, so PGI ranged from 0.8 (*B. oleracea* var. *alboglabra*) to 1.6 (*B. rapa* ssp. *pekinensis*) (Table 1, Fig 1a). Two remaining species *B. napus* and *B. carinata* showed better germination of pollen grains and pollen tubes growth after SP was 2.6; 2.3 respectively (Fig. 1b). The reciprocal crosses of *B. napus* cultivar Californium with the pollen of *B. rapa* ssp. *pekinensis* showed different intensity of pollen tubes growth and the value of PGI depended on which form was used as a maternal parents, e.g. after cross-pollination of *B. napus* with pollen grain of *B. rapa* ssp. *pekinensis* the intensity of pollen tube growth was higher and pollen tubes were observed in the ovary (PGI higher than 2). Quite different situation was observed in the case of crosses in which *B. rapa* ssp. *pekinensis* plants were used as a maternal and *B. napus* cultivar Californium as a pollen donor. In this cases the intensity of pollen tubes growth was low and PGI was less than 1. The crossing between *B. napus* and one variety of *B. oleracea* var. *alboglabra* showed that this two species do not suit to each other concerning their compatibility. The germination of *B. oleracea* var. *alboglabra* pollen grains on the stigma of *B. napus* was very weak and PGI was lower than 2. In the case of reciprocal crossing i.e. *B. oleracea* var. *alboglabra* x *B. napus*, the germination of pollen grains on the stigma was also weak and PGI equal 1. In contrast *B. oleracea* var. *gongyloides* showed better germination of pollen grains than *B. oleracea* var. *alboglabra* and in analyzed two ways of pollination PGI was higher than 2 after cross-pollination (Fig. 1c). Similar situation was observed in reciprocal crosses between *B. napus* x *B. fruticulosa* and *B. carinata* (Fig. 1d).

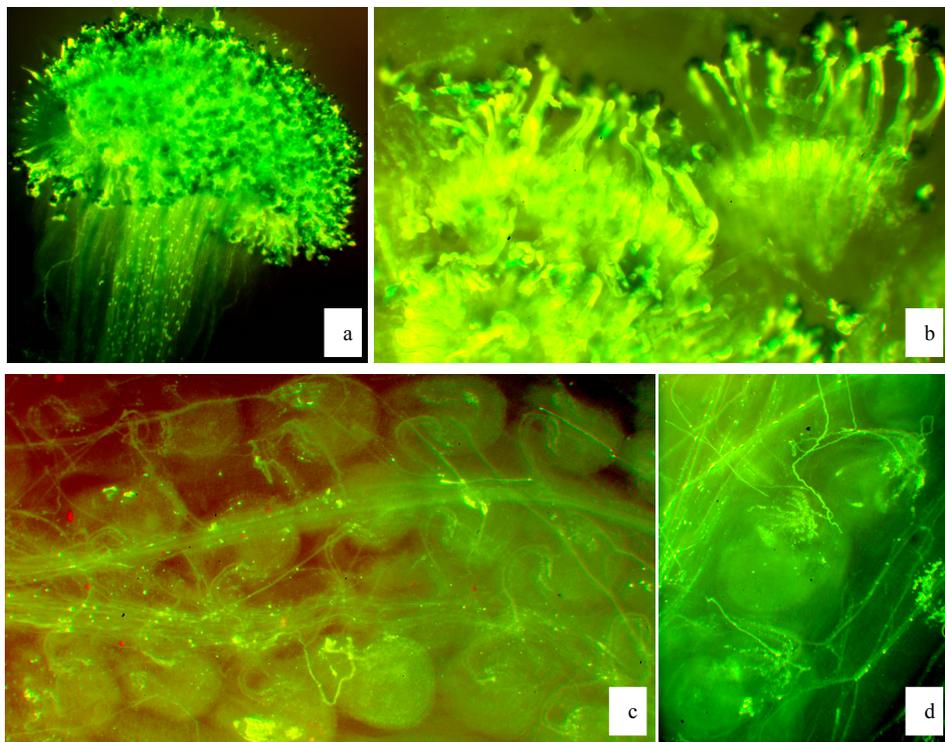


Fig. 1. Pollen germination and pollen tube elongation in pistils of analyzed *Brassica* species; a – the pistil of SP *B. rapa* ssp. *pekinensis* with germinating pollen grains on the stigma, b – pollen grains germinating on the stigma, 48 h after self-pollination of *Brassica napus*, c – pollen tubes in the ovary, 48 h after cross-pollination *Brassica napus* x *B. oleracea* var. *gongyloides*, d – pollen tube penetrating the ovule, 48 h after cross-pollination: *Brassica napus* x *Brassica carinata*

Rys. 1. Kielkowanie pyłku i wzrost łagiewek pyłkowych w słupkach analizowanych gatunków *Brassica*, a – słupek SP *B. rapa* ssp. *pekinensis* z kielkującymi ziarnami pyłku na znamieniu, b – ziarna pyłku kielkujące na znamieniu, 48 h po samozapyleniu *Brassica napus*, c – łagiewki pyłkowe w zalążni, 48 h po obcozapyleniu *Brassica napus* x *B. oleracea* var. *gongyloides*, d – łagiewki pyłkowe wnikające do zalążków, 48 h po obcozapyleniu: *Brassica napus* x *Brassica carinata*

2. The crossability evaluation based on the observation of seed set in particular cross combinations

Between self-pollinated species, the highest number of seeds to the number of pollinated flowers was observed in the case of *B. napus* and *B. carinata* (224 and 168 respectively). This is consistent with the PGI index value, which was the highest from all tested self-pollinated genotypes. No seeds were obtained for self-pollinated *B. oleracea* var. *alboglabra* and *gongyloides*. This observation is connected with their PGI which confirmed the self-incompatibility of those species. In the remaining two analyzed genotypes i.e. *B. rapa* ssp. *pekinensis* and *B. fruticulosa*, despite of the nearly 2 PGI, only a small amount of seeds were obtained. In the case of cross-pollinated combinations the highest number of seeds per pollination was obtained from *B. napus*

x *B. carinata* (3.4 ± 3.2^a) and *B. napus* x *B. rapa* ssp. *pekinensis* (2.4 ± 1.6^a). The number of obtained seeds and PGI confirmed the bilateral cross compatibility between *B. napus* x *B. carinata* and unilateral cross compatibility between *B. napus* x *B. rapa* ssp. *pekinensis*. The lowest number of seeds was observed in the case of crossing between *B. napus* and two tested varieties of *B. oleracea* (*alboglabra* and *gongyloides*). In reciprocal crosses between that species there were no seeds at all. Similarly, no seeds were also for *B. fruticulosa* x *B. napus* cross combination, although in the reverse crossing (*B. napus* x *B. fruticulosa*) there were.

DISCUSSION

The results of our investigation on pollen grain germination and penetration of pollen tubes into particular parts of the pistil showed that only two among five tested *Brassica* species are SC. The remain tested genotypes belonging to three *Brassica* species are SI. In recent works different methods of overcoming pre-zygotic barriers among *Brassica* species were used. For example through treatment in appropriate dosage of laser and high-voltage electrostatic field, the budding quantity of pollen of *Brassica oleracea* var. *gongyloides* was increased, the self-compatibility index and the quantity of seed were increased [Zhang *et al.* 2007]. In our work both crosses made between SC *B. napus* and SC *B. carinata* and between SC *B. napus* and two analyzed SI genotypes e.g. *B. fruticulosa* and *B. oleracea* ssp. *gongyloides* were bilaterally compatible. This has been confirmed by the seed set in almost all combinations with the exception of crosses between *B. fruticulosa* x *B. napus* and *B. oleracea* var. *gongyloides* x *B. napus*. In this cross combinations no seeds were obtained. This experiments revealed that there are strong postzygotic barriers between that two above mentioned combinations. This interspecific incompatibility can be overcome by embryo rescue techniques, where in, the embryo or the maternal flower organs are cultured in vitro on artificial media [Springer *et al.* 2007]. However, it is interesting that crosses between SC cultivar Californium (*B. napus*) and SI *B. rapa* ssp. *pekinensis* were successful only when SC cultivar was used as maternal form. Reciprocal crosses (SI x SC) failed. These results are consistent with those obtained by Yoshinbu *et al.* [2005].

Similar observation related with unilateral incompatibility was made by Antkowiak *et al.* [2006]. Reciprocal crosses made by these authors between SC cultivars from families Solanaceae and Brassicaceae showed unilateral interspecific incompatibility. Previously another researcher [Kemp and Doughty 2003] stated that the most remarkable feature of interspecific incompatibility is that it usually occurs unilaterally. The pollen tubes of certain SC species are inhibited in the styles of SI species but the reciprocal crosses are compatible. Moreover, the data obtained by Antkowiak and Wojciechowski [2006] were indicative of unilateral incompatibility also in the genus *Pyrus* when two SI species were crossed.

CONCLUSIONS

1. Three of five tested *Brassica* species are self-incompatible. Only two species i.e. *B. napus* and *B. carinata* are self-compatible.

2. Unilateral interspecific incompatibility occurred in the crosses between SI *B. rapa* ssp. *pekinensis* and SC *B. napus* cv. Californium (*B. napus* – paternal form).

3. Bilateral interspecific incompatibility occurred in the crosses between *B. oleracea* var. *alboglabra* x *B. napus*.

4. The crossability evaluated on the seed set confirmed the existence of strong post-zygotic incompatibility barriers between *B. fruticulosa* x *B. napus* and *B. oleracea* var. *gongyloides* x *B. napus*. Therefore, it is appropriate to use *in vitro* culture of isolated embryos in such a cross combinations.

5. The results show that selection of parental components for hybridization is an important step for obtaining breeding success.

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OCENA SAMONIEZGODNOŚCI I ZGODNOŚCI KRZYŻOWEJ U WYBRANYCH GATUNKÓW Z RODZAJU *Brassica* NA PODSTAWIE OBSERWACJI KIEŁKOWANIA ŁAGIEWEK PYŁKOWYCH I WIĄZANIA NASION

Streszczenie. Wiele gatunków roślin kwiatowych, w tym również gatunki z rodzaju *Brassica*, ma genetycznie determinowaną skłonność do obcozapylenia (samoniezgodność gametofitowa lub sporofitowa). U *Brassica* samoniezgodność jest sporofitycznie kontrolowana przez pojedynczy, wysoce polimorficzny locus zwany S loci. Z punktu widzenia praktyki hodowlanej, zapylenie krzyżowe jest niezbędne do uzyskania wysokiego plonowania. Dlatego celem pracy było oszacowanie samoniezgodności i zgodności krzyżowej u pięciu testowanych gatunków z rodzaju *Brassica*. Materiał roślinny użyty do przeprowadzonych krzyżowań oddalonych stanowiły: *B. napus* var. *oleifera* cv. *Californium*, *B. carinata*, *B. fruticulosa*, *B. rapa* ssp. *pekinensis* i dwie odmiany *B. oleracea* (var. *alboglabra* and var. *gongyloides*). Wszystkie badane obiekty zapyłano w szklarni Katedry Genetyki i Hodowli Roślin w Poznaniu w układzie diallelicznym. Pyłek poszczególnych zapylaczy наносzony był na znamiona słupków w kwiatach kastrowanych w stadium zamkniętego pąka kwiatowego. Obserwacje kiełkowania ziaren pyłku i wnikania łagiewek pyłkowych do zalążków przeprowadzono analizując słupki komponentów macecznych po 48 godzinach od momentu zapylenia, przy użyciu mikroskopu fluorescencyjnego. Zgodność krzyżową oceniono na podstawie indeksu kiełkowania pyłku (PGI) oraz wiązania nasion we wszystkich kombinacjach krzyżowania. Przeprowadzone obserwacje wykazały jednokierunkową niezgodność w przypadku krzyżowań pomiędzy *B. rapa* ssp. *pekinensis* and *B. napus* var. *oleifera* cv. *Californium* (użyta jako forma ojcowska) oraz dwukierunkową niezgodność w krzyżowaniach pomiędzy *B. napus* and *B. oleracea* var. *alboglabra*. Generalnie lepsze kiełkowanie ziaren pyłku i wnikanie łagiewek pyłkowych obserwowano w kombinacjach,

gdzie jako komponent maticzny użyta była forma *B. napus*. Pomędzy analizowanymi gatunkami zaobserwowano zarówno pre- jak i postzygotyczne bariery krzyżowalności.

Słowa kluczowe: Brassicaceae, krzyżowania oddalone, łagiewki pyłkowe, mikroskopia fluorescencyjna, samoniezgodność

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