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Mendelian inheritance of introrse orientated anthers in *Brassica rapa*

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Abstract: The inheritance of anther orientation of 154 individuals from two B_1 populations of *Brassica rapa* (syn. *Brassica campestris*) was evaluated under controlled conditions in a greenhouse. The anther orientation was evaluated visually at the time of fully open flowers. The observed extrorse:introrse ratios were 1 : 1 in R-o-18 × (R-o-18 × RM29) population and 3 : 1 in R-o-18 × (R-o-18 × R c-50) population. It was concluded that this trait is controlled by two duplicated pairs of genes (A_1, A_2) for extrorse anthers, either of which can produce extrorse anthers when a single dominant allele is present. Introrse anthers result when all alleles at both loci are recessive.

Keywords: anther orientation; *Brassica campestris*; chi-square test; Chinese cabbage; mating system; turnip rape; yellow sarson

Considerable efforts have recently been made to obtain hybrid varieties of vegetables and oilseeds from the genus *Brassica*. The main advantages of these varieties are their higher yield, earliness, uniformity and resistance to environmental conditions (Riggs 1988). In the production of hybrid seed using self-incompatibility, a lot of time is devoted to evaluating the degree and stability of self-incompatibility. The currently used methods of pollen tube staining and the seed test are time consuming, and in addition, pollen tube staining does not provide for *Brassica napus* very reliable results (Havel 1994). Therefore, we were interested in the occurrence of introrse anthers (anthers facing inwards toward the stigmatic surface at dehiscence, so that pollen transfer to the stigma is easily possible), which occur in *B. rapa* ssp. *trilocularis* (Mohammad et al. 1942) and all newer varieties of *B. napus*, which are self-pollinating. The position of these anthers in the flower gives a greater chance to get the pollen to the stigma of own flower. After comparing these materials with *B. rapa* ssp. *oleifera* and ssp. *chinensis*, which are cross-pollinated and have extrorse anthers (facing outward the flower at dehiscence), we conclude that there may be a correlation between self-compatibility and introrse anthers. In this case, introrse anthers could serve as a morphological marker of self-compatibility, resp. self-incompatibility to complement and simplify their assessment.

To date, little is known about gene(s) responsible for stamen twisting in any plant. Searle et al. (in Madawala 2020) performed reciprocal crosses between *B. rapa* accessions R500 (extrorse) and DS17 (introrse) only produced F_1 plants with extrorse stamens when grown under controlled environmental conditions, demonstrating the extrorse oriented stamen phenotype is dominant to introrse. After selfing F_1 plants, in the F_2 generation of 512 plants

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a 3 : 1 (extrorse : introrse) segregation ratio was observed, which suggested the phenotype is controlled by a single gene or locus.

The present study was undertaken to more clearly define the inheritance of introrse anthers in *B. rapa* and to study the possibility of using this trait as a morphological marker of self-compatibility.

MATERIAL AND METHODS

Plant material. A description of the lines used for crosses is given in Table 1. To study the inheritance of introrse anthers, two B_1 populations were created by backcrossing: R-o-18 × (R-o-18 × RM29) and R-o-18 × (R-o-18 × R c-50). Repeated spraying of 1.5% NaCl was used to overcome self-incompatibility. Several plants were pollinated in a bud.

Conditions for growing plants. The plants were grown in a temperature-controlled greenhouse (20 °C during the day and 12 °C overnight). The lighting time in the greenhouse with day light length control was 16 hours. The plants were grown in pots with a diameter of 15 cm filled with a mixture of loam, peat, grit, limestone and the slowly releasing fertilizer Osmocote (Scotts-Sierra Horticultural Products Co., USA).

Evaluation of anthers. The anther orientation was evaluated visually at the time of fully flowering plants on 10 randomly selected flowers throughout the plant. The plants were divided into two groups according to the anther orientation: into plants with introrse anthers (T) and into plants with extrorse anthers (A) (Figure 1).

Statistical analysis. Simple chi-square (χ^2) tests (Paterson 1939) for goodness-of-fit based on an assumed genetic model were used to evaluate the experimental results.

RESULTS

All *B. rapa* lines evaluated had six anthers and the same anther arrangement, with two tall pairs of anthers and two short single anthers. In the flower bud of all lines, all anthers were introrse, or facing inwards toward the stigma. The two short single anthers in the flower of all lines evaluated did not rotate and remained introrse after dehiscence.

Table 1. Description of *Brassica rapa* lines used

Code	Subspecies	Genetic characteristics	Anther orientation
R-o-18	*trilocularis*	line	introrse
RM29	*oleifera*	inbred line	extrorse
R-c-50	*chinensis*	inbred line	extrorse

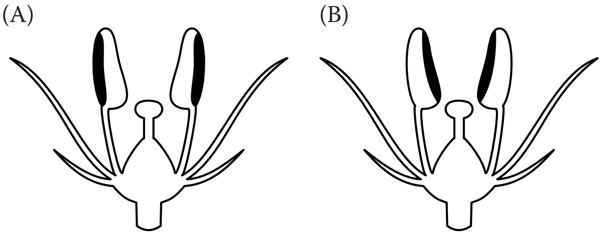

Figure 1. A flower showing introrse or extrorse oriented anthers: introrse oriented anthers facing inwards toward the stigmatic surface at dehiscence (A), extrorse oriented anthers facing outward the flower at dehiscence (B)

Crosses between lines R-o-18 × (introrse) and RM29 and R-c-50 (both extrorse) only produced F_1 plants with extrorse anthers. It demonstrated that the extrorse orientated anther phenotype (A) was dominant to introrse one (T) in *B. rapa*. After backcrossing F_1 plants, in both B_1 populations plants with extrorse and introrse anthers were observed. Anther orientation of the B_1 plants was either extrorse or introrse. The proportion of introrse anthered plants within B_1 population appeared to be either 1/2 or 1/4. The segregation ratios in both B_1 populations, χ^2 test values and the probability of agreement of these segregation ratios with the expected segregation ratios (P) are given in Table 2.

Thus, it was hypothesized that a duplicate gene existed in the parental lines. In such a situation three classes of true breeding extrorse anther plants are possible. And these may be designated $A_1A_1a_2a_2$, $a_1a_1A_2A_2$ and $A_1A_1A_2A_2$. These genotypes would give rise to three genotypic classes within the F_1 opulation, $A_1a_1A_2a_2$, $A_1a_1a_2a_2$ and $a_1a_1A_2a_2$. On the basis of such a hypothesis, ratio 1 : 1 and 3 : 1 would be expected to occur in the B_1 generation.

Table 2. Chi-square (χ^2) analysis of the segregation for anther orientation in B_1 populations

Population	Segregation for anther orientation		χ^2	*P*
	extrorse	introrse		
	expected segregation ratio in B_1 1 : 1			
RM29	28	30	0.069	0.793
	expected segregation ratio in B_1 3 : 1			
R-c-50	79	17	2.722	0.099

Both B_1 populations were tested for goodness of fit to a 1 : 1 or 3 : 1. Of two populations tested, two gave a significant χ^2 value. Both B_1 populations did not fail to fit the tested ratio. We were satisfied therefore that all the evidence was in favour of the hypothesis of a duplicate gene situation.

DISCUSSION

Segregation ratios in B_1 populations did not confirm our original hypothesis that the anther orientation in *B. rapa* lines used is controlled by a single dominant gene with dominance of extrorse anthers to introrse ones. Although the segregation ratio in the RM29 B_1 population showed good agreement with the expected 1 : 1 segregation ratio, this was not the case in the R-c-50 B_1 population and the segregation ratio was closest to a 3 : 1 segregation ratio, which suggested that the phenotype is controlled by a pair of duplicated (A_1, A_2) genes for extrorse orientation, either of which can produce extrorse anthers when a single dominant allele is present. Introrse anthers result when all alleles at both loci are recessive. According to this model, we could write the genotypes of the lines used in our crosses, and the plants in the F_1 and B_1 populations as follows in Table 3.

During the evolutionary history of *Brassica* a whole genome triplication and subsequent partial genome elimination occurred, and therefore genes within the *B. rapa* range from being triplicated, duplicated or single copy (Mun et al. 2009).

A previous study indicated that a single gene action for anther orientation might be expected in the segregating populations examined (Madawala 2020). Anther orientation of the F_2 plants was either extrorse or introrse. The proportion of introrse anther plants within the F_2 population appeared to be 1/4.

Table 3. Hypothesis of the inheritance of anther orientation in *Brassica rapa*

Generation	Code	Hypothetic genotype	Anther phenotype
P	R-o-18	$a_1a_1a_2a_2$	introrse
	RM29	$A_1A_1a_2a_2$	extrorse
	R-c-50	$A_1A_1A_2A_2$	extrorse
F_1	RM29	$A_1a_1a_2a_2$	extrorse
	R-c-50	$A_1a_1A_2a_2$	extrorse
B_1	RM29	$A_1a_1a_2a_2$	extrorse
		$a_1a_1a_2a_2$	introrse
	R-c-50	$A_1a_1A_2a_2$	extrorse
		$A_1a_1a_2a_2$	extrorse
		$a_1a_1A_2a_2$	extrorse
		$a_1a_1a_2a_2$	introrse

The findings of our study differ from conclusions of Madawala (2020). It is likely that the accession R500 used by Madawala already had a locus in the homozygote recessive condition. R-c-50 is a line which apparently consists of the genotype $A_1A_1A_2A_2$, whereas RM29 although also pure extrorse, contains other genotype in which one of the loci (A_1 or A_2) is always in the homozygote recessive condition, in order to behave as a true breeding line.

The line R-o-18 contains the recessive allele *a* at both loci. Thus true breeding extrorse lines may be homozygous dominant at either or both the A_1 or A_2 locus, with the possibility of the recessive allele occurring at the other locus. Introrse anther production is possible in the F_2 when intercrossing between lines carrying different recessive alleles.

Salisbury et al. (2017) investigated the usefulness of floral characters, including anther direction at dehiscence, as a potential indicator of breeding system in the *Brassicaceae*. None of the floral characteristics was able to clearly differentiate the self-compatible and self-incompatible species and allow prediction of the breeding system with absolute confidence. The floral characteristic which was most effective at differentiating the two groups was anther direction at dehiscence and timing of dehiscence.

Anther orientation is correlated with the mating system also in other members of the *Brassicaceae*. In the 1960 s, Rollins (1963) and Lloyd (1965) identified introrse anthers as one of several floral traits distinguishing selfing from outcrossing taxa of *Leavenworthia*. Two later studies have confirmed this notion and shown that anthers are more introrse in highly selfing populations than in populations with low or intermediate selfing rates in *Leavenworthia crassa* (Lyons & Antonovics 1991) and in *L. alabamica* (Busch 2005).

This has not been confirmed in our experiments. Variation in anther orientation in two B_1 *B. rapa* populations was not correlated with the mating system (data not shown). In both B_1 populations there were a number of self-compatible plants with extrorse anthers and also self-incompatible plants with introrse anthers. It could be due to the plant material used, repeated spraying of flowers with 1.5% NaCl and pollination of some plants in a bud to overcome self-incompatibility.

It is clear that direction of dehiscence alone can not be used to precisely predict breeding systems in the *Brassicaceae*. This is supported by the fact that the degree of rotation can be affected by weather and that sometimes differences are observed between different anthers in the same flower (Lloyd 1965). Madawala (2020) also demonstrated that lower ambient growth temperatures reduced stamen twisting in extrorse accession R500, which may reduce cross pollination rates in nature.

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