

Self-Incompatibility in Rapid Cycling *Brassica rapa*

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ABSTRACT

Self-incompatibility (SI) is a mechanism by which plants may insure the widest degree of variation possible within an inter-breeding population. It is defined in cruciferous vegetables as producing no more than one seed per open flower self-pollination. Rapid Cycling *Brassica rapa* (RCBr), was chosen mainly because it can be pollinated within two weeks of planting. The type of SI that brassicas exhibit is localized on the surface of the stigma. In order to overcome this, buds were opened between four and twelve hours prior to stigma emergence, and five treatment groups were hand pollinated. The two questions that we wished to answer were; first do RCBr's bud pollinated with self and outcross pollen have similar rates of seed production and second, will RCBr's choose outcross pollen over self pollen. Our data suggest that pollinations with self pollen, even when performed in the bud stage, produce smaller numbers of seeds. Work is still being performed to find out whether outcross pollen is selected for when provided a choice between self and outcross pollen.

INTRODUCTION

All plants exhibit varying degrees of success with self pollination. Self-compatibility (SC) and self-incompatibility (SI) are not absolute classifications and must often be distinguished quantitatively rather than qualitatively (Rigney, 1993). In some plants self pollen never penetrates the surface of the stigma, in others there is a reduced seed set and in yet others there is less reproductive vigor in future generations.

Self pollinations averaging no more than one seed per silique in open flower selfing are considered as good indications of SI (Hinata, 1988). This is an accepted and arbitrary standard in the case of cruciferous vegetables, however I used a different cultivar than that used in previously published material, and I hand pollinated buds prior to flower opening. SI is strongest in plants one day before and one day after flower opening (Hinata, 1988). As a result, seed setting varies with the flower age. The maximum number of seeds from SI lines is usually obtained by self-pollinating in the bud stage, pollinations performed at this time usually produce seed sets comparable to out-cross pollinations of open flowers (Tsujimoto, 1981).

Rapid Cycling *Brassica rapa* (RCBr), is the cultivar I worked with. After a literature search I have found no detailed information on the extent of SI in RCBr. The cultivar has been patented under Wisconsin Fast Plants (Williams, 1989).

Three types of SI have been described:

- (1) Genic male sterility - Nuclear genes inhibit the normal development of anthers and pollen. This is usually measured by the percentage of viable pollen grains.
- (2) Gametophytic SI - The hindrance to pollen tube growth is in the style. Plants exhibiting this type of SI usually have bi-nucleate pollen grains.

- (3) Sporophytic SI - The hindrance to pollen germination and tube growth is localized in the surface of the stigma, through the build up of callose. Pollen grains are generally tri-nucleate. This is the system employed in the Brassicaceae (Opena, 1988).

Many methods have been used to overcome both powerful and weak SI. Delayed pollination, in which flowers are allowed to age before pollination, this method is not a practical method of propagation except in the case of common cabbage. Recognition pollen, pollen grains are washed in a 10 to 50 percent solution of acetone prior to pollination, this method is considered unreliable. Chemically treating the stigma with various organic solvents is also not considered very practical method of over-coming SI. Various environmental controls have been tried. Humidity, temperature, and carbon dioxide have all successfully been used. Technological aspects which have been studied to counter SI include: mutilation of stigma, Gamma or X-ray irradiation, electric-aided pollination, and thermal-aided pollination, have all been used to help overcome SI. The standard method of overcoming SI is bud pollination. This is the method I will employ (Ito 1988).

The two null hypotheses that I wish to test are:

- (1) Do RCBr's bud pollinated with self and outcross pollen enjoy similar rates of successful seed production.
- (2) If a mixture of self and outcross pollen is provided on the stigmas, what percentage of offspring is sired by each pollen donor.

MATERIALS AND METHODS

Rapid Cycling *Brassica rapa* has been developed

from traditional or natural Brassicas, reducing the average life cycle from a normal six to twelve months down to approximately thirty- five days (Williams, 1989). Stigma surfaces are receptive to pollination approximately three to four days prior to the anthers releasing pollen. There is a form of RCB_r that does not produce the pigment anthocyanin this trait is a recessive trait which expresses itself as a green stem instead of the dominant purple stem expressed by the wild type. For the determination of paternity in pollinations using outcross pollen, pollen donors were homozygous for one trait and receptacles were homozygous for the other (Jones, 1994). Therefore anthocyanin-less receptacles are expected to produce offspring with purple stems. Individual plants were ready for pollen treatments at different times. It was therefore impossible to pollinate the first three individual flowers on all plants. Buds were selected for pollination roughly four to twelve hours before the stigma protruded from the tip of the bud.

The five relevant pollen donors were:

Single donors (Jones, 1994)

1. outcross (wildtype)
2. outcross (Anthocyaninless)
3. self

Dual donors (Rigney, 1988)

4. self followed by wildtype
5. outcross (Anthocyaninless) followed by wildtype

Groups one and two were designed to find the degree of SI exhibited by RCB_r. Groups four and five were designed to address whether or not RCB_r can possibly select outcross pollen over self pollen. Group three was to address the possibility that Anthocyaninless individuals might not be as reproductively vigorous when pollinated with wild type pollen. All pollen recipients were homozygous recessive for the Anthocyaninless trait. All double pollinations were separated by one hour. All plants were grown in a growth chamber at 26 C, with twenty four hour lighting (Williams, 1989).

The pollinations proceeded in this way:

1. sepals were opened.
2. a mature anther was selected from the relevant treatment group.
3. the anther was rubbed on the surface of the newly exposed stigma, depositing a visible powdery layer of pollen on each.
4. After the pollinations were performed all excess buds were trimmed off the plants in order to focus the plants reproductive energy on the pollinated.

The plants developed siliques, they were allowed to mature, and then were dried. The seeds were harvested and weighed. The seeds were set on wet paper in petri dishes to germinate. The number of seeds that germinated was recorded as well as the color of their stems.

Chi squared analysis of seed set size was used to analyze the statistical significance of pollen selection in

the dual pollinations. The expected ratios were calculated from the results of the single pollinations (# of wildtype seeds/ # of anthocyaninless seeds). An ANOVA was run on the data that was obtained from all the treatment groups.

RESULTS

In group one (wildtype pollen donor), a total of 100 seeds were produced, and 80% of them germinated. In group two (anthocyaninless outcross pollen donor), a total of 243 seeds were produced, and 77% of them germinated. In group three (self pollen donor), 124 seeds were produced, and 67% germinated. In group four (self followed by wildtype), a total of 131 seeds developed, of the 54% that germinated 11% were sired by wildtype pollen and 89% were sired by self pollen. In group five (anthocyaninless outcross pollen followed by wildtype), a total of 153 seeds were produced and of the 66% that germinated 3% were sired by wildtype pollen and 97% were sired by anthocyaninless pollen.

DISCUSSION

There was no significant difference between the single donor pollinations, as far as the number of seeds produced and the number that successfully germinated. There was quite a bit of variance in the number of seeds that were produced per plant within the treatment groups but not between the treatment groups. The expected definition of SI (Hinata, 1988) of no more than one seed per open flower selfing, is used to describe SI in the Brassicaceae. Excepting that definition, it would appear that bud pollination is an acceptable method for overcoming SI in RCB_r's. However it appears that anthocyaninless individuals are more receptive (on the basis of total seeds produced) of anthocyaninless outcross pollen than wildtype pollen. In order to use the anthocyaninless trait as a marker, anthocyaninless individuals should be equally receptive to wildtype or anthocyaninless outcross pollen.

Based on the results of the dual pollinations, it would appear that outcross pollen is not chosen over self pollen. However, no pollen treatment group was designed to control for order of dual pollination. In both dual pollinations, wildtype pollen was deposited on the stigma an hour after anthocyaninless pollen was deposited. It is very possible that the first pollen treatment had too much of an advantage temporally speaking.

If the the average seed size (g), is compared in the various treatment groups, than it appears that self pollen leads to the production of smaller seeds than outcross pollen. These results are comparable to a study of cryptic self fertility in *Campsis radicans* of the family Bignoniaceae (Bertin, 1989). In a separate study

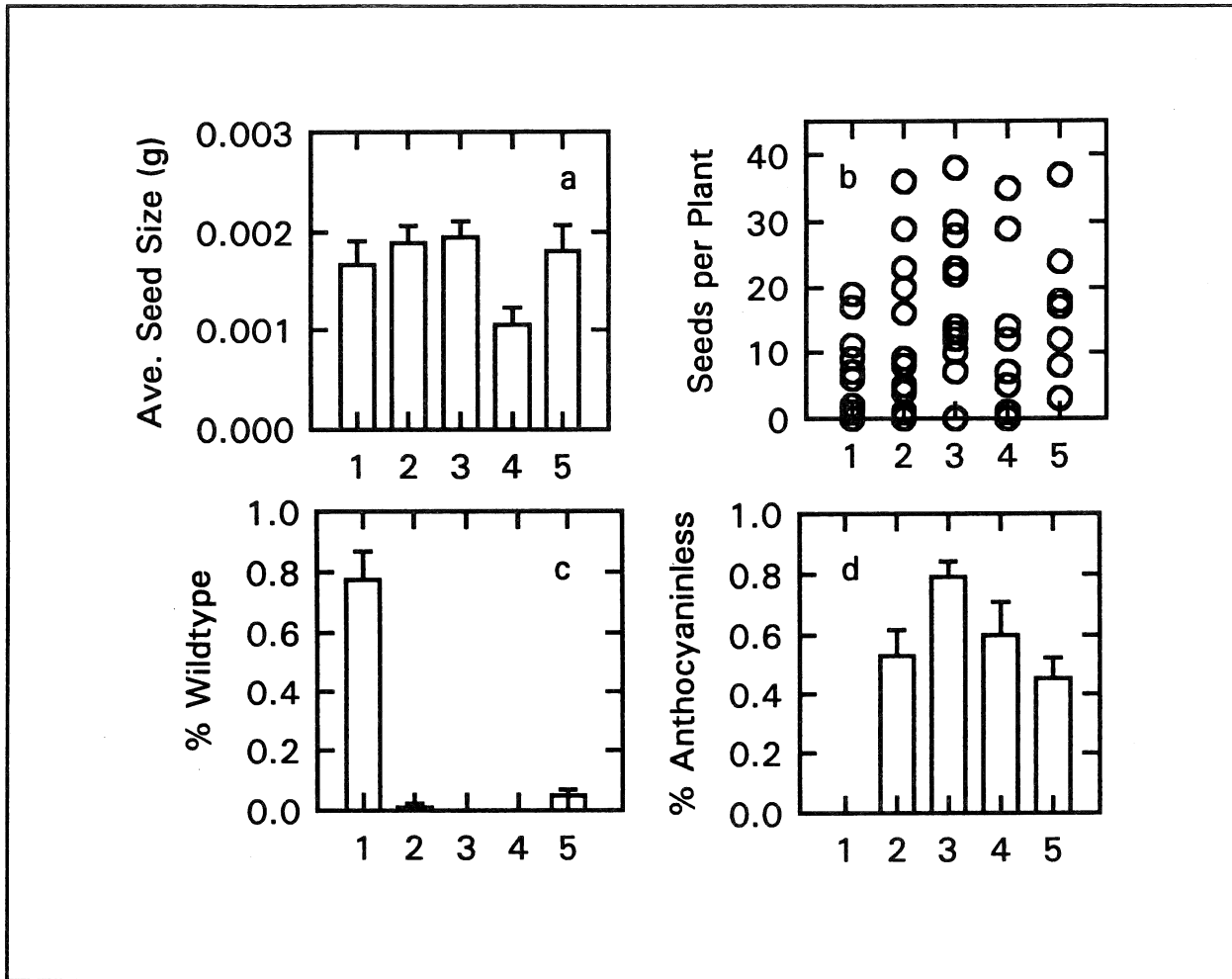


Figure 1. Results of the various pollination treatments, see text for description of the treatment groups: (a) average seed size in grams, (b) number of seeds produced per plant distributed through each treatment group, (c,d) results of the germination tests, (c) percent of seeds that show wildtype paternity, (d) percent of seeds that show anthocyaninless paternity.

of inbreeding depression in *Lythrum salicaria* from the family Lythraceae, (O'Neil, 1994) it was found that the only trait that was affected when self pollination occurred, was seed size (g).

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