RESULTS AND PROBLEMS IN BUILDING UP OF SELF- INCOMPATIBLE LINES AND THEIR USE IN HYBRID SEED PRODUCTION OF WINTER RAPE (Brassica napus L.)

E. Rudloff

Institute of Oil and Forage Crop Breeding "Hans Lembke"
D (O) - 2401 Malchow/Poel

INTRODUCTION

The sporophytically determined self-incompatibility (s.i.) is an efficient genetic mechanism for the prevention of self-fertilization in the Brassica family. It is controlled by a gene S which has a series of multiple alleles. The incompatibility reaction (i.r.) consists in the inhibition of pollen tube growth on the stigma surface thus preventing it's penetration into the stylar tissue. The i.r. occures if the S-allele of the pollen is identical with the active allele in the stigma.
These properties are making the s.i. very interesting for hybrid breeding. It is possible to develop s.i.-lines which are homozygous for different S-alleles. An S-homozygous line doesn't show any intra-line fertilization, but uninhibited inter-line fertilization is possible. There are imaginable both single crosses involving two different S-homozygous lines and double crosses involving four different S-homozygous lines as well as other combinations, too.
The work on s.i. at Malchow has started in the early seventies and at present there are about 30 s.i.-lines available as parents for experimental hybrids. This paper reports on the methods which are used to examine the i.r. as well as to develop S-homozygous lines and the results and the problems with s.i..

METHODS

Examination of Incompatibility Reaction(i.r.)

The i.r. is only to recognize as the result of a pollination. A screening method has been developed involving the examination of two different traits resulting from the same pollination. These are: **(1) the pollen tube growth** by fluorescence microscopy and **(2) the seed set.**
The procedure which is used since many years is as follows: For test pollination the inflorescence is bagged before the flowers open. After opening 5 flowers per combination are pollinated by hand and bagged again. In case of crossing the flowers are emasculated. All other flowers are removed.
24 hours later all the pistils of a combination are excised taking care that the seed vessels remain intact. The pistils are prepared for fluorescence microscopy in the following manner:

They are taken in a microtube and the staining solution is added. It consists of

1 part anilin blue (aqueous solution 1% w/w)
1 part "Fit" (a detergent)
7 parts K_3PO_4 x 3 H_2O 0.2m
1 part NaOH 1.0n

This solution has a combined effect: the tissue will be fixed, macerated, bleached, and stained in one step. For fixation the pistil will remain for some months in the solution, but in darkness. For preparation the pistils are cooked in a water bath for 11 minutes. Then they are slightly squashed in a droplet of glycerine. This is a very quick and harmless procedure. The preparation of 50 combinations each 5 pistils needs about 35 minutes. Up to 20 pollen tubes are counted exactly, more than 20 are appraised. The mean pollen tube number (p.t./pistil) of a combination must not exeed more than 5.0 to be called "incompatible".
The seed set is counted at the pods developing from the remaining seed vessels. It's possible to do this already with green pods. The mean seed set must not exeed 1.0 seeds per pollinated flower (seeds/fl.) to call this combination "incompatible". A combination is called "incompatible " only if neither the pollen tube number nor the seed set doesn't exeed the obove mentioned limits.

Development of S.I.-Lines

It is carried out in 3 steps:
(1) Selection of s.i.-plants. To select s.i.-plants the test pollinations are done on single plants in the field. Plants which are s.i. according fluorescence test are propagated by bud pollination (selfing). If the seed set doesn't agree with that result, the offspring will be eliminated.
(2) Identification of S-allele homozygous plants. For this purpose reciprocal diallele test crosses including 11-13 plants of the single plant's offspring are necessary. Only incompatible reactions without any reciprocal differences refer to an S-homozygous maternal plant. Reciprocal differences refer to an S-heterozygous maternal plant and they permit the identification of the different S-genotypes. The homzygous genotypes are reproduced by bud pollination.
(3) Detection of S-allele differences between S-homozygotes. Therefore the different S-homozygous groups from step 2 are testcrossed. Incompatibility indicates identical S-alleles and compatibility different S-alleles, respectively.

Hybrid Seed Production

The first concepts of commercial seed production have been based on the following assumptions: (1) the propagation of s.i.-lines is carried out by bud pollination;
(2) the number of different S-alleles is limited - at least in the first time. For this reason a top cross hybrid involving two crosses has been intended. In the first cross two s.i.-lines with different S-alleles are combined to a S-heterozygous single cross being also s.i.. In the second cross this single cross is combined with a compatible pollinator line. Hybrid seed is harvested from the s.i.-parent only. The experimental hybrids have been produced in the same way. To

produce single crosses both s.i.-lines are grown in alternating rows on isolation plots in barley. For the second cross the single crosses are grown each 30 single plants in drilled plots of the pollinator varieties. Usually the single crosses are flowering earlier than the pollinators. Therefore synchronization has to be achieved by daily removing the early flowering inflorescences.

RESULTS AND DISCUSSION

Development of S.I.-Lines

The production of s.i.-lines needs a great expense of test pollinations. Therefore the reliability of the measurement of the i.r. is very important. Both possible criterions (pollen tube growth as well as seed set) may be influenced by a number of effects. The comparing examination of both criterions should increase the certainty of i.r. measurement.The quick availability of the results on pollen tube growth - a pre-fertilization event - permits a great number of test pollinations and several succeeding tests in the same flowering season, resp..
On the other hand the check of the pollen tube result by the seed set - the result of fertilization - is helpfully in avoiding misselection. There is a good correlation between both traits of r = 0.7 - 0.8 , indeed, but the remaining uncertainty makes mistakes possible.
At present there are about 30 s.i.-lines originating from single plants which had been selected in varieties or breeders material. They show a varying degree of s.i as demonstrated in Table 1 with some characteristical lines.
The determination of S-allele differences is something difficulty because of irregularities in the respective test-crosses. The reason is not known; perhaps there exist non-linear relationships between or mutual influences of different S-alleles. All the s.i.-lines which have been developed so far seem to be of a recessive typ. At least the F_1 of s.i. x self-compatible didn't show any s.i. That's important for the commercial use because the hybrid varieties will show no problems with fertility.
The possibilities of evaluating the goodness of s.i.-lines has been completed recently by a test assay involving the erucic acid content as a genetic marker (RUDLOFF and SCHWEIGER 1986).

Table 1. Incompatibility measurement on some s.i.-lines

Line	Number of Pollen tubes (p.t./pistil)	Seed set (seeds/fl.)
6/86	2,3	0,3
9/86	0,2	0,1
10/87	1,1	0,2
11/87	1,0	0,0
13/87	0,2	0,1
15/87	9,7	3,1
24/88	0,8	1,4
36/89	0,9	0,1
38/89	0,3	0,1

This method gives an outcrossing percentage measured under conditions which are very similarly to to natural ones. In Table 2 the seed set and the outcrossing of some characteristical s.i.-lines is compared.

Table 2. Seed set and outcrossing of some characteristical s.i.-lines in 1990

Line	Seed set (seeds/fl.)	Outcrossing (%)
2/85	0.0	77
6/86	0.3	77
9/86	0.1	84
10/87	0.2	100
11/87	0.0	100
13/87	0.1	100
15/87	3.1	76
16/88	1.1	60
20/88	0.2	60
24/88	1.4	100
32/89	0.5	100
35/89	0.0	91
36/89	0.1	100
38/89	0.1	69

The disagreement between seed set and outcrossing in several lines may be due to changes in the strength of the i.r. during the course of flowering. The investigation in this phenomenon is going on. On the other hand there are cases of very good agreement showing the good suitability of the described method of measuring the i.r..
Unfortunately the screening in double-low rape for s.i.-individuals didn't show any success so far. There are two possible explanations: (1) the repated selfings wich are characteristically for breeding double-low varieties have resulted in a total loss of the s.i.; (2) the reduction of the glucosinolate content results in a weakening or an inhibition of the s.i.-reaction. To overcome this situation the introgression of the S-alleles from zero-erucic s.i.-lines as well as from Brassica campestris is tried. The results of these works will give the right explanation, too. But extensive attempts with S-allele homozygous B. oleracea didn't show any success.
The obove mentioned way to develop s.i.-lines is very expensive and difficult. It may be simplified by the use of s.i.-plants from the first step as donors for doubled haploids . It results in S-allele homozygous lines whose s.i.will not change in further generations. Doubled haploids may be helpfully, too, for the obove mentioned attempts of introduction of s.i. into double-low rape because of the very low frequency of the recombinant "s.i. + low glucosinolate".

Heterosis

Both crosses for the production of experimental topcross hybrids have been carried out in open pollination. In many cases te a striking increased vegetative vigour of the single crosses was to observe. There are many hybrids with a yield

significantly higher than the pollinator. Of 20 topcross hybrids tested in 1988 at one location 18 have been significantly better and exeeded the respective pollinator (48.8 and 49.3 q/ha, resp.) with 9 to 16 percent. But only three hybrids exeeded the check variety (Malux 52.6 q/ha) significantly with 6 to 8 percent. On the one hand certainly many more different combinations have to be checked to recognize more and better combinations. But on the other hand there is an unexpected event. The utilization of the obove mentioned test assay for the screening of outcrossing has shown an obvious decrease of outcrossing in S-heterozygotes as compared to their S-homozygous parents (Table 3).

Table 3. Outcrossing in s.i.-lines and corresponding single crosses (1990)

Combination A x B	Outcrossing (%) of A	B	A x B
5 x 6	78	77	43
5 x 9	78	75	68
6 x 9	77	84	68
16 x 17	61	100	46
17 x 21	100	66	64
17 x 23	100	75	52
19 x 23	100	75	87
21 x 25	66	88	70

In the most cases outcrossing has decreased below the lower parent. But it is to note that the outcrossing nevertheless is distinct higher than in normal rape. That refers to a relationship between S-heterozygosity and the i.r.. Possibly there exists a heterotic effect weakening the incompatibility. Another explanation may be the change of the strength of the i.r. during the course of flowering. It is known that this change exists. Perhaps it's pattern varies in heterozygotes resulting in more selfing at the end of flowering. At present this phenomenon is under investigation. It seems to be necessary to test the i.r. of a great number of single crosses to choose the high-s.i. ones for hybrid seed production. The reproduction and multiplication of s.i.-lines is of great importance for commercial hybrid breeding. During the experimental phase as well as in maintaining the base seed of the lines the manual bud pollination may be well suited. The production of topcross hybrid seed as described obove for 10.000 hectares needs about 60 grams per s.i.-line; it's manual production may be just possible. New perspectives are opened by such means as application of CO_2 or salt solution. That permits a large scale multiplication thus enableing the production of single cross hybrids for commercial use, i.e. in great amounts. And then the advantag of s.i. will be utilized to it's full extent - that is the harvesting of hybrid seed from **both** hybrid parents.

REFERENCES

RUDLOFF,E. 1990. Procedures to detect the incompatibility reaction and to make S-homozygous lines in winter rape (Brassica napus L.)
In: : Procedings of Symposium of China International Rapeseed Sciences,Shanghai ,4.24.-5.2. 1990. Q.R. CHU and G.H.FANG (eds.).Shanghai Scientific and Technical Publishers 1990,p. 40

RUDLOFF,E. und W. SCHWEIGER 1985: A method for the assess ment of the selfing ratio in zero-erucic winter rape (Brassica napus L.). Cruciferae Newsl. 10: 80-81