

INVESTIGATIONS ON SELF-INCOMPATIBILITY IN *BRASSICA NAPUS* L. TOWARDS HYBRID BREEDING

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ABSTRACT

Self-incompatible winter oilseed rape with double-low quality was selected. Self-incompatibility is inherited by a single recessive gene. A RFLP and RAPD marker were found to be linked with 9,9 % and 27,6 % recombination, respectively. In order to facilitate the propagation of the self-incompatible lines by selfing self-incompatibility was overcome by spraying NaCl solutions into the open flowers. First experimental hybrids were produced in the field. Their performance was at the level of the best cultivars.

INTRODUCTION

There are many efforts to produce hybrids in winter oilseed rape. In addition to cytoplasmic male sterility, self-incompatibility (SI) could be a mechanism to control pollination in hybrid seed production. Many attempts have been made to transfer self-incompatibility from *Brassica oleracea* or *B. campestris* into *B. napus* (Mackay 1977, Ripley and Beversdorf 1991, Beschorner and Odenbach 1991). Because interspecific crosses were used for the introgression of the *S* alleles, several backcrosses and an intensive selection are needed to develop lines which can be used in rapeseed breeding programmes.

Our aim was to select self-incompatible plants out of breeding stocks of winter oilseed rape with double-low quality and to analyse their suitability for hybrid seed production.

EXPERIMENTAL

Selection of self-incompatible lines and inheritance of self-incompatibility

For the identification of self-incompatible genotypes, plants from different origins showing poor seed set after selfing in the field (1 to 6 seeds) were selected. Fluorescent microscope assay of pollen tube growth (Kho and Baer 1968) and seed set after selfing were used to classify the progeny of these plants. Four different self-incompatible lines with at least two different *S* alleles were selected. The frequency of self-incompatibility alleles in the plant material used was only 0.08 %. All self-incompatible plants showed different levels of pseudo-compatibility caused by the influence of varying temperatures and the genetic background. Nevertheless, it seems to be possible to select lines with a stable expression of self-incompatibility.

To investigate the inheritance of self-incompatibility a plant showing the lowest level of pseudo-compatibility was crossed with 11 plants from different self-compatible cultivars and lines. All 55 F₁ plants were self-compatible indicating the incompatibility being recessive. Segregation in the F₂ generation was analysed using two different lines. One population showed a segregation of 116 self-compatible to 26 incompatible plants, which is consistent with a 3:1 ratio and suggests the inheritance by a single recessive gene. In the second population a high level of pseudo-compatibility occurred and therefore the identification of the phenotype of some genotypes was more difficult. This observation indicates a strong influence of the genetic background on *S* allele expression.

The recessiveness of self-incompatibility has a great advantage in hybrid breeding. Hybrids produced by a cross of a self-incompatible line with a self-compatible pollinator will be self-compatible and will show full seed set in the field under different environmental conditions.

Molecular markers for the tagging of self-incompatibility

In order to facilitate the introduction of self-incompatibility into different lines via a marker based selection, RAPDs and RFLPs were analysed for identifying linked marker loci. For the RAPD analysis decamer-primers from Operon Technologies, Alameda, were used according to Williams *et al.* (1990) with few modifications. We found one RAPD marker (OPA17-700) being linked to the locus controlling self-incompatibility with a recombination frequency of 27,6 %. Southern hybridisations were performed with a cDNA clone of the *SLG* gene of a dominant *S* allele from *Brassica oleracea* (Trick and Flavell 1989). It detected a dominant RFLP locus located on the other side of the self-incompatibility locus with a distance of 9,9 % recombination. Thus two flanking markers were identified for the locus controlling self-incompatibility.

Overcoming self-incompatibility and results on first experimental hybrids

To overcome self-incompatibility plants in the greenhouse were sprayed with a NaCl solution during flowering (Fu *et al.* 1992). Solutions with concentrations of 5 and 8 % were sprayed two times a week into the open flowers whereas the 8 % solution showed the best results. In all three lines tested self-incompatibility was completely suppressed, when the solution was applied at the right time. Daily application would, perhaps, increase the seedset further.

First experimental hybrids were produced in the field by planting single self-incompatible plants into plots of cultivars assigned as pollinators. Outcrossing rates were controlled through the analysis of isozyme markers. Different models for estimating the outcrossing rate were applied, because there was no isolation and plants from outside the trial were able to pollinate. The selfing rate of the five hybrids varied between 7 and 29 %. These hybrids were grown in a little field trial together with 7 other hybrids produced by hand crossing. Their performance was comparable to the performance of the best cultivars.

All lines selected and characterized in this study seem to be a good basis for

further investigations on the use of self-incompatibility for hybrid breeding in winter oilseed rape.

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