Development of improved winter rapeseed populations for quality breeding and QTL mapping of agronomic characters

Tobias Spiller *, Wilfried Lühs, Roland Baetzel, Rod Snowdon and Wolfgang Friedt
Institute of Crop Science and Plant Breeding I, Justus-Liebig-University, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany; Phone: +49-641-99-37421, Fax: +49-641-99-37429, e-mail: Tobias.H.Spiller@agrar.uni-giessen.de

ABSTRACT
In the course of a winter oilseed rape breeding programme emphasis is placed on the development of a separate gene pool for hybrid breeding, based on high-erucic acid and high-glucosinolate rapeseed (HEAR). In the first step, this material is used as tester in order to determine its combining ability with male sterile double-low rapeseed lines. Concomitantly, the HEAR material is improved towards double-low seed characters through a quality conversion procedure. The main aim of this project is to target the 'combining ability' for relevant quantitative traits, such as seed and oil yield as well as oil content, in a QTL mapping approach to maximise the expected heterosis effects in corresponding cross combinations.

Key words: Rapeseed – genetic diversity – combining ability – gene pool – QTL mapping

INTRODUCTION
Exploitation of genetic potential with regard to seed and oil yield is a primary breeding objective in order to further increase the economic value of winter rapeseed (Brassica napus L.) as an oilseed crop. With the advent of rapeseed hybrid breeding, seed yield has undergone considerable enhancement in recent years. In addition to individual yield performance the availability of useful genetic diversity between the potential crossing partners - as a prerequisite for a good combining ability - is necessary. The overall aim of the present project is the development of a new divergent gene pool based on high-erucic acid and high-glucosinolate rapeseed (HEAR) which can be utilised in hybrid breeding of double-low winter oilseed rape. Following test crosses, suitable HEAR lines were selected which formed the basic material for all project work. Regarding seed yield the general combining ability (GCA) was estimated for the parental HEAR lines selected due to their yield performance. Based on the results of GCA testing, a cross 'good combiner' (double-low quality pool) x 'poor combiner' (HEAR pool) was selected to develop a new mapping population doubled-haploid (DH) lines for QTL analysis.

MATERIALS AND METHODS
The starting material was a HEAR pool consisting of nine semi-synthetic lines, one old French cultivar ('Marcus') and 10 doubled-haploid (DH) lines. To estimate the general combining ability (GCA) the 20 HEAR lines were used as pollinators in order to develop intraspecific hybrids. For hybrid seed production the male sterile double-low lines 'MSL-Falcon' and 'MSL-Express' (Male Sterility Lembke-System, Norddeutsche Pflanzenzucht, Hohenlieth, Germany) were used. The inter-pool hybrids were tested in the growing season 2000/2001 for yield performance at three German locations, the Field Research Station of Rauischholzhausen (RH, near Marburg), Hohenlieth (Norddeutsche Pflanzenzucht, Hohenlieth) and Thüle (Deutsche Saatveredelung Lippstadt-Bremen GmbH, Lippstadt). Estimates for GCA effects were calculated according to Wricke and Weber (1986). Following the cross 'good combiner' ('Express', double low quality) x 'poor combiner' ('V8', HEAR quality) a mapping population of doubled-haploid (DH) lines was created using a haploid method described earlier (Weber et al. 1995). From all mapping individuals leaf material was taken, freeze-dried and DNA was extracted. For the molecular studies a screening of the cross parents was carried out with 256 AFLP and 72 SSR primers to determine the most efficient primers to be applied in the mapping population. Due to their yield performance 10 of the original HEAR male lines were selected for developing a new double-low gene pool by conducting a backcross procedure with the HEAR line as recurrent parent. Half-seed fatty acid analysis (Thies 1971, Lühs and Friedt 1994) was used to select suitable plants with low erucic acid content for further quality conversion.
RESULTS AND DISCUSSION

The yield performance of inter-pool hybrids (double-low quality x HEAR) in a three-location field trial (data not shown) were used to estimate the general combining ability (GCA) of 20 selected HEAR lines (V1 to V22, Fig. 1). In order to identify gene loci contributing to ‘combining ability’ with regard to relevant quantitative traits, such as seed and oil yield as well as oil content, a segregating mapping population of about 220 DH lines was developed by microspore culture. The population derived from a cross between a ‘good combiner’ (‘Express’, double-low quality) and a ‘poor combiner’ (‘V8’, HEAR quality), which is now used for subsequent genetic mapping by AFLP and SSR markers.

Figure 1. General combining ability (GCA) regarding seed yield of 20 HEAR rapeseed lines (V1 to V22) and two male sterile MSL-lines (‘Falcon’, ‘Express’). Data from field trials in 2001 at three locations (RH, Hohenlieth, Thüle).

In the second part of the project, the conversion of selected HEAR lines into a novel double-low gene pool, fatty acid samples of approx. 3,000 plants were analysed in order to improve the population. Selection for this conversion was made on the basis of low erucic acid content. For this purpose, a distribution into five phenotypic classes (EEEE, EEEe, EEee, Eeee, eeee) was carried out according to the genetic model of erucic acid inheritance (Fig. 2) in B. napus (cf. Lühs and Friedt 1995). Half-seed plants nearly free of erucic acid (eee) and those with one effective allele (Eeee, 15-25 % erucic acid) were further cultivated. Due to the additive inheritance of erucic acid content, which is controlled by two genes, a segregation of 1:4:6:4:1 with regard to erucic acid content is expected in F2 (Fig. 2). From these segregating plants only about 1/3 can be used for quality conversion. The selection on reduced glucosinolate (GSL) content, however, is more difficult because this feature is maternally determined and the selection can be conducted for the first time on F3S2 seed. Nevertheless, to attain a selection as early as possible of the remaining plants with low erucic acid content, they have to be examined for low glucosinolate content by analysing the plant material (leaves, buds) before pod development. Due to a correlation coefficient of approx. r=+0.8 (Jürges 1982, Schilling 1991) sufficient relation between the GSL content of seeds and leaves exists allowing the elimination of high GSL plants before flowering. Only the remaining plants are then used for the backcross with the respective recurrent parent (HEAR male line).

OUTLOOK

For the production of test hybrids a male sterile female line (‘MSL-Falcon’) was sown in the field at the end of August 2002. Furthermore, artificially vernalised clones of 90-110 DH individuals were planted in the field in spring 2003 in order to produce seed material of hybrids involving a
first partial set of individuals of the mapping population. These experimental hybrids will be tested in the field 2003/2004 for the assessment of the GCA effects of the DH lines and finally generating phenotypic data for the QTL analysis.

### ACKNOWLEDGEMENTS

This work is founded by the "Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung" (GFP), Bonn, Germany. The authors thank Sonja Weber, Rosa Allerdings, Petra Degen and Daniela Bernhardt for excellent technical assistance.

### REFERENCES


