Research Center for Bio Systems, Land Resources and Nutrition,
Department of Plant Breeding
Head: Prof. Dr. Dr. h.c. Wolfgang Friedt

Development of camelina (*Camelina sativa* Crtz.) genotypes and winter rapeseed (*Brassica napus* L.) hybrids for marginal locations

A dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Agricultural Science
in the Faculty of Agricultural Sciences, Nutritional Sciences
and Environmental Management
at Justus Liebig University, Giessen

submitted by
Dipl.- Ing. agr. Anke Gehringer
from Mömbris

Giessen, November 2009

This thesis was accepted as a doctoral dissertation in fulfillment of the requirements for the degree of Doctor of Agricultural Science by Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management Justus-Liebig-University Giessen

Date of defence: February 12th, 2010

Dekan: Prof. Dr. U.Leonhäuser

<u>Members of the examination committee:</u>

Chairman:	Prof. Dr. St.Hoy
Supervisor:	Prof. Dr. Dr. h.c. W.Friedt
Co-supervisor:	Prof. Dr. B. Honermeier
Examiner:	Prof. Dr. S. Schnell
Examiner:	Prof. Dr. K.-H. Kogel

1. Introduction and Aims

1.1 Oilseed crops as an alternative for low-input cropping systems

Oilseed crops play a major role both in human nutrition and as a protein source for animal feed. Furthermore they act as a valuable renewable resource for the oleo-chemical industry and for the production of hydraulic oil and lubricants. Moreover, during the past few decades biodiesel from oilseeds has become one of the major contributors of renewable fuel worldwide. The diesel demand of the European Union in 2004 comprised around 185 million t (Eurostat, 2006), with the highest consumption occurring in France and Germany. WOOD MACKENZIE (2006) projected annual increases of about 2.5% for the diesel market in Europe between 2003 and 2015. With limited quantities of fossil diesel, biodiesel is playing an important role in meeting this constant increase in demand. In the temperate climate of Western Europe, rapeseed oil or rapeseedoil methyl ester (RME, biodiesel) is the most suitable locally-available raw material for biodiesel production, meeting all required quality standards. In the European Union in 2005 a total of around 17.6 million t of plant oil were produced, 2.4 million t of which were utilized for the production of biodiesel. Since the majority of this production derived from rapeseed oil, this means that around half of the usable rapeseed oil in Europe was used for biodiesel (WALLA 2006).

At the current rate of yield increases through advances in breeding and agronomy, the production of key food and energy crops may not satisfy the growing worldwide demand in the coming decades without major increases in production intensity. However, a sustainable production of agricultural crops for bioenergy and/or food purposes can only be achieved by reduction of the production intensity, for example with reduced fertilization and pesticide applications. So-called *low-input crops* are of great importance in this regard. In particular, the production of some energy crops, including oilseed rape, is coming under increasing criticism with regard to atmospheric nitrogen oxide release caused by excessive nitrogen fertilisation requirements (KRÜTZEN et al. 2007). On the other hand, oilseed rape and related cruciferous oilseeds are a valued component in crop rotations, due to their positive influence on soil structure and soil nitrogen contribution to following cereal crops. In order to improve the energy balance of whilst sill providing the positive contribution to crop rotations, nitrogen-efficient oilseed crops with improved N-absorption and/or utilization efficiency are a major breeding goal for sustainable biodiesel production. Oilseed crops suitable for low input production systems would be a valuable alternative for high-value crop production in marginal agricultural locations (e.g. Figure 1) with poor soils or sub-optimal climatic conditions.

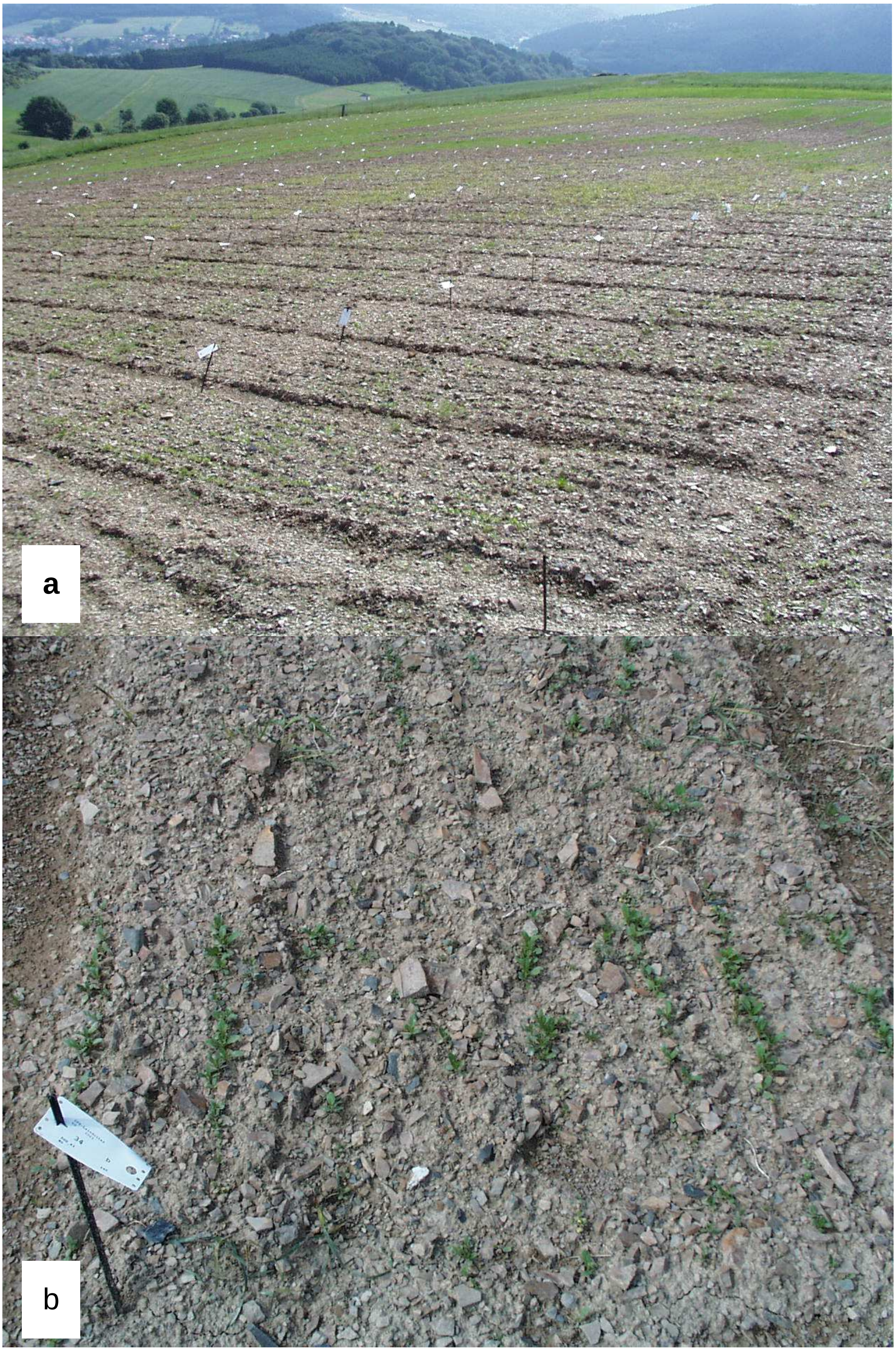

Figure 1: (a) Marginal location in Niederhörlen, Lahn-Dill District, with cool climate and poor soils (b) characterized by decomposed acidic slate soil with stones and a very poor nutrient balance

The work presented in this thesis is based on previous studies (MÜLLER et al. 1998, 1999, MÜLLER 2002, FRIEDT et al. 2003) that identified winter oilseed rape hybrids, on the one hand, and *Camelina sativa* on the other hand as promising alternatives for targeted breeding activities with regard to improved performance under low-input production conditions. Winter oilseed rape (*Brassica napus* L.) is presently the commanding oil-crop in Germany and Europe, due to its high seed and oil yield in temperate climates, and has a positive effect in crop rotations due to an improvement of soil fertility and the reduction of soil erosion damage. The related crucifer species *Camelina sativa* (also known as false flax, Gold of Pleasure, camelina or German sesame; see VOLLMANN et al. 1996) was a quite common crop in Europe and North America until the middle of the last century, but since then has continually lost in importance so that it is now virtually unknown in Europe. However, with an increased interest in renewable resources this summer annual oil plant has been re-discovered. Over the last years the importance of camelina as an alternative oilseed crop with special oil quality is rising. Camelina is particularly interesting as an alternative spring-sown oilseed crop because of its adaptability to adverse environmental conditions and its comparatively short vegetation time. In some areas of North America the seed and oil yields of camelina are comparable to those of spring canola, although camelina has considerably lower nutrient and plant protection requirements.

1.2 Oilseed rape (Brassica napus ssp. napus)

Oilseed rape (*Brassica napus* ssp. *napus*; Figure. 2) is the most important oilseed crop in Europe, followed by sunflower and soybean. Worldwide oilseed rape is the second most important oilseed crop after soybean.

Figure 2: Flower of oilseed rape (*Brassica napus* ssp. *napus*)

The production area of oilseed rape in Germany in 2007 was 1.5 million ha, compared with 1.43 million ha in 2006, 1.3 million ha in 2005 and less than 1 million ha during the 1990s. Due to this rapidly-growing economic importance, largely the result of political requirements for mixing of biodiesel into fossil diesel fuels, a further increase of rapeseed cultivation is anticipated in the coming years. Production areas as high as 1.7 million ha have been projected for Germany by the year 2010 (Workshop of the Society for the Promotion of Private German Plant Breeders – GFP, 2006).

The seed oil of *B. napus*, a member of the mustard family (Brassicaceae), naturally contains high levels of the anti-nutritive fatty acid erucic acid (C22:1) and is therefore unsuitable as a vegetable oil. However, almost all modern oilseed rape varieties carry a mutation in two copies of the fatty acid elongase gene *FAE1* (ECKE et al. 1995, DAS et al. 2002), which results in a seed oil almost free of C22:1 and instead containing large quantities of oleic acid (C18:1). This erucic-acid free oil is considered as one of the most nutritionally valuable edible plant oils available (DE LORGERIL et al. 2001), while at the same time it is also highly suitable for renewable products, e.g. as a feedstuff for oleochemicals and for biodiesel.

Oilseed rape is a facultative outcrosser, meaning that it can be bred using both inbreeding methods and via hybrid techniques based on male sterility. The worldwide first restored oilseed rape hybrid variety was released in Germany in 1995, and since then the importance of hybrids has grown continually. Hybrid oilseed rape cultivars tend to show a higher adaptability and yield stability under sub-optimal growth conditions, which makes them particularly interesting for use in low-input cropping systems.

However, the gene pool of double-low quality oilseed rape (zero erucic acid, low seed glucosinolate) is relatively narrow (HASAN et al 2006, 2008), meaning that the yield performance of currently available hybrids is not always considerably higher than that of the best open-pollinated varieties. On the other hand, combining the distinct genetic pools of conventional high yielding breeding lines or cultivars with novel high-erucic acid rapeseed (HEAR) lines as respective cross parents is a promising route to enhance hybrid vigour. Such new hybrids may be of particular interest for increasing nitrogen efficiency and hence for use in low-input farming systems. For maximizing the heterosis effect and achieving a consequent high grain yield and yield stability, it is important to consider not only the productivity of the parental lines *per se*. A sufficient genetic diversity between the potential cross parents and a superior combining ability are also of crucial importance.

1.3 Camelina sativa Crtz. (Camelina)

Camelina sativa Crtz. (Figure 3), a member of the *Brassicaceae* family, has been grown in Europe since the Bronze Age (SCHULTZE-MOTEL 1979). It was broadly cultivated throughout Europe and North America until the 1950s. The importance of camelina has diminished considerably over the last half century, however its positive agronomic attributes with regard to sustainable agriculture have recently rekindled interest in this spring-sown oilseed crop.

Figure 3: Flowering Camelina sativa Crtz. in field plots

Camelina is a high-value, multi-use crop with applications in food, feed and industry (Pilgeram 2007) It produces a seed oil rich in poly-unsaturated fatty acids, making it a valuable renewable feedstock for the oleochemical industry. At present, the oil is used mainly in non-food applications as a drying oil, however recently it has gained increasing attention as a potential alternative bio-fuel crop to spring-sown canola. A revival of interest in camelina oil for food purposes has also occurred in recent years, mainly due to its comparatively high concentration (35-40%, see Figure 4) of α-linolenic acid, an ω3 fatty acid that is generally found in substantial quantities only in linseed and fish oils. Development of camelina as an alternative spring oilseed for modern crop rotations offers an opportunity to diversify crop production while supplying the growing demand for edible oils rich in ω3 fatty acids (Matthäus 2004).

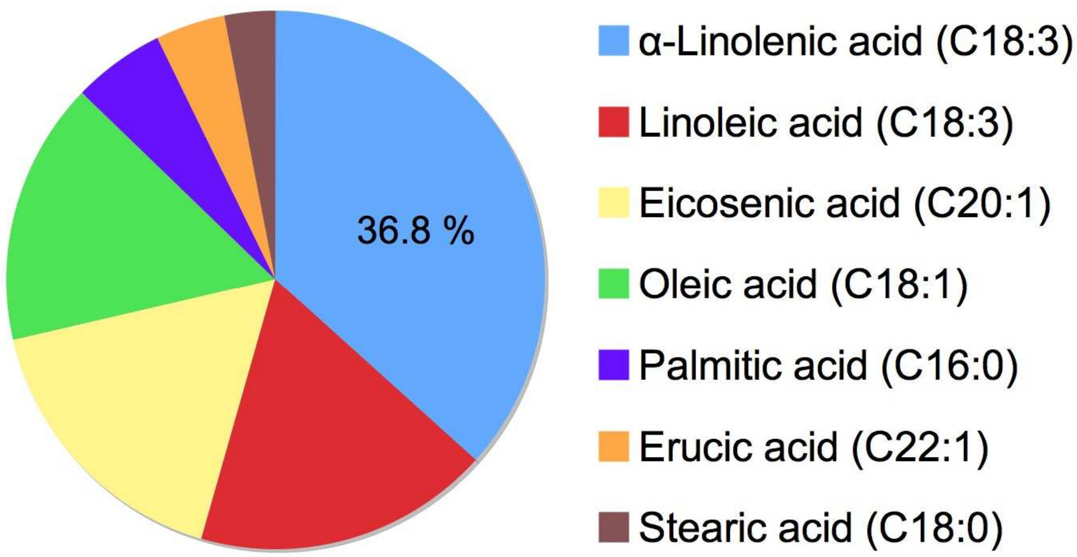

Figure 4: Fatty acid composition of camelina oil

Camelina possesses numerous valuable agronomic attributes that make it attractive as an alternative spring-sown crop both for tight crop rotations and marginal locations. It combines a good adaptability to adverse environmental conditions (Makowski 2003) and a short growing season (Müller et al. 1999) with a diverse food and non-food application of the seed oil. An important feature of camelina is its high level of resistance against insect pests and plant pathogens (Volmann et al. 2003). Its positive agronomic attributes also include high nutrient efficiency, which makes the crop suitable for low-input cropping systems with reduced N-fertilization and considerably reduced fungicide and pesticide applications (Schuster and Friedt 1995; Müller 2002). Due to the low interest in the crop throughout the late 20th century, breeding efforts for the crop have been limited. As a result very few registered varieties and advanced breeding lines are available. In preliminary work, however, a good degree of phenotypic variation could be observed among camelina accessions (Müller, 1999). This provides a basis for phenotypic selection towards further improvements of the major agronomic and quality characteristics.

In contrast to the vegetable and oilseed Brassica species, almost no information was available prior to the beginning of this study with regard to the genomic makeup of C. sativa and the genetic control of complex agronomic traits in this species. A genetic map of camelina would represent a valuable tool for future genomics-assisted improvement of this crop.

1.4 The principle of heterosis

The term heterosis, used to described the increased performance of an F1 hybrid compared with the mean performance of two parental homozygous lines, is a basic quintessence in plant breeding. Since its discovery at the beginning of the 20th century (EAST 1908; SHULL 1908), exploitation of heterosis has become one of the most important means for increasing yield performance in the breeding of outcrossing crop species. The general genetic basis of heterosis is still not completely clear, however in different situations it is thought that dominance (DAVENPORT 1908; BRUCE 1910; JONES 1917), overdominance (HULL 1945; CROW 1948) and epistasis (POWERS 1944; WILLIAMS 1959) play important roles.

As a facultative outcrossing species, utilisation of heterosis in oilseed rape by hybrid breeding is today an important means to improve the yield potential. Hybrid cultivars afford a systematic use of heterosis and therefore a better consumption of the yield potential and higher yield stability. Due to their heterozygous genotype, hybrid cultivars can also potentially achieve a higher yield stability under unfavorable conditions (LÉON 1991; DIEBENBROCK 2000). In rapeseed, a higher yield potential of 20 to 50% compared to the parents has been observed in experimental hybrids (SCHUSTER and MICHAEL 1976; LÉFORT-BUSON et al. 1987; BRANDLE and McVETTY 1990; McVETTY et al. 1991, SCHUSTER et al. 1999). According to SAUERMANN and FINKE (1998), winter oilseed rape hybrid varieties can reach 5-12% yield advantage compared to open-pollinated varieties.

Different researchers have reported substantial heterosis in the major oilseed Brassicas (for a review see Leon and Becker 1995) that stimulated a worldwide interest for developing hybrid cultivars. In Canada, China and Europe, hybrids are today exceeding open-pollinated varieties as the major winter and spring oilseed rape cultivar types (DIANRONG 1999; FRAUEN et al. 2003). This is particularly the case in Germany, where the world’s first restored rapeseed hybrids were released in 1995 and hybrid varieties meanwhile make up more than half of the seed production (SNOWDON et al. 2006). The increased yield potential of hybrids (heterosis) is influenced by two main factors: the individual performance of the parental lines and their combining ability. Besides the average ability of a crossing parent (general combining ability, GCA), the heterotic potential of two specific crossing parents is described by their specific combining ability (SCA). Both GCA and SCA are heritable, quantitative traits.

1.5 Quantitative Trait Loci (QTL) and low-input performance

Analysis of quantitative trait loci (QTL) helps to clarify the inheritance of complex, quantitative traits (e.g. seed or oil yield) with a continuous phenotype variation that does not allow the identification of defined gene effects. With the help of the genetic linkage maps developed on the basis of molecular marker screening in a segregating population, along with phenotypic data gathered in different environments, genome regions associated with effects on complex traits can be detected. To locate the most likely position of a QTL on a chromosome, the chromosome intervals between adjacent molecular markers are analyzed regarding phenotypic effects using multiple regression or maximum likelihood functions. The theory and methods of QTL detection in segregating populations are outlined in detail by LANDER and BOTSTEIN (1989), KNAPPS *et al*. (1990) and HALEY and KNOTT (1992).

Numerous seed quality traits, seed yield and yield parameters along with many other important agronomical traits like winter hardiness and important pathogen resistance have been investigated in various *B. napus* crosses by QTL analysis (see SNOWDON et al. 2006 for a detailed review). Particular attention has been paid to QTL detection for flowering time (CAMARGO and OSBORN 1996), oil content (BURNS et al. 2003; ZHAO et al. 2006), fatty acid content ad composition (ECKE et al. 1995; HU et al. 1995, 1999; JOURDREN et al. 1996b; SOMERS et al. 1998; RAJCAN et al. 1999), glucosinolate content (TOROSER et al. 1995; UZUNOVA et al. 1995, HOWELL et al. 2002) as well as disease resistance (e.g. DION et al. 1995; PILET et al. 1998, 2001; ZHAO and MENG 2003, RYGULLA et al. 2008). On the other hand very little is known about the genetic control of adaptability of oilseed rape to marginal cropping conditions, or of its performance under low-input agronomy systems. Field testing of appropriate mapping populations in multiple environments under normal and reduced-input conditions, respectively, could potentially generate suitable data for identification of important QTL involved in nutrient uptake and assimilation efficiency. In *C. sativa* no QTL studies had been published prior to the work described in this thesis. This is due to the absence of a genetic map for this species, which is a prerequisite for QTL detection.

1.6 Objectives

The papers presented in this thesis describe an analysis of yield performance in pre-selected rapeseed and camelina genotypes that were studied under reduced nitrogen regimes at different locations. The sites used for the study ranged from optimal agricultural conditions, under which oilseed rape already plays a key role in crop rotations, to marginal locations (e.g. Figure 4) with poor soils, low rainfall and a cool climate where oilseed crops are currently less widespread. The underlying objective of the work was to identify genotypes and breeding lines with superior adaptability to sub-optimal growth conditions, as a basis towards development of oilseed cultivars as a valuable alternative crop for marginal locations.

The key aims of the work were:

- Development of a population of camelina inbred lines and genetic analysis of their yield performance and seed quality traits under low-input cropping conditions;

- Generation of the first genetic map for *C. sativa* and localization of quantitative trait loci (QTL) for seed quality and yield traits under low-input conditions, as a basis for future marker-assisted improvement of camelina as a renewable resource;

- Development of new low glucosinolate, zero erucic acid (00 quality) winter oilseed rape hybrids with high performance under low-nutrient conditions and in marginal locations;

- Development of high erucic acid, low glucosinolate (+0-quality) winter oilseed rape hybrids and identification of hybrid combinations with consistently high seed and oil yields under low-nutrient conditions and in marginal locations.

I. Publication 1

Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. *sativa*)

Gehringer A., Friedt W., Lühs W. and Snowdon R.J.

Published 2006 in *Genome*, Vol. 49, No. pp. 1555-1563

Abstract

The crucifer oilseed plant false flax (*Camelina sativa* subsp. *sativa*) possesses numerous valuable agronomic attributes that make it attractive as an alternative spring-sown crop for tight crop rotations. The oil of false flax is particularly rich in polyunsaturated C18 fatty acids, making it a valuable renewable feedstock for the oleochemical industry. Due to the low interest in the crop throughout the 20th century breeding efforts for the crop have been limited. In this study a genetic map for *C. sativa* was constructed using AFLP markers in a population of recombinant inbred lines that were developed via single seed descent from a cross between the phenotypically distinct parental varieties 'Lindo' and 'Licalla'. Three *Brassica* SSR markers were also integrated into the map, and one of these shows linkage to oil content loci in both *C. sativa* and *Brassica napus*. Fifty-five further SSR primer combinations showed monomorphic amplification products, indicating partial genome homoeology with *Brassica* species. Using data from field trials with different fertilization treatments (0 and 80 kgN/ha, respectively) at multiple locations over a total of three years, the map was used to localise quantitative trait loci (QTL) for seed yield, oil content, 1000-seed weight and plant height. Some yield QTL were found only in the N0 treatment and may represent loci contributing to the competitiveness of camelina in low-nutrient soils. The results represent a starting point for future marker-assisted breeding.

Key words: False flax, Camelina sativa, genetic map, QTL

Introduction

Oil crops count among the most valuable basic agricultural trade materials, and as the world population expands and fossil resources decline the demand for refined edible oil products and renewable industrial oils continues to grow. Presently the international oilseed market is dominated by soybean, rapeseed and sunflower; however under increasing production areas the pressure on crop rotations in terms of sustainable production has increased the requirement for greater diversification of oil crops. Of growing interest in this respect are alternative crops that combine positive agronomical properties, for example nutrient efficiency, pathogen tolerance and a short growing season, with a diverse food and non-food application of the seed oil. The crucifer oilseed species Camelina sativa (variously known as camelina, false flax, gold of pleasure or German sesame; see Vollmann et al. 1996) is of particular interest in this regard.

Although camelina has been cultivated in Europe since the Bronze Age (Schultze-Motel 1979), it is presently an underexploited oilseed crop. Until the late 18th century false flax was cultivated throughout eastern, central and western Europe and to a limited extent in North America, however since the middle of last century cultivation has all but disappeared. In Germany and Austria *C. sativa*, an annual oilseed plant belonging to the mustard family (Brassicaceae), is grown on a limited scale mainly in mixed cropping systems (Paulsen et al. 2003; Makowski 2003). Compared to other oil plants it is particularly competitive in semi-arid regions and in low-fertility or saline soils (Budin et al. 1995). Like its polyploid relative oilseed rape (*Brassica napus*), false flax exhibits a high adaptability to adverse environmental conditions (Makowski 2003) and its competitive growth character means that herbicide requirements are also minimal (Zubr 2003). An important feature of camelina is its high level of resistance against insect pests and plant pathogens, which may be partly due to the production of anti-microbial phytoalexins (Vollmann et al. 2001). Its positive agronomic attributes also include high nutrient efficiency; hence false flax can be produced in low-input cropping systems with reduced N-fertilization and without fungicide or pesticide applications (Schuster and Friedt 1995; Müller 2002). The comparatively short vegetation period of approximately 120 days makes it particularly suitable as an alternative annual crop for renewable resource production within tight crop rotations (Agegnehu and Honermeier 1997; Müller and Friedt 1998; Müller et al. 1999) The seed oil of false flax is rich in polyunsaturated C18-fatty acids, making it a valuable renewable feedstock for the production of oleochemicals, particularly as a drying oil for paints and varnishes but also as an alternative source of biodiesel (Bernardo et al. 2003; Zubr 2003; Fröhlich and Rice 2005; Matthäus and Zubr 2000). On the other hand the high (35-40%) content of α-linolenic acid, a ω3 fatty acid otherwise found in substantial quantities only in linseed and fish oils (Matthäus 2004), has also led to a recent revival of interest in camelina oil for food purposes.

Mature *C. sativa* plants grow to a height of 30 to 120 cm and produce seed with an average 1000-seed weight (TSW) between 0.7 and 1.6 g depending on the variety and growing conditions (Putnam et al. 1993). Seed yields range from 2 to 3 t/ha, with an oil content ranging from 28 to 42%. Despite this relatively high variation in agronomic properties, little effort has been made to date with regard to the improvement of false flax through breeding. The main objective of the present study was to investigate the productive efficiency of false flax as a renewable primary resource for food and non-food oil production, particularly in low-input cropping systems. For this purpose yield performance and stability of a group of inbred lines were studied under different nitrogen fertilization regimes at three locations with major differences in soil characteristics in Hesse, Germany. Furthermore we produced a first genetic map of *C. sativa* based on AFLP markers, and used it to localise quantitative trait loci (QTL) related to agronomic characters including seed plant height, oil content, TSW and seed yield. The data allowed identification of potential genotypes for further improving the adaptability and performance of false flax as a crop for marginal locations and low-input systems, and will serve as a first basis for future marker-assisted breeding of improved varieties.

Material and Methods

Plant Material

A total of 187 recombinant inbred lines were created from a cross between the registered German false flax varieties ’Lindo’ and ’Licalla’ (Deutsche Saatveredelung, Lippstadt, Germany) using single-seed descent (SSD) as described by Seehuber et al. (1987). Plants were grown in the greenhouse under water and nutrient stress, enabling production of two to three generations per year. The SSD lines were continued to the F_6 generation. The parents used to produce the inbreds showed considerable variation in seed yield, TSW, plant height and seed oil content.

Field trials

The SSD lines and parental genotypes were grown in 3.75 m^2 plots with two replications of a 14 x 14 lattice experimental design in a total of four different environments (year x location) at three diverse locations in Hesse, Central Germany. The environments differed considerably in climate and soil type (Table 1).

Environ-ment	Location	Nmin (kgN/ha)	Mean annual precipitation (mm)	Mean annual temperature (℃)	Altitude (m ASL)	Soil classification[#]	Soil pH
RH03	Rauischholzhausen	43	581	9.4	200-295	70-80	6.8
RH04	Rauischholzhausen	50	626	9.0	200-295	70-80	6.6
NH04	Niederhörlen	21	834	7.1	340-380	25-35	5.5
GG05	Gross Gerau	30	573	11	91	20-25	7.0

Table 1: Climatic features, Nmin contents and soil properties of the four environments used for field trials.

[#] German soil classification scale (Görz and Hock 1939)

The sites at Rauischholzhausen (RH, around 70km north of Frankfurt) and Gross Gerau (GG, 30km south of Frankfurt) are situated on University of Giessen experimental farms. The RH site is characterised by good cropping conditions with mainly loess soils, whereas the GG site has a mild climate and predominantly sandy soil. The marginal location Niederhörlen (NH, situated in the Lahn-Dill District) has a considerably colder climate than the other two sites and is characterised by decomposed acidic slate soil with a poor nutrient balance. Field trials were performed in the years 2003 (RH03), 2004 (RH04, NH04) and 2005 (GG05).

For each location the experiments were repeated with two different nitrogen fertilization treatments, hereafter referred to as N0 and N80. The soil N_{min} content (measured at a depth of 0-90 cm) varied from 21 kgN/ha (NH04) to 50 kgN/ha (RH04). Independent of the N_{min} content no additional nitrogen fertilizer was applied to the N0 treatment, whereas in the N80 treatment 80 kg/ha calcium ammonium nitrate containing 27% N (KAS) was applied in two volumes of 40 kgN/ha at two and six weeks after sowing. During the course of the vegetation period the date of flowering and plant height at flowering were recorded, and seed yield, TSW and seed quality traits were determined after harvest.

Quality analysis

Oil and water contents of the harvested seed were determined by pulsed nuclear magnetic resonance (NMR) using a Bruker Minispec analyzer (Bruker Analytische Messtechnik, Rheinstetten, Germany). Fatty acid composition was determined by gas-liquid chromatography on a TRACE GC 2000 (Thermo Finnigan, Italy) with a flame-ionization detector and automatic injector using helium as a carrier gas.

Genetic mapping

Genomic DNA from all SSD lines and the parental genotypes was extracted using CTAB extraction, as described by Doyle and Doyle (1990), from young leaves from plants grown in the greenhouse. A total of 256 AFLP primer combinations were screened for polymorphisms between the two mapping parents, and the 44 primer combinations with the highest rate of polymorphism were used to genotype the 181 SSD lines. In addition a set of 400 publicly available *Brassica* SSR primers (www.brassica.info; Suwabi et al. 2002; Lowe et al. 2004) were screened in the parental lines. The majority of the SSR primers did not amplify loci in *C. sativa*, however eight polymorphic SSR markers were identified of which four could be integrated into the genetic map. The genetic linkage map was calculated with JoinMap® 3.0 (Kyazma Software, Wageningen, Netherlands; see Stam 1993) using only loci that showed the expected 1:1 allelic segregation ratio. Markers were assigned to linkage groups using the Kosambi mapping function with a minimum LOD score parameter of 2.00 and a maximum recombination frequency of 40cM.

Data analysis and QTL localisation

Analysis of variance (ANOVA, p=0.001) for all agronomic traits was performed using the mixed-model procedure in the statistical software package SAS 9.1, in order to test for significant differences among the SSD lines and to estimate variance components. Broad sense heritability for mean values over environments was calculated following Hill et al. (1998) from components of variance as:

$$h^2 = V_g/(V_g + V_{ge}/E + V_r/ER)$$

where V_g, V_{ge} and V_r represent the respective variance components for genotype (g), genotype*environment (ge) and residual variance (r), and E and R are the number of environments and replicates, respectively. Correlations among traits were calculated separately using the respective data from the N0 and N80 treatments, in order to detect possible effects of nutrient deficiency on trait interactions. Correlation analysis was performed with SPSS 12.0.1 for Windows.

For all phenotypic traits QTL analysis was performed using mean values from the two replicates of each genotype for each treatment and at each location. QTL were localised in the genetic map by composite interval mapping (CIM), after stepwise regression analysis based on single marker genotypes of markers with significant effects on the trait analysed, using Windows QTL Cartographer, Version 2.5 (Zeng et al. 1994; http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). LOD thresholds for QTL detection were established by permutation analysis using 1000 permutations and a significance threshold of p=0.05. The QTL analysis was repeated using first the mean phenotype data from individual locations, years and treatments, respectively, then with data averaged over the different locations or treatments, and finally using means from the cumulative data from all environments, treatments and years.

Chromosome counts

Because the chromosome number of *C. sativa* is variously reported as 2n=20 (Warwick et al. 2000), 2n=21 (Schuster 1992), and n=6 or 14, 2n=12, 26 or 40 (Canadian Biodiversity Information Facility, http://www.cbif.gc.ca/spp_pages/brass/index_e.php), chromosome counts were performed on the two parental genotypes used for the production of the SSD population. Chromosome preparations were generated from root-tip preparations treated with 2mM 8-hydroxyquinoline using the procedure described for *Brassica* chromosomes by Schelfhout et al. (2004), and after staining with the blue fluorescent dye DAPI the mitotic chromosomes were counted in ten metaphases from each line under a fluorescence microscope.

Results

Agronomic characters

Table 2 describes variation in mean seed yield, TSW, plant height and oil content in the SSD lines under different nitrogen fertilization treatments in the four environments RH03, RH04, NH04 and GG05.

Environment	Nitrogen treatment	Seed yield (t/ha)	Oil content (% DM)	Plant height (cm)	TSW (g)
RH03	N0	1.46^{a}	40^{a}	79^{a}	1.41^{a}
	N80	1.67^{b}	38^{b}	85^{b}	1.32^{b}
RH04	N0	1.49^{a}	44^{c}	70^{cd}	1.58^{cd}
	N80	2.05^{c}	42^{d}	75^{e}	1.57^{cd}
NH04	N0	1.17^{d}	40^{a}	67^{d}	1.71^{e}
	N80	1.86^{e}	40^{a}	73^{ce}	1.76^{f}
GG05	N0	1.15^{d}	39^{e}	50^{f}	1.55^{c}
	N80	2.20^{f}	38^{b}	59^{g}	1.60^{d}
	LSD (p=0.05)	0.12	0.58	3.26	0.05

Table 2: Variation in mean seed yield, oil content, plant height and TSW in the SSD lines under varying N-fertilization treatments (N0, N80) in the four environments RH03, RH04, NH04 and GG05, displayed as comparisons of means with respect to least significant d difference (LSD, p=0.05) for each variable. For each trait the environments and treatments with show non-significant differences are assigned the same superscript letter.

As expected, the mean yield was always significantly higher with N-fertilization (N80) than in the treatment with no added nitrogen (N0), and particularly strong yield improvements were obtained at the two locations with the lowest N_{min} values. Some SSD lines yielded up to 2.5 t/ha with N80 at both NH and RH in 2004, while the highest yield of a single line (3 t/ha) was achieved at GG in 2005. When seed yields were averaged over all treatments and environments, 45 SSD lines (24% of the population) showed higher grain yield than the best-performing cross parent, 'Lindo'. This indicates transgressive segregation of positive alleles for seed yield, which could be confirmed by a combination of positive alleles from both parents at the major QTL contributing to yield. When least significant differences (LSD, p=0.05) for mean seed yields over all environments and treatments were calculated, five SSD lines showed significantly higher grain yield than the best parent 'Lindo' (Table 3). These lines are interesting candidates for further improvement/breeding.

	Seed yield (t/ha)								
	RH03		RH04		NH04		GG05		Mean (all environments & treatments)
Genotype	N0	N80	N0	N80	N0	N80	N0	N80	
Lindo	1.64	1.88	1.55	1.84	1.10	1.84	1.22	1.81	1.61
SSD-10	2.06	2.27	1.66	1.96	1.21	1.94	1.38	2.65	1.89
SSD-88	1.93	1.93	1.61	2.10	1.56	2.25	1.56	2.20	1.89
SSD-177	1.71	2.13	1.58	1.96	1.47	2.12	1.30	2.25	1.82
SSD-186	1.68	2.09	1.55	1.98	1.20	1.88	1.80	2.24	1.80
SSD-241	1.71	2.02	1.71	1.92	1.36	1.90	1.28	2.48	1.80
LSD (p=0.05)	0.33	0.39	0.22	0.34	0.21	0.34	0.53	0.68	0.12

Table 3: Seed yields (t/ha) over all environments (RH03, RH04, NH04, GG05) and N-treatments (N0, N80) of five SSD lines with mean seed yields in excess of the best parent 'Lindo'. Yield values in italics represent lines with significantly greater yields (p=0.05) than the best parent calculated by the least significant difference (LSD).

Analysis of variance showed highly significant effects for seed yield (p=0.001) due to N-fertilization, genotype and the interaction environment*genotype, respectively. Environment and the interaction environment*N-fertilization had significant effects on yield (p=0.05); however no significant effects for seed yield were observed in the interactions N-fertilization*genotype or environment*N-fertilization*genotype. Heritability for grain yield was calculated to be h^2=0.54. TSW showed a very high heritability of h^2=0.94, and the large differences in TSW among SSD lines (0.95 to 2.10 g) were consistent at the different locations without being significantly affected by the fertilization treatment. Significant differences in TSW between the N0 and N80 treatments were observed in all environments except RH04. The highest TSW (2.10g) was achieved at the marginal location NH. This indicates that TSW increases as a response to sub-optimal nutrient supply, possibly in association with a reduced number of seeds per plant. For plant height considerable variation was observed among the SSD lines (30-100 cm), and the heritability for plant height was relatively high at h^2=0.68. In all environments significant differences in plant height were seen between the N0 and N80 treatments (Table 2).

Analysis of variance for seed oil content revealed significant effects (p=0.05) from the environment and from the interaction environment*N-fertilization. Highly significant genetic variation in oil content (p=0.001) was achieved between N-fertilization levels, between genotypes and due to the interaction genotype*environment, whereas no significant environment*N-fertilization*genotype interaction could be observed. The very high heritability for oil content of h^2=0.89 emphasises the strong dependence of this trait on the genotype and underlines the potential for improvement through breeding. The minimum oil content was 32%, whereas the best SSD lines achieved maximum oil contents of up to 49% of seed dry weight. This was only achieved in the N0 treatment, however, suggesting that the high oil content was probably obtained at the expense of a reduction in seed protein due to a nitrogen deficit during seed ripening.

Fatty acid composition

A relatively large variation was observed in the contents of the main fatty acid components. Using mean data over the N0 and N80 treatment the content of oleic acid (C18:1) ranged from 13% to 20%, whereas the unsaturated linoleic acid (C18:2) varied from 13% to 22% and linolenic acid (18:3) from 30% to 40%. The content of eicosenic acid (20:1) ranged from 10% to 17%, whereas erucic acid (22:1) content varied between 2% and 9%. This comparatively low natural content of erucic acid in *C. sativa* is unusual for a member of the Brassicaceae.

Correlations between agronomic traits

Correlations among seed yield, oil content, TSW and plant height for the N0 and N80 treatments are shown in Figure 1.

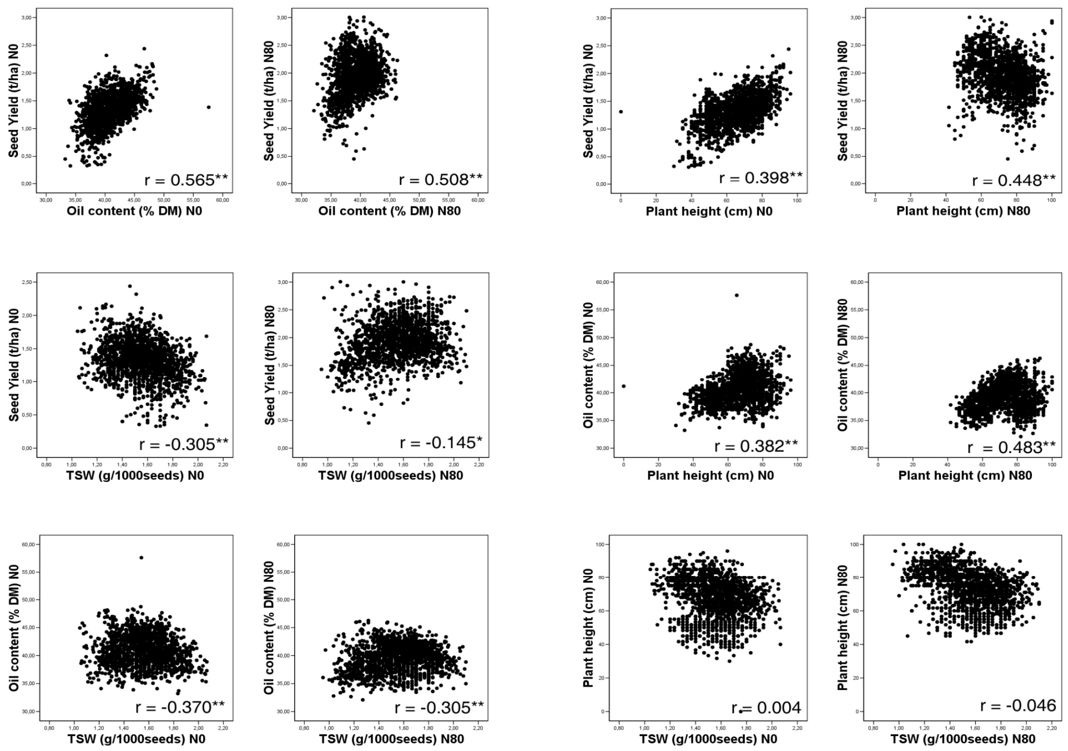

Figure 2: Correlations among seed yield, oil content, TSW and plant height for the N0 and N80 treatments averaged over all environments. Correlation values (r) with one asterisk are significant at p=0.05, while correlations with two asterisks are significant at p=0.01.

With the exception of TSW and plant height all other trait combinations showed significant correlations in both treatments. As expected the N0 and N80 treatments showed noticeable differences in the correlations among the traits. The correlations between plant height and the other traits were always stronger in the N80 treatment than with N0, while the respective correlations between seed yield and oil content with all traits except plant height were always stronger in the N0 treatment. Seed yield and TSW were found to be negatively correlated, and seed oil content was positively correlated to seed yield. Interestingly, plant height was found to have a positive correlation with both seed yield and oil content. This suggests possible pleiotropic effects or a linkage between gene loci involved in these three traits, in which case plant height could be useful as a selection criterion for yield and oil content.

Chromosome counts, genetic mapping and QTL localisation

A chromosome number of 2n=40 was confirmed in all ten mitotic metaphases from each of the parental genotypes. Accordingly, the linkage map of *Camelina sativa* (Figure 2) was constructed containing 157 AFLP markers and three *Brassica* SSR markers on a total of 20 linkage groups corresponding to n=20. The map covers a total length of 1385.6 cM cM with an average marker interval of 8.6 cM. A total of 47 AFLP and four SSR markers deviated from the expected 1:1 segregation and were not included in the map.

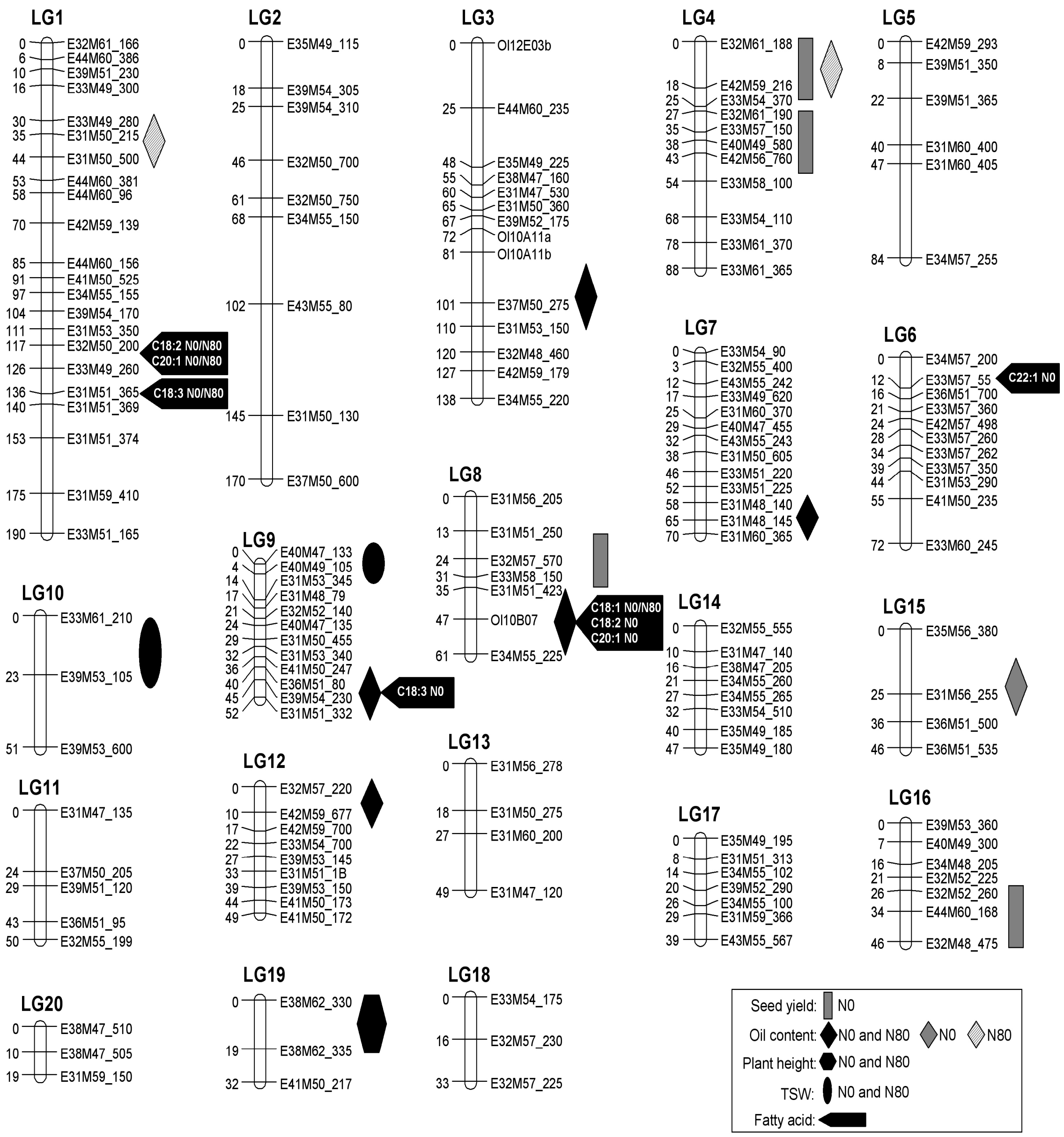

Figure 3: Genetic map of Camelina sativa, comprising 157 AFLP and 3 SSR markers, constructed using 181 single seed descent (SSD) lines from the cross 'Lindo' x 'Licalla'. Shown on the map are the positions of major QTL for seed yield, oil content, plant height, 1000-seed weight (TSW) and fatty acids (18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:1 eicosenic acid, 22:1 erucic acid) measured under different nitrogen fertilization levels (N0 and N80) in field trials at four different environments (RH03, RH04, NH04, GG05).

Details of all significant QTL detected for seed yield, oil content, plant height, TSW and fatty acid composition using mean data over all environments for the respective treatments are presented in Table 4.

Trait	Treatment	LG	Nearest associated marker	LOD	A	R2 (%)
Seed yield	N0	4	E32M61_188	3.89	0.40	10.51
	N0	4	E40M49_580	3.50	0.32	6.45
	N0	8	E33M58_150	3.18	0.30	5.81
	N80	8	E33M58_150	2.73	0.35	6.93
	N0	16	E32M52_260	3.17	0.31	6.50
Oil content	N80	1	E31M50_215	4.73	0.39	8.72
	N0	3	E37M50_275	5.36	0.46	9.81
	N80	3	E37M50_275	3.39	0.33	5.73
	N80	4	E32M61_188	3.03	0.28	4.42
	N0	7	E31M48_145	3.57	0.44	9.09
	N80	7	E31M48_145	3.67	0.39	8.14
	N0	8	Ol10B07	4.56	0.41	8.48
	N80	8	Ol10B07	3.33	0.32	5.71
	N0	9	E39M54_230	8.88	0.58	16.84
	N80	9	E39M54_230	7.45	0.49	13.40
	N0	12	E42M59_677	3.07	-0.34	5.57
	N80	12	E42M59_677	4.06	-0.34	6.49
	N0	15	E35M56_380	3.03	0.35	5.96
Oleic acid	N0	8	Ol10B07	3.72	0.50	8.10
	N80	8	Ol10B07	3.35	0.51	7.42
Linoleic acid	N0	1	E33M49_260	3.58	-0.43	6.82
	N80	1	E33M49_260	3.22	-0.41	6.17
	N0	8	Ol10B07	3.26	-0.41	6.40
Linolenic acid	N0	1	E31M51_369	3.13	0.45	8.05
	N80	1	E31M51_369	3.86	0.52	9.54
	N0	9	E39M54_230	3.45	0.44	7.79
Eicosenic acid	N0	1	E33M49_260	3.78	0.22	7.17
	N80	1	E33M49_260	3.35	0.25	6.33
	N0	8	Ol10B07	3.49	0.23	8.06
Erucic acid	N0	6	E33M57_55	3.66	-0.33	9.50
Plant Height	N0	19	E38M62_330	4.63	1.01	10.96
	N80	19	E38M62_330	2.64	0.81	6.36
TSW	N0	9	E40M49_105	5.85	49.28	13.48
	N80	9	E40M49_105	5.19	43.04	12.79
	N0	10	E33M61_210	3.55	-37.37	7.70
	N80	10	E33M61_210	3.32	-31.53	7.03

Table 4: Linkage groups (LG), nearest associated marker, LOD value (logarithm of odds), additive main effect (A) and percentage of phenotypic variation explained by the locus (R^2) for all significant QTL for seed yield, oil content, plant height, TSW and fatty acids detected in the N0 and N80 treatments. The QTL marked in italics were detected over all locations in both the N0 and N80 fertilization treatments.

A total of eight significant QTL were detected for oil content, four for seed yield, one for plant height, two for TSW, one for oleic acid, two for linoleic acid, two for linolenic acid, two for eicosenic acid and one for erucic acid.

Seven of the QTL for oil content were detected with mean phenotype data from the normal (N80) fertilization treatment, whereas one QTL for oil content on LG15 (LOD=3.03, R^2= 5.96) was only detected using data from the N0 treatment. Because protein content in the seed is negatively correlated to oil content, these QTL may represent loci involved in the interaction of oil and protein content with nitrogen treatment, for example via regulation of nitrogen use efficiency with a subsequent influence on protein content. In LG8 and LG9 two QTL for oil content co-localized with QTL for long-chain fatty acids, as is expected due to the correlation of the oil volume with the extension and unsaturation of the fatty acid carbon chains. Despite the low variation in erucic acid in the cross we used, a significant QTL for C22:1 could be localised on LG6 with the pooled N0 data. For both the N0 and N80 treatments one QTL for oleic acid co-localized on LG8 with QTL for linoleic acid and eicosenic acid, whereas on LG1 a co-localization of QTL for linoleic, linolenic and eicosenic acid was observed in both fertilization treatments. Interestingly, the QTL on LG8 localised at the position of the *Brassica* SSR marker Ol10B07. In *Brassica napus* an SSR locus amplified by Ol10B07 is localised on chromosome N13 adjacent to a QTL for oil content, oleic acid and erucic acid content (own unpublished results) that presumably corresponds to the copy of the FAE1 gene for erucic acid biosynthesis on N13 (Ecke et al. 1995, Zhao et al. 2005).

A total of four QTL for seed yield (two loci on LG4 and one each on LG8 and LG16) were identified with the pooled data from all N0 treatments. None of these QTL were detected above the significance level under the N80 treatment, however with the pooled N80 data a clear peak also occurred on LG8 at the same position as the QTL for yield at N0. The three remaining QTL for yield were detected at the same positions at N80 in the different environments, albeit with LOD values below the permuted significance threshold. The fact that these QTL consistently co-localised suggests that they may represent contributing loci with only a small effect on yield. Two of these QTL, on LG4 and LG8, co-localised with QTL detected in multiple environments for oil content and presumably contribute to the high correlation observed between these two traits.

Four significant QTL for plant height were detected for the individual locations and treatments. Two of these QTL were located very close to two significant QTL for seed yield localized on LG4 and LG16. An interaction between plant height and seed yield is also implied by the relatively high correlation between these traits. For TSW two significant QTL on LG9 and LG10 were detected in both the N0 and N80 treatments at multiple locations in all years, whereas further QTL on LG1 (two loci), LG12, LG14 and LG20 were only detected in individual environments. One of the QTL for TSW on LG12 was located at the same position as one of the major QTL for oil content and may contribute to the negative correlation among these traits.

Discussion

Interest in cultivation of the alternative crucifer oilseed species *C. sativa* as a renewable resource has been rekindled in recent years. In the present study the high yield potential of camelina could be confirmed, and even in a marginal location with poor soils it was possible with moderate fertilization levels (80kg N/ha) to achieve competitive yields of more than 1.6 t/ha. Furthermore, the maximum seed yield of 3.0 t/ha achieved by some of the SSD lines we developed demonstrates that further breeding for seed yield has the potential to further improve the profitability of camelina as a spring oilseed crop. In particular, six SSD lines significantly showed stable high yields in excess of the best cross parent across a range of environments. This shows the potential for further yield improvements in camelina through combination of positive alleles from different sources. The yields observed with improved lines in the present study were comparable with mean yields of other summer annual oil plants like spring rapeseed (*Brassica napus*), which typically yields around 2-3 t/ha at the best locations used in the present study (own unpublished data).

A significant negative correlation was observed between TSW and oil content. An increase in seed size without a corresponding increase in oil content could be explained either by an increase in protein content, or alternatively by a thicker seed coat with an associated reduction in the relative proportion of both oil and protein in the seed. In the former case, however, a different effect would be expected in the N0 treatment, where N-deficiency should lead to reduced protein and a corresponding increase in oil content. This was not the case, suggesting that the high oil content of some genotypes may be due to a thinner seed coat. Further analyses of protein and fibre contents in the seeds may assist in selection of varieties with high oil combined with reduced fibre content due to reduction of the seed coat.

Molecular breeding today plays an important role in the genetic improvement of Brassica oilseed crops (Snowdon and Friedt 2004), however to our knowledge no molecular genetic resources have been developed to date for C. sativa and little is known about the inheritance of major agronomical traits in this species. To a limited scale intertribal somatic hybridization and RFLP analyses have been used to investigate and utilize disease resistance properties of camelina (Hansen 1998; Sigareva and Earle 1999; Khadhair et al. 2001), and genetic diversity within the species was investigated via RAPD markers (Vollmann 2005). This study is the first we know of to generate a molecular genetic map for C. sativa, and is also the first report of QTL for agronomic characters this species. Based on these data we are now in a position to fine-map significant QTL for identification of closely linked markers, as a first step towards marker-assisted breeding for important yield and quality traits. In previous work we were able to positively influence yield performance and oil content in camelina by selection (Müller and Friedt 1998; Müller 2002). Although we were only able to detect seed yield QTL above the significance threshold in the N0 treatment and not in the N80 treatment, the yield was positively correlated with both plant height and oil content in both treatments. Hence the major QTL we detected for oil content on LG4, which also co-localises with a QTL for seed yield, may be a promising target for simultaneous marker assisted improvement of seed yield and oil content.

A moderate level of DNA sequence conservation between C. sativa and the Brassica A, B and C genomes was demonstrated by the ability of 55 out of 406 tested Brassica SSR primer combinations to amplify microsatellite loci in C. sativa. On the other hand, only three polymorphic SSR markers could be integrated into the C. sativa genetic map, while five further polymorphic markers remained unlinked to the C. sativa linkage groups, so that the degree of genome co-linearity between camelina and other Brassica oilseeds cannot yet be compared. Nevertheless, the small amount of data available revealed a first putative genome similarity associated with genes involved in fatty acid biosynthesis. A Brassica SSR marker that in oilseed rape is linked to a QTL for erucic acid biosynthesis and oil content was the nearest marker to a QTL for oleic acid, linoleic acid, eicosenic acid and oil content in C. sativa. Interestingly, a large proportion (31 of 55) of the homoeologous SSR primers amplified more than one locus in camelina, and the large proportion (21%) of AFLP markers that had to be excluded from the genetic mapping due to a skewed segregation also suggests a polyploid or duplicated structure of the C. sativa genome. Polyploidy may also explain the differing reports of chromosome number in the literature. Among the tribe Brassiceae a base chromosome number as high as n=20, as was determined for C. sativa in the present study, has only been found among known alloploids or autoploids (Warwick et al. 2000). Amphidiploidy and extensive gene duplication in B. napus are thought to contribute to a certain level of so-called “fixed heterosis” in oilseed rape (Osborn et al. 2003; Abel et al. 2005), and could be a factor in the high degree of phenotypic plasticity and adaptability observed in camelina crops grown under marginal or low-input cropping conditions.

Acknowledgements

This work was funded by the German Research Society (DFG) within the framework of the Special Research Area SFB 299 “Land use options for peripheral regions”. The authors thank Karlheinz Balzer, Petra Degen, Juliane Döhrel, Birgit Keiner, Annette Plank and Mario Tolksdorf for technical assistance.

References

Abel, S., Möllers, C., and Becker, H.C. 2005. Development of synthetic *Brassica napus* lines for the analysis of “fixed heterosis” in allopolyploid plants. Euphytica **146:** 157-163.

Abramovič, H., and Abram, V. 2004. Physio-chemical properties, composition and oxidative stability of Camelina sativa oil. Food Technol. Biotechnol. 43 (1): 63-70.

Agegnehu, M., and Honermeier, B. 1997. Effects of Seeding rates and Nitrogen Fertilization on Seed Yield, Seed Quality and Yield Components of False Flax (*Camelina sativa* Crtz.). Die Bodenkultur **48**: 15-21.

Bernardo, A., Howard-Hildige, R., O’Connell, A., Nichol, R., Ryan, J., Rice, B., Roche, E., and Leahy, J.J. 2003. Camelina oil as a fuel for diesel transport engines. Ind. Crops Prod. **17**: 191-197.

Budin, J.T., Breene, W.M., and Putnam, D.H. 1995. Some Compositional properties of camelina (*Camelina sativa* L. Crantz) seeds and oil. JAOCS **72** (3): 309-315.

Doyle, J.F., and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin **19**: 11-15.

Ecke, W., Uzunova, M., and Weißleder, K. 1995. Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor. Appl. Genet. **91**: 972–977.

Fröhlich, A., and Rice, B. 2005. Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production. Ind. Crops Prod. **21**: 25–31.

Görz, G. and Hock, A. 1939. Reichsbodenschätzung und Bodenkunde. Zeitschrift der Deutschen Gesellschaft für Geowissenschaften **91**: 612-616.

Hansen, L.N. 1998. Intertribal somatic hybridziation between rapid cycling *Brassica oleracea* L. and *Camelina sativa* (L.) Crantz. Euphytica **104**: 173-179.

Hill, J., Becker, H.C., and Tigerstedt, P.M.A. 1998. Quantitative and Ecological Aspects of Plant Breeding. Chapman and Hall, London.

Khadhair, A.-H., Tewari, J.P., Howard, R.J., and Paul, V.H. 2001. Detection of aster yellows phytoplasma in false flax based on PCR and RFLP. Microbiol. Res. **156**: 179-184.

Lowe, A.J., Moule, C., Trick, M., and Edwards, K.J. 2004. Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor. Appl. Genet. **108**: 1103-1112.

Lynch M., and Walsh, B. 1998. Genetics and analysis of quantitative traits. Sinauer Assoc, Sunderland, Mass.

Makowski, N. 2003. Anbau von Leindotter – Alternativen im ökologischen und konventionellen Landbau? Raps **2**: 73-77.

Matthäus, B., and Zubr, J. 2000. Variability of specific components in *Camelina sativa* oilseed cakes. Ind. Crops Prod. **12**: 9-18.

Matthäus, B. 2004. Leindotteröl – ein altes Öl mit neuer Zukunft? Ernährungs-Umschau **51**: 12-16.

Müller, M. 2002. Untersuchungen zur Anbaueignung und zur Verbesserung der Stickstoffeffizienz von Leindotter (*Camelina sativa* Crtz.), Öllein (*Linum usitatissimum* L.) und Winterraps (*Brassica napus* L.). Dissertation, JLU-Giessen, Shaker Verlag, Aachen.

Müller, M., Ordon, F., and Friedt, W. 1999. Perspektiven einer leistungsfähigen Getreide- und Ölsaatenproduktion an ackerbaulichen Grenzstandorten - Perspectives for efficient cereal and oilseed production in marginal areas. Z. f. Kulturtechnik und Landentwicklung **40**: 275-281.

Müller, M., und Friedt, W. 1998. Entwicklung von Low-input-Ölpflanzen – eine Chance für marginale Standorte? Vortr. Pflanzenzüchtg. **42**: 143-145.

Osborn, T.C., Butrulle, D.V., Sharpe, A.G., Pickering, K.J., Parkin, I.A.P., Parker, J.S., and Lydiate, D.J. 2003. Detection and Effects of a Homeologous Reciprocal Transposition in *Brassica napus*. Genetics **165**: 1569-1577.

Paulsen, H.M., Dahlmann, C., und Pscheidl, M. 2003. Anbau von Ölpflanzen im Mischanbau mit anderen Kulturen im ökologischen Landbau *In* Freyer, Bernhard, (Hrsg.) Ökologischer Landbau der Zukunft - Beiträge zur 7. Wissenschaftstagung zum Ökologischen Landbau. pp. 49-52.

Putnam, D.H., Budin, J.T., Field, L.A., and Breene, W.M. 1993. Camelina: A promising low-input oilseed.. *In* J.Janick and J.E. Simon (eds.), New crops: John Wiley and Sons, New York. pp. 314-322.

Schelfhout, C.J., Snowdon, R.J., Cowling, W.A., and Wroth, J.M. 2004. A PCR based B-genome specific marker in *Brassica* species. Theor. Appl. Genet. **109**: 917-921.

Schultze-Motel J. 1979. Die Anbaugeschichte des Leindotters, *Camelina sativa* (L.) Crantz. Archaeo-Physika **8**: 267-281.

Schuster, W.H. 1992. Ölpflanzen in Europa. DLG-Verlag, Frankfurt/Main.

Schuster A., and Friedt, W. 1995. *Camelina sativa*: old face – new prospects. Eucarpia Cruciferae Newsletter **17**: 6-7.

Seehuber, R., Vollmann, J., und Dambroth, M. 1987. Anwendung der Single-Seed-Descent Methode bei Leindotter (*Camelina sativa* Crtz.) zur Erhöhung des Ertragsniveaus. Landbauforschung Völkenrode **37**: 132-136.

Sigareva, M.A., and Earle, E.D. 1999. Camalexin induction in intertribal somatic hybrids between *Camelina sativa* and rapid-cycling *Brassica oleracea*. Theor. Appl. Genet. **98**: 164-170.

Snowdon R.J., and Friedt, W. 2004. Molecular markers in Brassica oilseed breeding: Current status and future possibilities. Plant Breeding **123**: 1-8.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. **3**: 739-744.

Suwabi, K., Iketane, H., Nunomi, T., Kage, T., and Hirai, M. 2002. Isolation and characterisation of microsatellites in *Brassica rapa* L. Theor. Appl. Genet. **104**: 1092-1098.

Vollmann, J., Damboeck, A., Eckl, A., Schrems, H., and Ruckenbauer, P. 1996. Improvement of *Camelina sativa*, an underexploited oilseed. *In* J. Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA. pp. 357-362.

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., and Lelley, T. 2005. Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. Plant Breeding **124**: 446-453.

Vollmann, J., Steinkellner, S., and Glauninger, J. 2001. Variation in resistance of camelina (*Camelina sativa* [L.] Crtz.) to downy mildew (Peronospora camelinae Gäum.). J. Phytopathology **149**: 129-133.

Warwick S.I., Francis, A.. and La Fleche, J. 2000. Guide to the wild germplasm of *Brassica* and allied crops (tribe Brassiceae, Brassicaceae) 2nd Edition [online]. Available from http://www.brassica.info/resources/crucifer_genetics/guidewild.htm [accessed 12 June 2006].

Zeng, Z.-B. 1994. Precision mapping of quantitative trait loci. Genetics **136**: 1457-1468.

Zhao, J., Becker, H.C., Zhang, D., Zhang, Y., and Ecke, W. 2005. Oil content in a European x Chinese rapeseed population: QTL with additive and epistatic effects and their genotype-environment interactions. Crop Sci. **45**: 51-59.

Zubr, J. 2003. Qualitative variation of *Camelina sativa* seed from different locations. Ind. Crops Prod. **17**: 161-169.

II. Publication 2

New oilseed rape (Brassica napus) hybrids with high levels of heterosis for seed yield under nutrient-poor conditions

Gehringer A., Spiller T., Basunanda P., Snowdon R.J. and Friedt W.

Published 2007 in Breeding Science, Volume 57, pages 315-320

Summary

Winter oilseed rape (*Brassica napus* L.) is the most important oil crop in Europe. Due to a continually increasing demand for rapeseed oil for food and non-food uses, the production of hybrid cultivars with higher seed and oil yields has become increasingly important in recent years. However, the systematic use of heterosis for hybrid breeding in oilseed rape is limited by the relatively narrow genetic basis of adapted germplasm, which can impede the generation of distinct heterotic pools. In the present study experimental hybrids were developed from a population of 190 DH lines derived from a cross between an elite, double-low seed quality (zero erucic acid, low glucosinolate content) winter oilseed rape variety and a semi-synthetic line derived from a genetically diverse resynthesised rapeseed line with high erucic acid and glucosinolate contents. The DH lines were crossed with a male sterile tester and the resulting test hybrids were examined for yield performance at two locations in Hesse, Germany, that exhibit extreme differences in climatic conditions and soil characteristics. Mid-parent heterosis for seed yield was determined at both the agronomically optimal location Rauischholzhausen and the marginal site Niederhörlen. A value of up to 43 % mid-parent heterosis for seed yield could be observed among selected test hybrids compared to that of their parental DH lines. The heterosis level for yield was particularly high at the nutrient-poor site, where the best test hybrids showed significantly higher yields than elite open-pollinating and hybrid varieties. This demonstrates the suitability and adaptability of highly heterotic rapeseed hybrids on marginal locations and suggests the existence of a strong heterotic effect on nutrient uptake efficiency.

Key Words: Oilseed rape, hybrids, heterosis, marginal conditions.

Introduction

Winter rapeseed (*Brassica napus* L.; genome AACC, 2n=38) is the most important oilseed crop in the European Union, with an acreage of 5.4 million ha in 2005 (data from FAOStat: http://faostat.fao.org/). Worldwide oilseed rape is the second most produced oilseed species after soybean, with extensive production in Europe, North America and China. The seed oil of rapeseed, a member of the mustard family (*Brassicaceae*), is widely used both as a high-quality edible oil and also for non-food purposes as an important renewable resource. The production of rapeseed has increased rapidly over the past decade due to a massively growing demand for renewable fuels and biodegradable lubricants as an alternative to mineral oils. Besides a major increase in production area, the best current winter rapeseed varieties also display improved yield performance and oil contents; in Germany a nationwide average seed yield of more than 4 t/ha was achieved in 2004.

In Germany more than 50 % of the current winter oilseed rape production is derived from hybrid cultivars, and similar trends towards hybrid production are apparent in all the other major rapeseed-producing countries. Identifying parental combinations with strong heterosis for yield is the most important step in the development of new hybrid cultivars (Diers *et al.* 1996, Becker *et al.* 1999, Melchinger 1999), and heterosis effects are generally more pronounced in crosses between genetically distinct materials. In oilseed rape, however, the level of genetic diversity in adapted winter oilseed rape breeding material with double-low seed quality (zero erucic acid, low glucosinolate content) is relatively low due to strong selection for these vital nutritional traits for the seed oil and meal, respectively. This severely hinders the generation of genetically diverse germplasm pools for hybrid development. On the other hand, *B. napus* genotypes containing high levels of erucic acid (C22:1) and seed glucosinolates (++ quality) represent a comparatively genetically divergent source of germplasm for hybrid breeding (Röbbelen and Nitsch 1975, Thompson 1983, Schuster 1987), and novel *B. napus* genotypes with ++ quality can also be resynthesised from interspecific crosses between the two diploid ancestors of *B. napus*, namely *Brassica rapa* (A genome, 2n=20) and *B. oleracea* (C genome, 2n=18).

Oilseed rape F1-hybrids generated from crosses between resynthesised rapeseed and elite breeding lines can exhibit high levels of yield heterosis (Seyis *et al.* 2006). Unfortunately, most primary resynthesised rapeseed genotypes are not well-adapted to the western European climatic conditions. On the other hand, since the so-called semi-synthetic lines derived by backcrossing resynthesised rapeseed to adapted oilseed rape material can still contain a high degree of genetic diversity combined with a more adapted oilseed phenotype, such material represents a potentially interesting source of germplasm for hybrid production.

In experimental rapeseed hybrids, yield increases of up to 20-50 % can be recorded compared to those of the parents (Brandle and McVetty 1990, McVetty *et al.* 1991, Schuster *et al.* 1999), while the yield potential of hybrid cultivars was reported by Sauermann and Finck (1998) at levels of 5-12 % above those of standard cultivars. Besides their generally better exploitation of yield potential, hybrid cultivars often also show a higher yield stability and improved adaptability; furthermore, their heterozygous genotype implies that this superior yield potential and yield stability can also be achieved under unfavourable conditions (Léon 1991, Diepenbrock 2000). The breeding of rapeseed hybrid genotypes that are adaptable to marginal locations is attracting increasing interest due to the rapidly growing acreage of oilseed rape production; highly heterotic rapeseed hybrids represent a potentially profitable alternative oilseed crop for less productive agricultural land that would enable further expansion in production without additional strain on already tight crop rotations.

In the present study a set of test hybrids was produced by crossing DH lines, derived from a cross between an elite winter oilseed rape cultivar and a genetically diverse semi-synthetic line, with an elite male sterile line from a different genetic pool. The intention was to combine different combinations of exotic alleles from the semi-synthetic line with alleles from two different elite, double-low quality winter oilseed rape genetic backgrounds, in order to select hybrid combinations showing the highest levels of heterosis for seed yield. Particular emphasis was placed on the identification of hybrids that showed high levels of mid-parent heterosis at marginal locations.

Material and Methods

Plant material

A population of 190 microspore-derived doubled haploid (DH) lines was generated from a cross between the elite, double-zero seed quality (zero erucic acid, low glucosinolate content) winter oilseed rape variety 'Express' (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG Hohenlieth, Germany) and the semisynthetic breeding line ‘V8’. The genetically diverse ++ quality ‘V8’ parent of the DH population was derived from a resynthesised *B. napus* produced via embryo rescue from an interspecific cross between the Indian *B. rapa* ssp. *triloculoris* (Yellow Sarson) variety ‘YSPb-24’ and the cauliflower (*B. oleracea L.* convar. *botrytis*) accession ‘Super Regama’ (Lühs and Friedt 1995a). To transfer this exotic germplasm into an adapted genetic background, the resynthesised rapeseed was backcrossed to a high erucic acid breeding line, resulting in the parental line ‘V8’ (Lühs and Friedt 1995b).

All the DH lines from ‘Express’ x ‘V8’ were crossed with the male sterile tester ‘MSL Falcon’. The MSL system (Male Sterility Lembke) is based on the use of spontaneous male sterile mutants that were selected in the nursery of the breeding company Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, Germany (Paulmann und Frauen 1998), who kindly provided seeds from ‘MSL Falcon’ which were used to produce a set of 190 test hybrids from the 190 DH lines. Oilseed rape hybrids produced with the MSL system are characterized by a high fertility restoration and a low glucosinolate content (Girke 2002). Seeds of the test hybrids were produced in 2003 and 2004 in 2.25 m² (150cm x 150cm x 180cm) isolation tents using colonies of solitary bees (*Osmia cornuta*). A total of 25 male sterile plants were grown together in each isolation tent with 10 plants from each respective DH line. The fertile pollinators were removed after flowering and F1 seeds from the MSL lines were harvested.

Field trials

To evaluate heterosis for yield, the experimental hybrids along with their parental DH lines were grown in 3.75 m^2 plots using a triple-replicated 14 x 14 lattice experimental design at two diverse locations in Hesse, Central Germany. The planting density was 64 seeds/m^2 for the experimental hybrids and 80 seeds/m^2 for the DH lines. As shown in Table 1, the two locations differed considerably in both climatic conditions and soil types.

Location	Nmin (kgN/ha)	Mean annual precipitation (mm)	Mean annual temperature (℃)	Altitude (m ASL)	Soil classific-ation[1)]	Soil pH
Rauischholzhausen	50	626	9.0	200-295	70-80	6.6
Niederhörlen	21	834	7.1	340-380	25-35	5.5

Table 1:Climatic features and soil properties of the two field trial locations, Rauischholzhausen and Nierhörlen

[1)] German classification scale for agricultural land (Görz and Hock 1939)

The field trial sites were evaluated according to the German classification scale for agricultural land (Görz and Hock 1939), which differentiates fields on a scale from 10 (extremely poor) to 100 points (excellent) based on soil characteristics and prevailing climatic conditions. The site at Rauischholzhausen (RH, around 70km north of Frankfurt), located on an experimental farm belonging to the University of Giessen, is characterized by good cropping conditions with high-quality loess soils (70-80 points), whereas the marginal location Niederhörlen (NH, situated in the Lahn-Dill District) has a considerably colder climate and is characterized by decomposed acidic slate soil with a poor nutrient balance (25-35 points). The soil N_{min} content (measured at a depth of 0-90 cm) ranged from 21 kgN/ha at the location NH to 50 kgN/ha at RH. At each location, the experimental hybrids were treated with 160 kg/ha calcium ammonium nitrate containing 27% N (KAS).

Field trials were performed in the growing season 2004/2005. To compare the yield performance, the experimental hybrids were cultivated together with a total of six registered German winter oilseed rape cultivars as standards, including the three open-pollinated varieties ‘Oase’ (Deutsche Saatveredelung GmbH, Lippstadt), ‘Express’ and ‘Falcon’ (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth) and the three hybrid cultivars ‘Titan’, ‘Trabant’ and ‘Talent’ (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth).

Yield determination and quality analysis

Plots were harvested in July 2005 using a plot harvester. Crude composition of intact seeds, including oil, protein, glucosinolate, erucic acid and moisture contents, was determined by near-infrared reflectance spectroscopy (NIR System 6500, WinISI II software, FOSS GmbH Rellingen, Germany), as described by Daun (1995) with a standardization and calibration from VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, Kassel, Germany; NIRS-networks for rapeseed).

Data analysis

Analysis of variance (ANOVA, p=0.001) for all the agronomic traits was performed using the mixed-model procedure with restricted maximum likelihood

(REML) in the statistical software package SAS 9.1, in order to test for significant differences in the measured traits among the DH lines and experimental hybrids. Heterosis for seed yield was estimated as follows: mid-parent heterosis (MPH) = test hybrid value (TH) - mid-parental value (MPV); relative MPH = ((TH – MPV)/MPV) x 100.

Results

Seed yield and oil content

The pooled analysis of variance (ANOVA) for seed yield of the DH population and experimental hybrids over the two locations revealed highly significant differences (p=0.001) between the genotype and location*genotype interaction. Significant genetic variation in seed yield (p=0.05) was achieved between locations (data not shown). Table 2 shows the variation (minimum, mean and maximum values) for seed yield and oil content of the experimental hybrids compared to those of the DH population and the six standards. At both locations, the mean yield averaged over all the test hybrids was higher than the mean yield averaged over all the parental lines, with an improvement of 1.9 dt/ha at NH and 2.6 dt/ha at RH, respectively. However, the seed yield of the best individual DH lines was comparable to, and in some cases significantly higher than the seed yield of the best test hybrids. At the marginal location NH, the best DH lines and the best test hybrids achieved significantly higher maximum yields than all the open-pollinating and hybrid varieties used as standards. At the agronomically optimal location RH, the best DH lines and test hybrids gave comparable yields to those of the two best-performing standard varieties 'Oase' (open-pollinating line variety) and 'Talent' (MSL-based hybrid variety), respectively.

		Seed yield (dt/ha)		Oil content (% DM)	
		NH	RH	NH	RH
DH population	Min	10.50	17.22	46.68	45.03
	Mean	33.93	53.01	51.54	52.50
	Max	48.81	74.24	56.56	57.17
Test hybrids	Min	20.64	45.02	43.49	48.46
	Mean	35.83	55.58	47.18	52.28
	Max	47.03	66.56	50.62	55.49
Standards	'Titan' (H)	39.34	58.13	47.42	50.59
	'Trabant' (H)	31.32	55.70	47.76	50.87
	'Talent' (H)	34.41	67.47	46.26	51.34
	Mean (H)	35.02	60.43	47.15	50.93
	'Oase' (L)	36.38	66.61	49.45	53.32
	'Express' (L)	33.08	52.97	47.95	50.91
	'Falcon' (L)	25.36	50.82	46.49	50.21
	Mean (L)	31.61	56.80	47.96	51.48
	LSD	4.88	5.21	1.35	1.51

Table 2: Minimum, mean and maximum values of seed yield and oil content of the experimental hybrids compared to the DH population and standards (H: hybrid cultivar; L: open-pollinated line cultivar) at the marginal location Niederhörlen (NH) and the optimal location Rauischholzhausen (RH). Differences among values are compared with respect to least significant difference (LSD, p=0.05) for each variable

Estimation of heterosis for yield

The magnitude of heterosis for seed yield varied considerably within the population of test hybrids and between the two locations. High levels of heterosis were observed in many of the test hybrids at the marginal location NH, where the absolute MPH values for seed yield ranged from -16.62 to 12.90 dt/ha, corresponding to a maximum relative MPH value of 43.38 %. At the superior site RH, the absolute MPH values ranged from -16.61 to 13.21, corresponding to a maximum relative MPH value of 35.7 %. The significantly higher MPH value at the marginal location NH suggested the existence of a higher adaptability of the experimental hybrids to poor soils or low-nutrient conditions.

A relative MPH value above 30 % was recorded in a total of twenty-three test hybrids at the marginal location NH (Fig. 1a). Although the absolute yield potential of these hybrids was obviously lower in the poor soils and under the unfavourable climatic conditions of the marginal location, compared to the yields observed at RH, in many of them a negligible or absent heterosis for yield was recorded at RH (Fig. 1b). This may suggest possible advantages of these hybrids in terms of capacity for nutrient uptake or assimilation, or a better pre-winter plant development resulting in an improved yield performance.

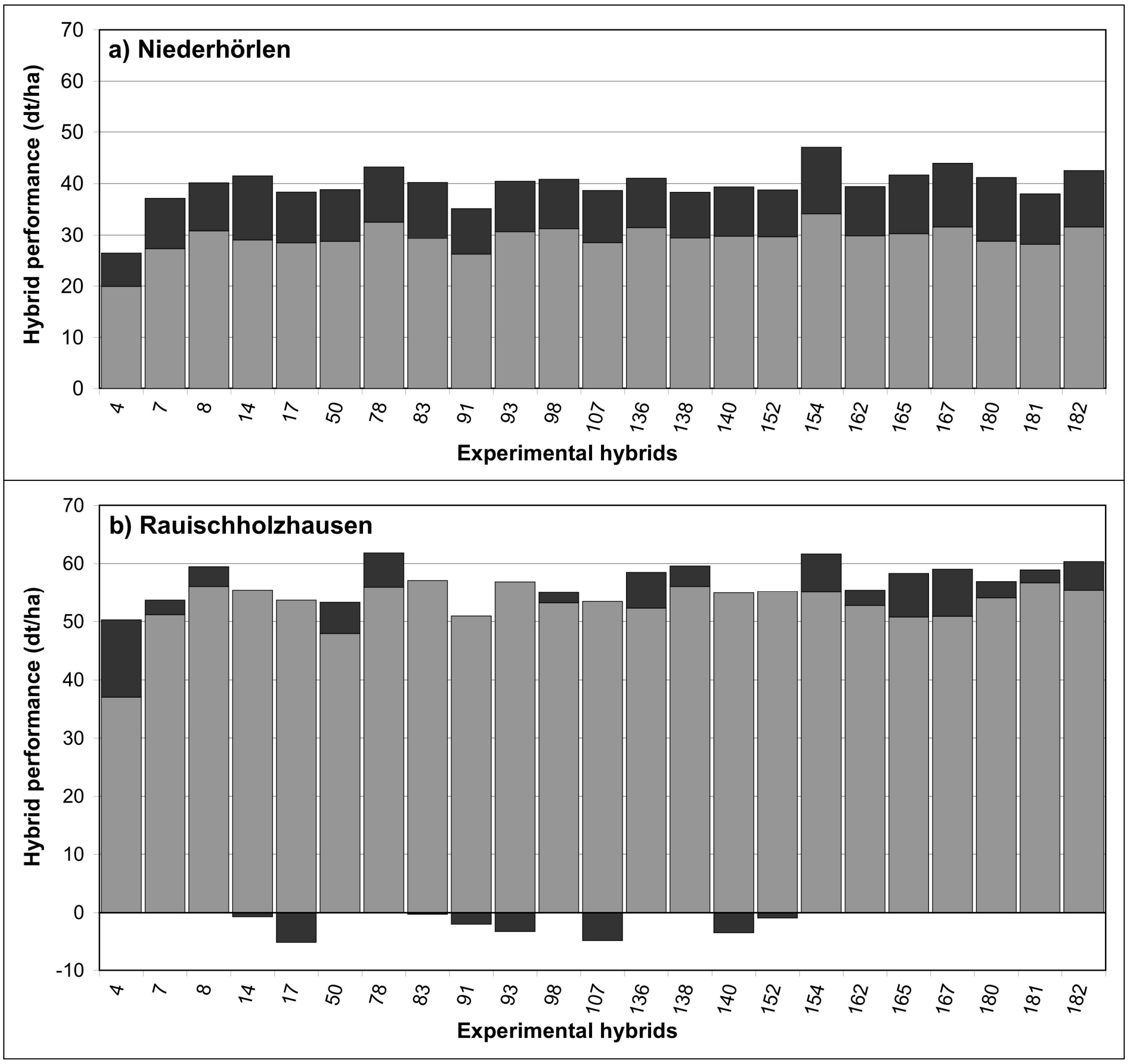

Figure 1: Hybrid performance. Seed yield (hybrid performance) of twenty-three experimental hybrids with relative mid-parent heterosis (MPH) values above 30 % at (a) the marginal location Niederhörlen (NH) compared to the (b) performance at the optimal location Rauischholzhausen (RH). Values of hybrid performance are separated into parental mean (grey bars) and mid-parent heterosis values (black bars)

Seed yield and oil content of the selected test hybrids at the two different locations NH and RH are listed in Table 3 along with the relative MPH values for seed yield. Heterosis for oil content was not calculated because the oil content is strongly influenced by the erucic acid content, which varied considerably among the DH lines.

	Location					
	Niederhörlen			Rauischholzhausen		
	Seed yield			Seed yield		
Test hybrid No.	Absolute (dt/ha)	MPH (%)	Oil content (% DM)	Absolute (dt/ha)	MPH (%)	Oil content (% DM)
4	26.41	32.63	43.72	50.28	35.66	51.00
7	37.13	36.08	43.15	53.65	4.88	48.46
8	40.15	30.31	48.79	59.45	6.08	54.33
14	41.50	43.29	45.92	54.72	-1.27	50.81
17	38.33	34.90	44.78	48.58	-9.47	51.54
50	38.83	35.44	45.51	53.30	11.18	52.30
78	43.21	32.88	43.83	61.81	10.53	51.34
83	40.20	37.11	45.57	56.82	-0.49	51.76
91	35.14	34.16	43.36	48.96	-3.91	50.34
93	40.44	31.92	48.19	53.63	-5.66	53.33
98	40.82	30.67	46.01	54.99	3.37	51.05
107	38.65	35.92	42.95	48.69	-8.91	50.74
136	41.04	30.51	45.69	58.48	11.86	53.01
138	38.32	30.33	45.45	59.58	6.29	52.13
140	39.35	32.17	45.95	51.48	-6.26	51.17
152	38.77	30.56	45.68	54.27	-1.65	53.05
154	47.03	37.79	47.67	61.65	11.95	54.29
162	39.41	31.96	47.74	55.41	5.06	53.34
165	41.65	37.53	47.02	58.29	14.83	53.71
167	43.93	39.02	46.39	59.02	15.98	52.04
180	41.16	43.38	43.49	56.89	5.27	50.78
181	37.99	35.08	47.15	58.92	3.93	53.41
182	42.54	34.73	46.06	60.35	8.92	51.32
LSD	4.16		1.41	9.62		1.92

Table 3: Absolute values for seed yield and oil content along with mid-parent heterosis (MPH) values for seed yield at the marginal location Niederhörlen and the optimal location Rauischholzhausen. The twenty-three experimental hybrids exhibited MPH values for yield above 30 % at Niederhörlen. Differences among values are compared in terms of least significant difference (LSD, p=0.05) for each variable

Discussion

In winter oilseed rape, elite breeding material displays a comparatively low genetic diversity (Hasan *et al*. 2006) due to the bottlenecks caused by intensive selection for double-low seed quality (zero erucic acid, low glucosinolate content) during the past two decades. Therefore, genetically diverse resynthesised rapeseed genotypes from interspecific crosses between the diploid parents of *B. napus*, namely *B. oleracea* and *B. rapa*, can exhibit a considerably higher heterosis potential than conventional rapeseed (Seyis *et al.* 2006). Girke (2002) obtained an improved heterosis effect of up to 22% in experimental hybrids by using resynthesised rapeseed as crossing parents. This is presumably because resynthesised rapeseed forms can harbor a comparatively high proportion of rare alleles (Seyis *et al.* 2003) that will potentially increase the degree of heterozygosity when they are used as parents for hybrid combinations with elite oilseed rape lines.

The location NH is characterized by soils with a low N_{min} content in autumn and a low nitrogen mineralization rate in spring. In the present study, we identified a number of new genetically diverse oilseed rape test hybrids that appeared to show a high heterosis level under these sub-optimal growing conditions, coupled with a *per se* yield performance that was superior to that of open-pollinating and hybrid varieties used as standards. These results suggest that F1 rapeseed hybrids display a comparatively wider adaptability to adverse soil and climatic conditions than open-pollinating lines, along with a high yield potential and yield stability in general. On the other hand, in some of the DH lines used as parents for the experimental hybrids, yields were higher than those of the best test hybrids. This might be ascribed to fixed heterosis within the amphidiploid oilseed rape genome (Abel *et al.* 2005), which can be expected to be comparatively high in DH offspring with a combination of alleles from genetically diverse parental lines. In the test hybrids this fixed heterosis may have been diluted because the tester ‘MSL-Falcon’ was derived from the narrow gene pool of 00 winter oilseed rape and, therefore, presumably harbored many of the same alleles as those of the 'Express' parent of the DH lines. In other words, the level of heterozygosity among homoeologous genes may be higher in some of the DH lines than in the corresponding test hybrids generated from them. The level of heterosis in the best test hybrids was comparable to the heterosis levels observed previously in the F1 hybrid from 'V8' x 'MSL-Falcon', indicating that the potential for enhancing the heterotic character of the genetically diverse parent 'V8' was exploited to near its maximum in the test hybrids from the DH lines. This is particularly interesting in the case of genetically diverse lines that exhibit 00 seed quality despite a genetically diverse background derived primarily from the ++ gene pool (Basunanda *et al.* 2007). The two test hybrids derived from the DH lines 14 and 78 exhibited low (<20µmol.g^{-1}) seed glucosinolate levels and low to moderate seed erucic acid levels (Basunanda et al. 2007). On the other hand, while the erucic acid content is controlled by only two genes in oilseed rape (Fourmann et al. 1998) and can be relatively easily eliminated by recurrent selection, the glucosinolate content is a complex trait that can often cause an undesired linkage drag in materials derived from synthetic rapeseed. Hence these two lines are particularly interesting candidates for further variety development. The genome of the DH line 78 was derived to almost 60% from that of the genetically diverse semisynthetic *B. napus* parent 'V8' (Basunanda et al. 2007) and showed a high heterosis level at both NH and RH in the present study.

In conclusion, our results demonstrated that *Brassica napus* displays a considerable potential for heterosis breeding using single-cross hybrids, based on a DH population obtained by using a semi-synthetic line with ++ quality as pollinator. This underlines previous suggestions (e.g. Röbbelen and Nitsch

1975, Thompson 1983, Schuster 1987) that *B. napus* genotypes containing high levels of erucic acid and GSL represent a comparatively genetically divergent source of germplasm for hybrid breeding.

Acknowledgements

The present study was funded by the German Research Society (DFG) within the framework of the Special Research Area SFB299 "Land use options for peripheral regions" and the priority program SPP 1149 "Heterosis in Plants". Technical assistance from Karlheinz Balzer, Petra Degen, Birgit Keiner and Liane Renno is gratefully acknowledged.

Literature Cited

Abel,S., C.Möllers and H.C.Becker (2005) Development of synthetic Brassica napus lines for the analysis of “fixed heterosis” in allopolyploid plants. Euphytica 146: 157-163.

Basunanda,P., T.H.Spiller, M.Hasan, A.Gehringer, J.Schondelmaier, W.Lühs, W.Friedt and R.J.Snowdon (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed. (in press).

Becker,H.C., H.Löptien and G.Röbbelen (1999) Breeding: an overview. In: “Biology of Brassica Coenospecies”. Gomez-Campo C (ed), Elsevier, Amsterdam: 413–460.

Brandle,J.E. and P.B.E.McVetty (1990) Geographical diversity, parental selection and heterosis in oilseed rape. Can. J. Plant Sci. 70: 935-940.

Daun,J.K. (1995) Seed analysis. In: “Brassica Oilseeds - Production and Utilization”. D. Kimber and D.I. Mcgregor (eds), CAB International, Wallingford, UK: 243-265.

Diepenbrock,W. (2000) Yield analysis of winter oilseed rape (Brassica napus L.): a review. Field Crops Research 67: 35-49.

Diers,B.W., P.B.E.McVetty and T.C.Osborn (1996) Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (Brassica napus L). Crop. Sci. 36: 79-83.

Fourmann,M., P.Barret, M.Renard, G.Pelletier, R.Delourme and D.Brunel (1998) The two genes homologous to Arabidopsis FAE1 co-segregate with the two loci governing erucic acid content in Brassica napus. Theor. Appl. Genet. 96: 852-858.

Girke,A. (2002) Neue Genpools aus resynthetisiertem Raps (Brassica napus L.) für die Hybridzüchtung. Dissertation, Göttingen.

Görz,G. and A.Hock (1939) Reichsbodenschätzung und Bodenkunde. Zeitschrift der Deutschen Gesellschaft für Geowissenschaften 91: 612-616.

Hasan,M., F.Seyis, A.G.Badani, J.Pons-Kühnemann, W.Friedt, W.Lühs and R.J.Snowdon (2006) Analysis of genetic diversity in the Brassica napus L. gene pool using SSR markers. Genetic Resources and Crop Evolution 53: 793-802.

Léon,J. (1991) Heterosis and mixing effects in winter oilseed rape. Crop. Sci. 31: 281-284.

Lühs,W. and W.Friedt (1995a) Natural fatty acid variation in the genus Brassica and its exploitation through resynthesis. Cruciferae Newsl. 17: 14-15.

Lühs,W. and W.Friedt (1995b) Breeding high-erucic acid rapeseed by means of Brassica napus resynthesis. In: “Proc. 9th Intern. Rapeseed Congr.”, 4-7 July 1995, Cambridge, United Kingdom, Vol. 2: 449-451.

McVetty,P.B.E., R.Scarth and S.R.Rimmer (1991) Hybrid canola breeding at the University of Manitoba. Proc. 8th Intern. Rapeseed Congress (GCIRC), Saskatoon, Sask., Canada, Vol. 1: 76-81.

Melchinger,A.E. (1999) Genetic diversity and heterosis. In: “Genetics and exploitation of heterosis in crops”. Coors, C.G. and Pandey, S. (eds), American Society of Agronomy, Madison: 99–118.

Paulmann,W. and M.Frauen (1998) Erfahrungsbericht zur Entwicklung und Saatguterzeugung von restaurierten Winterraps-Hybridsorten. Bericht über die 48. Tagung d. Arbeitsgem.d. Saatzuchtleiter. BAL Gumpenstein, 25.-27- Nov. 1997.

Röbbelen,G. and A.Nitsch (1975) Genetical and physiological investigations on mutants for polyenoic fatty acids in rapeseed, Brassica napus L. Z Pflanzenzuecht 75 : 93–105.

Sauermann,W. and M.Finck (1998) Bundes- und EU-Sortenversuch Winterraps 1996/97. UFOP-Schriften 7: 9-44.

Schuster,C., W.Sauermann and W.Diepenbrock (1999) Ertragsleistung und Unkrautkontrolle beim Anbau von transgenem herbizidtoleranten Hybridraps (Brassica napus L.). Planzenbauwissenschaften 3: 73-81.

Schuster,W. (1987) Die Entwicklung des Anbaues und der Züchtung von Ölpflanzen in Mitteleuropa. Fett Wissenschaft Technol. 89: 15-27.

Seyis,F., R.J.Snowdon, W.Lühs and W.Friedt (2003) Molecular characterisation of novel resynthesised rapeseed (Brassica napus L.) lines and analysis of their genetic diversity in comparison to spring rapeseed cultivars. Plant Breed. 122: 473-478.

Seyis,F., W.Friedt and W.Lühs (2006) Yield of Brassica napus L. hybrids developed using resynthesized rapeseed material sown at different locations. Field Crops Research 96: 176-180.

Thompson,K.F. (1983) Breeding winter oilseed rapeseed Brassica napus. Adv. Appl. Biol. 7: 1-104.

III. Manuscript 1

Increasing the sustainability of vegetable oil-based bio-fuel production: High-yielding erucic acid rapeseed hybrids

Gehringer A., Friedt W. and Snowdon R.J.[1]

Department of Plant Breeding, Research Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

Phone: +49-641-99-37421, Fax: +49-641-99-37429

e-mail: Rod.Snowdon@agrar.uni-giessen.de

[1] Corresponding author

Abstract

The seed oil from oilseed rape and canola (*Brassica napus* L.) cultivars with zero erucic acid (C22:1) and low seed glucosinolate content (00 quality) is widely used both as a high-quality edible oil and also as an important source of bio-fuel. In Europe and North America the production of 00-quality oilseed rape has expanded enormously over the past two decades due to a growing demand for renewable fuels. The great increase in growing area for bio-diesel production has raised concerns in recent years in the context of food-fuel competition, the energy balance of major bio-diesel crops and environmental considerations related to intensifying production inputs. In this context, high erucic acid rapeseed (HEAR) cultivars with a large increase in oil yield per hectare and a considerably improved nutrient efficiency may be an interesting alternative for bio-fuel production. Erucic acid vegetable oils can be co-refined with fossil oil in existing refineries, where isomerisation of the C22:1 chain results in a high-quality fuel with a renewable vegetable oil component. In this study we report on novel, high erucic acid rapeseed hybrids that combine very high oil content with high seed yield. The seed and oil yields of the new hybrids were compared with existing 00-quality and erucic acid rapeseed cultivars in multi-year, multi-location field trials with variable nitrogen fertilization inputs. Three new hybrid combinations were found to combine high seed yields with very high oil content, enabling oil yield per hectare gains of up to 20% compared to existing 00 rapeseed and open-pollinated HEAR cultivars. Furthermore, high oil yields in the HEAR hybrids were also achieved under reduced nitrogen input. Such high-performing erucic acid hybrid cultivars could potentially play an important role in the generation of renewable energy on less

productive soils or in low-input production systems, considerably improving the environmental sustainability of bio-fuel production in comparison to conventional bio-diesel.

Introduction

The seed oil from oilseed rape and canola (*Brassica napus* L.) cultivars with zero erucic acid and low seed glucosinolate content (00 seed quality) is a high-quality edible oil, but today it is particularly important as a source of renewable fuel in the form of either crude or purified rapeseed oil or bio-diesel (rapeseed methyl ester, RME). In Europe and North America the production of 00-quality oilseed rape has increased enormously over the past two decades, largely due to the massively growing demand for renewable, non-fossil fuels. For example, around half of the rapeseed oil produced in Europe in 2005 was used for fuel production (Walla 2006), and around 80% of the rapeseed oil produced in Germany, Europe’s largest producer of commodity rapeseed, is today used as RME or bio-diesel (UFOP, 2008). The inherent suitability of 00 rapeseed oil for both food and fuel production makes the crop particularly attractive for the oil processing industry. Conversely, the availability and market price of 00-quality food oil from rapeseed or canola is strongly influenced by both the food and fuel markets. On the other hand HEAR oil is a strictly non-food product that has practically no influence on food-fuel competition.

The production area for winter oilseed rape in Germany was 1.5 million ha in 2007, an almost two-fold increase in only around 15 years. Particularly in Eastern Europe further massive increases in rapeseed production are projected as the market demand for RME and bio-diesel continues to rise. This rapid and dramatic increase in growing area for bio-diesel production has raised concerns in recent years in the context of food-fuel competition, the energy balance of major bio-diesel crops and other environmental considerations related to intensifying production inputs. For example, Crutzen et al. (2008) claimed that bio-diesel production from oilseed rape has a negative rather than positive effect on greenhouse gas volumes due to atmospheric nitrous oxide (N_2O) release. This is because high seed yields are generally only achievable in oilseed rape with considerable input of mineral nitrogen fertilizers, and a relatively low nitrogen use efficiency of the crop causes high post-harvest N losses (Rathke et al. 2006).

Rapeseed cultivars with high contents of erucic acid (C22:1) tend to have an elevated volume of seed oil compared to erucic acid-free cultivars. This is because the latter possess oleic acid (C18:1) as the primary fatty acid with a shorter carbon chain. Disregarding potential variation in the quantity of triacylglycerol subunits, the extent of fatty acid elongation is one of the limiting factors governing the total oil volume in the seed. The zero-erucic acid rapeseed cultivars that today dominant the oilseed market resulted from spontaneous mutations in two copies of the gene *fatty acid elongase 1* (*FAE1*) which extends C18:1 fatty acids to C22:1 molecules. In nutritional oils erucic acid is a non-desired fatty acid due to its non-neutral flavor and an implication in the cause of cardiac health problems. On the other hand, the oil from high erucic acid rapeseed (HEAR) is an important renewable raw material in plastic film manufacturing, in the synthesis of nylon and in the lubricant and emollient industries (Barret et al. 1998, Taylor et al. 2001). However, the relatively small markets for these products mean that production is very limited. Due to the highly different end-use requirements for 00 and HEAR oils, the cropping and processing of 00-quality rapeseed oil is strictly separated from HEAR production and processing.

HEAR oil is not used at all for bio-diesel or RME production. In contrast to the C16 and C18 fatty acids that predominate in palm oil, soybean and rapeseed oil, respectively, C22 fatty acids have a poorer boiling behaviour (boiling curve), which prevents a uniform fuel injection into the engine. This can cause carbon accumulation at the injection nozzle and have a negative effect on the combustion behaviour of engines using HEAR oils. Furthermore, fuels with higher C22 contents tend to leak into the engine oil, necessitating a more frequent service and oil change. The preference of the automobile industry is for vegetable oils with C12 fatty acids, for example lauric acid-rich oil from genetically modified rapeseed (Voelker et al. 1996), however such oils cannot currently be produced in temperate climates. Alternatively, co-refining of C22:0-based HEAR oil together with petroleum-based fuels in conventional oil refineries leads to isomerisation (so-called "cracking") of the olefin chains, resulting in the desired shorter carbon chain lengths and consequently greatly improved properties as an automotive fuel. In this way HEAR oils can potentially be implemented as a vegetable oil-based bio-fuel supplement that is compatible with current refining and automotive technologies.

As a facultative outcrossing species, oilseed rape cultivars can be developed either by traditional inbreeding techniques or by hybrid breeding. In the latter case pure F1 hybrid seed is produced on male-sterile maternal plants using a selected line as pollinator. The F1 hybrid plants grown from this seed by the farmer tend to have an improved yield performance compared to their parental inbred lines, due to the expression of hybrid vigour (heterosis). Hybrid vigour is generally more strongly expressed in crosses between genetically diverse parental lines.

The degree of heterosis in 00-quality oilseed rape is low compared with classical hybrid crops like maize and sunflower. One reason for this is that modern oilseed rape cultivars derive from a relatively small gene pool, due to an intensive bottleneck selection for 00 seed quality in the 1970s: The zero erucic acid and low glucosinolate traits each derived from single cultivars that were used as the basis for international backcrossing programs to convert rapeseed cultivation to 00-quality variety types. On the other hand, HEAR material has a much broader genetic diversity (Hasan et al. 2006), hence the potential for exploitation of heterosis to increase yield potential is much greater. In this study a set of new, high-performing, genetically diverse HEAR inbred lines were used as pollinators to produce F1 hybrid seed in combination with a male-sterile mother line. The performance of the F1 hybrids in comparison to check cultivars with 00 and +0 seed quality was evaluated in field trials in six environments spanning three years at a total of four different locations. In each environment three variable nitrogen fertilization regimes were tested. The performance of the best hybrids is described in the context of sustainable bio-fuel production from oilseed rape under low-input cropping conditions.

Material and Methods

Plant Material

In this study, three HEAR inbred lines with good agronomical performance and low seed glucosinolate content (V23, V24 and V24) were selected from performance trials in a breeding nursery for use as female parents of crosses to produce HEAR experimental hybrids. Pure F_1 hybrid seed as generated by controlled pollination of the male-sterile HEAR maternal line ‘MSL-Eruca’ (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, Germany), which carries a genic male sterility known as Male Sterility Lembke (MSL; Frauen and Paulmann 1999). Pure F_1 hybrid seed was produced by controlled pollination of male-sterile maternal plants using colonies of solitary bees (*Osmia cornuta*) in insect-proof isolation tents; V23, V24 or V25 plants in the respective isolation tents served as pollen donors. The hybrids from V23, V24 and V25 were designated V23-H, V24-H and V25-H, respectively.

Two sets of registered winter oilseed cultivars were used as checks to evaluate the performance of the test hybrids. The first set comprised the hybrid cultivars ‘Artus’, ‘Panther’, ‘Pronto’ and ‘Talent’ and the two line varieties, ‘Express’ and ‘Falcon’. The MSL-hybrid ‘Talent’ was the top-selling winter oilseed rape cultivar in Germany in the mid 2000s, while ‘Express’ preceded ‘Talent’ as the top-selling cultivar in Germany during the late 1990s and early 2000s. The second set of checks comprised the HEAR line varieties cultivars, ‘Maplus’, ‘Maruca’ and ‘Zeruca’ along with the only current HEAR hybrid available in Germany, ‘Marcant’.

Field trials

For evaluation of seed quality traits, oil content, seed yield and oil yield under field conditions, the experimental hybrids V23-H, V24-H and V25-H were grown together with all check cultivars in performance trials in the years 2003-2006 (three vegetation periods) at four geographically distinct locations in the state of Hesse, Germany. All trials were performed in 10 m^2 plots in a lattice design, and three randomized replications per variant to account for neighbour effects. The trial sites at Rauischholzhausen (approx. 90km north of Frankfurt) and Wöllstadt (20 km north of Frankfurt) are characterized by good cropping conditions with predominantly loess soils, while Gross Gerau (30 km south-west of Frankfurt) has a warmer climate but sandy soil. The site at Niederhörlen (120km northwest of Frankfurt) has a considerably colder climate due to a higher altitude and is characterized by decomposed acidic slate soil with a poor nutrient balance. The soil N_{min} content (measured at a depth of 0-90 cm) varied from a minimum of 17 kgN/ha at Niederhörlen to a maximum of 60 kgN/ha at Rauischholzhausen. A total of 6 different environments (year x location combinations) were tested: Niederhörlen in 2004, Groß Gerau in 2005, Wöllstädt in 2006 and Rauischholzhausen in 2004, 2005 and 2006, respectively.

In order to evaluate the relative performance of the materials under low-input cropping conditions, three different nitrogen (N) fertilization levels were applied as variants in all six environments. The N fertilization variants are hereinafter referred to as N0, N100 and N200, respectively. Independent of the N_{min} content, no additional nitrogen fertilizer was applied in the N0 treatment, whereas in the N100 and N200 treatments 100 or 200 kg/ha of calcium ammonium nitrate (CAN) containing 27% N was applied, respectively. The nitrogen application was split into two equal applications over the growing season. An application of up to 200 kg/ha CAN is common practice in European winter oilseed rape production, while 100 kg/ha N can be regarded as a “low-input” application; production with no added nitrogen is not common practice due to the high N-demand of oilseed rape. The plots were harvested using a Nurserymaster plot combiner (Wintersteiger, Ried, Austria). Seed yields per plot were measured for each of the three replicate plots per variant and location, and individual plot samples were collected for laboratory tests to measure seed quality traits.

Seed quality analysis

The crude composition of intact seeds, including contents of oil, protein, glucosinolates and major fatty acids, were determined by near-infrared reflectance spectroscopy using the NIR System 6500 with WinISI II software (FOSS GmbH, Rellingen, Germany) using a standardization and calibration (NIRS-Networks for Rapeseed) from the German Agricultural Analysis and Research Organisation VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, Kassel, Germany).

Statistical analysis

Statistical analysis of the yield and quality data from the field trials was performed using the software packages SAS 8.02 (SAS Institute, Cary, North Carolina, USA) and SPSS 12.0.1 (SPSS Inc., Chicago, Illinois, USA). Relative performance data (% of mean reference value) for seed and oil yield values were generated using the following references: 1) ‘Talent’ (best-performing 00 cultivar check); 2) ‘Marcant’ (best-performing +0 cultivar check); 3) mean of all 00 cultivar checks; 4) mean of all +0 cultivar checks. In order to compare the potential of the new hybrids for improved oil production under reduced N-input conditions, compared with current optimal production levels under common-practice cropping conditions, the relative seed and oil yields of the N0 and

N100 variants were calculated in relation to the performance in the N200 treatment of the best 00 and +0 cultivars, and with the means of the 00 and +0 cultivars, respectively.

Results

An overview of the seed yield, oil content and oil yield of the three new experimental hybrids V23-H, V24-H and V25-H, along with the six 00 cultivar checks and the five +0 (HEAR) cultivar checks, is given in Table 1.

		Mean seed yield (t/ha)				Mean oil content (% DW)				Mean oil yield (t/ha)			
Type	Accession	N0	N100	N200	Overall	N0	N100	N200	Overall	N0	N100	N200	Overall
00 varieties	Artus	3.62	4.73	4.90	4.33	52.76	47.97	46.68	49.14	1.91	2.27	2.29	2.13
	Express	3.45	4.11	4.62	4.04	53.75	50.43	48.48	50.89	1.85	2.07	2.24	2.06
	Falcon	3.42	4.17	4.56	3.92	52.31	49.23	46.26	49.27	1.79	2.05	2.11	1.93
	Panther	3.18	4.37	4.83	4.18	53.72	48.42	47.69	49.86	1.71	2.11	2.30	2.08
	Pronto	3.58	4.56	5.04	4.30	52.94	49.11	46.37	49.47	1.89	2.24	2.34	2.13
	Talent	3.48	4.90	5.39	4.52	53.23	49.88	47.54	50.22	1.85	2.44	2.56	2.27
	Mean all 00	3.45	4.47	4.89	4.21	53.12	49.17	47.17	49.81	1.83	2.20	2.31	2.10
	Maplus	2.71	3.71	4.34	3.66	54.55	53.17	50.26	52.57	1.48	1.97	2.18	1.92
	Marcant	3.65	4.76	5.21	4.49	57.81	53.93	51.41	54.39	2.11	2.57	2.68	2.44
	Maruca	2.70	3.64	4.28	3.71	56.97	54.56	53.07	54.87	1.54	1.98	2.27	2.03
	Zeruca	2.92	3.74	4.68	4.14	57.78	53.94	52.03	54.59	1.68	2.02	2.43	2.26
	Mean all +0	2.99	3.95	4.63	4.00	56.78	53.90	51.69	54.10	1.70	2.14	2.39	2.16
New +0 hybrids	V23-H	3.44	4.49	5.23	4.36	57.79	55.86	53.84	55.83	1.99	2.51	2.81	2.44
	V24-H	3.76	4.75	5.11	4.56	57.88	55.03	53.06	55.32	2.17	2.61	2.71	2.52
	V25-H	3.42	4.70	5.14	4.34	58.61	55.36	53.66	55.88	2.01	2.60	2.76	2.43

Table 1: Mean seed yield, oil content and oil yield of the experimental HEAR hybrids V23-H, V24-H and V25-H along with six 00 quality winter oilseed rape cultivar standards and four +0 cultivar standards. Mean values were calculated over a total of six year-by-location combinations for each nitrogen fertilization treatment (N0, N100, N200; see text for details) and three replications per genotype per treatment. Fisher's least significant difference values (LSD; p = 0.05) were to identify groups of genotypes (denoted by common superscript letters) with significantly different means for each trait.

The performance is given as the mean of each accession over all replications, locations and years for each of the three N fertilization variants, along with the overall mean over all replications, years, locations and N variants. Figure 1 compares the overall mean seed yields and oil contents of the experimental hybrids and checks.

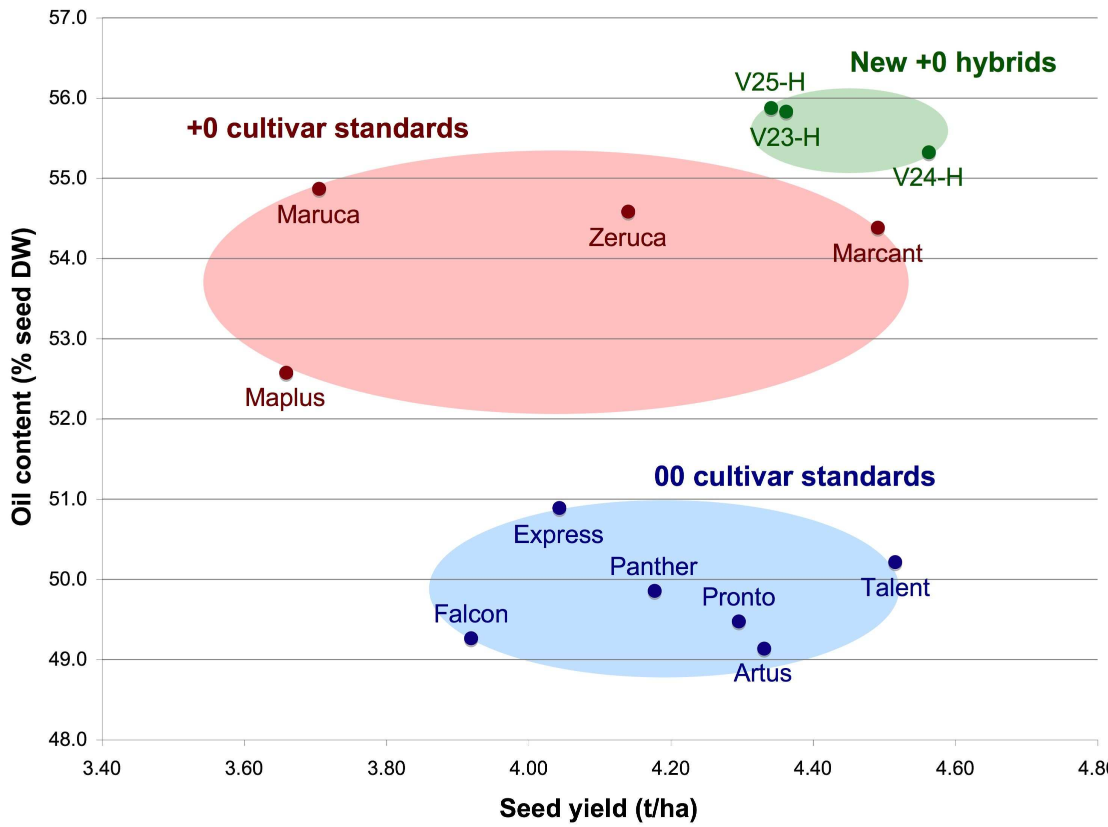

Figure 1: Average seed yields and oil contents from the new HEAR hybrids V23-H, V24-H and V25-H compared with six 00 quality winter oilseed rape cultivar standards and four +0 cultivar standards. Mean values were calculated over a total of six year-by-location combinations with three different fertilization treatments (N0, N100, N200) at each location and three replications per genotype per treatment.

The overall yield levels of V23-H, V24-H and V25-H were similar to or higher than the seed yields of the 00 and 0+ hybrid cultivar checks, with the top yield performance being achieved by V24-H. The overall seed yields of V23-H and V25-H were exceeded only by the top 00-hybrid ‘Talent’. This indicates a substantial degree of hybrid vigour in the experimental hybrids and an excellent overall agronomic performance.

As expected due to their high erucic acid content, the oil content per seed dry weight was significantly higher (LSD, $p<0.05$) in V23-H, V24-H, V25-H and the HEAR check cultivars than in the 00 checks. Whereas the generally poor seed yields of the open-pollinated HEAR line varieties resulted in a low oil yield, the three new HEAR hybrids and the registered HEAR hybrid ‘Marcant’ all combined high oil contents with a high seed yield and hence resulted in excellent oil yields per harvested area.

Tables 2 and 3 give the relative mean seed yield and oil yield, respectively, for each of the accessions tested with respect to (a) the best performing 00 hybrid cultivar ‘Talent’, (b) the best-performing HEAR hybrid cultivar ‘Marcant’, (c) the mean of all 00 cultivar checks, and (d) the mean of all HEAR cultivar checks under different N-fertilization treatments. Averaged over all treatments, years and locations, the four HEAR hybrids gave a relative oil yield per ha of between 115% and 120% compared to the mean of the 00 varieties. An even higher level of improvement in oil yield (118% to 122% compared to the mean performance of the 00 varieties) was seen when only the N200 treatment (standard practice in European winter oilseed rape production) was considered.

Type	Accession	Relative overall seed yield (%) compared to best 00 variety Talent	Relative seed yield at N0 (%) compared to best 00 variety Talent at N0	Relative seed yield at N100 (%) compared to best 00 variety Talent at N100	Relative seed yield at N0 (%) compared to best 00 variety Talent at N200	Relative seed yield at N100 (%) compared to best 00 variety Talent at N200	Relative seed yield at N200 (%) compared to best 00 variety Talent at N200	Relative overall seed yield (%) compared to best +0 variety Marcant	Relative seed yield at N0 (%) compared to best +0 variety Marcant at N0	Relative seed yield at N100 (%) compared to best +0 variety Marcant at N100	Relative seed yield at N0 (%) compared to best +0 variety Marcant at N200	Relative seed yield at N100 (%) compared to best +0 variety Marcant at N200	Relative seed yield at N200 (%) compared to best +0 variety Marcant at N200	Relative overall seed yield (%) compared to mean yield of all 00 varieties	Relative seed yield at N0 (%) compared to mean of all 00 varities at N200	Relative seed yield at N100 (%) compared to mean of all 00 varieties at N200	Relative seed yield at N200 (%) compared to mean of all 00 varieties at N200	Relative overall seed yield (%) compared to mean yield of all +0 varieties	Relative seed yield at N0 (%) compared to mean of all +0 varieties at N200	Relative seed yield at N100 (%) compared to mean of all +0 varieties at N200	Relative seed yield at N200 (%) compared to mean of all +0 varieties at N200
00 varieties	Artus	95.92	104.01	96.66	67.15	87.73	90.90	96.44	99.24	99.40	69.46	90.75	94.03	102.79	74.15	96.88	100.37	108.41	78.26	102.24	105.93
	Express	89.54	98.96	83.96	63.89	76.21	85.65	90.03	94.42	86.35	66.09	78.83	88.60	95.96	70.55	84.15	94.58	101.20	74.46	88.82	99.82
	Falcon	86.80	98.21	85.11	63.40	77.25	84.51	87.27	93.70	87.52	65.59	79.91	87.42	93.01	70.01	85.30	93.32	98.10	73.89	90.02	98.49
	Panther	92.51	91.22	89.21	58.90	80.98	89.59	93.01	87.04	91.74	60.92	83.76	92.67	99.13	65.03	89.41	98.92	104.56	68.64	94.37	104.40
	Pronto	95.13	102.69	93.09	66.30	84.50	93.41	95.65	97.98	95.73	68.58	87.40	96.62	101.94	73.21	93.30	103.14	107.52	77.26	98.47	108.86
	Talent	100.00	100.00	100.00	64.56	90.77	100.00	100.54	95.42	102.84	66.78	93.89	103.44	107.16	71.29	100.22	110.42	113.02	75.24	105.78	116.54
+0 varieties	Maplus	81.04	77.88	75.69	50.28	68.70	80.42	81.48	74.31	77.83	52.01	71.06	83.19	86.84	55.52	75.86	88.80	91.59	58.59	80.06	93.72
	Marcant	99.46	104.81	97.24	67.67	88.26	96.67	100.00	100.00	100.00	69.99	91.30	100.00	106.58	74.71	97.46	106.75	112.41	78.85	102.86	112.66
	Maruca	82.06	77.57	74.27	50.08	67.41	79.42	82.51	74.02	76.38	51.81	69.73	82.16	87.94	55.30	74.43	87.70	92.74	58.37	78.56	92.56
	Zeruca	91.69	83.73	76.44	54.06	69.39	86.72	92.19	79.89	78.61	55.92	71.77	89.70	98.26	59.69	76.61	95.75	103.63	63.00	80.86	101.06
New +0 hybrids	V23-H	**96.61**	**98.77**	**91.79**	**63.77**	**83.32**	**96.89**	**97.14**	**94.24**	**94.40**	**65.96**	**86.18**	**100.22**	**103.53**	**70.41**	**92.00**	**106.98**	**109.19**	**74.31**	**97.09**	**112.91**
	V24-H	**101.05**	**107.90**	**97.00**	**69.66**	**88.05**	**94.82**	**101.61**	**102.95**	**99.76**	**72.06**	**91.08**	**98.08**	**108.29**	**76.92**	**97.22**	**104.69**	**114.21**	**81.18**	**102.61**	**110.50**
	V25-H	**96.14**	**98.29**	**96.10**	**63.46**	**87.23**	**95.30**	**96.66**	**93.78**	**98.83**	**65.64**	**90.23**	**98.58**	**103.03**	**70.07**	**96.31**	**105.23**	**108.66**	**73.95**	**101.65**	**111.06**

Table 2: Relative seed yields of the experimental HEAR hybrids V23-H, V24-H and V25-H along with five 00 quality winter oilseed rape cultivar standards and four +0 cultivar standards. Relative values are given as the percentage of the mean value compared to the respective standard

Type	Accession	Relative overall oil yield (%) compared to best 00 variety Talent	Relative oil yield at N0 (%) compared to best 00 variety Talent at N0	Relative oil yield at N100 (%) compared to best 00 variety Talent at N100	Relative oil yield at N0 (%) compared to best 00 variety Talent at N200	Relative oil yield at N100 (%) compared to best 00 variety Talent at N200	Relative oil yield at N200 (%) compared to best 00 variety Talent at N200	Relative overall oil yield (%) compared to best +0 variety Marcant	Relative oil yield at N0 (%) compared to best +0 variety Marcant at N0	Relative oil yield at N100 (%) compared to best +0 variety Marcant at N100	Relative oil yield at N0 (%) compared to best +0 variety Marcant at N200	Relative oil yield at N100 (%) compared to best +0 variety Marcant at N200	Relative oil yield at N200 (%) compared to best +0 variety Marcant at N200	Relative overall oil yield (%) compared to mean of all 00 varieties	Relative oil yield at N0 (%) compared to mean of all 00 varities at N200	Relative oil yield at N100 (%) compared to mean of all 00 varieties at N200	Relative oil yield at N200 (%) compared to mean of all 00 varieties at N200	Relative overall oil yield (%) compared to mean of all +0 varieties	Relative oil yield at N0 (%) compared to mean of all +0 varieties at N200	Relative oil yield at N100 (%) compared to mean of all +0 varieties at N200	Relative oil yield at N200 (%) compared to mean of all +0 varieties at N200
00 varieties	**Artus**	93.86	103.10	92.96	74.52	88.52	89.25	87.13	90.57	88.41	71.28	84.67	85.37	101.41	82.93	98.52	99.33	98.42	79.87	94.87	95.66
	Express	90.75	99.93	84.90	72.24	80.85	87.35	84.24	87.80	80.74	69.09	77.33	83.55	98.04	80.39	89.98	97.21	95.16	77.42	86.65	93.61
	Falcon	85.16	96.52	84.00	69.77	79.99	82.23	79.06	84.79	79.89	66.73	76.51	78.65	92.01	77.64	89.02	91.51	89.30	74.77	85.73	88.12
	Panther	91.85	92.07	86.59	66.55	82.46	89.86	85.27	80.89	82.36	63.66	78.88	85.95	99.23	74.07	91.77	100.00	96.32	71.33	88.38	96.31
	Pronto	93.73	102.14	91.66	73.83	87.29	91.11	87.01	89.73	87.17	70.62	83.49	87.15	101.27	82.16	97.14	101.40	98.29	79.12	93.55	97.65
	Talent	100.00	100.00	100.00	72.28	95.23	100.00	92.84	87.85	95.11	69.14	91.09	95.65	108.04	80.44	105.98	111.29	104.87	77.47	102.06	107.17
+0 varieties	**Maplus**	84.85	79.82	80.69	57.70	76.84	85.02	78.77	70.12	76.74	55.19	73.50	81.32	91.67	64.21	85.51	94.62	88.98	61.83	82.35	91.12
	Marcant	107.72	113.82	105.15	82.28	100.13	104.55	100.00	100.00	100.00	78.70	95.78	100.00	116.38	91.57	111.43	116.35	112.96	88.18	107.31	112.05
	Maruca	89.66	83.03	81.24	60.02	77.37	88.66	83.24	72.95	77.27	57.41	74.00	84.81	96.88	66.80	86.10	98.67	94.03	64.33	82.92	95.02
	Zeruca	99.67	90.90	82.67	65.70	78.73	94.92	92.53	79.86	78.62	62.84	75.30	90.79	107.68	73.12	87.61	105.63	104.52	70.42	84.37	101.72
New +0 hybrids	**V23-H**	**107.42**	**107.24**	**102.81**	**77.52**	**97.90**	**109.72**	**99.72**	**94.21**	**97.78**	**74.14**	**93.64**	**104.95**	**116.05**	**86.27**	**108.96**	**122.11**	**112.64**	**83.08**	**104.93**	**117.59**
	V24-H	**111.33**	**117.33**	**107.02**	**84.81**	**101.92**	**105.82**	**103.36**	**103.08**	**101.78**	**81.12**	**97.48**	**101.22**	**120.29**	**94.39**	**113.42**	**117.77**	**116.75**	**90.90**	**109.23**	**113.41**
	V25-H	**106.98**	**108.24**	**106.66**	**78.24**	**101.57**	**107.57**	**99.32**	**95.09**	**101.44**	**74.83**	**97.15**	**102.89**	**115.58**	**87.07**	**113.04**	**119.71**	**112.19**	**83.85**	**108.86**	**115.28**

Table 3: Relative oil yields of the experimental HEAR hybrids V23-H, V24-H and V25-H along with five 00 quality winter oilseed rape cultivar standards and four +0 cultivar standards. Relative values are given as the percentage of the mean value compared to the respective standard

If the mean of the 00 varieties is taken as a rough estimate of current yield potential in European winter oilseed rape production, this indicates that HEAR hybrids could potentially enable between 15 and 22% greater oil production per production area with no additional increase in production costs or energy input. Even in comparison to the best-performing 00 hybrid cultivar 'Talent' the HEAR hybrids achieved an overall oil yield increase of between 6 and 11%, with an increase of between 5 and 9% under optimal N-fertilization. These comparative data represent a remarkable potential improvement in oil production per unit cropping area with no additional input costs; hence HEAR hybrids could contribute significantly to improving the energy balance of rapeseed oil production as a feedstock for renewable fuel.

On the other hand, the energy and environmental balance of rapeseed oil production could also be significantly improved by reducing the input of mineral nitrogen fertilizer during rapeseed production. Even with a reduction of the standard N application level by 50% to 100 kg N/ha, the HEAR hybrids still achieved oil yields that were 8 to 13% higher than the mean oil yields of all 00 varieties, and equivalent to the oil yield of the best 00 hybrid 'Talent' with 200 kg N/ha. Furthermore, even with no additional N fertilizer (N0 treatment) the HEAR hybrid still achieved a remarkable 86 to 94% of the mean oil yields of the 00 varieties.

The seeds of all three new HEAR hybrids consistently contained very high contents of erucic acid (Figure 2). Besides having a positive impact on the oil volume, the long C22:1 fatty acid chain of erucic acid is a valuable raw material in the form of erucamide for the production of industrial lubricants and biodegradable plastics, for example.

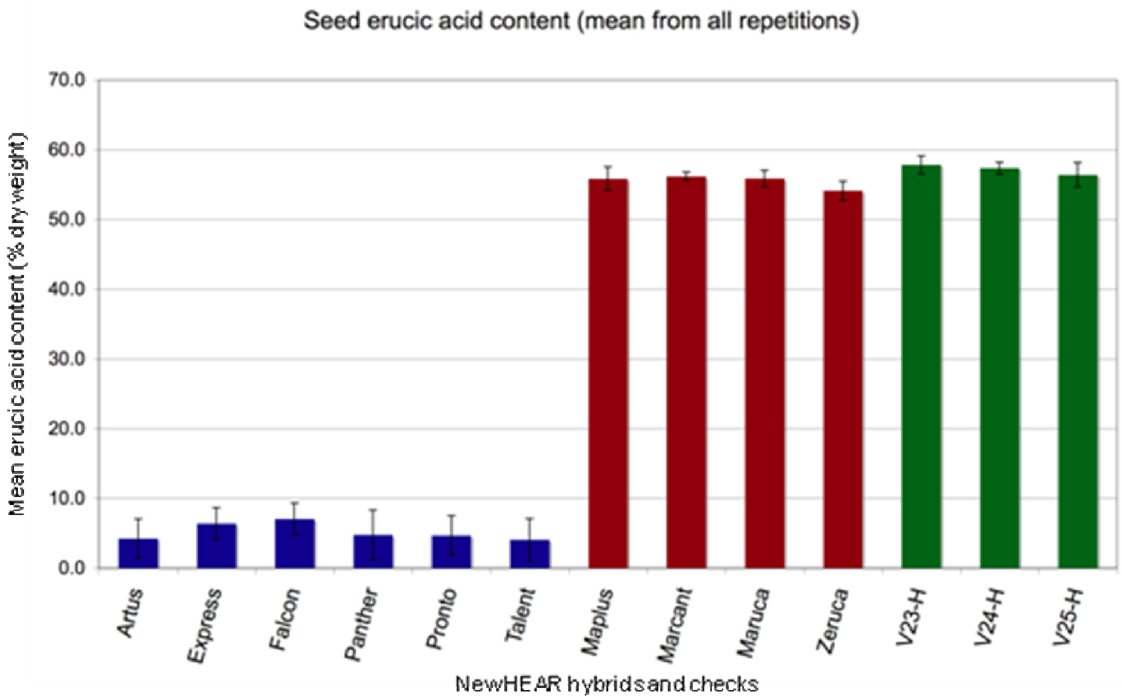

Figure 2: Mean erucic acid (C22:1) content (% dry weight) of the new HEAR hybrids V23-H, V24-H and V25-H (green bars) in comparison to six 00 cultivar controls (blue bars) and four HEAR cultivar controls (blue bars). The whiskers on the bars show standard deviation in each genotype over a total of 56 replicates (six environments with three different fertilization treatments each, three replications per genotype per environment and treatment).

The two hybrids V23-H and V24-H showed levels of erucic acid that were significantly higher than all of the HEAR cultivar checks. Interestingly, the seeds of the V23-H hybrid also contained very low levels of glucosinolates (GSL; Figure 3), with a mean seed GSL content of 7.28 µMol/g dry weight over all environments and a maximum of 9.65 µMol/g. Only the HEAR cultivar 'Maruca' (mean GSL content 8.10 µMol/g, maximum 11.50 µMol/g) and the 00 cultivar 'Pronto' (mean GSL content 10.08 µMol/g, maximum 13.35 µMol/g) had GSL levels which were not significantly higher than V23-H.

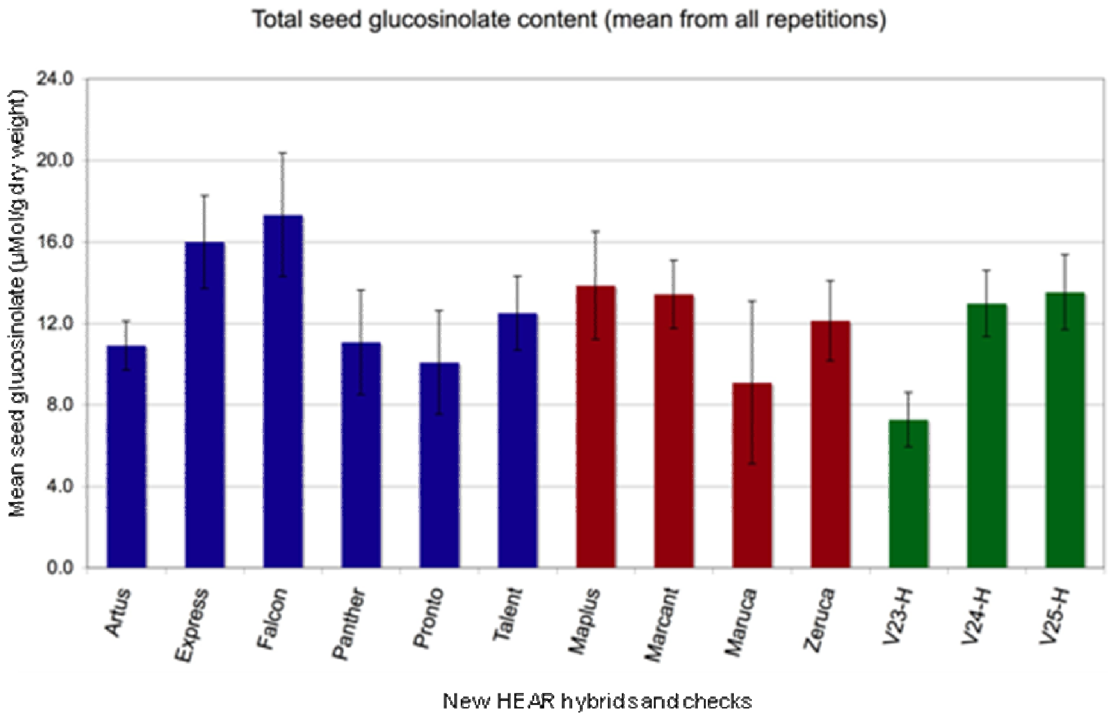

Figure 3: Mean seed glucosinolate content (µMol/g dry weight) of the new HEAR hybrids V23-H, V24-H and V25-H (green bars) in comparison to five 00 cultivar controls (blue bars) and four HEAR cultivar controls (blue bars). The whiskers on the bars show standard deviation in each genotype over a total of 56 replicates (six environments with three different fertilization treatments each, three replications per genotype per environment and treatment).

The content of antinutritive seed GSL is a particularly important trait with respect to utilization of the seed meal in animal feed mixtures, a factor which is of considerable importance to the overall value of seed products used for large-scale bio-diesel production. The current standard for the maximum accepted level of GSL in canola seed meal is is 30µMol/g DW (see http://www.canola-council.ca/). Depending on the oil content this corresponds to approx. 18 µMol/g of total seed DW, which is the current limit in European 00 rapeseed varieties (Sauermann and Gronow 2008). A reduction to levels close to zero would be extremely beneficial to enable increased supplementation of rapeseed meal in animal feed mixtures.

Discussion

At the current rate of yield increases through advances in breeding and agronomy, the production of key food and energy crops will not satisfy the growing worldwide demand in the coming decades without major increases in production intensity (Ewert et al. 2005). On the other hand, energy crops represent an important component of currently available strategies for increased independence from fossil fuels. A more efficient and simultaneously more environmentally sustainable production of major crops for energy use is today a central theme in agriculture. To date only a limited range of suitable, high-yielding temperate crops are available for energy utilization in temperate climates. Under European cropping systems, the major adapted energy crops are maize, for ethanol or biogas production, and oilseed rape for bio-diesel or rapeseed methyl ester. Due to the available infrastructure for processing and utilization of these two crops, it seems obvious that the greatest short-term gains in environmental sustainability and energy-use efficiency for bio-fuel production in Europe can be achieved by significantly increasing the production efficiency of these major existing crops. In the long term, alternative crops and other innovative strategies will doubtless replace many existing bio-energy sources, but optimization of existing possibilities is an important priority until such alternatives are realized.

Winter oilseed rape achieves high seed yields of up to 5 t/ha in Western Europe (FAOstat data), and the high oil content of almost 50% of seed dry weight makes this crop the most prolific sources of bio-diesel under temperate cropping conditions. On the other hand, high-intensity winter oilseed rape production can lead to considerable post-harvest N losses by nitrate leaching, in the range of 50-100 kg N ha^{-1} a^{-1} (Aufhammer et al. 1994, Colnenne et al. 1998, Gabrielle et al. 1998, Sieling & Christen 1999, Sieling et al. 1997, Trinsoutrot et al. 2000), and the consequent release of atmospheric N_2O can arguably have a negative impact on net greenhouse gas emissions (Crutzen et al. 2007). Improving the nitrogen use efficiency of winter oilseed rape to allow lower fertilizer applications would therefore an immediate impact on the greenhouse gas balance of bio-diesel production from rapeseed. The reduced necessity for N fertilization would also considerably improve the energy balance of winter rapeseed production, both in terms of the energy invested in fertilizer production and in terms of the fossil fuel usage during fertilizer applications.

The results presented in this paper present compelling arguments for high erucic acid rapeseed hybrids as an alternative source of bio-diesel in terms of improved energy balance, reduced soil N leaching and potentially reduced atmospheric N_2O pollution. Being a hydrocarbon of high calorific value with a very low flash point, high cetane rating and good lubrication qualities, erucic acid can be a valuable component of bio-diesel. On the other hand, production of erucic acid-containing rapeseed must be strictly separated from 00-quality rapeseed production for nutritional purposes due to the risk of outcrossing. This problem could be overcome by allocation of specific production areas for HEAR cropping, for example in regions currently not used for oilseed rape production due to suboptimal soils are climatic conditions. As seen in the current study, an environmentally sustainable low-input production of HEAR oil for bio-diesel or industrial usage is also a feasible option under sub-optimal cropping conditions. Broadening of the geographical scope for energy crop production to include land areas not currently used for arable agriculture has the further advantage of avoiding the so-called food-fuel controversy often associated with energy crops. Bio-diesel from high-yielding HEAR hybrids with very high oil content could potentially play a significant role in future, environmentally sustainable bio-fuel production strategies without further compromising agricultural diversity.

Acknowledgements

The authors thank Karl-Heinz Balzer, Mechthild Schwarte und Malte Luh for excellent technical assistance. The father lines for the HEAR hybrid production were provided by Dr. Wilfried Lühs, University of Giessen, while Norddeutsche Pflanzenzucht Hans-Georg Lembke KG kindly provided the male sterile maternal line ‘MSL Eruca’. The work was funded by the Deutsche Forschungsgemeinschaft within the special research area “SFB 299: Land use concepts for peripheral regions”.

References

Aufhammer W, Kübler E, Bury M, (1994). Stickstoffaufnahme und Stickstoffrückstände von Hauptfrucht- und Ausfallrapsbeständen. J. Agron. & Crop Sci. 172 : 255-264.

Barret P, Delourme R, Renard M, Domergue F, Lessire R, Delseny M, Roscoe TJ, (1998). A rapeseed FAE1 gene is linked to the E1 locus associated with variation in the content of erucic acid. Theor. Appl. Genet. 96: 177-186.

Colnenne C, Meynard JM, Reau R, Justes E, Merrien A, (1998). Determination of a critical nitrogen dilution curve for winter oilseed rape. Ann. Bot. 81: 311-317.

Crutzen PJ, Mosier AR, Smith KA, and Winiwarter W, (2008). N2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. Atmos.Chem.Phys. 8: 389-395

Ewert F, Rounsevell MDA, Reginster I, Metzger MJ, Leemans R (2005). Future scenarios of European agricultural land use I. Estimating changes in crop productivity. Agriculture Ecosystems & Environment 107: 101-116.

Gabrielle B, Denoroy P, Gosse G, Justes E, Andersen MN, (1998). Development and evaluation of a CERES-type model for winter oilseed rape. Field Crops Res. 57: 95-111.

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ, (2006). Diversity in the Brassica napus L. Gene Pool Using SSR Markers. Genetic Resources and Crop Evolution 53: 793-802.

Rathke GW, Behrens T, and Diepenbrock W, (2006). Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (Brassica napus L.): A review. Agriculture, Ecosystems & Environment 117: 80-108.

Sauermann W, Gronow J. (2008) Bundes- und EU-Sortenversuch 2. Prüfjahr Winterraps 2008. UFOP.

Sieling K, Christen O, (1999). Yield, N-uptake and N-use efficiency of and N leaching after oilseed rape grown in different crop management systems in northern Germany. In: New Horizons for an Old Crop. Eds.: N. Wratten, P.A. Salisbury, Proc. 10th Intern. Rapeseed Congr., 26-29 Sept. 1999, Canberra, Australia (http://www.regional.org.au/au/gcirc/2/95.htm).

Sieling K, Günther-Borstel O, Hanus H, (1997). Effect of slurry application and mineral nitrogen fertilization on N leaching in different crop combinations. J. Agric. Sci. Cambridge 128: 79-86.

Taylor DC, Katavic V, Zou J, MacKenzie SL, Keller WA, An J, Friesen W, Barton DL, Pederson KK, Giblin EM, Ge Y, Dauk M, Sonntag C, Luciw T, Males D, (2001). Field testing of transgenic rapeseed cv. Hero transformed with a yeast sn-2 acyltransferase results in increased oil content, erucic acid content and seed yield. Molecular Breeding 8: 317-322.

Trinsoutrot I, Nicolardot B, Justes E, Recous S, (2000). Decomposition in the field of residues of oilseed rape grown at two levels of nitrogen fertilisation. Effects on the dynamics of soil mineral nitrogen between successive crops. Nutrient Cycling in Agroecosystems 56: 125-137.

Voelker TA, Hayes TR, Cranmer AM, Turner JC, Davies HM (1996). Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed. Plant J 9:229–241.

Walla C, (2006). Biodiesel als Chance für den Ölsaatenanbau. Erschienen 2006 in: I. Darnhofer, C. Walla und H.K. Wytrzens (Hrsg.). Alternative Strategien für die Landwirtschaft. Wien: Facultas, S. 165-171.

http://www.ufop.de/

http://www.canola-council.ca/

http://faostat.fao.org/

2. Discussion

A major objective for sustainable agriculture is the development of crops with improved yield and yield stability under simultaneously reduced production input. So-called “low-input” crops, with reduced applications of chemical plant protection and fertilizers, are particularly relevant with regard to the generation of renewable resources (e.g. biofuels, oleochemicals), where a positive energy balance requires minimal chemical input and reduced fossil fuel consumption during production. Low-input crops can also serve as an important option for peripheral agricultural regions where poor soils or suboptimal climatic conditions prevent an economically viable agricultural food production. Due to their relatively good yield stability on substandard locations, the oilseed crops rapeseed and camelina could be viable options for a competitive biofuel and oleochemical oil production in marginal areas. One prerequisite for this is the development, testing and identification of breeding lines with improved performance under low-fertilisation conditions.

2.1 *Camelina sativa* as an alternative crop for marginal locations

Today molecular breeding plays an important role in the genetic improvement of Brassica oilseed crops (SNOWDON and FRIEDT 2004). The genome of the major oilseed crop rapeseed and other major *Brassica* crops has been extensively investigated, however in *Camelina sativa* very few molecular genetic resources have been developed to date and little is known about the inheritance of major agronomical traits in this species. Intertribal somatic hybridization and RFLP analyses have been used to investigate and utilize disease resistance properties of camelina (HANSEN 1998; SIGAREVA and EARLE 1999; KHADHAIR et al. 2001), and VOLLMANN (2005) investigated genetic diversity within the species via RAPD markers. No previous study reported a genetic map of camelina and even the size of the genome was reported differently in various studies.

In this study, a first genetic map for *C. sativa* was constructed using AFLP markers in a population of recombinant inbred lines that were developed via single seed descent from a cross between the phenotypically distinct parental varieties ’Lindo’ and ’Licalla’. The map was used to publish the first analysis of quantitative trait loci (QTL) in this species (GEHRINGER et al. 2006). Three Brassica SSR markers were integrated into the genetic map and five further polymorphic SSR markers remained unlinked to the *C. sativa* linkage groups. One of the integrated SSR markers that in oilseed rape is linked to a QTL for erucic acid biosynthesis and oil content was the nearest marker to a QTL for oleic acid, linoleic acid eicosenic acid and oil content in *C. sativa*. Furthermore, 55 further SSR primer combinations showed monomorphic amplification products, indicating partial genome homoeology with *Brassica* species. Interestingly, a large proportion of these SSR primers (31 of 55) amplified more than one locus in camelina suggests a polyploid or duplicated structure of the *C. sativa* genome. This may explain the different reports of chromosome number in the literature, which range from n=6 till n=21. In this study a base chromosome number for camelina of n=20 was determined – a number which among the Brassicaceae has only been found among known alloploids or autoploids (WARWICK et al. 2000). Amphidiploidy and extensive gene duplication e.g. in *B. napus* are thought to contribute to a certain level of so-called “fixed heterosis” in oilseed rape (OSBORN et al. 203; ABEL et al. 2005) and could be a factor in the high degree of phenotypic plasticity and adaptability observed in camelina crops grown under marginal or low-input cropping conditions.

The analysis and evaluation of the data collected in this study and the availability of a first genetic map of camelina allowed the identification of a total of 23 QTLs related to oil content, seed yield, plant height, oleic acid, linoleic acid, linolenic acid, eicosenic acid and erucic acid. Seed yield QTL above the significance threshold were only detected in trials with no added nitrogen fertilizer, and may represent loci contributing to the competitiveness of camelina in low-nutrient soils. The seed yield was also positively correlated with both plant height and oil content. The high yield potential of camelina was confirmed, whereby even on sandy soils a maximum seed yield potential of up to 19 dt/ha without any N-fertilization and up to 30 dt/ha with 80 kgN/ha was achieved. This represents a significantly higher seed yield potential than both of the parental varieties of the SSD population, Lindo and Licalla, and demonstrates that further breeding for seed yield has the potential to further improve the profitability of camelina as a spring oilseed crop. In particular, six SSD lines showed stable, high yields in excess of the best cross parent across a range of environments, including the marginal locations Niederhörlen and Groß-Gerau under an N0 regime. These high-yielding SSD lines show comparable yields as high as mean yields of other summer annual oil plants like spring rapeseed (*Brassica napus*) which reaches seed yields around 20-30 dt/ha at the best locations used in the present study (own unpublished data). Especially under sandy soil conditions camelina outperforms other summer annual crops and shows a particularly high yield potential (MAKOWSKI 2003). The current study succeeded in identification of potential genotypes for improving the adaptability of this crop to marginal locations and low-input systems, and it also showed the potential for further yield improvements in camelina through combination of positive alleles from different sources. The genet map and QTL data that were generated are a valuable tool for future marker-assisted breeding efforts. Since the publicaiton of the genetic map the SSD population has been distributed to research groups in Canada and the USA and is being used as a reference population for further QTL analysis studies, genetic mapping and genome analysis.

2.2 Heterosis for seed yield of rapeseed hybrids on marginal areas

The importance of restored hybrid varieties with improved yield stability and adaptability has increased dramatically during the past decade, and today hybrids have the largest market share in most major oilseed rape and canola growing areas worldwide. However, the yield gain of hybrid varieties compared to homozygous varieties is relatively low compared to classical hybrid crops like maize or rice. In official plot trials in Germany, winter oilseed rape hybrids achieve a mean yield advantage of only around 6% compared to open-pollinated varieties (cumulative data from state variety trials), while in practical winter oilseed rape production the 10-year mean yield advantage of hybrids is currently around 11% (data from Kleffmann Group/Norddeutsche Pflanzenzucht H.-G. Lembke KG). A significant improvement in yield performance through more efficient exploitation of heterosis is therefore an important breeding aim. Correspondingly, the identification of parental combinations with strong yield heterosis is a vital step in developing hybrids (DIERS et al. 1996; BECKER et al. 1999; MELCHINGER 1999).

In winter oilseed rape, elite breeding material has a comparatively low genetic diversity (HASAN *et al*. 2006) due to bottlenecks caused by intensive selection for double-low seed quality (zero erucic acid, low glucosinolate content) during the past two decades. To widen the genetic pool, new resynthesized rapeseed genotypes developed from interspecific crosses between the diploid parents of *B. napus*, namely *B. oleracea* and *B. rapa*, could be used to provide a higher genetically diversity with considerably higher heterosis potential for seed and oil yield than conventional rapeseed (SEYIS *et al.* 2006). GIRKE (2002) obtained an improved heterosis effect of up to 22% in experimental hybrids by using resynthesised rapeseed as crossing parents. In our study we could reach up to 43% mid-parent heterosis for seed yield, under reduced N-fertilization, in test-hybrids produced from semisynthetic rapeseed father lines. A total of 23 experimental hybrids attracted attention regarding their heterosis potential, particularly under low-input conditions. This suggested the existence of a higher adaptability of the experimental hybrids to poor soil or low-nutrient conditions. These results demonstrate that considerable potential exists in *Brassica napus* for heterosis breeding using single cross hybrids on the basis of high erucic acid, semi-synthetic DH lines as pollinator. This underlines previous suggestions (e.g. RÖBBELEN and NITSCH 1975, THOMPSON 1983, SCHUSTER 1987) that *B. napus* genotypes containing high levels of erucic acid and GSL represent a comparatively genetically divergent source of germplasm for hybrid breeding. Responsible for this enhanced performance could be a probably comparatively high proportion of rare alleles (SEYIS *et al* 2005) in resynthesised rapeseed forms, which leads to a potential increase in the degree of heterozygosity when they are used as parents for hybrid combinations with elite oilseed rape lines.

Nitrogen is the most limiting nutrient for plant production. Considering the relatively high cost of N fertilizer and environmental concerns associated with excessive N application, increasing N use efficiency of cropping systems is an imprtant consideration for sustainable production of field crops (SZUMIGALSKI AND VAN ACKER 2006). The production of winter oilseed rape has been characterized by a comparatively high input of nitrogen (N). The high intensity of production leads to a considerable risk of post-harvest N losses by nitrate leaching in the range of 50-100 kg N ha-1 a-1 (AUFHAMMER *et al*. 1994, COLNENNE *et al*. 1998, GABRIELLE *et al*. 1998, SIELING & CHRISTEN 1999, SIELING *et al.* 1997, TRINSOUTROT *et al*. 2000). A reduction of nitrogen input and/or an increase in N efficiency of oilseed rape cultivars is expected to reduce the risk of nitrate leaching (RIEMER *et al.* 1998, KESSEL & BECKER 1999A, 1999B, KESSEL 2000, SEIFFERT 2000, ZHOU 2000, MÖLLERS *et al.* 2000, MÜLLER 2002). Use of crop varieties with a lower demand for resources (in particularly reduced fertilization and plant protection, coupled with reduced fossil fuel inputs) will contribute to the maintenance of a high efficiency of agricultural plant production with a simultaneously more sustainable agriculture. Breeding varieties with an improved N-use efficiency is attracting attention world-wide, due to (1) economical grounds, such as the increasing prices of fertilizer, (2) ecological reasons, such as pollution of groundwater resulting from nitrate leaching, and (3) socio-economical reasons, such as the need to expand agricultural production to marginal lands for non-food production in order to avoid competition with agricultural food production.

The location Niederhörlen (NH) is a good example for a marginal region because it is characterized by a low N_{min} content in autumn and a low nitrogen mineralization rate in spring, plus additional lower mid-year temperature. Especially under these sub-optimal growing conditions, a number of new genetically diverse oilseed rape test hybrids showed high heterosis coupled with a *per se* yield performance that was superior to existing open-pollinating and hybrid varieties used as standards. These results tend to underline the supposition that F1 rapeseed hybrids have a comparatively wider adaptability to adverse soil and climatic conditions than open-pollinating lines, along with a high yield potential and yield stability in general. On the other hand, some of the DH lines used as parents for the experimental hybrids showed a better yield performance than the best test hybrids. ABEL *et al.* (2005) explained such a phenomen with so-called “fixed heterosis” within the amphidiploid oilseed rape genome. Intergenomic heterozygosity leading to fixed heterosis can be expected to be comparatively high in DH offspring that carry a combination of alleles from genetically diverse parental lines. In the present case it appears that some combinations of alleles from the semisynthetic parental line ‘V8’ with alleles from the recurrent parent ‘Express’ result in considerable fixed heterosis. On the other hand the combination of DH lines from ‘Express’ x ‘V8’ with ‘MSL-Falcon’ may not show exteremely high levels of heterosis due to the relatively narrow gene pool of 00-rapeseed from which both ‘Express’ and ‘MSL-Falcon’ are derived. In conclusion the may be higher level of heterozygosity among homoeologous genes in some of the DH lines outperforming the level in the corresponding test hybrids generated from them. The level of heterosis in the best test hybrids was comparable to heterosis levels observed previously in the F1 hybrid from ‘V8’ x ‘MSL-Falcon’, indicating that the potential for increasing the heterotic potential of the genetically diverse parent ‘V8’ was exploited to near its maximum in the test hybrids from the DH lines. This is particularly interesting in the case of genetically diverse lines that exhibit 00 seed quality despite a genetically diverse genetic background derived primarily from the ++ gene pool (BASUNANDA *et al.* 2007). The two test hybrids derived from DH lines 14 and 78 exhibited low seed glucosinolate levels (<20 µmol.g^{-1}) and low to moderate seed erucic acid levels (BASUNANDA et al. 2007). Whereas erucic acid content is controlled by only two genes in oilseed rape (FOURMANN et al. 1998) and can be relatively easily eliminated by recurrent selection, glucosinolate content is a complex trait that can often cause undesired linkage drag in materials derived from synthetic rapeseed. Hence these two lines are particularly interesting candidates for further development with regard to variety development. The DH line 78 has a genome composition of almost 60% from the genetically diverse semisynthetic *B. napus* parent ‘V8’ (BASUNANDA et al. 2007) and showed high heterosis at both NH and RH in the present study.

2.3 High-erucic acid rapeseed (HEAR) hybrids as an alternative resource for sustainable biofuel production

The great increase in growing area for bio-diesel production has raised concerns in recent years in the context of food-fuel competition, the energy balance of major bio-diesel crops and environmental considerations related to intensifying production inputs. In this context, high erucic acid rapeseed (HEAR) cultivars with a large increase in oil yield per hectare and a considerably improved nutrient efficiency may be an interesting alternative for bio-fuel production. Erucic acid vegetable oils can be co-refined with fossil oil in existing refineries, where isomerisation of the C22:0 chain results in a high-quality fuel with a renewable vegetable oil component.

The proportion of rapeseed oil produced in Germany that is used for non-food purposes has grown during the past decade to around 80%. From the total rapeseed oil produced and processed in Germany, currently around 2.5 million tonnes per year, only about 0.5 Mio. t are used for nutritional purposes (FRIEDT and SNOWDON 2009). Around 1.5 Mio. t are processed into biodiesel (rapeseed oil methyl ester, RME), while some 0.5 Mio. t of processed oil are directly used in engines of tractors or lorries. (source http://www.biofuelstp.eu/fuelproduction.html). This fact emphasize the increasing relevance of non-food rapeseed production as important source of revenue for farmers. The utilization of the rapeseed oil as non-food oil is manifold and it is suited for renewable products as biofuel, as bio-lubricant, as feedstock for oleo chemicals and for the production of industrial valuable fatty acids like erucic acid (C22:1). Oil with high erucic acid has broad industrial applications (TAO and HE 2005) with a high viscosity and a high smoke point (LAZZERI et al., 2004) and its found in many Brassica species. High-erucic acid rapeseed (HEAR) varieties contain a high proportion of erucic acid (45-60 % of the oil), and this long chain fatty acid and its derivates are important renewable raw materials used in plastic film manufacture, in the synthesis of nylon 13,13 and in the lubricant and emollient industries (BARRET et al. 1998; TAYLOR et al. 2001). Regarding this a varied use combined with a high yield potential underline the status of rapeseed as an outstanding renewable resource.

At the current rate of yield increases through advances in breeding and agronomy, the production of key food and energy crops will not satisfy the growing worldwide demand in the coming decades without major increases in production intensity (EWERT *et al.* 2005). To date only a limited range of suitable, high-yielding temperate crops are available for energy utilization in temperate climates. In Europe the key energy crops are maize for ethanol and biogas production, and oilseed rape for bio-diesel or rapeseed methyl ester. By significantly increasing the production efficiency of these major existing crops, an environmental sustainable and energy-efficient bio-fuel production could be achieved.

The current cultivation area in Germany of high-erucic acid rapeseed is estimated at 20.000 to 30.000 ha (SAUERMANN 2006). Correspondingly the variety spectrum is small as well, with only three registered varieties currently on the market. These three varieties, ‘Maplus’, ‘Maruca’ and ‘Hearty’, all derive from the same breeding company (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG Hohenlieth). Whereas for 00-quality oilseed rape the cultivation area of hybrids exceeds that of open-pollinated varieties, hybrid HEAR varieties are of only secondary importance with only a single hybrid cultivar, ‘Maruca’, being released to date. In the present study it was clearly demonstrated that through breeding of new HEAR hybrids the seed and oil yield per hectare could be significantly increased, and a high adaptability of the crop to marginal areas and low-input growing condititons could be achieved. Three new HEAR hybrid combinations were found in multi-year, multi-location field evaluations to combine high seed yields with very high oil content. This enabled oil yield per hectare gains of up to 20% compared to existing 00 rapeseed and open-pollinated HEAR cultivars. Furthermore, high oil yields in the HEAR hybrids were also achieved under reduced nitrogen input. Such high-performing erucic acid hybrid cultivars could potentially play an important role in the generation of renewable energy on less productive soils or in low-input production systems, considerably improving the environmental sustainability of bio-fuel production in comparison to conventional bio-diesel. HEAR hybrids therefore represent a highly interesting alternative source of bio-diesel in terms of improved energy balance, reduced N-fertilizer input, reduced soil N leaching and potentially reduced atmospheric N2O pollution.

2.4 Conclusions

This thesis presents materials and data from three oilseed crops that show great potential for production of food and non-food vegetable oils on marginal lcoations or under low-input cropping conditions. The alternative oilseed crop camelina was shown to be a suitable summer annual crop with high yields on locations with dry or nutrient-poor soils, and a basis was laid for further breeding of this rediscovered crop using modern molecular breeding tools. New winter rapeseed hybrids with 00 seed quality were found to show high hybrid vigour under low-input conditions and could serve as a resource for broadening the narrow genetic basis of current 00 oilseed rape varieties. The high potential of hybrids for marginal locations as an enrichment for crop rotations was demonstrated. Finally, a set of extremely high-performing, high erucic acid rapeseed (HEAR) hybrids was generated that could potentially play an important role in the generation of renewable energy on less productive soils or in low-input production systems. In future the materials generated in this work might play a role in improving the environmental sustainability of bio-fuel production and increasing agricultural options for marginal locations.

3. Summary

In this study the productive efficiency and stability of winter oilseed rape (*Brassica napus* L.) and camelina (*Camelina sativa* Crtz.) genotypes has been tested for production under low-input conditions (reduced N-fertilization and plant protection) in the Lahn-Dill region (Hesse, Germany). Furthermore these studies aimed at the identification of superior varieties and breeding lines regarding adaptability to marginal conditions (locations, N-fertilization) as a starting material for subsequent breeding programmes.

The crop species *Camelina sativa* is basically suitable for low input production systems because of its good adaptability to adverse environmental conditions and its comparatively short vegetation time. On the basis of the achieved selection progress concerning productivity further improvements of the major agronomic and quality characteristics should be possible by breeding. In this study a first genetic map for *C. sativa* was constructed using AFLP and 3 *Brassica* SSR markers in a population of recombinant inbred lines resulting from a cross of phenotypically distinct parents. The map was used to localize QTLs for different agronomical traits of interest (1,000 seed weight, seed yield, oil content, and plant height) and additional promising lines from the yield tests with improved yield performance could be selected. The results represent a starting point for future marker-assisted camelina breeding.

Oilseed rape is the most important oil crop in Europe and in particular, winter rape is very well suitable for low input production systems, since it produces the highest grain yield of all adapted oil crops even under less intensive agronomical conditions. Today, the high yield potential of oilseed rape is successfully exploited by hybrid breeding. In this study a value of up to 43% mid-parent heterosis for seed yield could be observed among selected test hybrids compared to that of their parental DH lines particularly at the nutrient-poor site. This demonstrates the suitability and adaptability of highly heterotic rapeseed hybrids on marginal locations and suggests the existence of a strong heterotic effect on nutrient uptake efficiency.

But regarding renewable fuels not only rapeseed with 00-quality attracts attentions. In this context high erucic acid rapeseed (HEAR) cultivars with a large increase in oil yield per hectare and a considerably improved nutrient efficiency may be an interesting alternative for bio-fuel production. In this study three new hybrid combinations were found to combine high seed yields with very high oil content, enabling oil yield per hectare gains of up to 20% compared to existing 00 rapeseed and open-pollinated HEAR cultivars. Furthermore high oil yields in the HEAR hybrids were also achieved under reduced nitrogen input. Such high-performing erucic aid hybrid cultivars could potentially play an important role in the generation of renewable energy on less productive soils or in low-input production systems, considerably improving the environmental sustainability of bio-fuel production in comparison to conventional bio-diesel.

4. Zusammenfassung

Ziel der vorliegenden Untersuchungen war zunächst die Erfassung von Kriterien der Leistungsfähigkeit der einheimischen Ölpflanzen Winterraps (*Brassica napus* L.) und Leindotter (*Camelina sativa* Crtz.) für die Produktion an marginalen Standorten und unter low-input Bedingungen (reduzierte Stickstoffdüngung und minimaler Pflanzenschutz) in der Lahn-Dill Region (Hessen, Deutschland). Ferner sollten durch diese Untersuchungen Potentiale zur züchterischen Verbesserung der Anpassungsfähigkeit an Grenzstandorte und verminderte N-Düngung identifiziert und entsprechende Zuchtprogramme initiiert werden.

Leindotter ist aufgrund seiner guten Anpassungsfähigkeit an widrige Bedingungen und kurzen Vegetationszeit prinzipiell für low-input Produktionssysteme geeignet. Auf der Basis der erzielten Selektionsfortschritte bzgl. Ertragsfähigkeit sollten weitere Verbesserungen der Nutzungseigenschaften durch Kombinationszüchtung möglich sein. In der vorliegenden Untersuchung wurde eine erste genetische Karte für Leindotter erstellt, im wesentlichen bestehend aus AFLP-Markern sowie drei *Brassica* SSR-Markern. Die dafür verwendete Population resultierte aus einer Kreuzung von phänotypisch unterschiedlichen Eltern. Auf der genetischen Karte konnten verschiedene QTLs für agronomisch relevante Merkmale (Ertrag, Ölgehalt, Pflanzenhöhe und Tausendkorngewicht) lokalisiert und zusätzlich aus den Feldversuchen vielversprechende Leindotterlinien mit verbesserter Ertragsleistung selektiert werden. Die Ergebnisse bilden einen Ansatzpunkt für eine zukünftige Marker-gestützte Leindotter-Züchtung.

Winterraps ist die bedeutendste Ölpflanze in Europa und die zweitwichtigste Ölsaat weltweit. Raps ist gut geeignet für low-input Produktionssysteme, da er die höchsten Kornerträge aller Ölpflanzen selbst an weniger günstigen Standorten zeigt. Das Ertragspotential von Raps wird heutzutage durch die systematische Nutzung der Heterosis in Form der Hybridzüchtung im besser ausgeschöpft. In dieser Arbeit konnten aus einer Serie von Testhybriden bis zu 43% Heterosis für den Kornertrag im Vergleich zum Mittel der elterlichen DH-Linien erzielt werden. Dabei war die Überlegenheit der Hybriden unter extensiven Standortbedingungen besonders hoch, so dass man von einem ausgesprochenen Heterosis-Effekt für Stickstoffaufnahmeeffizienz sprechen kann. Dieser Befund verdeutlicht die gute Eignung der Rapshybriden für eine sowohl ökonomisch tragfähige als auch ökologisch akzeptable Ölsaatproduktion an marginalen Standorten.

Aber hinsichtlich erneuerbarer Brennstoffe ist nicht allein Qualitätsraps mit 00-Qualität von Interesse sondern insbesondere auch Hoch-Erucasäure Raps (HEAR, high erucic acid rapeseed). HEAR-Hybriden zeigen einen signifikant höheren Ölertrag pro Hektar und eine bessere Stickstoffeffizienz als 00-Raps, so dass HEA-Raps ggf. eine Alternative zu 00-Raps für die Biokraftstoffproduktion sein könnte. In der vorliegenden Studie konnten drei neue Hybridkombinationen identifiziert werden, die bei reduzierter Stickstoffdüngung einen sehr hohen Ertrag erreichen: der Ölertrag dieser Hybriden lag mehr als 20% über dem Durchschnittsertrag gängiger 00-Rapssorten und offen abblühenden HEAR-Linien Solche hochleistungsfähigen Hoch-Erucaraps Sorten könnten künftig ggf. eine größere Rolle in der Produktion von erneuerbarer Energie in Low-input Produktionssystemen unter marginalen Bedingungen spielen. Damit könnte letztendlich die Energie- und Umweltbilanz der Produktion von Biokraftstoffen im Vergleich zur heutigen Biodieselerzeugung möglicherweise verbessert werden.

5. References

Abel, S., Möllers, C., Becker, H.C., (2005). Development of synthetic *Brassica napus* lines for the analysis of “fixed heterosis” in allopolyploid plants. Euphytica 146**:** 157-163.

Aufhammer, W., Kübler, E., Bury, M., (1994). Stickstoffaufnahme und Stickstoffrückstände von Hauptfrucht- und Ausfallrapsbeständen. J. Agron. & Crop Sci. 172: 255-264.

Barret, P., Delourme, R., Renard, M., Domergue, F., Lessire, R., Delseny, M., Roscoe, T.J., (1998). A rapeseed FAE1 gene is linked to the E1 locus associated with variation in the content of erucic acid. Theor. Appl. Genet. 96: 177-186.

Basunanda, P., Spiller, T.H., Hasan, M., Gehringer, A., Schondelmaier, J., Lühs, W., Friedt, W., Snowdon, R.J., (2007). Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed. 126 (6): 581-587.

Becker, H.C., Löptien, H., Röbbelen, G., (1999). Breeding: an overview. In: “Biology of Brassica Coenospecies”. Gomez-Campo C (ed), Elsevier, Amsterdam: 413–460.

Brandle, J.E. and McVetty, P.B.E., (1990). Geographical diversity, parental selection and heterosis in oilseed rape. Can. J. Plant Sci. 70: 935-940.

Bruce, A.B., (1910). The Mendelian theory of heredity and the augmentation of vigour. Science 32: 627-628

Burns, M.J., Barnes, S.R., Bowman, J.G., Clarke, M.H.E., Werner, C.P., Kearsey, M.J., (2003). QTL analysis of an intervarietal set of substitution lines in *Brassica napus*: (i) Seed oil content and fatty acid composition. Heredity 90: 39–48.

Camargo, L.E.A., Osborn, T.C., (1996). Mapping loci controlling flowering time in *Brassica oleracea*. Theor Appl Genet 92: 610-616

Colnenne, C., Meynard, J.M., Reau, R., Justes, E., Merrien, A., (1998). Determination of a critical nitrogen dilution curve for winter oilseed rape. Ann. Bot. 81: 311-317.

Crow, J. F., (1948). Alternative hypotheses of hybrid vigor. Genetics 33: 477–487.

Das, S., Roscoe, T.J., Delseny, M., Srivastava, P.S., Lakshmikumaran, M. (2002). Cloning and molecular characterization of the Fatty Acid Elongase 1 (FAE1) gene from high and low erucic acid lines of *Brassica campestris* and *Brassica oleracea*. Plant Sci 162: 245–250.

Davenport, C. B., (1908). Degeneration, albinism and inbreeding. Science 28: 454–455.

De Lorgeril, M., Salen, P., Laporte, F., Foulon, T., Payen, N., De Leiris, J. (2001). Rapeseed oil and rapeseed oil-based margarine for the prevention and treatment of coronary heart disease. Eur J Lipid Sci Technol 103:490–495.

Dianrong, L., (1999). Hybrid seed production in rapeseed. Heterosis and hybrid seed production in agronomic crops. In: Basra AS (ed) Food product press. New York, pp 217–260.

Diepenbrock, W., (2000). Yield analysis of winter oilseed rape (*Brassica napus* L.): a review. Field Crops Research 67: 35-49.

Diers, B.W., McVetty, P.B.E., Osborn, T.C., (1996). Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). Crop Sci 36: 79–83.

Dion,.Y., Gugel, R.K., Rakow, G.F.W., Sguin-Swartz, G., Landry, B.S., (1995). RFLP mapping of resistance to the blackleg disease [causal agent, *Leptosphaeria maculans* (Desm.) Ces. et de Not.] in canola (*Brassica napus* L.). Theor. Appl. Genet. 91: 1190-1194.

East, E.M., (1908). Inbreeding in corn. Rep.Conn.Agric.Exp.Sta. for 1907: 419-428.

Ecke, W., Uzunova, M., Weissleder, K., (1995). Mapping the genome of rapeseed (*Brassica napus* L.). 2. Localization of genes controlling erucic acid synthesis and seed oil content. Theor. Appl. Genet. 91:972–977.

Ewert, F., Rounsevell, M.D.A., Reginster, I., Metzger, M.J., Leemans, R., (2005). Future scenarios of European agricultural land use I. Estimating changes in crop productivity. Agriculture Ecosystems & Environment 107: 101

Fontana, F., Lazzeri, L., Malaguti, L., Galletti, S., (1998). Agronomic characterisation of some Crambe abyssinica genotypes in a locality of the Po Valley. Europ. J. of Agr. 9: 117–126.

Fourmann, M., Barret, P., Renard, M., Pelletier, G., Delourme, R., Brunel, D., (1998). The two genes homologous to Arabidopsis FAE1 co-segregate with the two loci governing erucic acid content in *Brassica napus*. Theor. Appl. Genet. 96: 852-858.

Frauen, M., Noaack, J., Paulmann, W., Grosse, F., (2003). Development and perspective of MSL-hybrids in winter oilseed rape in Europe. In: Proceedings of 11th international rapeseed conference, 6–10 July 2003, Copenhagen, Denmark, pp 316–318.

Friedt, W., Lühs, W., Müller, M., Ordon, F., (2003). Utility of winter oilseed rape (*Brassica napus* L.) cultivars and new breeding lines for low-input cropping systems. German J. Agron. 7:49–55.

Friedt, W., Snowdon, R.J., (2009). Oilseed rape. In: Vollmann J, Istvan R (eds) Handbook of Plant Breeding, Vol. 4: Oil crops breeding. Springer Verlag, in press.

Gabrielle, B., Denoroy, P., Gosse, G., Justes, E., Andersen, M.N., (1998). Development and evaluation of a CERES-type model for winter oilseed rape. Field Crops Res. 57: 95-111.

Gehringer, A., Friedt, W., Lühs, W., Snowdon, R.J., (2006). Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. *sativa*). Genome 49: 1555-1563.

Girke, A., (2002). Neue Genpools aus resynthetisiertem Raps (*Brassica napus* L.) für die Hybridzüchtung. Dissertation, Göttingen.

Haley, C.S., Knott, S.A., (1992). A simple method for mappin quantitative trait loci in line crosses using flanking markers. Heredity, 315-324.

Hansen, L.N., (1998). Intertribal somatic hybridziation between rapid cycling *Brassica oleracea* L. and *Camelina sativa* (L.) Crantz. Euphytica 104: 173-179.

Hasan, M., Friedt, W., Pons-Kühnemann, J., Freitag, N.M., Link, K., Snowdon, R.J., (2008). Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). Theoretical and Applied Genetics 116: 1035-1049.

Hasan, M., Seyis, F., Badani, A.G., Pons-Kühnemann, J., Friedt, W., Lühs, W., Snowdon, R.J., (2006). Analysis of genetic diversity in the Brassica napus L. gene pool using SSR markers. Genetic Resources and Crop Evolution 53: 793-802.

http://www.biofuelstp.eu/fuelproduction.html)

Hu, J., Li, G., Struss, D., Quiros, C.F., (1999). SCAR and RAPD markers associated with 18-carbon fatty acids in rapeseed, *Brassica napus.* Plant Breeding 118 (2): 145-150.

Hu, J., Quiros, C., Arus, P., Strass, D., Röbbelen, G., (1995). Mapping of a gene determining linolenic acid concentration in rapeseed with DNS-based markers. Theor. Appl. Genet. 90 (2): 258-262.

Hull, F.H., (1945). Overdominance and corn breeding where hybrid seed is not feasible. J.Amer.Soc.Agron. 38: 1100-1103.

Jones, D.F., (1917). Dominance of linked factors as a means of accounting of heterosis. Genetics 2: 466-479.

Jourdren, C., Barret, E., Brunel, D., Delourme, R., Renard, M., (1996). Specific molecular marker of the genes controlling linolenic acid content in rapeseed. Theor. Appl. Genet. 93:512-518.

Kessel, B., (2000). Genetische Variation und Vererbung der Stickstoff-Effizienz bei Winterraps (*Brassica napus* L.). Dissertation, Universität Göttingen, Cuvillier Verl. Göttingen.

Kessel, B., Becker, H.C., (1999a). Genetic variation of nitrogen-efficiency in field experiments with oilseed rape (*Brassica napus* L.). In: New Horizons for an Old Crop. Eds.: N. Wratten, P.A. Salisbury, Proc. 10th Intern. Rapeseed Congr., 26-29 Sept. 1999, Canberra, Australia (http://www.regional.org.au/au/gcirc/4/360.htm).

Kessel, B., Becker, H.C., (1999b). Genetic variation of nitrogen efficiency in field experiments with oilseed rape (*Brassica napus* L.). In: Plant Nutrition – Molecular Biology and Genetics. Eds.: G. Gissel-Nielsen and A. Jensen, Kluwer Academic Publ., Dordrecht, Netherlands, 391-395.

Khadhair, A.-H., Tewari, J.P., Howard, R.J., Paul, V.H., (2001). Detection of aster yellows phytoplasma in false flax based on PCR and RFLP. Microbiol. Res. 156: 179-184.

Knapp, S.J., Bridges, W.C., Birkes, D., (1990). Mapping quantitative trait loci using molecular marker linkage maps. Theor. Appl. Genet. 79 (5): 583-592.

Krützen, M., van Schaik, C., Whiten, A., (2007). The animal cultures debate: response to Laland and Janik. Trends in Ecology and Evolution 22: 6.

Lander, E. S., Botstein, D., (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Lazzeri, L., Errani, M., Leoni, O., Venturi, G., (2004). *Eruca sativa* spp. *oleifera*: a new non-food crop. Industrial Crops and Products 20: 67-73.

Lefort-Buson, M., Guillot-Lemoine, B., Dattee, Y., (1987). Heterosis and genetic distance in rapeseed (*Brassica napus* L.): crosses between European and Asiatic selfed lines. Genome 29: 413-418.

Léon, J., Becker, H.C., (1995). Rapeseed (*Brassica napus* L.), Genetics. In: W. Diepenbrock and H.C. Becker (eds.) Physiological Potentials for Yield Improvement of Annual Oil and Protein Crops. Advances in Plant Breeding 17, Blackwell Wissenschaftsverlag Berlin, pp. 53-90.

Léon, J., (1991). Heterosis and mixing effects in winter oilseed rape. Crop. Sci. 31 : 281-284.

MacKanzie, W., (2006). The long and short of it - European product imbalances and their implications. Multi-client Study. Eigenverlag.

Makowski, N., (2003). Anbau von Leindotter – Alternativen im ökologischen und konventionellen Landbau? Raps 2: 73-77.

Matthäus, B., (2004). Leindotteröl – ein altes Öl mit neuer Zukunft? Ernährungs-Umschau 51: 12-16.

Mazzoncini, M., Vannozzi, G.P., Megale, P., Secchiari, P., Pistoia, A., Lazzeri, L., (1993). Ethiopian mustard (Brassica carinata A. Braun) crop in central Italy. Note 1: characterisation and agronomic evaluation. Agric. Med. 123: 330–338.

McVetty,P.B.E., Scarth, R., Rimmer, S.R., (1991). Hybrid canola breeding at the University of Manitoba. Proc. 8th Intern. Rapeseed Congress (GCIRC), Saskatoon, Sask., Canada, Vol. 1: 76-81.

Melchinger, A.E., (1999). Genetic diversity and heterosis. In: Coors CG, Pandey S (eds) genetics and exploitation of heterosis in crops. American society of Agronomy, Madison, pp 99–118.

Möllers, C., Kessel, B., Kahlmeyer, M., Ossenkop, O., Becker, H.C., (2000). Untersuchungen zur genotypischen Variabilität der Stickstoff-Effizienz bei Winterraps. In: Stickstoffeffizienz landwirtschaftlicher Kulturpflanzen. Hrsg.: C. Möllers, Erich Schmidt Verl. Berlin, 30-47.

Müller, M., (2002). Untersuchungen zur Anbaueignung und zur Verbesserung der Stickstoffeffizienz von Leindotter (*Camelina sativa* Crtz.), Öllein (*Linum usitatissimum* L.) und Winterraps (*Brassica napus* L.). Diss. JLU-Giessen, Shaker Verlag, Aachen.

Müller, M., Ordon, F., Friedt, W., (1999). Perspektiven einer leistungsfähigen Getreide- und Ölsaatenproduktion an ackerbaulichen Grenzstandorten - Perspectives for efficient cereal and oilseed production in marginal areas. Z. f. Kulturtechnik und Landentwicklung 40: 275-281.

Müller, M., Friedt, W., (1998). Entwicklung von Low-input-Ölpflanzen – eine Chance für marginale Standorte? Vortr. f. Pflanzenzüchtg. 42: 143 – 145.

Osborn, T.C., Butrulle, D.V., Sharpe, A.G., Pickering, K.J., Parkin, I.A.P., Parker, J.S., Lydiate, D.J., (2003). Detection and Effects of a

Homeologous Reciprocal Transposition in Brassica napus. Genetics 165: 1569.

Pilet, M.L., Duplan, G., Archipiano, M., Barret, P., Baron, C., Horvais, R., Tanguy, X., Lucas, M.O., Renard, M., Delourme,R., (2001). Stability of QTL for Field Resistance to Blackleg across Two Genetic Backgrounds in Oilseed Rape. Crop Science 41: 197-205.

Pilet, M. L., Delourme, R., Foisset, N., Renard, M., (1998). Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent Leptosphaeria maculans (Desm.) Ces. et de Not., in Winter rapeseed (*Brassica napus* L.). Theor Appl Genet 96: 23-30.

Pilgeram, A.L., Sanda, D.C., Boss, D., Dale, N., Wichmann, D., Lamb, P., Lu, C., Barrows, R., Kirkpatrick, M., Thompson, B, Johnson, D.L., (2007). *Camelina sativa*, A Montana Omega-3-and Fuel Crop. In: Janick.J. and Whipkey, A. (Eds.). Issues in New Crops and New Uses , ASHS Press, Alexandria. VA.p. 129-131.

Pourdad, S.S., Sachan, J.N., (2003). Study on heterosis and inbreeding depression in agronomic and oil quality characters of summer rape (*Brassica napus* L.) In: Proceedings of 11th international rapeseed conference, Copenhagen, Denmark, 6–10 July 2003, pp 343–346.

POWERS, L., (1944). An expansion of Jones' theory for the explanation of heterosis. Am. Nat. 78: 275–280.

Rajcan, I., Kasha, K. J., Kott, L.S., Beversdorf, W.D., (1999). Detection of molecular markers associated with linolenic and erucic acid levels in spring rapeseed (*Brassica napus* L.). Euphytica 105 (3): 173-181.

Riemer, H.M., Kessel, B., Klemisch, M., Link, W., Maidl, F.X., Möllers, C., Becker, H.C., (1998). Genotypic variability of N efficiency in winter barley, winter rapeseed and grain legumes. In: Sustainable Agriculture for Food, Energy and Industry - Strategies Towards Achievement. Eds.: N. El-bassam, R.K. Behl, B. Prochnow, James & James Science Publishers, London, Vol. 1, 412-417.

Röbbelen, G., Nitsch, A., (1975). Genetical and physiological investigations on mutants for polyenoic fatty acids in rapeseed, *Brassica napus* L. Z Pflanzenzuecht 75 : 93–105.

Rygulla, W., Snowdon, R.J., Friedt, W., Happstadius, I., Cheung, W.Y., Chen, D., (2008). Identification of Quantitative Trait Loci for Resistance Against Verticillium longisporum in Oilseed Rape (*Brassica napus*). Phytopathology 98 (2): 215-221.

Sauermann, W., Finck, M., (1998). Bundes- und EU-Sortenversuch Winterraps 1996/97. UFOP-Schriften 7: 9-44.

Sauermann, W., (2006). Erucaraps-Sorten im EU-Sortenversuch. Rapszeitschrift 2: 89-91.

Schultze-Motel, J., (1979). Die urgeschichtlichen Reste des Schlafmohns (*Papaver somniferum* L.) und die Entstehung der Art. Genetic Resources and Crop Evolution 27 (2): 207-215.

Schuster, W., (1987). Die Entwicklung des Anbaues und der Züchtung von Ölpflanzen in Mitteleuropa. Fett Wissenschaft Technol. 89: 15-27.

Schuster, A., Friedt, W., (1995). *Camelina sativa*: old face – new prospects. Eucarpia Cruciferae Newsletter 17: 6-7.

Schuster, W., Michael, J. (1976). Untersuchungen über Inzuchtdepressionen und Heterosis Effekte bei Raps (Brasica napus oleifera). Z. Pflanzenzüchtung 77: 56-66.

Schuster, C., Sauermann, W., Diepenbrock, W., (1999). Ertragsleistung und Unkrautkontrolle beim Anbau von transgenem herbizidtoleranten Hybridraps (*Brassica napus* L.). Planzenbauwissenschaften 3: 73-81.

Seiffert, B., (2000): Molekularbiologische Untersuchungen zur Stickstoffeffizienz bei *Brassica napus* L. Dissertation, Universität Göttingen, Cuvillier Verl. Göttingen.

Seyis, F., Friedt, W., Lühs, W., (2005). Development of resynthesized rapeseed (*Brassica napus*) forms with low erucic acid content. Asian Journal of Plant Sciences 4: 6-10.

Seyis, F., Friedt, W., Lühs, W., (2006). Yield of *Brassica napus* L. hybrids developed using resynthesized rapeseed material sown at different locations. Field Crops Research 96: 176-180.

Shen, J., Lu, G., Fu, T., Yang, G., (2003). Relationship between heterosis and genetic distance based on AFLPs in *Brassica napus*. In: Proceedings of 11th international rapeseed conference, Copenhagen, Denmark, 6–10 July 2003, pp 343–346.

Shull, G.H., (1908). The composition of a field of maize. Rep.Amer.Breed.Ass. 4: 296-301.

Sieling, K., Christen, O., (1999). Yield, N-uptake and N-use efficiency of and N leaching after oilseed rape grown in different crop management systems in northern Germany. In: New Horizons for an Old Crop. Eds.: N.

Wratten, P.A. Salisbury, Proc. 10th Intern. Rapeseed Congr., 26-29 Sept. 1999, Canberra, Australia (http://www.regional.org.au/au/gcirc/2/95.htm).

Sieling, K., Günther-Borstel, O., Hanus, H., (1997). Effect of slurry application and mineral nitrogen fertilization on N leaching in different crop combinations. J. Agric. Sci. Cambridge 128: 79-86.

Sigareva, M.A., Earle, E.D., (1999). Camalexin induction in intertribal somatic hybrids between *Camelina sativa* and rapid-cycling *Brassica oleracea*. Theor. Appl. Genet. 98: 164-170.

Snowdon, R.J., Friedt, W., (2004). Molecular markers in Brassica oilseed breeding: Current status and future possibilities. Plant Breeding 123: 1.

Snowdon, R.J., Lühs, W., Friedt, W., (2006). Oilseed rape. In Kole C, ed., Genome Mapping and Molecular Breeding, Vol. 2: Oilseeds. Berlin: Springer Verlag, pp. 55–114.

Somers, D.E., Devlin, P.F., Kay, S.A., (1998). Phytochromes and Cryptochromes in the Entrainment of the Arabidopsis Circadian Clock. Science 282: 1488-1490.

Szumigalski, A.R., Van Acker, R.C., (2006). Nitrogen Yield and Land Use Efficiency in Annual Sole Crops and Intercrops. Agron J 98: 1030-1040.

Tao, C., He, B.B., (2005). Enzymatic isolation and enrichment of erucic acid from HEA seed oils: Current status. Transactins of the ASEA 48: 1471-1479.

Taylor, D.C., Katavic, V., Zou, J., MacKenzie, S.L., Keller, W.A., An, J., Friesen, W., Barton, D.L., Pederson, K.K., Giblin, E.M., Ge, Y., Dauk, M., Sonntag, C., Luciw, T., Males, D., (2001). Field testing of transgenic rapeseed cv. Hero transformed with a yeast sn-2 acyltransferase results in increased oil content, erucic acid content and seed yield. Molecular Breeding 8: 317-322.

Thompson, K.F. (1983). Breeding winter oilseed rapeseed *Brassica napus*. Adv. Appl. Biol. 7: 1-104.

Toroser, D., Thormann, C.E., Osborn, T.C., Mithen, R., (1995). RFLP mapping of quantitative trait loci controlling seed aliphaticglucosinolate content in oilseed rape (*Brassica napus* L.). Theor Appl Genet 91: 802-808.

Trinsoutrot, I., Nicolardot, B., Justes, E., Recous, S., (2000). Decomposition in the field of residues of oilseed rape grown at two levels of nitrogen fertilisation. Effects on the dynamics of soil mineral nitrogen between successive crops. Nutrient Cycling in Agroecosystems 56: 125-137.

Uzunova, M., Ecke, W., Weissleder, K., Röbbelen, G., (1995). Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet (1995) 90: 194-204.

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., Lelley, T., (2005). Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. Plant Breeding 124: 446-453.

Vollmann, J., Damboeck, A., Eckl, A., Schrems, H., Ruckenbauer, P., (1996). Improvement of *Camelina sativa*, an underexploited oilseed. In J. Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA. pp. 357-362.

Vollmann, J., Steinkellner, S., Glauninger, J., (2001). Variation in resistance of camelina (*Camelina sativa* [L.] Crtz.) to downy mildew (*Peronospora camelinae* Gäum.). J. Phytopathology 149: 129-133.

Walla, C., (2006). Biodiesel als Chance für den Ölsaatenanbau. Erschienen 2006 in: I. Darnhofer, C. Walla und H.K. Wytrzens (Hrsg.). Alternative Strategien für die Landwirtschaft. Wien: Facultas, S. 165-171.

Warwick, S.I., Francis, A., La Fleche, J., (2000). Guide to the wild germplasm of *Brassica* and allied crops (tribe *Brassiceae, Brassicaceae*) 2nd Edition [online]. Available from http://www.brassica.info/resources/crucifer_genetics/guidewild.htm [accessed 12 June 2006].

Williams, W., (1959). Heterosis and the genetics of complex characters. Nature 184: 527–530.

Zhao, J., Meng, J., (2003). Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.) Theor. Appl. Genet. 106: 759–764.

Zhao, J., Becker, H.C., Zhang, D., Zhang, Y., Ecke, W., (2006). Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield Theor. Appl. Genet. 113: 33–38.

Zhou, Z., (2000). Untersuchungen zum Blatt- und Wurzelmetabolismus sowie zum Phloem- und Xylemtransport in Zusammenhang mit der Stickstoff-Effizienz bei Raps (*Brassica napus* L.). Dissertation, Universität Göttingen (http://webdoc.gwdg.de/diss/2000/zhou/).

Danksagung

Ich bedanke mich in erster Linie und von ganzem Herzen bei Herrn Prof. Dr. Dr. h.c. Wolfgang Friedt für die Überlassung des Themas, die Möglichkeit am Institut für Pflanzenbau und Pflanzenzüchtung I zu arbeiten und die professionelle Betreuung. Herrn Prof. Dr. Bernd Honermeier als zweitem Gutachter danke ich sehr für die Übernahme des Koreferates.

Ein weiteres herzliches „Danke schön“ geht an Herrn Dr. Rodney Snowdon, der mir hilfsbereit und tatkräftig unter die Arme gegriffen und mich über die ganze Zeit hinweg produktiv und kreativ begleitet hat. Ohne seine engagierte Unterstützung wäre die Arbeit nie zu einem Ende gekommen.

Weiterhin möchte ich mich bei zeitweisen Betreuern und Kollegen für rege Diskussionen und Anregungen bedanken: Dr. Wilfried Lühs, Dr. Roland Baetzel, Dr. Jutta Ahlemeyer, Panjisakti Basunanda, Maen Hasan, Dr. Stephan Rönicke, Dr. Ana Gloria Badani-Dehmer, Mechthild Schuppener, Benjamin Wittkop, Markus Riehl, Dr. Katrin Humbroich, Dr. Christoph Stoll und Dr. Wiebke Rygulla. Wir haben so manche fröhliche, teilweise feucht-fröhliche Zeit verbracht, an die ich mich immer wieder gerne zurück erinnern werde.

Einen ganz besonderen Dank möchte ich an die LTAs, die „fleissigen Bienchen“ dieser Arbeit senden - Karl-Heinz Balzer, Petra Degen, Juliane Döhrel, Werner Gipper, Birgit Keiner, Annette Plank, Liane Renno, Mechthild Schwarte und Mario Tolksdorf - ohne deren Engagement meine Arbeit schwer zu bewältigen gewesen wäre.

Meinen beiden früheren Bürokollegen Dr. Katrin Link und Dr. Tobias Spiller möchte ich im Besonderen ein herzliches Dankeschön aussprechen, da sie mir mit ihrer liebevollen und ehrlichen Art in guten und in schlechten Zeiten immer zur Seite gestanden haben und ihre Freundschaft über diese Arbeit hinausgeht.

Meiner Familie und meinem Partner danke ich für die Geduld, selbstlose Unterstützung und den Glauben an mich!

Eidesstattliche Erklärung

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten."

Mömbris, 17.11.2009

Anke Gehringer