Genetic diversity based on SSR markers, heterosis and yield performance of *Brassica rapa* for biomass production

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Presented by

Atta Ofori
born in Kukurantumi (Eastern Region), Ghana

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Referee: Prof. Dr. Heiko C. Becker

Co-referee: Prof. Dr. Rolf Rauber

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Acronyms and Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AHPH	Absolute Mid Parent Heterosis
AMPH	Absolute High Parent Heterosis
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
CO_2	Carbon dioxide
DBY	Dry Biomass Yield
DMC	Dry Matter Content
DNA	Deoxyribonucleic Acid
DTF	Days to Flowering
EU	European Union
FAO	Food and Agriculture Organisation
FBY	Fresh Biomass Yield
F_1	First filial
FS_b	between cultivar Full-Sibs
FS_w	within cultivar Full-Sibs
GenAlEx	Genetic Analysis in Excel
GCA	General Combining Ability
GSL	Glucosinolate
HPH	High Parent Heterosis
MPH	Mid Parent Heterosis
MPV	Mid Parent Value
PH	Plant Height
RAPD	Random Amplified Polymorphism DNA
RES	Renewable Energy Sources
RFLP	Restriction Fragment Length Polymorphism
SCA	Specific Combining Ability
SNP	Single Nucleotide Polymorphisms
Syn-1	Synthetic-1

SSR	Simple Sequence Repeat
UPGMA	Unweighted Pair-Group Method with Arithmetic Mean Algorithm

1. General Introduction

1.1 Biomass as renewable energy source

Fossil fuels from coal, natural gas and petroleum (oil) have been the main sources of energy since the mid 1800s. In 2005, fossil energy's contribution to total energy consumption was about 79% in the European Union (EU) countries and 81% worldwide (EC 2007). However, there is worry about using up all of the earth's resource of fossil fuels in future. Also the mining, processing, and combustion of fossil fuels produce CO_2 and other gases which are contributing significantly to the increase in atmospheric CO_2 concentration (IPCC 2001).

The first global attempt to search for solutions to these problems was the Kyoto accord which was introduced in 1997 by the United Nations. There after, renewable energy sources (RES) of bio-, water-, wind- and solar- energy is being promoted because they are neutral to the production of CO_2 and replaceable. Presently, RES have gained much support politically and factors such as subsidies, tax exemptions, and research grants to increase the share of RES in energy sectors have been adopted. The EU target is to increase its RES from 5.4 % in 1997 to 12.0% by 2010.

Recently, Biomass among other RES is highly being promoted. This is because the resources are vastly abundant, can be generated in a short period of time and obtained from different sources (waste products, forest, annual, biennial and perennial crops). Biomass production would provides employment opportunities through the cultivation, harvesting, transporting and conversion to bioenergy (Rosillo-Calle 2006). Also, it can be used to generate different forms of energy including heat, electricity and fuel. In 2000, 79.8% out of 13.8% of RES used worldwide was from biomass (IEA 2002) and in Europe, 66.1% of RES used was biomass (EC 2006).

Traditionally, bioenergy has been the main source of energy in developing nations, particularly in the native form when used as firewood for cooking and heating. Presently, modern technologies are increasing rapidly and both total biomass and grains are utilized for a number of different bioenergy products. Different biomass crops ranging from herbaceous annuals (alfalfa, sorghum, maize, barley, rapeseed, rye, triticale and wheat) to perennials which can be grouped into herbaceous (*Miscanthus* and grasses), and woody (forest trees) are cultivated purposely for bioenergy (Sims et al. 2006).

Also, the technology used in the conversion of biomass to bioenergy is advancing rapidly and methods such as pyrolysis to produce liquid fuels, combustion alone or in combination with fossil fuels to produce heat or electricity, gasification to produce combustible gas, and anaerobic fermentation (biogas) to produce heat and electricity are employed (Rosillo-Calle 2006).

In the past five years, anaerobic fermentation (biogas) is gaining interest as a biomass conversion technology in Europe (Wellinger 2007). This is because it has low establishment cost, is flexible in its operation and variable substrates such as organic and industrial waste, animal slurries, agricultural residues and a variety of biomass energy crops can be used (Svensson et al. 2006). Biogas production is a way of managing organic waste products by producing methane for bioenergy and organic fertilizers obtained from the digest (Abraham et al. 2007; Börjesson and Berglund 2007).

The biogas produced from biomass depends on substrates that can be degraded to CH_4 and CO_2. Therefore, content of organic matter and lignin in plant substrate is important (Stewart et al. 1984). A substrate with higher amount of organic matter and with low level of lignin implies less decomposition time. Lignin is a complex phenolic polymer with phenyl propane units cross-linked to each other by different chemical bonds. These complex bonds make it difficult for plant material to decompose and they in turn increase with increasing maturity in plants (Grabbers 2005).

Amon et al. (2007) mentioned that the quality of energy crops for biogas production is mainly influenced by field conditions. The content and availability of substance that influence methane production depends on crop variety, cultivation and the stage of maturity at harvesting. In maize, they reported a range of 22 to 62 % dry matter content (DMC) at different harvesting periods. Anaerobic digestion showed that on specific methane yield production basis, methane decreases with increasing DMC. However, methane yield per hectare basis, thus the product of DMC, volatile solids and specific methane yield increases with increasing DMC up to about 50% DMC after which a further increase is not significant.

1.2 *Brassica rapa* as potential biomass crop for biogas production

The use of plant biomass as substrate for biogas production has recently become an important and popular practice in Europe. The number of biogas plants has been increasing rapidly and in Germany, about 3.500 were in operation at the end of 2006 (Weiland 2007), which is an increase of 75% between 2004 and 2006. Presently, over 350.000 ha, representing 2% of agricultural land in Germany, is used for the cultivation of biogas energy crops. However, about 80% of the biogas substrate in Germany comes from maize (Weiland 2007), a crop of sub-tropical origin with low cold tolerance that can not be sown before May, and is harvested in September/October.

For maximum utilisation of land and availability of biogas substrate through out the year, biomass crops that are winter hardy and can be harvested early in the year are required. This will give growers the option to grow two crops in one season. The first one sown in autumn and harvested in spring followed by a second crop sown in May and harvest in autumn. Examples of crops adapted to higher temperatures are sunflower or the C_4 crops maize and sorghum, and those with high biomass production even under low temperatures are C_3 plants as rye and triticale, forage grasses, and also *Brassica* crops.

Among the *Brassicas*, *B. rapa* is of special interest because it has a higher early biomass and is flowering earlier than *B. napus* with fewer frost free days requirement to complete its life cycle (CFIA 1999; Pertl et al. 2002; Halfhill et al. 2005). Today, *B. rapa* is mainly grown as spring oilseed crop in Canada and in some marginal regions in Northern Europe where the growing season is very short. Traditionally, also winter *B. rapa* was grown as oilseed crop in Northern and Central Europe, but cultivation has nearly ceased.

The European winter *B. rapa* is the first among winter crops to develop early biomass because of its high growth rate at low temperature during spring. As source for bioenergy, also older cultivars of winter *B. rapa* can be used. They have sometimes low grain yield and relatively poor seed quality. The open pollinated nature of *B. rapa* has made it difficult to introduce seed quality genes into cultivars. However, they might be suitable for biomass production because seed yield and seed quality are not important for biomass production. Also, this could positively contribute to increased biodiversity.

Winter *B. rapa* is an herbaceous winter annual crop and sown in Europe between September/October. It is stress tolerant and can be cultivated on a variety of soils. On the average, it can grow to a height of 1.5 m with profuse branching. Winter *B. rapa* blooms in April or May since it can grow under lower temperatures (CFIA 1999). It could be harvested during flowering where the biomass yield is nearly as high as the maximum (Diepenbrock 2000). Though water content at flowering period is high, it could either be stored or used directly after harvesting for biogas production since it has high soluble cell and low lignin contents which is suitable for anaerobic digestion (Stewart et al. 1984).

1.3 Origin and importance of winter *Brassica rapa*

The *Brassica* genus consists of three monogenomic diploid species, *B. rapa* (A genome n=10), *B. oleracea* (C genome n=9), *B. nigra* (B genome n=8) and three amphidiploids, *B. napus* (AC genome n=19), *B. carinata* (BC genome n=17), *B. juncea*, (AB genome n=18) (U

1935). The amphidiploid species originated through spontaneous inter-specific hybridization of the diploids and are believed to be of recent origin with narrow genetic base (Song and Osborn 1992). The diploid species have a long history of domestication and *B. rapa* was cultivated already during the Bronze Age in Northern Europe (Persson et al. 2001), towards the end of the sixteenth century in Holland and Belgium, and in the eighteenth century in Britain (Riddet 1925).

Molecular and morphological studies have proposed that *B. rapa* originated from two independent centers; Europe and Asia (Song et al. 1988; He et al. 2003; Zhao et al. 2005). The Asian types consist of several subgroups of species which are mainly used as leafy vegetables, while the European types are used as oilseed (Reiner et al. 1995). Based on vernalization requirement before flowering, *B. rapa* can be grouped into winter and spring types and presently for oilseed production, mainly spring type is cultivated.

The *Brassica* species together are the second largest oilseed crop produced worldwide (FAO 2006). The most important *Brassica* species is *B. napus*, but *B. rapa* is also of special interest as a progenitor of *B. napus* and *B. juncea*. The oil is presently processed as a renewable energy in the petrochemical industry for biodiesel and over 3.9 million tonnes of biofuel was produced by the EU in 2005 (EC 2006).

The subspecies *rapifera* of *B. rapa* is cultivated either for its turnips or leaves. In Northern Spain, Portugal and Southern Italy (Padilla et al. 2005), it is used either as leafy vegetable for human consumption or fodder for feeding animals, depending on the morphotype. The swollen root is consumed by both human and animals. In China, different morphotypes of *B. rapa* are vegetable cultivars, which includes Chinese cabbage (subsp. *pekinensis*) characterized by its large leaves with wrinkled surfaces, and Pak choi (subsp *chinensis*) which does not form heads (Zhao et al. 2005).

1.4 Genetic diversity of *B. rapa*

The maximum utilization of any species for breeding and its adaptation to different environments or stress conditions depend on the level of genetic diversity it holds. In out-crossing species, a more variable germplasm implies high heterozygosity levels (Mohammedi and Prasanna 2003), with high stability to changing environments. For breeding, information about germplasm diversity in cultivated species is important to identify diverse parental combinations for hybrid breeding or to create variable segregating progenies for inbred lines.

Also, information on the identity and genetic diversity of accessions is necessary for the management, conservation and utilization of crop germplasm (Cruz et al. 2006). Genetic diversity in plants can be investigated with either data from pedigree, morphology, isozymes, storage proteins, or DNA markers. Examples of DNA markers presently used in *Brassica* are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphism DNA (RAPD), simple sequence repeat (SSR) and single nucleotide polymorphisms (SNP) (Snowdon and Friedt 2003). Genetic diversity may be studied at the level of individual genotypes, populations or species, and a number of studies on genetic diversity in *B. rapa* have been carried out with different methods.

Padilla et al. (2005) using 34 morphological and agronomical traits differentiated 120 populations of *B. rapa* landraces collected throughout northern Spain into five groups; worst agronomic potential, rosette growth habits, without rosette growth habits, highest early vigour and number of secondary stems per plants, large flowering period and large seed weight. Mukhlesur et al. (2004) with seed protein and isozymes distinguished clearly between yellow sarson (self-compatible) and brown sarson (self-incompatible) in 32 *B. rapa* cultivars collected from China, Bangladesh and Japan.

Genetic diversity in *B. rapa* in relation to crop type (oilseed, turnip and vegetable) and geographical origin (Central Asia, India, and Europe) was also investigated with isozymes and RFLP (McGrath and Quiros 1992). The RFLP diversity within populations was higher

than variation in isozymes and a clear separation was observed between European accessions whereas the Chinese and Indian accessions were more similar. The Asian accession and the Indian types showed the highest genetic diversity followed by the European. The within populations diversity accounted for about 70% of the total population variation.

Persson et al. (2001) employing allozymes reported 81% of the genetic diversity within accessions and 19% among accession in turnip *B. rapa* coming from Northern Europe. Zhao and Becker (1998) also with isozymes observed in cultivars of winter and spring types obtained from Europe, China and Canada a high genetic diversity of which 70% was attributed to within cultivar variation.

Das et al. (1999) compared the performance of AFLP and RAPD markers in detecting genetic diversity between different oilseed morphotypes (self-compatible and self-in compatible) of *B. rapa*. Genetic similarities based on Jaccard coefficient ranged from 0.42 to 0.73 for RAPD and 0.48 to 0.93 for AFLP, indicating a large genetic diversity and both AFLP and RAPD separated the self-compatible and self-incompatible cultivars. Simonsen and Heneen (1995) in Chinese accessions and Swedish *B. rapa* cultivars, observed a larger genetic diversity within the Chinese accessions than the Swedish cultivars, even though both were larger than in *B. oleracea* when compared with isozymes.

1.5 Mating system and self incompatibility

Brassica rapa is a cross pollinated crop with the exception of yellow sarson which is self pollinated (Becker et al. 1999; Das et al. 1999). The cross pollination is the result of self-incompatibility (SI) which is the inability to produce zygotes when stigma is self pollinated. Self-incompatibility in *B. rapa* is of sporophytic type, where the incompatibility phenotype in the pollen is determined by the pollen producing plant and controlled by a single multi-allelic S locus (Franklin-Tong and Franklin 2000). To induce self pollination in SI plants, treatments such as high humidity, high temperature, bud pollination, CO_2, chloroform and salt could be

applied (Johnson 1972).

Self-incompatibility in *B. rapa* influences the agronomical performance by increasing the out-crossing rate and minimizing inbreeding; the mating between closely related individuals. Inbreeding in turn increases homozygosity which leads to the exposure of deleterious alleles that have been masked by dominant effect. This consequently negatively affects the development of seed set, germination, survival and resistance to stress (Keller and Waller 2002). Self-incompatibility mechanism results in complete cross-pollination in *B. rapa* (Becker et al. 1999). It can also be used as mechanism for producing F_1 hybrids (Sakamoto and Nisio 2001; Shen et al. 2005). However, this requires the development of SI inbred lines by one of the artificial methods mentioned above to induce self pollination.

1.6 Breeding methods

Different methods are used to test for performance in cross pollinated crops and these have resulted in the development of different breeding methods. Examples are mass selection, recurrent selection, half-sib selection, full-sib selection and synthetic cultivars, and all these methods have been applied in *Brassica* breeding (for review see Becker et al. 1999).

Mass selection is a selection method where individual plants are selected based on their phenotypic (mother) performance. The seed of selected plants are bulked and sown for the next generation. The cycle of selection, bulking and sowing continues until no further improvement is achieved. It is an oldest system of selection, very simple, easy and completes its cycle in one year. However, its selection response is very low because pollen flow is not controlled and each plant is randomly mated with the population. It is suitable for traits that are of high heritability and controlled by few genes (Falconer and Mackey 1996).

Recurrent selection involves the evaluation of individual plants which have been selected from a base population. After evaluation, best plants identified are mated randomly before used for the next generation. Thus it is a cyclic breeding procedure designed to

increases the frequency of favourable alleles while maintaining genetic variation in breeding populations and was proposed by Hull (1954). It may be applied in both self and cross pollinated crops and has been extended to different mating systems. Examples are full-sib recurrent selection, half-sib recurrent selection and S_1 recurrent selection (Hallauer et al. 1988). The different recurrent selection methods differ in their cycle length and are mostly effective when the genetic variance is controlled by mainly general combining ability (GCA).

A half-sib progeny is the results of random mating of an individual plant as female pollinated by many other plants. In population improvement, a portion of seed produced from selected half-sibs is evaluated based on progeny performance. The best productive progeny seed is bulked and used for the next generation. Mating can be either polycross where all lines to be tested are allowed to randomly mate or top cross which is the mating of test lines with their base population (Falconer and Mackey 1996). The variance of general combining ability of half-sibs is mostly small because pollen movement is not controlled and only between family variations is utilized (Aastveit and Aastveit 1990). Half-sib family selection is simple and has been used in population improvement of *B. rapa* (Bradshaw et al. 2002)

In full-sib, crosses between two plants are produced and evaluated. Based on results of progeny evaluation, the high yielding full-sibs seed are sown and used for the next generation. Thus pollen movement is controlled and combining ability and heterosis are directly utilized (Lambeth et al. 2001). In addition, it utilizes both within and between family variation (Aastveit and Aastveit 1990) and population improvement is quite fast because only high yielding plants are allowed to cross. It has commonly been used in population improvement of cross pollinated crops and examples are maize (Pixley et al. 2006) and forage grasses (Aastveit and Aastveit 1990; Fang et al. 2004).

Synthetic cultivars are specific kinds of population cultivars, typically produced through random mating of selected components based on their performance and combining ability (Becker et al. 1999). The mixtures of parental components are referred to as Syn-0 and

their offspring as Syn-1. The following generations are produced by random mating without selection and are referred to as Syn-2, Syn-3, and so on. Synthetics could be utilized even at Syn-1 level, where for two parental cultivars it composes of 25% each of plants from crosses within the parental components and of 50% of plants from crosses between the two populations. Yield performance of synthetic has been experimentally demonstrated in *B. rapa* (Falk et al. 1994; Falk et al. 1998) and is effective in cases where genetic variance is controlled by specific combining ability with high out crossing rate (Becker et al. 1998).

1.7 Combining ability

Selection of parents for synthetic or hybrids breeding is based on their combining ability. Combining ability is the ability of a parent to produce superior progeny and has been divided into general combining ability (GCA) and specific combining ability (SCA). The GCA effect of a population is an indicator of the relative value of the population in terms of frequency of favourable genes and of its divergence, as compared to the other populations. The SCA effect of two populations expresses the differences of gene frequencies between them and their divergence, as compared to the diallel populations (Viana 2000).

The mating design most often employed in the assessment of combining ability is the diallel (Griffing 1956; Gardner and Eberhart 1966). This allows the selection of superior pure lines for hybridization and, in cross-pollinating species, to screen populations for use in within and between population breeding programs.

Studies on combining ability for traits such as yield and other agronomic traits are available in different *Brassica* species with diallel analysis. Qian (2003) evaluated intraspecific hybrid between *B. rapa* x *B. napus* for biomass yield in two years. Significant variation was observed for both GCA and SCA, indicating that both additive and non additive effects influenced biomass yield production. The ratios of variance component for GCA to SCA were 89% in 1999 and 88% in 2000, showing that GCA played a more important role

though both were significant.

Wang et al. (2007) studied combining ability for different traits in subspecies of Chinese *B. rapa*. They observed that yield per plant and length of main inflorescence were mainly controlled by SCA; plant height, number of primary branches, siliques of primary branches, seed per silique and 1000-seed weight were controlled by both GCA and SCA; and number of secondary branches, siliques of secondary branches and siliques per plant were mainly controlled by GCA.

Combining ability of 15 *B. rapa* subspecies yellow sarson was estimated by using diallel including reciprocals for 12 characters related to yield and oil content (Singh and Murty 1980). Gene action was predominantly controlled by SCA effects with GCA effects playing a minor role in oil content and 50% flowering. Yadav et al. (1988) in nine inbred lines of brown sarson used as females and three other cultivars as male examined the combining ability of their 27 hybrids. Specific combining ability was observed to control all traits when the hybrids were evaluated for plant height, number of branches per plant, number of seed per pot, 1000-seed weight and seed yield per plant.

1.8 Heterosis utilization

Heterosis is the difference in performance between F_1 generation and mid parent or high parent and has been a major breeding tool for plant productivity improvement. Preferably, inbred lines with genetically distinct backgrounds are used as parent for F_1 production. It makes maximum use of heterosis by combining favorable alleles of the individual homozygous parents. In populations such as *B. rapa,* a part of heterosis is already utilized in base population due to their open pollination with plants being partly heterozygous.

However, it can take advantage of the homozygous plants within the population for heterosis, and also ‘heterotic increase’ which could result by crossing heterozygous plants. Parental populations with different genetic make-up such as cultivars (Shuler et al. 1992)

synthetics (Falk et al. 1998), and subspecies (Wang et al. 2007) have been used in heterosis studies in *B. rapa*. For estimating heterosis in crosses between population, Lamkey and Edwards (1999) suggested the term "panmictic mid parent heterosis" for the difference between the mean of two random mating populations and the mean of a hybrid population produced by crossing individual plants of the two populations.

Dominance, over dominance and epistasis are the three principal genetic explanations for heterosis. The dominance hypothesis stipulated that heterosis is contributed by favorable alleles of both parents. Over dominance is a condition where loci in the heterozygous state are superior to parents and epistasis is the complex interactions of favorable alleles of the two parents (Crow 1999). Heterosis can only occur when parental cultivars used for F_1 production differ in gene frequencies (Falconer and Mackay 1996).

Heterosis for different agronomic traits has been reported. Schuler et al. (1992) in inter-cultivar F_1s of *B. rapa* reported mid parent heterosis (MPH) of 18% for seed yield. Falk et al. (1998) in cultivars of spring *B. rapa* reported 25% MPH in seed yield. Kaur et al. (2007) in *B. rapa* subspecies of toria, brown sarson and yellow sarson observed 31% heterosis in intra group crosses and 17% in inter group crosses for seed yield. Wang et al. (2007) in Chinese *B. rapa* vegetables reported MPH of 10% for plant leaves, 44% for petiole fresh weight and 17% for the length of biggest leaf.

One of the most expensive steps in heterosis utilization is the identification of parental combinations that produce F_1 with superior yield. Therefore, the prediction of F_1 performance with accuracy from morphology or molecular data is important. This could reduce the cost involved in evaluating parent and crosses in field trials to identify parental combinations that will give high F_1 performance. The predictions of heterosis from parental genetic distance have been widely studied in many crops though hardly utilized. It is estimated by calculating distances of molecular or phenotypic data and comparing it with heterosis from field experiments (Teklewold and Becker 2005).

Reports on the extent of correlation between genetic distance and heterosis have varied for traits and studies. Liu et al. (2002) and Qian et al. (2003) in interspecific hybrids between *B. rapa* and *B. napus* reported a larger genetic distance based on molecular marker resulted in a higher biomass yield. Qian et al. (2007) observed a weak correlation between genetic distance and heterosis for interspecific crosses of European spring and Chinese semi winter lines. Kaur et al. (2007) observed a negative correlation between genetic diversity and hybrid performance in diverse morphotypes of *B. rapa*.

1.9 Objectives of the study

A winter crop widely grown in Europe for oil is *Brassica*. It is able to develop high early biomass because of its high growth rate under low temperatures during spring. It could be used as a pre-crop harvested earlier in the year for biogas followed by a second crop such as maize. Nevertheless, to date *B. rapa* has been bred primarily to enhance its nutritional value (seed quality) as an oilseed crop for humans and animals consumption, where zero erucic acid and low glucosinolate (GSL) are important. These targets are quite different from the criteria for bioenergy (biogas) for which high biomass yield is required.

The improvement of seed quality in winter *B. rapa* by introducing genes for low erucic acid and glucosinolate content implies that its germplasm had to go twice through a breeding bottleneck, possibly causing a reduction in genetic diversity. For a successful application of *B. rapa* as a biogas crop, we need to broaden our knowledge on the level of genetic diversity in the different seed quality groups.

The general objective of the study was to determine the genetic variation, heterosis and genetic diversity in *B. rapa* for biomass yield. This will be used as bases for selection of appropriate breeding strategy and cultivars for biomass production of European winter *B. rapa* which can be used for biogas production.

The specific objectives were;

1. To develop a breeding strategy for biomass production of European winter *Brassica rapa* for biogas production: Heterosis and combining ability for biomass yield,
2. To determine the biomass yield and heterosis of crosses within and between European *Brassica rapa* cultivars,
3. To examine the effect of crop improvement on genetic diversity in oilseed *Brassica rapa* cultivars detected by molecular markers.

2. Breeding of *Brassica rapa* for biogas production: Heterosis and combining ability of biomass yield

2.1 Abstract

The use of plant biomass as substrate for biogas production has recently gained major interest in Europe. Winter *Brassica rapa* produces high early biomass and could be grown as a pre-crop harvested early in the year followed by a second crop such as maize. The objectives of this study were to estimate heterosis and combining ability of present and older 15 European winter *B. rapa* cultivars for biomass yield at flowering. A half-diallel without reciprocals was conducted among the cultivars to produce 105 crosses. These crosses and their parents were evaluated in two years at two locations in Northern Germany. Data collected were days to flowering (DTF), fresh biomass yield (FBY), dry matter content (DMC), dry biomass yield (DBY) and plant height (PH). The mean DBY was 5.3 t/ha for the parental cultivars and 5.6 t/ha for their crosses. The crosses surpassed in average their parents by 7.6 % for FBY and 5.9% for DBY whereas DMC was 1.4 % higher in the parents. Maximum mid parent heterosis was 21.0 % for FBY and 30.4 % for DBY. Analysis of variance showed that genetic variance was mainly due to specific combining ability (SCA). The correlation between parental performance and general combining ability (GCA) was 0.42** for FBY and 0.53** for DBY. In conclusion, the amount of heterosis in crosses between European winter *B. rapa* cultivars is not very high on average, but can be up to 30 % in the best crosses. Selection of parents with high specific combining ability to produce synthetic cultivars can rapidly improve biomass yield.

Key words: biogas, diallel, full-sibs, general combining ability, synthetic cultivars, specific combining ability

2.2 Introduction

Different technologies to convert biomass to bioenergy have been developed and biogas production among them has become of major interest in the past years in Europe. The number of biogas plants operating in Germany at the end of 2006 was 3.500 (Weiland 2007), which is an increase of 75% between 2004 and 2006. Presently, over 350.000 ha, representing 2% of agricultural land in Germany is used for the cultivation of biogas energy crops (Weiland 2007).

The majority of the biogas substrate in Germany comes from maize, a sub-tropical crop, which can not be sown before May and is harvested in September/October. For maximum utilisation of land and availability of biogas substrate through out the year, biomass crops that are winter hardy and can be harvested early in the year are required. This will give growers the option to grow two crops in one season: the first one sown in autumn and harvested in spring, followed by a second crop adapted to higher temperatures like maize, sorghum or sunflower. Crops with high biomass production even under low temperatures are rye, some forage grasses, and also *Brassica* crops. Among the *Brassicas, B. rapa* is of special interest, because it has a higher early biomass than *B. napus* (CFIA 1999; Halfhill et al. 2005).

Today, *B. rapa* is mainly grown as spring oilseed crop in Canada and in some marginal regions in Northern Europe. Traditionally, also winter *B. rapa* was grown as oilseed crop in Northern and Central Europe, but cultivation has nearly ceased. However, there is a renewed interest in the cultivation of winter *B. rapa* in Europe to produce biomass, because of its high growth rate under low temperatures during early spring. For biomass production, older cultivars of winter *B. rapa* can be used. They have low grain yield and relatively poor seed quality which is not important for biomass production. Also, this could positive contribute to increased biodiversity.

Brassica rapa is a cross-pollinated and self-incompatible crop with high genetic diversity within cultivars (Zhao et al. 2005). Different methods such as hybrid breeding, full-sib selection, recurrent selection and development of synthetic cultivars have been exploited in *Brassica* population improvement (for review see Becker et al. 1999). From these methods, full sib selection makes direct use of combing ability and heterosis (Lambeth et al. 2001).

It utilizes both within and between family genetic variation (Aastveit and Aastveit 1990). For heterosis determination in population, Lamkey and Edwards (1999) suggested the term "panmictic mid parent heterosis" for the difference between the mean of two random mating populations and the mean of a hybrid population produced by crossing individual plants of the two populations.

A number of studies on combing ability and heterosis for yield and yield components have been carried out. Singh and Murty (1980) in *B. rapa* subspecies yellow sarson and Wang et al. (2007) among different subspecies of *B. rapa* observed predominance of non additive genetic effects. Yadav et al. (1988) in nine inbred lines of brown sarson used as females and three other cultivars as males also observed predominance of specific combining ability for seed yield and other related traits. In heterosis studies, Falk et al. (1998) observed a mid parent heterosis of 25 % for seed yield, Schuler et al. (1992) in inter-cultivar F_1s of *B. rapa* reported mid parent heterosis of 18% for seed yield. Kaur et al. (2007) in *B. rapa* subspecies toria, brown sarson and yellow sarson observed heterosis of 17% for seed yield in intra group crosses.

The aim of this study is to analyse the genetics of biomass yield of 15 winter *B. rapa* cultivars and F1s derived from crosses amongst them. The specific objectives were: (i) to evaluate the biomass yield at end of flowering, (ii) to estimate the effects of general and specific combining ability (GCA and SCA), and (iii) to estimate the magnitude of heterosis in population crosses. The results will allow the development of efficient breeding strategies for *B. rapa* as new bioenergy crop in Europe.

2.3 Material and methods

2.3.1 Parental cultivars

The plant materials used in this experiment consisted of 15 winter *B. rapa* cultivars (Table 2.1). They were of European origin and were obtained from either genebanks or breeding companies. They represent a large range of genetic material from different geographical regions within Europe and both forage and oilseed types are included. Their seed quality differed with respect to erucic acid and seed glucosinolate content.

2.3.2 Material development

The 15 winter *B. rapa* cultivars were crossed in a half-diallel manner without reciprocals at Reinshof experimental station in May 2005 and 2006. Three full sibs (FS) were produced from each of the 105 parental combinations by isolating two plants each under one large bag before beginning of flowering. In this way nearly complete cross-pollination can be achieved. Bags were gently shaken every other day during flowering. To control hybridization, 40 seeds each were randomly selected from the crosses; Largo x Weibull Storrybs (ee x EE) and Lemkes Winterrübsen x Opava (EE x EE) and analyzed with near infrared reflectance spectroscopy (NIRS). Seeds were further analyzed with the gas chromatography to confirmation results.

2.3.3 Field Evaluation

For evaluation, equal amounts of seed from the three FS of each combination were bulked. The 15 parental cultivars, their 105 crosses, and the check cultivar ‘Perko’ were grown in two years at the two locations Göttingen and Einbeck in Northern Germany. The FS produced in 2005 were tested in 2005/06, the FS produced in 2006 were tested in 2006/07. The experimental design was an 11 x 11 lattice with two replications.

Table 2.1 *Brassica rapa* cultivars used in this study with their country of origin

Cultivar name	Country of origin	Seed quality[a]	Sources/ Breeder	Accession No
Steinacher	Germany	++	BAZ, Braunschweig, Germany	BAZ 18101
Weibulls Storrybs	Sweden	++	CZ, Czechoslovakia	
BRA 245	Bulgaria	++	IPK, Gatersleben, Germany	BRA 245
Lemkes Winter	Germany	++	BAZ, Braunschweig, Germany	BAZ 34349
Lemkes Malchower	Germany	++	BAZ, Braunschweig, Germany	BAZ 34342
Arktus	Germany	++	BAZ, Braunschweig, Germany	BAZ 34354
Schneiders Sprengel	Germany	++	IPK, Gatersleben, Germany	BRA 11
Hege's Winter	Germany	++	BAZ, Braunschweig, Germany	BAZ 34335
Janetzki's	Germany	++	BAZ, Braunschweig, Germany	BAZ 31204
Opava	Czechoslovakia	++	BAZ, Braunschweig, Germany	BAZ
Grubes Winter	Germany	++	BAZ, Braunschweig, Germany	BAZ 34346
Wild accession [b]	Germany	++	Bonn, Germany	
Orbit	Sweden	0+	SW Seed, Sweden	-
Largo	Sweden	00	SW Seed, Sweden	-
Rex	Germany	0+	NPZ, Germany	-

[a] ++ high erucic acid, high glucosinolate; 0+ - zero erucic acid, high glucosinolate; 00 – zero erucic acid, low glucosinolate

[b] by courtesy of the collector, Dr. Thomas Gladis, University of Kassel,

Sowing dates were 24th of August at Einbeck and 31st of August at Göttingen in 2005 and 31st of August at Einbeck and 5th of September at Göttingen in 2006. Rate of sowing was between 90 and 110 seeds m^{-2}. Plot sizes were 11.25 m^2 in Göttingen and each plot consisted of 6 rows, 7.5 m long and 0.3 m between rows. In Einbeck, plot size was 9.0 m^2 and consisted of 5 rows, 6.0 m long and 0.3 m between rows. Standard crop management practices for weed control and fertilization were followed.

Data were recorded on days to flowering (DTF, from the day of sowing until 50% of plants were flowering), fresh biomass yield (FBY, kg/m^2), dry matter content (DMC, %), dry biomass yield (DBY, g/m^2) and plant height (PH, cm). Plots were harvested on the 8th of May

at Einbeck and 15th of May at Göttingen in 2006 and 2ed of May at Einbeck and 21st of May at Göttingen in 2007. This was at end of flowering, using a harvester that cut at 5 cm above ground and the total fresh biomass yield was measured. From each plot a sub-sample of 300 g fresh weight was dried at 60°C for 6 days to determine the dry matter content. Based on this, total dry biomass yield per plot was calculated.

2.3.4 Statistical analysis

Analyses of variance (ANOVA) were first run separately for each experiment using PLABSTAT software (Utz 2001) based on the model: $Y_{ijk}= u + r_i + g_j + ß_k + e_{ijk}$, where Y_{ijk} = observation of genotype j in block k and replication i; u = general mean; r_i = effect of replication; g_j = effect of genotype j; $ß_k$ = effect of blocking; e_{ijk} = error of observation. A combined analysis of variance of the adjusted means was then computed individually for years 2006 and 2007, and then for the four environments with model: $Y_{ij}= u + l_i + g_j + lg_{ij} + e_{ijk}$, where Y_{ij} = observation of means of genotype j in location i; u = general mean; l_i = effect of location; g_j = effect of genotype j; lg_{ij} is the interaction effect between location i and genotype j and e_{ijk} = error of observation of lattice.

Random effects model was assumed for years and locations. The sum of squares for entry effects were partitioned into parents, crosses and parents vs. crosses effects. The variance of crosses was further partitioned into general combining ability (GCA) and specific combining ability (SCA), according to analyses III of Gardner and Eberhart (1966) using PZ14 software (Utz 1992).

Heterosis increase was calculated as follows: absolute mid-parent heterosis MPH = (Crosses – MP), relative mid-parent heterosis MPH % = (Crosses – MP)/MP*100, absolute high-parent heterosis HPH = (Crosses – HP) and relative high-parent heterosis (HPH %) = (Crosses – HP)/HP*100, where MP is mid parent and HP is high parent. To test for significant differences in heterosis, analysis of variance (ANOVA) was performed for MPH % and HPH

% values. The error of the variance of heterosis was calculated as follows;

Taken the variance of a component X_1 to be

$$\text{Variance } (X_1) : \sigma^2_{(x1)} = \sigma^2_e \qquad (1)$$

where, σ^2_{x1} is the error variance of X_1

For variance of the different or the sum of two components X_1 and X_2 and assuming that the error variance of the two components is equal and not correlated,

$$\text{Variance of } (X_1\text{-}X_2) : \sigma^2_{x1\text{-}x2} = \sigma^2_{x1} + \sigma^2_{x2} = 2\,\sigma^2_e \qquad (2)$$

where $\sigma^2_{x1\text{-}x2}$ is error of X_1 - X_2.

$$\text{Variance } (X_1 + X_2) : \sigma^2_{x1+x2 =} \sigma^2_{x1} + \sigma^2_{x2} = 2\,\sigma^2_e \qquad (3)$$

where σ^2_{x1+x2} error of X_1 + X_2.

In the case of dividing the variance of a component X_1 of (1) by a factor n

$$\text{Variance } (X_1)/n : \sigma^2_{(x1)/n} = \sigma^2_e/n \qquad (4)$$

According to (Pers. com. Utz 1988) the variance of a component X divided by another component Y and assuming that the errors are not correlated is

$$\text{Variance } (x/y) : \sigma^2 (x/y) \sim (^x/_{\hat{y}})^2 [\text{var. } (x)/_x{}^2 + \text{var. } (y)/_{\hat{y}}{}^2] \qquad (5)$$

From the above definitions, with equal variance for all components, the following can be deduced. From (2) and (4), variance of MPH = σ^2 [Cross – (P1+P2/2)] =

$$\sigma^2_e + \sigma^2_e/2 = 1.5\,\sigma^2_e \qquad (6)$$

From (2), error of HPH = σ^2 [Cross – P_H] =

$$\sigma^2_e + \sigma^2_e = 2\,\sigma^2_e \qquad (7)$$

For MPH % = [Cross – (P1+P2)/2)] / (P1+P2)/2)* 100, let [Cross – (P1+P2)/2)] = X and (P1+P2)/2) = Y, From (4), (5) and (6), error of MPH % =

$$(^x/_{\hat{y}})^2 [(1.5\sigma^2_e)/_x{}^2 + (0.5\,\sigma^2_e)/_{\hat{y}}{}^2] \qquad (8)$$

(1), (4) and (6), error HPH % = (Crosses – HP)/HP*100 ~ Crosses/HP=

$$(^{x}/_{\hat{y}})^2 \left[(2\sigma^2_e)/_x{}^2 + (\sigma^2_e)/_{\hat{y}}{}^2 \right] \quad (9)$$

2.4 Results

2.4.1 Parental cultivars and crosses

The mean values for traits and GCA for the 15 parents, their 105 crosses and SCA effects are listed in appendix. An overview of the results is given in Table 2.2. Comparison of locations for the year 2006 showed a higher performance at Göttingen than Einbeck for all traits except fresh biomass yield (FBY). This ocuured in both parents and full-sibs (FS). Full-sibs were higher than the parents whereas flowering was late in the parents at both locations. In the year 2007, all traits showed higher values in Göttingen than in Einbeck including plant height which was not measured in 2006. Full-sibs were also higher than the parents at both locations except dry matter content (DMC) and FBY which were higher in the parents at Einbeck.

Flowering was almost at the same time for parents and FS in both locations. Full sib means in 2007 were higher than 2006 by 40% for DMC, 8% for FBY, 42% for dry biomass yield (DBY) and flowering was earlier by 24 days. In the parents, yields in 2007 were also higher than 2006 by 45% for DMC, 9% for FBY, 46% for DBY and flowering was 25 days earlier.

The mean biomass yield over two years and two locations were higher in the crosses than in the parents (Table 2). The crosses out yielded the parents for fresh biomass yield (FBY) by 7.5% and for dry biomass yield (DBY) by 5.8%. Dry matter content was 1.5 % higher in the parents. Flowering was earlier in the crosses than in the parents. The variation of parent and crosses, differences in maximum and minimum values, were low in DTF but relatively higher in DMC, DBY and FBY. The parents with highest DBY were Opava (605

g/m²), Arktus (576 g/m²) and Lembkes Malchower (554 g/m²). The highest yielding crosses were Orbit x Lembkes Winter (666 g/m²), Orbit x Opava (665 g/m²) and Orbit x Arktus (657 g/m²).

Table 2.2 Minimum, maximum, mean, least significant difference (LSD) for 15 winter *B. rapa* cultivars and their 105 diallel crosses at Göttingen, Einbeck and across the four environments

Traits/	Parents			Crosses			LSD
locations	Min	Max	Mean	Min	Max	Mean	(5%)
2006							
Göttingen							
DTF(days)	240.50	246.00	243.54	240.00	247.00	242.90	3.09
DMC (%)	12.11	15.27	13.93	11.46	16.00	14.14	1.59
FBY (kg/m^2)	2.64	3.70	3.22	2.68	4.55	3.37	0.49
DBY(g/m^2)	336.04	503.49	449.75	372.58	577.80	475.5	80.89
PH(m)	129.77	147.97	139.21	115.00	152.32	140.1	12.88
Einbeck							
DTF(days)	251.54	255.84	253.06	249.51	256.47	252.8	2.65
DMC (%)	9.55	11.93	10.90	9.08	14.32	11.04	2.03
FBY(kg/m^2)	2.96	4.49	3.86	2.54	5.25	4.12	0.63
DBY(g/m^2)	351.91	498.63	419.61	274.20	572.71	451.3	99.37
2007							
Göttingen							
DTF(days)	219.50	230.50	225.40	219.50	230.50	225.6	1.45
DMC (%)	17.21	21.51	19.32	15.52	22.61	19.37	2.60
FBY(kg/m^2)	2.86	4.19	3.58	2.98	4.99	3.96	0.83
DBY(g/m^2)	555.39	779.37	691.13	576.20	1023.82	764.9	158.4
PH(m)	125.00	152.50	138.67	125	157.50	142.3	8.69
Einbeck							
DTF(days)	218.89	223.11	221.77	218.94	223.13	221.9	1.61
DMC (%)	12.48	24.37	17.31	10.68	23.08	15.86	6.67
FBY(kg/m^2)	3.02	3.90	3.40	2.71	4.43	3.59	0.66
DBY(g/m^2)	369.69	778.93	577.09	345.77	982.91	553.4	206.3
PH(m)	121.78	152.20	140.95	130.38	155.04	143.5	7.26
Across environments							
DTF(days)	232.95	238.32	235.95	232.69	238.30	235.8	2.05
DMC (%)	13.67	17.32	15.33	12.86	16.88	15.10	2.34
FBY(kg/m^2)	3.14	3.85	3.49	3.26	4.29	3.75	0.46
DBY(g/m^2)	447.18	605.19	530.55	442.66	666.35	561.30	105.40

2.4.2 Analysis of variance for traits

The climatic conditions in the two years were very different. Therefore, the results are presented for each year separately and combined over all environments. The combined ANOVA for 2006 showed highly significant variance between the two locations for all traits (Table 2.3). Significant differences among genotypes were also observed for all traits except DTF.

Separation of entries into parents, parents vs. crosses and within crosses indicated significant variation for FBY and DBY in parents whereas the parents vs. crosses effects were highly significant for DTF, FBY and DBY. The crosses were also highly significant for DMC, FBY and DDY. Partitioning of crosses into GCA and SCA showed significant GCA for DMC and FBY whereas SCA showed significant differences for DBY, DMC and FBY. The variance components were larger in SCA than GCA for all traits. The different sources (entries, parents, crosses, SCA and GCA) significantly interacted with the environment for all traits, except DMC.

In the year 2007, highly significant variation between locations was observed for all traits. With the exception of DMC, genotypic variance was significant for all traits including PH. Separation of genotypes into parents, parents vs. crosses and crosses indicated significant variation for only DBY in parents and the parents vs. crosses effects was highly significant for FBY, DDY and PH. The crosses were also highly significant for DTF, FBY, DBY and PH.

Partitioning of crosses into GCA and SCA showed significant GCA for only DTF and PH whereas SCA showed significant differences for all traits except DMC. The variance components were larger in SCA than GCA for FBY and DBY, and larger in GCA than SCA for DTF and DMC. The different sources showed different levels of significance for interaction with the environment for all traits. The means squares of error were higher in 2007 than in 2006.

The combined ANOVA pooled over two years and two locations showed highly significant variation across environments for all traits (Table 2.3). Partitioning of environment into locations, years and location x year interactions showed highly significant variation for all traits in the year x location interactions. Significant differences among entries were observed for all traits accept DMC and entries x environments were significant for all traits.

Partitioning of entries into parents and crosses showed significant variation of the parents only for DTF whereas the parent x environments were highly significant for all traits. The variances due to crosses were significant for all traits except DMC and their interactions with environments were highly significant for all traits. The effect of parent vs crosses, indicating the presence of heterosis, was significant for FBY and DBY, and parent vs crosses x environments were highly significant for all traits.

Seperation of the variance among crosses into GCA and SCA gave different results for different traits. Significant differences were for FBY in GCA and for FBY and DBY in SCA. For DTF and DMC the variance component for GCA was larger than for SCA, whereas for FBY and DBY the SCA was of larger importance. The GCA significantly interacted with environments only for DTF and the SCA showed highly significant interactions with environments for all traits.

Table 2.3 Mean squares and variance components from combined analysis of variance for 15 winter *B. rapa* cultivars and their 105 diallel crosses for four traits in 2006, five traits in 2007 and four traits over environments

Source/ Year	df	DTF (days)		DMC (%)		FBY (kg/m^2)		DBY (g/m^2)	
		MS	Vc	MS	Vc	MS	Vc	MS	Vc
2006									
Environment (E)	1	5741.5**	47.91	565.52**	4.71	31.27**	0.260	37454.52**	300.66
Genotypes (G)	119	2.23	0.174	11.1**	0.324	0.19**	0.06	2666.83**	645.94
Parents	14	2.39	0.544	0.84	0.136	0.06+	0.061	2857.84+	804.54
Parent vs Crosses	1	4.69**		0.33		1.79**		29164**	
Crosses	104	2.18	0.109	1.15**	0.352	0.17**	0.049	2386.35**	491.65
GCA	14	4.39	0.039	3.91**	0.123	0.26+	0.004	2253.73	26.40
SCA	90	1.81	0.041	0.72*	0.137	0.15**	0.041	2406.98*	445.45
G x E	119	1.86**	0.811	0.46	0.042	0.08**	0.035	1374.95*	337.31
Parent x E	14	1.30	0.252	0.571	0.150	0.09**	0.053	1248.77	211.14
Parent vs Crosses x E	1	1.01		0.38		0.44**		219.00	
Crosses x E	104	1.94**	0.893	0.44	0.028	0.71**	0.031	1403.05**	365.42
GCA x E	14	3.30**	0.121	0.45	0.000 [a]	0.07	0.000	676.41	0.000
SCA x E	90	1.72**	0.681	0.45	0.028	0.07**	0.032	1516.09*	478.45
Error	198	1.05		0.42		0.04		1037.65	

Continuation of table 2.3 for the year 2007

2007 Sources	df	DTF (days)		DMC (%)		FBY (kg/m^2)		DBY (g/m^2)		PH (m)	
		MS	Vc	MS	Vc	MS		MS	Vc	MS	Vc
Environment (E)	1	817.80**	6.804	679.33**	5.62	7.00**	0.057	2401496**	19945.7	108.83**	0.80
Genotypes (G)	119	7.04**	2.853	5.02	0.038	0.17**	0.037	13586.55**	2789.45	66.59**	27.22
Parents	14	12.23**	5.169	5.99	0.00	0.12	0.016	9158.81	0.00	140.17**	62.28
Parent vs Crosses	1	0.69		8.61		1.86**		20903.00**		237.73**	
Crosses	104	6.40**	2.57	4.86	0.077	0.15**	0.032	14112.25**	3453.42	55.05**	21.68
GCA	14	28.78**	0.833	5.06	0.051	0.26	0.003	13843.51	0.00	234.79**	7.96
SCA	90	2.92**	1.108	4.82	0.000	0.14**	0.027	14154.05**	3797.00	27.07**	7.76
G x E	119	1.33**	1.036	4.94**	1.703	0.09*	0.024	8007.65**	3783.33	12.14**	3.98
Parent x E	14	1.89**	1.594	6.39*	3.148	0.09	0.020	10641.42**	6417.09	15.62*	7.46
Parent vs Crosses x E	1	0.17		10.01+		0.28**		54570.07**		11.55	
Crosses x E	104	1.27**	0.973	4.7*	1.46	0.09+	0.022	7.205.39**	2981.07	11.68*	3.52
GCA x E	14	4.91**	0.323	3.75	0.000	0.13+	0.004	11354.39+	368.80	12.41	0.065
SCA x E	90	0.71**	0.407	4.85*	1.608	0.8	0.015	6559.99**	2335.67	11.56*	3.412
Error	198	0.30		3.24		0.07		4224.33		8.16	

Continuation of table 2.3 over environments

Over environments	df	DTF (days)		DMC (%)		FBY (kg/m^2)		DBY (g/m^2)	
Sources		MS	Vc	MS	Vc	MS		MS	Vc
Environments (E)	3	25533.2**	212.75	1460.75**	12.14	12.80**	0.106	2356116**	19586.4
Years (Y)	1	70031.14	269.08	3137.38+	13.06	0.13	0.00	4629399	15457.61
Locations (L)	1	1115.63	0.00	1242.24+	5.17	4.34	0.00	1519386	2499.26
Y x L	1	5452.93**	45.43	2.61	0.01	33.93**	0.28	919563**	7622.86**
Genotypes (G)	119	5.91**	0.934	3.05	0.05	0.20**	0.023	8373.36**	654.78
Parents	14	9.79**	1.781	3.90	0.149	0.14	0.045	4998.32	0.00
Parent vs Crosses	1	0.87		2.79		3.57**		49722.1**	
Crosses	104	5.44**	0.831	2.93	0.048	0.17**	0.017	8430.11**	717.78
GCA	14	24.92**	0.358	5.48*	0.061	0.35*	0.004	9496.28	9.209
SCA	90	2.41**	0.205	2.54	0.00	0.16*	0.011	8264.27**	701.66
G x E	357	2.18**	1.500	2.83**	1.000	0.11**	0.053	5754.21**	3123.23
Parent x E	42	2.67**	1.999	3.30**	1.468	0.12**	0.070	6302.84**	3671.86
Parent vs Crosses x E	3	5.61**		16.52**		0.45**		55131.32**	
Crosses x E	312	2.11**	1.44	2.74**	0.910	0.10**	0.049	5558.98**	2927.00
GCA x E	42	5.48**	0.300	2.56	0.00	0.12	0.002	6210.163	57.92
SCA x E	270	1.59**	0.915	2.77**	0.938	0.10**	0.047	5457.61**	2826
Error	390	0.67		1.83		0.05		2630.98	

a – negative estimates of variance component

+, *, ** statistically significantly different from zero at P = 0.10, P = 0.05 and P = 0.01, respectively

2.4.3 Heterosis measurement

The mean values MPH% and HPH% for 105 crosses are listed in appendix. An overview of the results is given in Table 2.4. The AMPH, AHPH, MPH% and HPH% estimates varied for the different traits. In 2006, positive mean heterosis was observed for FBY DBY, DMC and DTF flowered earlier in the crosses. The average effects of heterosis for DMC was 1% and small compared to FBY and DBY which were about 8% and 3%, for MPH% and HPH%, respectively.

In 2007, the amount of heterosis was not much different from the previous year except for DBY which was about 3% lower and for HPH% even negative. Flowering in the crosses was later than for the parents. Over all environments, positive mean heterosis was observed for FBY and DBY and negative for DTF and DMC in all cases. Negative heterosis in DTF might be desirable as it expresses the earlier flowering time of crosses compared to their parents. The effects of heterosis were generally low; for FBY the average mid parent heterosis (MPH) was 8.0% and ranged from -8.0 to 21.0, and for DBY the average MPH was 6.0% and ranged from -15.2 to 30.4. The average high parent heterosis (HPH) was 4.5% for FBY and 2% for DBY.

2.4.4 Variance analysis for heterosis

Analysis of variance for AMPH, AHPH, MPH% and HPH% showed the level of variation for location, crosses and their interaction (Table 5). In 2006, significant variation in heterosis over locations was observed for all traits except FBY and DBY. Crosses were also significant for all traits except DTF. The interaction between crosses and locations were significant for most traits.

In 2007, significant variation in heterosis over locations was observed for all traits except DTF. Variation among crosses was also significant for all traits except DMC. The interactions between crosses and locations were significant for all traits. Over all environments, both MPH% and HPH% showed significant variation among environments. The variation among crosses was significant only for DTF, and the crosses x environment interactions were significant for all

traits.

Table 2.4 Minimum, maximum, mean and standard error (SE) of AMPH, AHPH, MPH% and HPH% for four traits in 2006, five traits in 2007 and four traits over environments

Trait/year	Min.	Max	Mean	SE	Min.	Max	Mean	SE
2006		AMPH				AHPH		
DTF (days)	-3.27	3.05	-0.42	0.11	-4.44	2.49	-1.02	0.13
DMC (%)	-2.13	2.27	0.12	0.07	-1.93	2.26	-0.20	0.08
FBY (kg/m^2)	-0.68	1.01	0.26	0.03	-0.96	0.78	0.07	0.03
DBY (g/m^2)	-92.28	130.32	33.32	3.90	-92.65	114.50	11.69	3.99
		MPH%				HPH%		
DTF (days)	-1.32	1.23	-0.17	0.05	-1.77	1.23	-0.40	0.05
DMC (%)	-16.10	18.11	1.02	0.56	-14.19	18.02	-2.63	0.61
FBY (kg/m^2)	-18.89	29.36	7.65	0.96	-24.60	23.80	2.22	0.92
DBY (g/m^2)	-20.70	32.76	8.04	0.92	-20.70	27.20	2.90	0.99
2007		AMPH				AHPH		
DTF (days)	-3.95	4.33	0.16	0.13	-6.47	3.84	-1.22	0.16
DMC (%)	-4.88	3.45	-0.57	0.17	-6.47	2.18	-1.56	0.19
FBY (kg/m^2)	-0.53	0.86	0.27	0.03	-0.75	0.82	0.12	0.03
DBY (g/m^2)	-208.19	265.58	28.22	8.89	-252.53	200.45	-9.74	9.16
PH (m)	-15.125	18.10	2.02	0.73	-20.19	17.50	-1.98	0.76
		MPH%				HPH%		
DTF (days)	-1.75	1.97	0.07	0.06	-2.86	1.74	-0.54	0.07
DMC (%)	-24.20	20.02	-2.85	0.93	-29.72	13.09	-7.73	0.94
FBY (kg/m^2)	-14.32	26.27	7.81	0.89	-19.28	24.85	3.76	0.94
DBY (g/m^2)	-30.97	42.75	4.92	1.43	-35.24	35.98	-0.96	1.39
PH (m)	-10.55	13.99	1.56	0.52	-13.61	13.35	-1.23	0.53
Over environments		AMPH				AHPH		
DTF (days)	-2.19	2.55	-0.13	0.09	-4.63	2.47	-1.03	0.12
DMC (%)	-2.56	2.01	-0.23	0.09	-3.16	1.16	-0.79	0.10
FBY (kg/m^2)	-0.28	0.7%	0.26	0.02	-0.35	0.74	0.15	0.02
DBY (g/m^2)	-79.59	155.37	30.77	4.59	-105.94	140.47	10.38	4.83
		MPH%				HPH%		
DTF (days)	-0.93	1.09	-0.05	0.04	-1.95	1.06	-0.43	0.05
DMC (%)	-16.63	13.52	-1.42	0.56	-18.25	8.04	-4.78	0.61
FBY (kg/m^2)	-8.04	21.02	7.56	0.62	-9.19	20.85	4.37	0.62
DBY (g/m^2)	-15.241	30.41	5.93	0.88	-18.75	28.03	2.10	0.89

Table 2.5 Mean squares of analysis of variance for AMPH, AHPH, MPH% and HPH% of 105 crosses of winter *B. rapa* cultivars for four traits in 2006, five traits in 2007 and four traits over environments

Source/year	df	AMPH					AHPH				
		DTF	DMC	FBY	DBY	PH	DTF	DMC	FBY	DBY	PH
2006											
Environment (E)	1	8.50*	2.97*	1.28**	1747.47		12.09*	5.04*	0.05	2060.73	
Crosses (C)	104	2.64	1.04*	0.22**	13195.12**		3.33	1.21**	0.21**	3318.63**	
C x E	104	2.21*	0.71	0.09**	1929.26+		2.68+	0.76	0.11*	2258.79	
Error	198	1.57	0.63	0.06	1556.45		2.09	0.84	0.08	2075.26	
2007											
(E) Environment	1	1.09	76.0**	2.30**	436571.7**	92.19*	41.29**	324.1**	0.98*	815660**	228.76*
Crosses (C)	104	3.66**	6.18	0.20	16596.44*	42.33**	5.30**	7.81	0.22*	17683.57*	60.85**
C x E	104	0.77**	6.93*	0.16**	10666.42**	21.65**	1.33**	9.11*	0.16	12456.63*	33.75**
Error	198	0.44	4.86	0.10	6336.48	12.24	0.59	6.48	0.16	8448.64	16.3
Across Environments											
Environment (E)	3	15.13**	41.9**	1.20**	147021.6**		17.94**	180.8**	0.41+	316873**	
Crosses (C)	104	3.46**	3.09	0.19	8850.35		5.17**	4.17	0.19	9856.74	
C x E	312	1.94**	3.92**	0.16**	7845.63**		2.49**	4.91*	0.17**	8620.30**	
Error	398	1.01	2.74	0.08	3746.47		1.34	3.66	0.11	5260.0	

Continuation of table 2.5

Source/year	df	%MPH					%HPH				
		DTF	DMC	FBY	DBY	PH	DTF	DMC	FBY	DBY	PH
2006											
Environment (E)	1	1.48*	140.6+	676.53**	209.66		3.33*	409.6**	33.76	244.74	
Crosses (C)	104	0.43	69.23*	191.96**	185.79**		0.54	74.39**	155.1**	170.47*	
C x E	104	0.36*	48.43+	82.86**	114.44*		0.43+	46.12	81.92*	116.67+	
Error	198	0.25	40.61	50.13	84.30		0.34	54.81	60.43	91.29	
2007											
Environment (E)	1	0.22	1792**	1873**	8538.87**	49.83*	7.65**	7142**	780.26*	15810**	121**
Crosses (C)	104	0.73**	203.98	167.59	470.84*	22.22**	1.04**	194.28	169.53*	408.99**	28.94**
C x E	104	0.15**	225**	136.07**	318.83**	11.22**	0.25**	220.05+	120.7+	283.60**	15.79**
Error	198	0.09	136.97	83.55	156.25	6.25	0.12	180.55	104.17	190.74	8.08
Over environments											
Environment (E)	3	2.65**	1079**	852.39**	3315.94**		3.51**	3663**	323.7+	6568.73**	
Crosses (C)	104	0.64**	114.22	162.01	283.36		0.98**	119.57	140.66	263.66	
C x E	312	0.35**	144.1*	138.83**	268.85**		0.44**	138.43	128.9**	238.69**	
Error	390	0.18	116.89	67.24	140.36		0.24	146.48	84.85	174.28	

+, *, ** statistically significantly different from zero at P = 0.10, P = 0.05 and P = 0.01, respectively

3.4.5 Correlations among parameters and traits

Highly significant correlation was observed for the different parameters studied (Table 2.6). In 2006, highly significant positive correlation (P = 0.01) between GCA and parent were observed for all traits. Also, mid parent value and MPH% significantly correlated negatively and MPH% and crosses significantly correlated positively for all the traits. The correlations between mid parent value and the crosses were weak though positive for the traits.

Table 2.6 Correlation coefficient between different parameters in winter *B rapa* for four traits in 2006, five traits in 2007 and four traits over environments

Traits/ year	GCA vs PV	Crosses vs MPV	MPH% vs MPV	MPH% vs Crosses
2006				
DTF (days)	0.35**	0.19+	-0.46**	0.79**
DMC (%)	0.55**	0.36**	-0.21*	0.84**
FBY (kg/m^2)	0.36**	0.17+	-0.51**	0.76**
DBY (g/m^2)	0.36**	0.13	-0.52**	0.78**
2007				
DTF (days)	0.89**	0.69**	-0.29**	0.49**
DMC (%)	0.47**	0.18+	-0.49**	0.77**
FBY (kg/m^2)	0.13	0.06	-0.46**	0.86**
DBY (g/m^2)	0.28**	0.10	-0.40**	0.87**
PH (m)	-0.16	-0.13	-0.72**	0.78**
Over environments				
DTF (days)	0.83**	0.65**	-0.29**	0.53**
DMC (%)	0.69**	0.34**	-0.41**	0.72**
FBY (kg/m^2)	0.42**	0.22*	-0.37**	0.83**
DBY (g/m^2)	0.53**	0.21*	-0.29**	0.87**

+, *, ** statistically significantly different from zero at P = 0.10, P = 0.05 and P = 0.01, respectively

The pattern in 2007 was not different from 2006 with highly significant positive correlation (P = 0.01) between GCA and parent whereas mid-parent value and MPH% significantly correlated negatively. The MPH% and crosses significant correlated positively for all the traits. The correlations across environment between GCA and parent were positive and highly significant for all traits (Table 2.6).

The correlations between crosses and parents were also always positive and significant. However, mid parent value and MPH% significantly correlated negative for all traits. The correlations between MPH% and crosses were positive and significant for all the traits, ranging from $r = 0.53^{**}$ for DTF to $r = 0.87^{**}$ for DBY.

Correlations for the different traits varied for the individual locations and years (Table 2.7). The DBY significant correlated positively with FBY with a mean of 0.57**, ranging from 0.22* to 0.83**. Significant correlation ranging from 0.23* to 0.76** and with a mean of 0.59** was also observed between DBY and DMC whereas between FBY and DMC were negative and ranged from -0.07 to -0.40** with a mean of -0.16. The FBY correlated negatively with DMC and DTF also correlated negatively with DMC.

Table 2.7 Correlation coefficient between 5 traits of *B. rapa* for locations over 2006, 2007 and across environments (bold)

Traits/ locations	DTF (days)	DMC (%)	FBY (kg/m^2)	DBY (g/m^2)	PH (m)
DTF (days)					
2006 Göttingen		0.003	-0.086	-0.112	-0.076
2006 Einbeck		-0.320**	0.060	-0.197*	-
2007 Göttingen		-0.366**	0.003	-0.190*	0.238**
2007 Einbeck		-0.115	-0.048	0.048	0.165
over environments		**-0.310****	**0.039**	**-0.215***	-
DMC (%)					
2006 Göttingen			-0.351**	0.374**	0.117
2006 Einbeck			-0.401**	0.230*	-
2007 Göttingen			-0.213*	0.343**	-.103
2007 Einbeck			-0.077	0.764**	-0.063
over environments			**-0.160**	**0.595****	-
FBY (kg/m^2)					
2006 Göttingen				0.659**	0.170
2006 Einbeck				0.741**	-
2007 Göttingen				0.832**	0.289**
2007 Einbeck				0.22*	0.263**
over environments				**0.569****	-
DBY (g/m^2)					
2006 Göttingen					0.207*
2006 Einbeck					-
2007 Göttingen					0.251**
2007 Einbeck					0.027
Over environments					

+, *, ** statistically significantly different from zero at P = 0.10, P = 0.05 and P = 0.01, respectively

2.5 Discussion

2.5.1 Self incompatibility

The crosses were produced without emasculation, assuming that the material used is self-incompatible. Erucic acid in *B. rapa* is a qualitative trait and controlled by one gene (Downey 1964) Therefore, it is a very suitable marker to control the success of the crossing. Results from both near infrared reflectance spectroscopy and gas chromatography confirmed the self-incompatibility of the material. Crosses between high erucic acid cultivars showed high erucic acid content, between high erucic acid and zero erucic acid cultivars showed intermediate erucic acid content, and between zero erucic acid cultivars showed low erucic acid content.

2.5.2 Parents and crosses performance

The higher yield performance recorded at Göttingen than Einbeck may be attributed to environmental and management practices at each location. Differences between years observed for the traits may be due to the relatively long winter in 2006 compared to the warm early spring in 2007. Therefore, flowering started about three weeks earlier in 2007.

The crosses surpassed on average their parents for FBY and DBY indicating the presence of heterosis (Table 2.2). However, mid parent heterosis was only 7.6 % for FBY and 5.9 % for DBY. This observation was not unexpected since parents used in the study were not chosen on the basis of genetic relatedness but rather geographical regions (Germany, Sweden, Czechoslovakia, and Bulgaria), and the extent of heterosis is influenced by the level of genetic distances between parents (Falconer and Mackay 1996). Heterosis for grain yield of *B. rapa* has been reported to be much higher with 18% for seed yield (Schuler et al. 1992), 25% for seed yield (Falk et al. 1998) and 17% for fresh leaves in inter group crosses (Kaur et al. 2007).

However, all these experiments were conducted with spring type *B. rapa*, which might show a higher genetic diversity. The gene pool of European winter oilseed *B. rapa* is narrow (Zhao and Becker 1998). When analyzing the diversity of three cultivars Rex, Largo and Steinacher by molecular markers based on 32 individual plants sampled from each population, we observed most of the variation within populations and only a relatively small part of the variation between populations (Chapter 4).

This can explain the relative low amount of heterosis in crosses between different cultivars. Variation in heterosis was low (Table 2.5) and significant only for DTF. The possible explanation may be the very high crosses x environment interactions and also the different years in producing the full-sibs crosses used. This interaction is also large because, for each cross three different full-sibs were used in the two years.

2.5.3 Genetic effects

The experiment showed that the genetic variance is mainly due to variation in specific combining ability (SCA), indicating the predominance of non-additive gene action and the importance of specific cross combinations. This is in agreement with the observation that in specific crosses heterosis can be up to 30 % for DBY. The three crosses with the highest biomass yield were always between cultivars from different European countries. Further improvements in yield can therefore be made by identifying specific high yielding crosses among good combiners. To select parents with high general combining ability (GCA), the always positive correlation between GCA and parental performance can be helpful.

The lower crosses x environment variance compared to the parents x environment variance for most traits (Table 2.3) agrees with the philosophy, that hybrids are more stable than parents. However, Singh and Murty (1980) in *B. rapa* observed a higher crosses x environment variance compared to the parents x environment and concluded that stability of a cultivar is influenced by a lot of factors other than heterozygosity alone.

2.5.4 Correlations among traits

The correlations among traits are important for selection. There were no or only small correlations between flowering time and FBY and DBY, indicating that selection for early flowering does not necessarily improve biomass yield in *B. rapa.* The correlation between FBY and DBY over all four locations was only 0.57**, which is much lower than the value of 0.95** reported in biomass yield of interspecific crosses of *B. rapa* and *B. napus* by Liu et al. (2002). When analyzing the two locations separately, correlations were 0.85** in Göttingen, but only 0.22 in Einbeck in 2007, perhaps indicating a technical problem with taking a representative sample of leaves and stem for DMC determination.

Correlations between mid-parent and MPH% were negative for all traits. After observing 50% less MPH% for seed yield in inter population F_1s when compared with inbred parent derived F_1s in *B. carinata,* Teklewold and Becker (2005) concluded that populations used as parents already utilize a considerable level of heterozygosity.

Brassica rapa is a diploid species, but tetraploid cultivars with the double number of chromosomes have been developed. For comparison, we included the tetraploid cultivar "Perko" in the experiments. This cultivar had a FBY of 4.42 kg/m² and a DBY of 600 g/m². These yields are only surpassed by one of the parents and by seven of the crosses for FBY and 21 for DBY. Future winter *B. rapa* biomass breeding programmes should therefore consider also the potential of tetraploid genotypes.

2.6 Conclusions

In conclusion, the high importance of SCA implies that identifying the best combinations among parents is an efficient way to increase biomass yield. The production of hybrid cultivars will be probably too expensive at the moment, due to the self incompatibility of *B. rapa* and the lack of an easily available hybridizing system. However, large quantities of seed can be produced by the approach of synthetic cultivars when mixing different parents

and propagating them under open pollination. When starting with two populations, the first generation after random mating (syn-1) should theoretically be composed of 25% each of plants from crosses within the parental populations and of 50% of plants from crosses between the two populations. In this way, heterosis can be at least partly utilized for yield improvement in *B. rapa* cultivars for biomass production. The first *B. rapa* synthetic cultivars were Hysyn 100 and Hysyn 110 released in Canada in 1994 (Falk and Stoenescu 1996a; 1996b).

3. Biomass yield and heterosis of crosses within and between European *Brassica rapa* cultivars

3.1 Abstract

The use of plant biomass as substrate for biogas production has gained major interest in recent years in Europe. Winter *B. rapa* produces high early biomass and could be used as a pre-crop harvested earlier in the year for biogas followed by a second crop adapted to higher temperatures like maize. A promising strategy for *B. rapa* breeding is the development of synthetic cultivars that utilize the heterosis by combining genetically diverse parents. The objective of this study was to estimate the performance of full-sib crosses between and within three cultivars and to compare it with the performance of corresponding synthetic cultivars. Nine full-sibs each coming from the three possible combinations, three mixtures of ten full-sibs each within the three parental cultivars, and synthetics composed of the three possible cultivar combinations were produced. These different groups and their parents were evaluated at two locations for two years in Northern Germany. Data recorded were days to flowering, fresh biomass yield, dry matter content, dry biomass yield and plant height. The mean of full-sibs were higher than the mean of the parents for most traits. Analysis of variance showed significant variation for environments and genotype x environment interactions for all traits. The full-sibs within and between cultivars differed significantly for fresh biomass yield and dry biomass yield. Relative mid parent heterosis estimated as superior of between full-sibs over within fill-sibs was 9.2% for dry biomass yield, 4.4% for fresh biomass yield and 3.1% for dry matter content over environments. The correlation between dry biomass yield and fresh biomass yield was 0.61** and between dry biomass yield and dry matter content was 0.86**. In conclusion, heterosis for biomass production observed in cultivar crosses

was only 9 % or less, which indicates a relatively low genetic diversity between the three cultivars though they largely differ in breeding history. However, performance of synthetics was comparable to full-sibs between cultivars. The development of synthetic cultivars is a possibility to utilize heterosis in biomass production.

Key words: between cultivar full-sibs, biogas, winter *Brassica rapa*, within cultivar full-sibs, synthetics

3.2 Introduction

Maize has been the main biogas substrate in Germany because of its high yield per hectare. It is sown in May and harvested in September/October. The growing of other crops that can be harvested earlier in the year could give the possibility for growing two crops in one season: the first one sown in autumn and harvested in spring, followed by a second crop adapted to higher temperatures like maize, sorghum or sunflower. Crops with high biomass production even under low temperatures include winter triticale, rye, some forage grasses, and also *Brassica* crops. *B. rapa* is of special interest among the *Brassica* because it has a higher early biomass than *B. napus* (CFIA 1999; Halfhill et al. 2005).

Previous experimental results on early biomass yield of *B. rapa* showed an average dry biomass yield between 440 to 600 g/m^2 (Chapter 2). This can be increased through breeding, and information on variation between and within cultivar heterosis is important for determining a breeding method to be applied. A possibility mating system which utilizes both within and between family variation is full-sibs (Aastveit and Aastveit 1990) and it has large selection response because best plants are allowed to cross. It has commonly been used in population improvement of the cross pollinated crops maize (Pixley et al. 2006) and forage grasses (Aastveit and Aastveit 1990; Fang et al. 2004).

Heterosis has been a major breeding tool for plant productivity improvement and in population, Lamkey and Edwards (1999) suggested the term "panmictic mid parent heterosis" for the difference between the mean of two random mating populations and the mean of a hybrid population produced by crossing individual plants of the two populations. Studies in *B. rapa* have indicated high levels of heterosis between 10% and 25% (Schuler et al. 1992; Falk et al. 1998; Kaur et al. 2007).

The development of superior cultivars is expensive and time consuming. This involves the selection, crossing and testing of many parental cultivars to identify parental combinations that can produce F_1s with superior yield. In a breeding program, parental cultivars are mostly selected based on genetic relatedness and combining ability (Melchinger and Gumber 1998). In cases where genetic effect of cultivars is controlled by general combining ability, many parental combinations are considered for breeding whereas specific combinations are used when genetic effects is controlled by specific combining ability.

Different breeding methods have been development and examples of those that utilize specific combinations are hybrid development, full-sibs and synthetic breeding. The results presented in chapter 2 showed that variation in crosses among European winter *B. rapa* cultivars is mainly due to specific combining ability. Therefore, we investigated the performance of synthetic populations among cultivars since hybrid development will be probably too expensive at the moment, due to the self incompatibility of *B. rapa*.

Synthetic populations may be produced by simply mixing different parents and propagating them under open pollination. When starting with two populations, the first generation after random mating, which is called synthetic-1 should theoretically be composed of 25% each of plants from crosses within the parental populations and of 50% of plants from crosses between the two populations. The commercial use of synthetic cultivars for seed yield has been experimentally demonstrated in *B. rapa* (Falk et al. 1994) for which up to 60% heterosis was observed.

The aim of this study was to estimate the early biomass yield of winter *B. rapa* with the following objectives; (1) to determine the biomass yield performance and genetic variation in between full-sibs and within full-sibs; (2) measure heterosis of between full-sibs calculated over within full-sibs and (3) compare the performance of between full-sibs, within full-sibs and synthetic in three European winter *B. rapa* cultivars.

3.3 Material and methods

3.3.1 Parental cultivars

The three European *B. rapa* winter oilseed cultivars Largo, Rex and Steinacher used in this experiment were released in the years 1954, 1984 and 2002, respectively (Table 3.1). Steinacher was obtained from the genebank BAZ Braunschweig (accession BAZ 18101) and multiplied with about 800 plants under isolation in a cage with pollinators. For the other two cultivars, breeders' seed was used. Diploidy of each cultivar was confirmed by using a Partec Flow Cytometer (Münster Germany).

Table 3.1 Characteristics of the *B. rapa* cultivars used and their country of

Cultivar	Breeder	country	Year of release	Seed quality [a]
Steinacher	Saatzucht Steinach	Germany	1954	++
Rex	Norddeutsche Pflanzenzucht	Germany	1984	0+
Largo	SW Seed	Sweden	2002	00

[a] ++ high erucic acid, high glucosinolate; 0+ - zero erucic acid, high glucosinolate; 00 – zero erucic acid, low glucosinolate

3.3.2 Material development

The three parental cultivars were crossed to produce both between cultivar full-sibs (FS_b) and within cultivar full-sibs (FS_w) seed at Reinshof experimental station in the years 2005 and 2006. For FS_b, two plants from different parental cultivars were isolated under one

large bag before beginning of flowering. In this way nearly complete cross-pollination can be achieved. In the case of FS_w, isolation was between two plants of the same parental cultivar. Nine crosses each of Rex x Largo, Rex x Steinacher and Largo x Steinacher were produced for FS_b.

For FS_w, ten crosses each of Rex x Rex, Largo x Largo and Steinacher x Steinacher were produced. In addition, three plants of each parental cultivar were selfed to determine their self-incompatibility level. The bags were gently shaken every other day during flowering, to enhance pollen transfer and seed set.

3.3.3 Field evaluation

Three different FS_w mixtures were formed by bulking equal amounts of seed from each of the ten FS_w produced. To evaluate the performance of synthetic (Syn-1), 50% FS_b and 25% FS_w of each of the two parental cultivars was composed for the three possible cultivar combinations

The 27 FS_b, the three FS_w mixtures, the three syn-1 produced in 2005 and their parents were sown at two locations; Göttingen and Einbeck in northern Germany in 2005/06. Sowing dates were 24th and 31st of August 2005 at Einbeck and Göttingen, and at a rate of 90- 110 seeds m^{-2}. Experimental design was a 6x6 lattice with two replications. Plot sizes in Göttingen were 11.25 m^2 and each plot consisted of 6 rows, 7.5 m long and 0.3 m between rows. In Einbeck, plot sizes were 9.0 m^2 and each plot consisted of 5 rows, 6.0 m long and 0.3 m between.

Standard crop management practices such as weed control and fertilization application were followed. Data were recorded on days to flowering (DTF, from the day of sowing until 50% of plants were flowering), fresh biomass yield (FBY, kg/m^2), dry matter content (DMC, %), dry biomass yield (DBY, g/m^2) and plant height (PH, cm). Plots were harvested on 8th and 15th of May 2006 at Einbeck and Göttingen, respectively. This was done

at end of flowering, using a harvester that cut at 5 cm above ground and the total fresh biomass yield was measured. From each plot a sub-sample of 300g fresh weight was dried at 60^{o}C for 6 days to determine the dry matter content. Based on this, total dry biomass yield per plot was calculated.

In 2006/07, the remaining 27 FS_b, the three FS_w mixtures, the three syn-1 produced in 2005 and their parents were evaluated. However, in cases where the 2005 seed were not enough, they were supplimented by seed produced in 2006. Sowing was on 31st of August 2006 in Einbeck and 5th of September 2006 in Göttingen. Plot sizes, sowing rate, experimental design, replications, crop management practices, data recorded and harvesting methods were the same as that of 2005/06 experiment. Harvesting was on 2nd of May 2007 at Einbeck and 4th of May 2007 at Göttingen.

3.3.4 Statistical analysis

Separate analysis of variance (ANOVA) for each location was run for the 36 entries using PLABSTAT software (Utz 2001) based on the model: $Y_{ijk}= u + r_i + g_j + ß_k + e_{ijk}$, where Y_{ijk} = observation of genotype j in block k and replication i; u = general mean; r_i = effect of replication; g_j = effect of genotype j; $ß_k$ = effect of blocking; e_{ijk} = error of observation. A combined analysis of variance using adjusted means obtained from each location was then computed individually for years, 2006 and 2007, and over the four environments with the model: $Y_{ij}= u + l_i + g_j + lg_{ij} + e_{ijk}$, where Y_{ij} = observation of means of genotype j in location i; u = general mean; l_i = effect of location; g_j = effect of genotype j; lg_{ij} is the interaction effect between location i and genotype j and e_{ijk} = error of observation of lattice.

Random effects were assumed for years and locations in the analysis. Entries sum of squares was partitioned into parental cultivars, syn-1, FS_w, FS_b and FS_w vs FS_b. The FS_b vs FS_w effects were calculated as follows; SSwvb = SSe - SSw - SSb; where SSwvb is the sum of squares for FS_b vs FS_w, SSe is the sum of squares for entries, SSw is the sum of squares

for FS_w and SSb is the sum of squares for FS_b. The FS_b was further partitioned into between crosses (variation between crosses) and within crosses (variation between FS within crosses).

Heterosis was estimated for percent mid-parent heterosis (MPH %) and percent high-parent heterosis (HPH %). In order to avoid overestimation of heterosis that might have been caused by unconscious selection during FS_b production, FS_w was used in the calculation instead of parents. The formulae were MPH% = (FS_b – mean FS_w) / mean FS_w x 100, and HPH% = (FS_b – highest FS_w) / highest FS_w x 100. To test for significant differences in heterosis for the studied traits, combined ANOVA was computed using MPH% and HPH % values obtained from individual locations. For model and error calculations, see statistical analysis of chapter 2.

3.4 Results

The climatic conditions in the two years were very different. Therefore, the results are presented for each year separately and combined over all environments.

3.4.1 Biomass yield among full-sib groups

The different full-sib groups were higher than the parents for most traits (Table 3.2). In 2006, the pattern at Göttingen was in the order syn-1 > FS_w > FS_b > parental cultivars for DBY, FS_w > syn-1 > FS_b FS_w > parental cultivars for FBY and FS_b > syn-1 > FS_w > parental cultivars for DMC. At Einbeck, the pattern observed was FS_b > FS_w > syn-1 > parental cultivars for DBY, FS_b > syn-1 > FS_w > parental cultivars for FBY and FS_w > FS_b > syn-1 > parental cultivars for DMC.

In 2007, the pattern was FS_b > syn-1 > parents = FS_w for DBY, syn-1 > FS_b > parental cultivars > FS_w for FBY and FS_b > syn-1 > FS_w > parental cultivars for DMC at Einbeck. At Göttingen, the pattern was syn-1 > FS_b > FS_w > parental cultivars for DBY, FS_w > FS_b > parental cultivars > syn-1 for FBY and FS_w > parental cultivars > syn-1 > FS_b for DMC. The

mean over environments were FS_b > syn-1 > FS_w > parental cultivars for DBY, syn-1 > FS_b > FS_w > parental cultivars for FBY and FS_b > FS_w > syn-1 > parental cultivars for DMC (Table 3.3).

3.4.2 Biomass yield within full-sibs

The individual crosses varied among the different full-sib groups (Table 3.2). In 2006, the FS_w (S x S) was highest for DMC and DBY, and FS_w (R x R) for FBY and Synthetics (R x Ssyn-1) for PH at Göttingen. In Einbeck, the FS_b (R x L) was highest for FBY and DBY, and FS_b (S x S) for DMC.

In 2007, the Synthetics (L x Ssyn-1) were highest for FBY, DBY and PH. The parental cultivars Steinacher was highest for DMC in Göttingen. At Einbeck, FS_w (R x R) was highest for PH, Synthetics (L x Ssyn-1) for DBY, Synthetic (R x Lsyn-1) for FBY and FS_w (S x S) for DMC. The mean over environments showed the Synthetics (L x Ssyn-1) being highest for DBY, FS_b (R x L syn-1) for FBY whereas FS_w (S x S) was highest for DMC (Table 3.3).

Table 3.2 Means of between cultivar full-sibs, within cultivar full-sibs, synthetics cultivars and parents cultivars evaluated for five traits at Göttingen in 2006 and 2007 and four traits at Einbeck in 2006 and five traits in 2007

Groups/		Göttingen					Einbeck			
years	No	BTF(days)	DMC (%)	FBY(k/m^2)	DBY(g/m^2)	PH (m)	BTF(days)	DMC (%)	FBY(k/m^2)	DBY(g/m^2)
2006										
FS_b										
R x L	9	244.67	13.66	3.30	451.67	135.00	252.07	10.68	3.86	408.83
R x S	9	242.99	14.46	3.09	463.14	137.50	251.35	10.92	3.69	404.39
L x S	9	242.79	14.17	3.24	440.64	130.83	251.21	11.12	3.48	388.46
FS_b mean		243.48	14.09	3.21	451.82	134.44	251.52	10.96	3.66	400.74
FS_w										
R x R	1	245.39	13.19	3.44	456.74	135	252.12	10.25	3.44	359.18
L x L	1	244.98	12.45	3.40	404.34	127.5	251.88	10.72	3.42	362.42
S x S	1	241.73	16.02	3.16	513.24	137.5	251	12.11	3.32	402.75
FS_w mean		244.03	13.89	3.33	458.11	133.33	251.44	11.42	3.37	382.58
Synthetics										
R x L syn-1	1	244.89	14.75	3.29	467.75	135	252.8	10.83	3.73	399.4
R x S syn-1	1	241.68	13.51	3.18	467.06	142.5	251.06	10.43	3.40	354.57
L x S syn-1	1	241.59	13.69	3.33	458.24	130	251.06	10.42	3.66	386.35
Syn-1 mean		242.72	13.98	3.27	464.35	135.83	251.06	10.43	3.53	370.46
Parental cultivars										
Parent R	1	243.41	14.11	2.74	391.32	135	250.12	10.66	3.27	346.78
Parent L	1	246.00	12.27	2.88	332.41	117.5	252.06	10.89	3.25	356.51
Parent S	1	242.25	15.99	3.79	400.00	130	251.18	11.85	3.44	399.46
parental mean		243.89	14.12	3.14	374.58	127.50	251.62	11.32	3.35	377.98

Continuation of table 3.2

Groups/ years	No	Göttingen					Einbeck				
		BTF(days)	DMC (%)	FBY(k/m^2)	DBY(g/m^2)	PH (cm)	BTF(days)	DMC (%)	FBY(k/m^2)	DBY(g/m^2)	PH (cm)
2007											
FS_b											
R x L	9	226.78	17.26	2.60	449.63	133.56	222.83	15.89	3.88	621.32	142.34
R x S	9	224.39	16.55	2.90	476.51	130.99	222.00	17.27	4.04	715.28	140.22
L x S	9	225.44	17.36	2.51	432.69	132.38	222.66	17.44	4.00	719.82	142.56
FS_b mean		225.25	17.03	2.69	456.51	132.04	222.51	16.85	3.97	684.28	141.72
FS_w											
R x R	1	226	18.11	2.76	507.9	132.2	223.11	13.13	3.93	523.25	148.44
L x L	1	226	17.29	2.36	416.9	123.76	223.10	12.12	3.38	410.31	126.82
S x S	1	223	18.29	2.40	406.89	126.29	221.05	18.22	3.88	708.79	144.74
FS_w mean		225	17.89	2.51	443.89	127.42	222.39	14.49	3.73	547.45	140.00
Synthetic											
R x L syn-1	1	225.5	17.7	2.89	498.55	130.71	223.05	15.09	4.29	594.33	143.18
R x S syn-1	1	224	17.58	2.85	451.01	127.1	222.05	11.53	4.00	458.11	147.09
L x S syn-1	1	225	16.97	2.93	503.19	140.55	222.89	19.21	3.90	742.17	138.54
syn-1 mean		224.83a	17.42	2.89	484.25	132.79	222.66	15.28	4.06	598.20	142.94
Parental cultivars											
Parent R	1	226.5	16.84	2.85	467.7	141.46	223.05	14.76	4.17	616.5	151.2
Parent L	1	227	16.72	2.35	380.55	123.02	223	12.62	3.52	454.43	139.89
Parent S	1	223	18.88	2.53	499.89	129.16	221.05	15.43	3.62	573.7	138.54
parental mean		225.5a	17.48	2.58	449.38	131.21	222.37	14.27	3.77	548.21	143.21

Table 3.3 Mean of between cultivar full-sibs, within cultivar full-sibs, synthetic cultivars and parental cultivars evaluated for four traits over environments

Groups /years	n[1]	BTF(days)	DMC (%)	FBY(k/m^2)	DBY(g/m^2)
FS_b					
R x L	9	236.49	14.36	3.42	482.37
R x S	9	235.18	14.8	3.43	514.83
L x S	9	235.53	15.02	3.31	495.40
FS_b mean		235.73	14.70	3.38	495.50
FS_w					
R x R	1	236.65	13.67	3.39	461.77
L x L	1	236.46	13.15	3.14	398.49
S x S	1	234.19	16.16	3.19	507.92
FS_w mean		235.77	14.32	3.24	456.06
Synthetic					
R x L syn-1	1	236.56	14.59	3.55	490.01
R x S syn-1	1	234.7	13.26	3.36	432.69
L x S syn-1	1	235.13	15.07	3.45	522.49
Syn-1 mean		235.46	14.31	3.45	481.73
Parent cultivars					
Parent R	1	235.77	14.09	3.26	455.58
Parent L	1	237.01	13.13	3.00	380.98
Parent S	1	234.37	15.54	3.35	468.26
parental mean		235.72	14.25	3.21	434.94

n[1] number of crosses among groups

3.4.3 Analysis of variance

Combined ANOVA showed highly significant variation between the four environments for all traits (Table 3.4). In the year 2006, significant variation among genotypes were observed for DTF (p = 0.01), FBY (p = 0.10) and DBY (p = 0.10). Partitioning of genotypes into parents, synthetic, FS_b, FS_w and FS_w vs FS_b showed no significant differences for any of the traits except DTF for FS_b, Syn-1 and FS_w. Nevertheless,

highly significant variation for DBY and FBY was observed when the contrast was calculated between parents vs FS_b effects (data not shown). The genotype x environment interactions were significant for DBY, FBY and DMC.

In the year 2007, highly significant variation between environments for all traits was observed (Table 3.4). The genotypes were significantly different for all traits except DMC. Partitioning of genotypes into parents, synthetics, FS_b, FS_w and FS_w vs FS_b showed significant variation for all traits except DMC in the parents and FS_b. The synthetics and FS_w showed significance for only DTF. The FS_w and FS_w vs FS_b were significant for DMC, FBY, DBY and PH. The genotypes x environment interactions were significant for DTF and DMC.

The combined ANOVA over environments were highly significant for all traits. Partitioning of environment into locations, years and location x year interactions showed highly significant variation for all traits in the year x location. The genotypes were significantly different for only DTF. Separation of genotypes into parents, synthetics, FS_b, FS_w and FS_w vs FS_b showed significant variation for only DTF in the synthetics and FS_b. The parents and FS_w showed significant difference for DTF and DMC, and FS_w vs FS_b was also significantly different for FBY and DBY. The genotype x environment interactions were highly significant for all traits.

3.4.4 Heterosis determination

Percent mid parent heterosis (MPH %) and percent high parent heterosis for FS_b calculated over FS_w varied between the different crosses groups for the two locations and years (Table 3.5). In 2006, MPH% among FS_b ranged from -3.95 to 5.65 with a mean of 2.2 for DBY and -6.39 to -1.15 with a mean of -3.7 for FBY at Göttingen. At Einbeck, it ranged from 1.53 to 13.76 with a mean of 7.15 for DBY and 3.33 to 13.25 with a mean of 8.65 for FBY. At both locations, FS_b (R x L) was highest for DMC and PH, and FS_b (R x S) for FBY.

In 2007, MPH% among the different FS_b, ranged from -2.76 to 5.05 with a mean of 2.1 for DBY and 1.82 to 12.36 with a mean of 6.61 for FBY at Göttingen. In Einbeck, it ranged from 16.11 to 32.34 with a mean of 25.69 for DBY and 3.51 to 10.29 with a mean of 6.58 for FBY. The FS_b (R x L) was lowest in performance for FBY and DBY at Göttingen and FS_b (R x S) for DMC, FBY, DBY and PH at Einbeck.

The MPH% over environments was 9.21, ranging from 9.31 to 12.14 for DBY and 4.41, ranging from 4.22 to 4.65 for FBY (Table 3.6). Mean MPH% determined over parents was 29.66 for DBY, 7.47 for FBY, 0.50 for DMC and -0.11 for DTF (data not show).

Table 3.4 Mean squares from combined analysis of variance for parents and the three full-sib groups for five traits at tow locations in 2006 and 2007 and across environments

Source/years	df	DTF (days) MS	DMC (%) MS	FBY (kg/m^2) MS	DBY (g/m^2) MS	PH (m) MS
2006						
Environment (E)	1	1172.1**	181.6**	2.69**	2.69**	
Genotypes (G)	35	3.29**	0.97	0.09+	0.09+	
Parents	2	4.31	2.42	0.002	0.002	
Synthetics	2	4.18+	0.38	0.002	0.002	
FS_w	2	3.47	3.95	0.02	0.02	
FS_b vs FS_w	1	0.61	0.02	0.07	0.07	
FS_b	26	3.44**	0.78	0.08	0.08	
Between crosses	2	1.16	0.17	0.04	0.04	
Within crosses	24	2.86**	0.71	0.06	0.06	
G x E	35	0.54	0.73+	0.06*	0.06*	
Error	50	0.37	0.48	0.03	0.03	
2007						
Environment (E)	1	141.65**	14.04**	29.19**	29.19**	1783**
Genotypes (G)	35	4.07**	7.49	0.128*	0.128*	39.48**
Parents	2	5.49+	3.00	0.18*	0.18*	127.6+
Synthetic	2	0.84*	7.05	0.02	0.02	4.26
FS_w	2	4.18*	6.79	0.11	0.11	117.85
FS_b vs FS_w	1	0.33	4.50+	0.226+	0.226+	60.00*
FS_b	26	4.65**	8.47	0.13+	0.13+	31.33**
Between crosses	2	0.97	0.35	0.03	0.03	3.11
Within crosses	24	5.04**	9.18	0.139+	0.139+	33.94**
G x E	35	0.91**	8.18**	0.06	0.06	13.91
Error	50	1.42	1.34	0.06	0.06	11.39
Over environments						
Environment (E)	3	7128.79**	283.1**	10.82**	10.82**	
Year (Y)	1	20080.77	654.677	0.48	644272.43	
Locations (L)	1	246.75	147.74	25.40	195199.76	
L x Y	1	1058.85**	46.92**	6.77**	573590.4**	
Genotypes (G)	35	4.59**	4.77	0.09	0.09	
Parents	2	7.00*	5.89**	0.24	0.24	
Synthetics	2	3.79*	3.52	0.04	0.04	
FS_w	2	7.49**	10.38*	0.07	0.07	
FS_b vs FS_w	1	0.02	1.80	0.22*	0.23*	
FS_b	26	4.73**	4.70	0.07	0.07	
Between crosses	2	1.83*	0.45	0.01	0.01	
Within crosses	24	5.13**	5.10	0.078	0.078	
G x E	105	1.39**	4.41**	0.09**	0.09**	
Error	100	0.25	0.92	0.049	0.049	

+, *, ** statistically significantly different from zero at P = 0.10, P = 0.05 and P = 0.01, respectively

Table 3.5 Mean of MPH% and HPH% for between cultivar full-sibs calculated over within cultivar full-sibs for five traits at Göttingen in 2006 and 2007 and four traits at Einbeck in 2006 and five traits in 2007.

	Göttingen					Einbeck				
Crosses /years	BTF (days)	DMC (%)	FBY (k/m^2)	DBY (g/m^2)	PH (m)	BTF (days)	DMC (%)	FBY (k/m^2)	DBY (g/m^2)	PH (m)
2006										
MPH%										
R X L	-0.21	6.52	-3.57	4.91	2.86	0.05	1.66	13.25	13.76	
R X S	-0.23	-0.97	-6.40	5.65	0.92	-0.08	-2.34	9.37	6.15	
L X S	-0.23	-0.55	-1.15	-0.40	-1.25	-0.09	-2.59	3.33	1.54	
Mean	-0.23	1.67	-3.71	2.20	0.84	-0.04	-1.09	8.65	7.15	
HPH%										
R X L	-0.30	3.52	-.4134	-1.11	0.003	0.002	-0.57	12.92	13.25	
R X S	-0.98	-9.72	-10.21	-9.76	-0.01	-0.304	-9.84	7.46	0.41	
L X S	-0.89	-11.6	-4.64	-14.10	-4.84	-0.265	-8.18	1.82	-3.55	
Mean	-0.72	-5.94	-6.33	-8.34	-1.61	-0.189	-6.19	7.40	3.37	
2007										
MPH%										
R X L	0.12	-2.47	1.82	-2.76	4.36	-0.08	25.52	5.94	32.34	3.46
R X S	0.05	-9.07	12.36	4.18	1.36	-0.04	10.15	3.51	16.11	-4.35
L X S	0.42	-2.44	5.65	5.05	5.88	0.28	14.93	10.3	28.64	4.99
Mean	0.16	-4.66	6.61	2.16	3.86	0.06	16.86	6.58	25.69	1.37
HPH%										
R X L	0.12	-4.68	-5.56	-11.3	1.03	-0.11	20.69	-1.47	18.1	-4.07
R X S	-0.71	-9.52	5.03	-6.18	-0.91	-0.50	-5.23	2.85	0.92	-5.54
L X S	-0.25	-5.11	4.77	3.79	4.82	-0.15	-4.31	3.18	1.56	-1.51
Mean	-0.28	-6.44	1.42	-4.62	1.64	-0.25	3.72	1.52	6.84	-3.71

Table 3.6 Mean of MPH% and HPH% for between cultivar full-sibs calculated over within cultivar full-sibs for four traits over environments

	BTF(days)	DMC (%)	FBY (k/m^2)	DBY (g/m^2)
MPH%				
R X L	-0.03	7.06	4.65	12.14
R X S	-0.10	-0.77	4.22	6.18
L X S	0.09	2.84	4.55	9.31
Mean	-0.01	3.04	4.41	9.21
MPH%				
R X L	-0.07	5.02	0.77	4.46
R X S	-0.62	-8.42	1.15	1.36
L X S	-0.40	-7.07	3.73	-2.46
Mean	-0.35	-3.71	1.00	0.33

3.4.5 Variation among heterosis

Significant variation based on MPH% and HPH% for environments was observed for all traits except DMC in 2006 (Table 3.7). The crosses were significant for DTF in MPH% and DTF and DMC in MPH%. In 2007, environments showed significant variation for all traits except FBY whereas only DTF and PH were significant in the crosses for both MPH% and HPH%.

The mean over environments were highly significant for MPH% and HPH% in all traits. Crosses were significant only for DTF in MPH% and HPH% whereas crosses x environment interactions were significant for all traits in MPH% and for DTF and DMC in HPH%.

Table 3.7 Mean squares of analysis of variance for MPH% and HPH% for four traits in 2006, five traits in 2007 and four traits over environments

		MPH%					HPH%				
Source/years	df	DTF	DMC	FBY	DBY	PH	DTF	DMC	FBY	DBY	PH
2006											
Location (L)	1	0.456*	102.5	2061**	936.91**		4.28**	0.894	2544.10**	936.1**	
Crosses (C)	26	0.439**	55.32	65.46	96.52		0.55**	94.54*	73.48	135.68	
C x L	26	0.064	47.49	66.27+	92.46		0.07	44.77	61.78	107.91	
Error	50	0.08	46.79	43.72	71.73		0.12	55.27	55.7	90.34	
2007											
Location (L)	1	0.1608	6258**	0.0122	7482.64**	83.99**	0.024	1392+	0.152	1774.5+	386**
Crosses (C)	26	0.857**	435.69	129.299	792.03	34.59**	0.93**	402.18	134.98+	584.129	23.4**
C x L	26	0.211**	445**	99.06	661.39**	7.93	0.22**	404.5**	75.387	537.4**	5.09
Error	50	0.04	77.20	102.03	270.61	9.56	0.06	91.35	123.55	281.55	11.89
Across environments											
Location (L)	3	0.738+	2425**	840.3**	3884.76**		1.73**	663.85*	855.897**	925.54*	
Crosses (C)	26	0.67**	265.76	67.937	481.27		0.88**	322.82+	62.94	389.04	
C x L	78	0.30**	239.5**	97.38+	387.05**		0.29**	207.7**	94.22	325.35	
Error	100	0.09	91.42	70.78	187.98		0.09	83.13	96.22	289.52	

+, *, ** statistically significantly different from zero at P = 0.10, P = 0.05 and P = 0.01, respectively

3.4.6 Correlation among traits

Correlations for the different traits studied varied for the individual locations and years (Table 3.8). Significantly positive correlation between DBY and DMC of r = 0.86**, ranging from -0.001 to 0.95**, and between DBY and FBY of r = 0.61**, ranging from 0.34* to 0.86** were observed over the environments. The over environment correlations between DTF and the other traits were negatively and weak, and with DMC was r = - 037*, FBY was r = 0.02 and DBY was r = 0.29. The correlation between DMC and FBY was r = - 0.25.

Table 3.8 Correlation coefficient between five traits of winter *B. rapa* for two locations in 2006 and 2007, and over environments (bold)

	DTF (days)	DMC (%)	FBY (Kg/m^2)	DBY (g/m^2)	PH (m)
DTF (days)					
2006 Göttingen		-0.403*	0.010	-0.367*	-0.117
2006 Einbeck		-0.457**	0.390*	0.027	-
2007 Göttingen		-0.424*	0.054	-0.120	0.220
2007 Einbeck		0.053	-0.111	0.019	-0.042
over Environments		**-0.370***	**0.017**	**0.287**	-
DMC (%)					
2006 Göttingen			-0.289	0.677**	0.172
2006 Einbeck			-0.252	0.358*	-
2007 Göttingen			-0.449	-0.001	-0.140
2007 Einbeck			0.244	0.950**	-0.129
over Environments			**-0.245**	**0.864****	-
FBY (kg/m^2)					
2006 Göttingen				0.343****	0.108
2006 Einbeck				0.794**	-
2007 Göttingen				0.867**	0.054
2007 Einbeck				0.481**	0.516**
over Environments				**0.614****	-
DBY (kg/m^2)					
2006 Göttingen					0.295
2006 Einbeck					-
2007 Göttingen					0.046
2007 Einbeck					0.011

3.5 Discussion

3.5.1 Performance of full-sib groups

Theory and earlier studies have demonstrated that between cultivar heterosis is higher than within cultivar heterosis (Ali et al. 1995; Falconer and Mackey 1996). Synthetic (syn-1), which composes of 25% within cultivar full-sibs (FS_w) of each parental cultivar and 50% between cultivar full-sibs (FS_b) are expected to show heterosis within the range of FS_w and FS_b.

This was the case for DMC at Göttingen and FBY at Einbeck in 2006, and for DMC at Einbeck in 2007 and for DBY over environments. For unknown reasons, FS_w yields were higher than syn-1 in DMC, and syn-1 was also higher than FS_b in FBY over environments. Higher heterosis in within-group hybrids than between group hybrids in diverse morphotypes of *B. rapa* has however been reported (Kaur et al. 2007).

The mean performance of FS_w developed randomly by crossing individuals plants within population, is expected to be the same as the parental cultivar mean (Falconer and Mackay 1996). The observed yields in FS_w were higher than the parents for all traits over environments except DBY. This deviation may have been caused by the unconscious selection during the production of FS_w. Basis of population improvement is the selection and mating of superior plants within populations to produce progenies whose mean performance is higher than their parents.

Generally, significant variation was observed for all traits except DTF in the different components and heterosis was low. A random model that allows the different components to be tested against their environment x genotype interactions was used for the analyses. The observed interactions were high since the two years were very different in climatic conditions. Differences between years may be due to the relatively long winter in 2006 compared to the warm early spring in 2007. Therefore, flowering started about three weeks earlier in 2007.

An alternative would have been to test the genotypes against the error by using a fixed model. In that case, mores sources of variation would have become significant. However, to get results that can be generalized, the more conservative random model was chosen.

3.5.2 Heterosis determination

To avoid the overestimation of heterosis, FS_w was used for the determination because unconscious selection may have taken place during the bagging of plants for FS_b and FS_w seed production. Heterosis observed for traits were quite low and this was unexpected, because parental cultivars were selected based on differences in seed quality (high erucic acid, high glucosinolate; low erucic, high glucosinolate; low erucic, low glucosinolate). Also, the extent of heterosis is expected to be influenced by the level of genetic distances between parents (Falconer and Mackay 1996).

However, this was supported by 17% between cultivars diversity observed when genetic diversity of 32 individual plants sampled from each of Rex, Largo and Steinacher, were analysed (chapter 4) and earlier study asserted that the gene pool of European winter oilseed *B. rapa* is narrow (Zhao and Becker 1998).

The high yielding cross combination FS_b (R x S) was surpassed by both FS_b (R x L) and FS_b (L x S) when MPH% was determined, indicating that higher *per se* performance of crosses doesn't imply higher heterosis. Percent mid parent heterosis depends on the mean of parents involved and a cross between parents with high *per se* performance will give low heterosis (Hegde et al. 2007). Also, the issue of heterosis is complex and involves the interaction of genes with dominance, over dominance or epistasis (Crow 1999).

Heterosis for grain yield of *B. rapa* has been reported to be much higher with 18 % MPH for seed yield observed in inter-varietal hybrids (Schuler et al. 1992), 25% for dry matter yield in turnip *B. rapa* after six generations of cultivar improvement by half-sib family selection (Bradshaw et al. 2002) and 25% for seed yield in *B. rapa* spring cultivars (Falk et al.

1998).

The correlations among traits are important for selection. There were no or only small correlations between flowering time and FBY and DBY, indicating that selection for early flowering does not necessarily improve biomass yield in *B. rapa*. The correlation between FBY and DBY over locations was only 0.62**, which is much lower than the value of 0.95** reported by Liu et al. (2002) in biomass yield of interspecific crosses of *B. rapa* and *B. napus*. The traits DBY relates linearly to both FBY and DMC and will increase with increasing DBY or DMC and vice versa. However, the effect is large if correlation between FBY and DMC is high.

When the two locations were separately analyzed for the different years, we observed a correlation between FBY and DBY of 0.79** at Einbeck and 0.34* at Göttingen in 2006. In 2007, correlation of 0.87** at Göttingen and 0.48* at Einbeck were observed between FBY and DBY. These low correlations reported indifferently in the two locations may probably be due to a weak correlation between FBY and DMC, indicating a technical problem with taking a representative sample of leaves and stem for DMC determination.

3.6 Conclusions

In summary, the mean yields of the different full-sibs were higher than the parents at the four locations. Heterosis of FS_b measured over FS_w was also quite low and most traits deviated from the expected order FS_w > syn- > FS_b = parents except for DBY. However, the yields of syn-1 were comparable with FS_b and could be used in large scale biomass production. Synthetic is envisaged as simple, less laborious and high amounts of seed could be produced compared to other methods such as full-sibs selection which requires the isolation of two plants.

The seeds of two parents with high specific combing ability are mixed and then cultivated under isolation. At this stage, the parental seed is referred to as Syn-0 and after

random mating, their offspring as syn-1 which produces high amounts of seed. The syn-1 is composed of 25% each of parental cultivar full-sibs and 50% of between cultivar full-sibs could be utilized for biomass production. Previous generations of random mating without selection are referred to as Syn-2, Syn-3 etc, and FS_b cultivars increases with increasing synthetic generation. In this way, heterosis can be at least partly utilized for yield improvement in *B. rapa* cultivars for biomass production.

4. Effect of crop improvement on genetic diversity in oilseed *Brassica rapa* cultivars detected by SSR markers

4.1 Abstract

With the improvement of seed quality, *Brassica rapa* oilseed germplasm went through two major breeding bottlenecks during the introgression of genes for zero erucic acid content and low glucosinolate content, respectively. This study investigates the impact of these bottlenecks on the genetic diversity in European winter *B. rapa* by comparing three open pollinated cultivars, each representing a different breeding period. Diversity was estimated on 32 plants per cultivar with 16 simple sequence repeat (SSR) markers covering each of the *B. rapa* linkage groups. Loss of genetic diversity over the three cultivars was indicated by a slight non significant (α = 0.05) decrease in allele number (59 – 55), alleles mean number (3.68 – 3.50), information index (0.94 – 0.87) and expected heterozygosity (0.53 – 0.48). Eighty three percent of the total variation was attributed to within cultivar and the remaining 17% to between cultivar variations by the analysis of molecular variance (AMOVA). Individual plants were separated into the according cultivars by both principal coordinate analysis (PCoA) and dendrogram based on Dice's similarity coefficient. In conclusion, genetic diversity within cultivars was high and quality breeding in *B. rapa* did not significantly reduce genetic diversity of *B. rapa* winter cultivars. To a large extent, there is no risk of decline in performance due to quality improvement.

Key words: *Brassica rapa* - breeding bottlenecks - erucic acid - germplasm - glucosinolate - genetic diversity - simple sequence repeat

4.2 **Introduction**

Brassica rapa (2n = 20) has been proposed to originate from two independent centres in Asia and Europe (Zhao et al. 2005) and domesticated as leafy vegetables, roots, fodder and oilseed cultivars. Oilseed cultivars dominate the European centre (Reiner et al. 1995) and extracted oil is either used for human consumption or further processed as a renewable resource in the petro-chemical industry. A by-product of oil extraction is the meal that is a valuable protein source for animal production.

While erucic acid is a valuable resource for the non food industry, this long chain fatty acid is not desired in oil for human consumption. To improve edible oil quality, breeding for erucic acid free cultivars was initiated in Canada in the early 1960's. By selecting zero erucic acid strains within *B. rapa* germplasm, the first low erucic acid cultivar was released soon after (Downey 1964). To improve meal quality, low glucosinolate (GSL) genes were introgressed from *B. napus* into *B. rapa* during the late 1960's (Krzymanski 1970) which drastically increased the economic value of the crop.

The improvement of seed quality in *B. rapa* implies that its germplasm had to go twice through a breeding bottleneck possibly causing a reduction in genetic diversity. Such reduction has been observed in Canadian oats cultivars (Fu et al. 2003), French bread wheat (Roussel et al. 2004) and Canadian hard red spring wheat (Fu et al. 2005). Nevertheless, no significant change was observed in current and historical maize inbreds (Lu and Bernardo 2001), European and Asian wheat accessions (Khlestkina et al. 2004) and British barley cultivars (Koebner et al. 2003).

For *B. rapa*, up to date no studies have been reported on the effects of different breeding periods on genetic diversity. However, studies on diversity within and between cultivar groups have been reported. Zhao et al. (2005) applying AFLP markers reported comparable genetic diversity between and within different *B. rapa* accession groups (leafy

vegetables, oilseed, roots and fodder) collected world-wide. Das et al. (1999) using AFLP and RAPD markers detected wide genetic diversity between different oilseed morphotypes of *B. rapa*.

Zhao and Becker (1998) with isozymes observed high genetic diversity of which 70% of the total diversity was attributed to within cultivars of winter and spring types obtained from Europe, China and Canada. Persson et al. (2001) also with allozymes reported 81% within genetic diversity in turnip *B. rapa* coming from Northern Europe. The high genetic diversity within cultivars of *B. rapa* is important for performance and minimizing inbreeding due to its open pollination nature.

Today, *B. rapa* is mainly grown as spring oilseed crop in Canada, some marginal regions in Northern Europe, and in Asia as well (Gu et al. 2003; Zhang et al. 2004). Traditionally, winter *B. rapa* was grown also as oilseed crop in Northern and Central Europe, but the cultivation nearly ceased. However, there is a renewed interest in cultivation of winter *B. rapa* in Europe to produce biomass, because of its high growth rate under low temperatures during early spring. To design breeding programs for the development of winter *B. rapa* cultivars for biomass production it is very important to know more about the genetic diversity of the European winter *B. rapa* gene pool.

The gene pool of European winter oilseed *B. rapa* is narrow (Zhao and Becker 1998). There are many old cultivars with high erucic acid and glucosinolate content, which however are genetically rather similar (Chapter 3). Only few erucic acid free cultivars have been developed, and for canola quality, there has been only one breeding program to our knowledge. Therefore we selected three cultivars for comparison, each representing one of the periods of winter *B. rapa* breeding in Europe.

The objectives of this study were (i) to genotype three open-pollinated *B. rapa* cultivars released in different breeding periods between 1954 and 2002 with SSR markers, (ii) to estimate the genetic diversity within and between cultivars and (iii) to test the effect of crop

improvement on within cultivar diversity.

4.3 Material and methods

4.3.1 Plant material

Three European winter cultivars of *B. rapa*, namely Largo (modern cultivar), Rex (older forage cultivar) and Steinacher (old oil seed cultivar) were used in this study. The materials have been described in detail in chapter 3. Briefly, Largo is a Swedish cultivar with zero erucic acid; low glucosinolate and released in 2002. Steinacher and Rex originated from Germany and were released in 1954 and 1984, respectively. Steinacher is of high erucic acid; high glucosinolate seed quality whereas Rex is zero erucic acid; high glucosinolate seed quality.

4.3.2 DNA extraction

DNA was extracted from young leaflets of two weeks old seedlings by using DNeasy Plant Mini Kits (Hilden Germany). Seeds of the different populations were sown in the green house and after three weeks, 32 individual plants from each population were randomly sampled and young leaflets harvested for marker DNA extraction. Approximately, 0.1g of leaf was taken for each plant. The fluorescent spectroscopy using Hoechst 33258 calf thymus DNA as standard was used to determine plant DNA concentration. Accordingly, DNA samples were diluted to a 5ng/ul working concentration.

4.3.3 Genetic marker analysis

The markers employed covers each of the *B. rapa* linkage groups according to previous works done in *B. napus* (Piquemal et al. 2005; Mladen Radoev and Wolfgang Ecke pers. com.). BRAS and CB denoted primer pairs were developed by Celera AgGen with funding provided by a consortium of the seed companies Advanta, Calgene, Caussade, Danisco, DLF, Euralis, Koipesol, KWS, Limagrain, Monsanto PGS, Pioneer Seminis,

Serasem, SW seeds and Syngenta. Primer pairs denoted MR and MD were developed by the Department of Crop Sciences at the University of Göttingen (Uzunova and Ecke 1999; Rudolph 2001).

Table 4.1 Number of different alleles observed at 16 SSR loci in three open pollinated *B. rapa* oilseed cultivars, each representing a breeding period. Genotyping was done on 32 plants per cultivar

Linkage Group	Markers	Allele number within cultivars			Allele number across cultivars
		Steinacher (1954 [a])	Rex (1984)	Largo (2002)	
1	CB1099	4	3	4	4
1	CB10206	4	5(1 [b])	5	6(1)
2	BRAS037	5	5	4	5
2	CB10416	4	3	3	4
3	MR197	2	2	2	2
4	CB10484	2(1)	3	4(1)	5(2)
5	CB10051	2	3	3(1)	4(1)
5	BRAS095	4	4(1)	3(1)	6(2)
7	CB10439	4	4	4	4
7	MD20	4	4	5	5
8	BRAS039	4(2)	2	2	4(2)
8	CB10448	4	5	4(1)	6(1)
9	BRAS020	3	3	2	3
9	CB10373	6(2)	4	4(1)	8(3)
10	CB10109	3	2	3	3
10	MR156	4(1)	5(1)	3	6(2)
Total	16	59(6)	57(3)	55(5)	75(14)

[a] year of release

[b] number of alleles that are unique to the respective cultivar

In an initial screen, 55 SSR primer pairs were used on 5 plants per population (data not shown). Sixteen of them were then selected based on their amplification strength, polymorphism, and resolution (Table 2). SSR analysis were done following the 13-tailing

ppolymerase chain reaction (PCR) techniques, where each forward primer carries a nucleotide tail which is complementary to a fluorescent labelled M13-universal primer. The amplification primed products with the M13-universal and the reverse primers are detected, after the fluorescence is excited with a laser beam. The universal M13 was labelled with the florophone (6FAM^{TM}), (VIC^{TM}) (NED^{TM}) and (PET^{TM}) which fluoresce in blue, green, yellow and green respectively.

4.3.4 Polymerase chain reactions (PCR**)**

The PCR was carried out in a final volume of 25µl containing 5 µl DNA template (5ήg/µl), 2 µl dNTPs (2mM), 1 µl 10x PCR buffer, 2 µl $MgCl_2$ (25 mM), 0.2 µl *Taq* polymerase (5 U/µl), 0.2 µl of each of the two primers (5 ρM) and 0.2 µl fluorescent labeled M13 primers (5 ρM).

Amplifications were performed in a thermocycler (Perkin Elmer 480) under the following conditions: 2 min at 95 ^{o}C, followed by a touchdown profile consisting of 10 cycles of 45 sec at 95 ^{o}C, 1 min at 68 ^{o}C and 1 min at 72 ^{o}C. Then 27 cycles of 45 min at 95 ^{o}C, 1 min at 47 ^{o}C and 1 min at 72 ^{o}C, and a final extension step at 75 ^{o}C for 10 min.

The different colors used make it possible to load four different PCR products at the same time. Markers with non overlapping fragment size were labeled with the same dye. Multiplexed PCR products were then separated and visualized on an ABI-3100 capillary sequencer (Applied Biosystems). The protocol used was, 0.5ul of internal line size standard (GeneScan-500 LIZ), 7.5ul of HiDi formamide and 0.2ul of the multiplex PCR product. The mixture was denatured for 2min at 95 ^{o}C and analyzed on an ABI 3100 genetic analyzer (Applied Biosystems).

4.3.5 Data analysis

To partition genetic diversity, an analysis of molecular variance (AMOVA) was

computed with the software GenAlEx v.6 (Peakall and Smouse, 2006) and significance was determined with 9999 permutations. A similarity matrix of the 96 genotypes based on Dice's similarity coefficient (Dice, 1945) was used for dendrogram construction using unweighted pair-group method with arithmetic average (UPGMA) and for principal coordinate analysis (PCoA) by employing the computer program NTSYS-pc version 2.1 (Rohlf, 2001). Bootstrap values to ascertain the internal support of clusters were calculated with the software WINBOOT (Yap and Nelson, 1996).

Based on the estimated fragment sizes of each marker and each genotype, the genetic diversity parameters of mean number alleles (An), Shnnon information index (I) and expected heterozygosity (He) were estimated for each *B. rapa* cultivar with GenAlEx v.6 (Peakall and Smouse, 2006). In addition, pair wise similarity distances of Dice's was transformed to dissimilarity coefficient (1- similarity coefficient) for each *B. rapa* cultivar with the software WINDIST (Yap and Nelson, 1996). Confidence levels for above parameters were determined based on standard deviation of all pair-wise combinations after adjustment for the actual population size of 32.

4.4 Results

4.4.1 Distribution of alleles

The number of detected alleles per SSR marker across cultivars ranged from two to eight and summing up to a total of 75 alleles and an average of 4.69 alleles per primer pair (Table 4.1). For the individual cultivars with respect to breeding period, the number of alleles per primer pair ranged from two to six with a total of 59 for Steinacher, two to five with a total of 57 for Rex and two to five with a total of 55 for Largo. Six alleles were unique for Steinacher, three for Rex and five for Largo. Common alleles were 44.1% for all three populations, 18% for Steinacher and Largo, 14.7% for Rex and Largo, and 11.4% for

Steinacher and Rex.

4.4.2 Genetic diversity within cultivars

Results from the AMOVA method of variance component analysis are presented (Table 4.2). As expected with cross pollinated species, genetic diversity within cultivars was high and accounted for 83% of the total variation while between cultivar variation was moderate but highly significant (P = 0.001) with 17%.

Table 4.2 AMOVA performed with 16 SSR loci in three open pollinated *B. rapa* oilseed cultivars, each representing a breeding period

Source	df	SS	MS	Var. comp	%	Fst	P
Between cultivars	2	116.323	58.161	0.843	17%	0.167	0.001
Within cultivars	189	794.234	4.202	4.202	83%		
Total	191	910.557	62.364	5.045			

4.4.3 Genetic diversity between cultivars

Across individual cultivars, total number of allele decreased from 59 to 55, allele mean number from 3.68 to 3.50, information index from 0.91 to 0.87 and expected heterozygosity from 0.53 to 0.48. A slight decrease in mean genetic distances across individual cultivars was also observed by the Dice's similarity coefficient (Table 4.3). However, these decreases were not significant (α= 0.05) for any of the parameters.

Table 4.3 Genetic diversity parameter values (± confidence intervals of the means) observed at 16 SSR loci in three open pollinated *B. rapa* oilseed cultivars, each representing a breeding period

Diversity parameters	Cultivars			Across cultivars
	Steinacher (1954 [a])	Rex (1984)	Largo (2002)	
Mean number of alleles (*An*)	3.68 (±0.37 [b])	3.56 (±0.37)	3.5 (±0.35)	4.68
Information index (*I*)	0.94 (±0.11)	0.91 (±0.10)	0.87 (±0.13)	1.14
Expected heterozygosity (*He*)	0.53 (±0.05)	0.51 (±0.05)	0.48 (±0.07)	0.58
Dissimilarity index (1-Dice)	0.45 (±0.032)	0.42 (±0.029)	0.42 (±0.033)	

[a] year of release

[b] 95% confidence interval

4.4.4 Genetic relationships based on principal coordinate analysis

A PCoA based on Dice similarity was carried out to show the genetic similarity of the three cultivars and a plot of the first and second coordinates are shown (Figure 4.1). The first two principal coordinates explained 50.3 % of the total variance and separated the 96 genotypes into the three cultivars with a slight overlap between Steinacher and Rex.

4.4.5 Genetic relationships based on dendrogram

The relationship among the 96 individuals of *B. rapa* comprising of the three cultivars based on genetic similarity (genetic distance) values were further determined with UPGMA cluster analysis (Figure 4.4). Cluster analysis showed a good fit with distance matrix as reflected in cophenetic correlation coefficient (r = 0.62). With the exceptions of two cultivars of Steinacher that each clustered with Rex or Largo, dendrogram perfectly separated the three cultivars into three main groups at about 0.48 genetic similarities. However, bootstrap values in support for individual cultivar groups were less than 1% in all cases (data not shown).

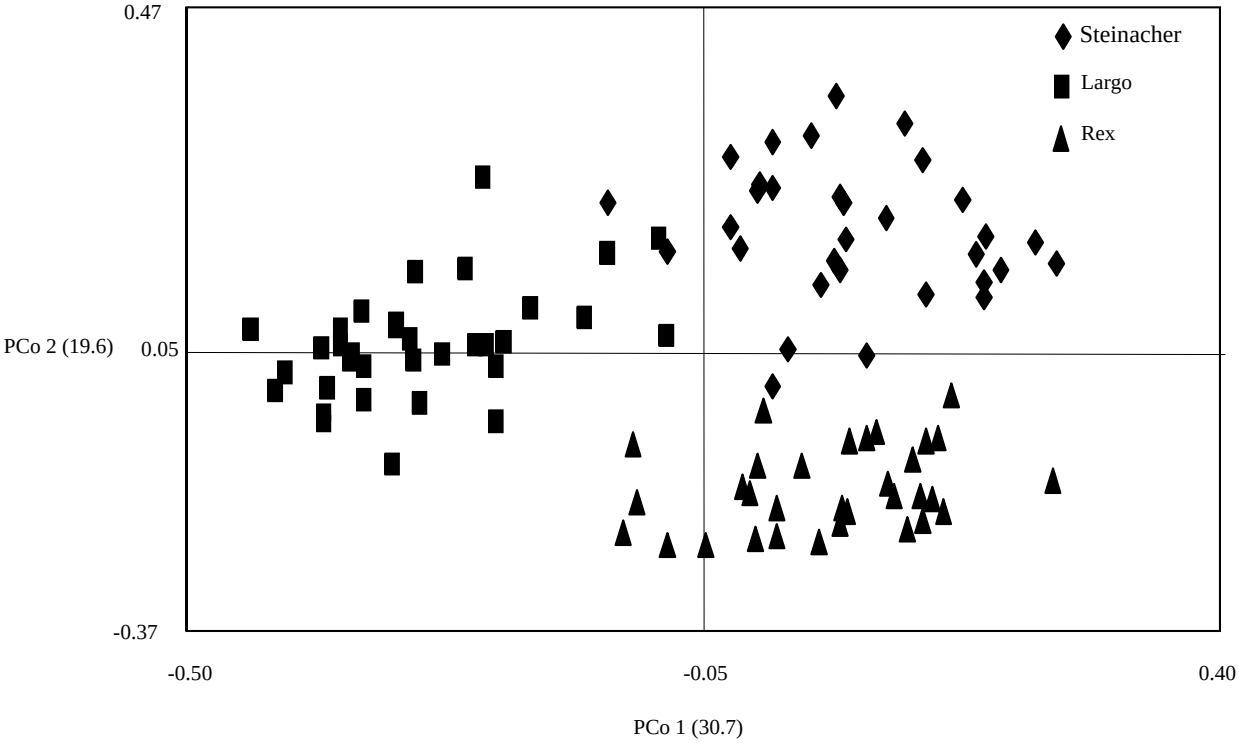

Figure 4.1 Association among 3 open pollinated *B. rapa* oilseed cultivars revealed by principal coordinates analysis at 16 SSR loci. Genotyping was done on 32 plants per cultivar

On average, within the cultivar Steinacher the genetic similarity values (cluster) ranged from 0.80 to 0.46 and a mean of 0.63, 0.97 to 0.45 and a mean of 0.66 for Rex, and 0.83 to 0.47 and mean of 0.67 for Largo. All 96 genotypes were genetically unique with the exception of two plants of Steinacher that. Confidence values for determining the degree of support for major nodes were generally low and those above 30% are indicated in the dendrogram.

4.4.6 Relationship between genetic distance and heterosis

The mean Dice genetic distances (Table 4.4) calculated from the 16 SSR markers, ranged from 0.54 to 0.56 with mean of 0.55 for among cultivars. For distances between cultivars, it was highest in L x S and lowest in R x S. Heterosis ranged from 6.18 to 12.14 for dry biomass yield (DBY) and from 4.22 to 4.65 for fresh biomass yield (FBY).

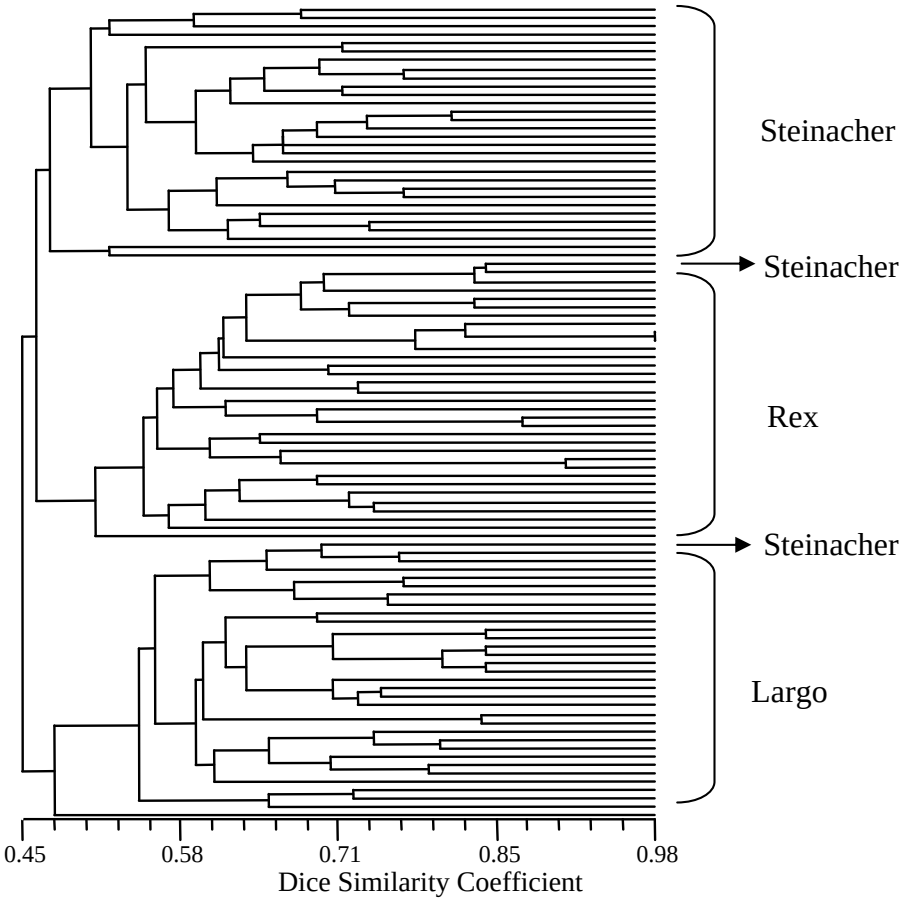

Figure 4.2 UPGMA dendrogram showing genetic relationships among 96 individuals of three *B. rapa* cultivars using 16 SSR markers. Numbers on branches are bootstrap values and only those above 30 are indicated

Table 4.4 Mean performance of between cultivar full-sibs and %MPH for four traits across environments.

Across environments									
Crosses	BPFS				%MPH				Genetic distance
	BTF (days)	DMC (%)	FBY (k/m^2)	DBY (g/m^2)	BTF (days)	DMC (%)	FBY (k/m^2)	DBY (g/m^2)	
R x L	236.5	14.4	3.42	482.3	-0.03	7.06	4.65	12.14	0.55
R x S	235.2	14.8	3.43	514.8	-0.10	-0.77	4.22	6.18	0.54
L x S	235.5	15.0	3.31	495.4	0.09	2.84	4.55	9.31	0.56
Mean	235.7	14.7	3.38	495.5	-0.01	3.04	4.41	9.21	0.55

4.5 Discussion

4.5.1 Genetic diversity within and between cultivars

With the advance of DNA markers, fingerprinting has been widely used to estimate genetic diversity on a molecular level. Out of the different marker technologies employed in *Brassica* crops (Snowdon and Friedt 2004), SSR markers have the advantage of being robust, cost effective and high informative due to their co-dominant expression (Zhou et al. 2006). However, they have hardly been used for diversity studies in *B. rapa*, though been employed in other species of *B. napus* (Plieske and Struss 2001), *B. oleracea* (Tonguc and Griffiths 2004) and *B. nigra* (Westman and Kresovich 1999).

An obvious limitation of our study is that only three cultivars were compared. However, the gene pool of European winter oilseed *B. rapa* is rather narrow (Zhao and Becker 1998) and due to the small present interest mainly old cultivars are available, which are high in erucic acid and glucosinolate content. A diallel cross among 15 old European *B. rapa* cultivars from Germany, Sweden, Czechoslovakia, and Bulgaria showed only 8% average heterosis increase across between cultivars, indicating their genetic similarity (Chapter 3).

There are very few erucic acid free winter *B. rapa* cultivars registered. The available germplasm for winter *B. rapa* of Canola quality is even much more limited because only SW Seed, Sweden, developed such cultivars. Therefore we consider the three selected cultivars as a representation of the available winter *B. rapa* breeding material, and principally do not expect different results if a study with a large number of accessions was to be performed.

The moderate genetic diversity of 17% between cultivars showed that the cultivar origin (country and breeding company) had only a minor impact on the outcome of our study. This is also indicated by the partly overlapping cultivar cluster derived from PCoA (Figure 4.1) and in the dendrogram (Figure 4.2). In in a study with winter and spring types obtained

from Europe, China and Canada (Zhao and Becker 1998), only 30% of the genetic diversity was between cultivars and for turnip *B. rapa* from the Northern Europe (Persson et al. 2001), 19% of the genetic diversity between cultivar was observed.

Our results are supported by a field experiment with the same three cultivars, where we compared full-sib progenies produced within cultivars with full-sib progenies between cultivars (Chapter 3). The biological yield of between cultivar crosses was only 2.6 % higher than the yield of within cultivar crosses. These results support the marker data reported here; genetic distance between plants of two different cultivars is not much larger than the average distance between two plants of the same cultivar.

4.5.2 Effect of crop improvement on genetic diversity

We observed a slight but non significant decrease of genetic diversity at the molecular level across three different *B. rapa* winter cultivars, each representing a different stage of seed quality improvement towards double low quality. This slight decrease in genetic diversity could be attributed to continuous plant breeding activities which is expected to narrow down crop germplasm, sometimes referred to as ‘genetic erosion’ (Harlan 1972).

With the two major breeding bottlenecks thus breeding for low erucic acid content (Downey 1964) followed by the introgression of low GSL genes from *B. napus* (Krzymanski 1970), it is surprising that we observed only a slight decrease in genetic diversity indicating that allele diversity at erucic acid and GSL loci has little effect on the average genetic diversity of *B. rapa* oilseed cultivars. Bottlenecks may not necessarily result in reduced diversity within open pollinated cultivars, because breeders probably selected unconsciously for heterozygous allele frequencies at loci expressing heterosis, they maintain some level of diversity (Falconer and Mackay 1996).

Fu et al. (2003) in Canadian oats cultivars, Roussell et al. (2004) in French bread wheat and Fu et al. (2005) in Canadian hard red spring wheat showed significant effect of modern plant breeding on genetic erosion in different crops. However, other studies by Lu and Bernardo (2001) in current and historical maize inbreds, Khlestkina et al. (2004) in European and Asian wheat accessions and Koebner et al. (2003) in British barley cultivars failed to show such effect.

4.5.3 Relationship between genetic distance and heterosis

The highest genetic distance was observed between 00 and ++ quality, supporting that crop improvement may reduce genetic diversity. However, no significant differences among the different quality pools were observed. Genetic distances did not translate to performance and cross between 0+ and ++ were better in yields than the cross between 00 and ++ quality.

Nevertheless, the distances between parental cultivars for hybrid performance has limit, above which performance may decreased (Falconer and Mackay 1996). The differences in genetic distance among the cultivars were low. This was the case in heterosis for FBY and DTF but not for DMC and DBY.

4.6 Conclusions

Our results also do not support concerns about major genetic erosion caused by quality breeding in *B. rapa*. Genetic diversity within cultivars was high and quality breeding in *B. rapa* did not significantly reduce genetic diversity *B. rapa* winter cultivars. To a large extent, there is no risk of reduction in performance due to inbreeding. With respect to relationship between marker distances and heterosis, correlations were higher for DTF and FBY compared to DMC and DBY.

5. General Conclusions

The study investigates the genetic diversity, heterosis and general combining ability of winter *Brassica rapa* biomass yield for which results and discussion have been presented in the three previous chapters. This concluding section relates the different chapters and proposes some recommendations based on major findings for future consideration.

5.1 *Brassica rapa* for biomass production

For biomass production winter *B. rapa* can be rotated with crops adapted to higher temperatures like maize, sorghum or sunflower. Our trials were sown in September even though sowing could have been delayed. Plant biomass was harvested in early May during end of flowering. Until that time a dry biomass yield (DBY) of up to 6.6 t/ha could be produced. Results of days to flowering (DTF) showed that some genotypes flowered earlier than others and could be utilized for early harvesting of biomass because no or only small correlations exist between flowering time and FBY and DBY. This indicate that selection for early flowering does not necessarily improve biomass yield in *B. rapa*.

Of importance to biogas production is methane and methane yield that has been observed to increase with increasing dry matter content (DMC) up to 50% DMC on per hectare basis in maize (Amon et al. 2007). The DMC in the range of 14-18 % at the time of harvesting (at end of flowering) was low and would economically increase the cost of biogas production due to the cost for transportation and silage.

A diallel cross among 15 old and newer European *B. rapa* cultivars showed only 8% average heterosis, indicating a low genetic diversity between cultivars. Grouping of germplasm into heterotic structure before crossing increases hybrid performance (Melchinger and Gumber 1998). Later studies should consider grouping cultivars into distinct groups by either morphology or molecular markers before heterosis utilization. Nevertheless, specific

crosses showed percent mid parent heterosis (MPH %) of 21% for DBY and could be utilized for biogas production through the development of synthetic cultivars.

5.2 Genetic diversity and heterosis

Genetic diversity study based on SSR markers was efficient and characterized the different cultivars into different groups, though distances among cultivars were not significant. This indicates that no major genetic erosion was caused by quality breeding in European winter *B. rapa*. Breeders' should be credited for preventing genetic reduction in the improved cultivars. They may have unconsciously selected for heterozygous allele frequencies at loci expressing heterosis, by maintaining some level of diversity (Falconer and Mackay 1996) This in part explains the high genetic diversity within cultivars, which is important for open pollinated crops such as *B. rapa* to offset inbreeding and for adaptation to different environmental conditions.

Alternatively, the between genetic diversity could be increased by germplasm introduction. The European gene pool is narrow and their genetic base could be broadened by the exchange of breeding material between the Europe and Chinese gene pool (Zhao and Becker 1998). The Chinese material that would be introduced should be checked for adaptation to European conditions, because experiments from *B. napus* (Qian et al. 2007) showed that the Chinese cultivars do not adapt well to European environment.

The order of FS_b > syn-1 > FS_w = parental cultivars observed for DBY, was in accordance with theory and has important implications for our understanding of population breeding (Ali et al. 1995; Falconer and Mackay 1996) and synthetic utilization. This indicates that population improvement depends not only on the parental cultivars, but also on their combining ability. Performance of crosses between populations is higher than crosses within population. The performance of mixture of seeds from two populations under random mating will range between within populations and between population crosses performance.

The genetic distances did not translate to performance and crosses between 0+ and ++ were better in yields than the cross between 00 and ++ quality. The differences in genetic distances among the cultivars correlated with traits, FBY and DTF compared to DMC and DBY. Thus, differences in genetic distances among the cultivars were low and this was the case in heterosis measured among crosses for FBY and DTF but not DBY and DMC, indicating that correlation between genetic distance and heterosis could vary from trait to trait.

5.3 Breeding methods

The breeding of cross-pollinated crops have involved methods such as mass selection, recurrent selection, half-sib selection, full-sib selection and synthetic cultivars. The methods of mass selection, recurrent selection and half-sib selection are effective when the genetic variation is due to general combining ability (GCA) whereas full-sib selection and synthetics are effective when the genetic effect is controlled by specific combining ability (SCA). Our results showed high significant genetic variation of SCA for biomass yield and other components, implying that the best breeding method to increase biomass yield is to identify the best combinations among parents.

This could be utilized through hybrid, full-sib or synthetic breeding. However, the production of hybrid cultivars will be probably too expensive at the moment, due to the self incompatibility of *B. rapa*. There is also lack of an easily available hybridizing system. Even some cytoplasm male sterility methods developed in *B. rapa* are hardly used for seed production because of the lack of stable maintainers or restorer lines (Verma et al. 2000).

Production of full-sibs between different parental populations could be utilized, but it is however very laborious. Plants need to be isolated for seed production, and hardly suitable for large scale seed production. In the case of synthetics, large quantities of seed can be produced by mixing different parents and propagating them under open pollination.

When starting with two populations, the first generation after random mating (syn-1) should theoretically be composed of 25% each of plants from crosses within the parental populations and of 50% of plants from crosses between the two populations. In this way, heterosis can be utilized for yield improvement in *B. rapa* cultivars for biomass production. The practical utilization of heterosis in synthetic breeding of *B. rapa* will rely on self-incompatibility to promote out crossing and the identification of best specific combinations.

6. Summary

Biogas production has recently become of major interest in Europe. The substrate mainly used is biomass from energy crops and 2% of agricultural land in Germany is presently used for the cultivation of energy crops. However, about 80% of the biogas substrate is today coming from maize, a crop of sub-tropical origin with low cold tolerance which can not be sown before end of April.

For maximum biomass production per year, cold tolerant crops like some cereals, forage grasses, and also *Brassica* with high biomass production even under low temperatures could be rotated with higher temperatures crops like maize, sorghum or sunflower that are adapted to higher temperature. This will give the possibility for growing two crops in one season: the first one sown in autumn and harvested in spring, followed by a second crop sown in spring and harvested in autumn.

Brassica rapa was traditionally grown as winter oilseed crop in Northern and Central Europe, but the cultivation has nearly deceased. However, there is a renewed interest in cultivation of winter *B. rapa* in Europe to produce biomass, because of its high growth rate under low temperatures during early spring. To design breeding programs for the development of winter *B. rapa* cultivars for biomass production, a better knowledge on the genetic diversity of the European winter *B. rapa* gene pool is required.

To date, the European winter *B. rapa* has been bred primarily to enhance its nutritional value for human and animal consumption, for which seeds with zero erucic acid and low glucosinolate content are important. These targets are quite different from the criteria for bioenergy (biogas) for which high biomass yield is required. The improvement of seed quality in *B. rapa* implies that its germplasm had to go twice through a breeding bottleneck possibly causing a reduction in genetic diversity. Therefore it is of interest to evaluate also the potential of older cultivars.

This study was carried out with the following objectives; 1. To develop a breeding strategy for biomass production of European winter *Brassica rapa* for biogas production, 2. To determine the biomass yield and heterosis of crosses within and between European *Brassica rapa* cultivars, 3. To examine the effect of crop improvement on genetic diversity in oilseed *Brassica rapa* cultivars detected by molecular markers.

To analyze heterosis and combining ability, 15 winter *B. rapa* cultivars of European origin were used. These cultivars were crossed in a half-diallel without reciprocals to produce 105 combinations. The parents and the 105 combinations were evaluated for days to flowering (DTF), fresh biomass yield (FBY), dry matter content (DMC), dry biomass yield (DBY) and plant height (PH) in a lattice design with two replicates at two locations in Germany for two years.

The crosses surpassed in average their parents by 7.6 % for FBY and 5.9% for DBY. Maximum mid parent heterosis was 21.0 % for FBY and 30.4 % for DBY. Analysis of variance showed that genetic variance was mainly due to specific combining ability (SCA). The correlation between parental performance and general combining ability (GCA) was 0.42** for FBY and 0.53** for DBY. Selection of parental combination with high specific combining ability to produce synthetic cultivars could rapidly improve biomass yield.

Based on predominance of SCA and the high within cultivar diversity, the performance of synthetics, within cultivar full-sibs and between cultivar full-sibs were studied in three European winter cultivars of *B. rapa*. The mean of full-sibs were higher than the mean of the parents for most traits. The full-sibs within and between cultivars differed significantly for fresh biomass yield and dry biomass yield.

Relative mid parent heterosis of between cultivar full-sibs calculated over within cultivar full-sibs was 9.2% for DBY, 4.4% for FBY and 3.0% for DMC across environments. The correlation between DBY and FBY was 0.61** and between DBY and DMC was 0.86**. Heterosis for biomass production observed in cultivar crosses was low, indicating a relatively

low genetic diversity between the three cultivars.

To investigate the impact of bottlenecks in *B. rapa* breeding on the genetic diversity, three open pollinated cultivars were compared, each representing a different breeding period. Diversity was estimated on 32 plants per cultivar with 16 simple sequence repeat (SSR) markers covering each of the *B. rapa* linkage groups. Loss of genetic diversity over the three cultivars was indicated by a slight, but non significant decrease in allele number, information index and expected heterozygosity. This indicates that no major genetic erosion caused by quality breeding in European winter *B. rapa*.

Eighty three percent of the total genetic variation was attributed to within cultivar variation and the remaining 17% to between cultivar variation by analysis of molecular variance (AMOVA). Individual plants of the three cultivars were characterized by both principal coordinate analysis (PCA) and a dendrogram from cluster analysis. These show that high genetic diversity exists mainly within cultivars which explain the relative small amount of additional heterosis in crosses between cultivars. However, the performance of synthetic cultivars was comparable to between cultivar full-sibs and could be utilized for biomass production.

7. Zusammenfassung

Die Erzeugung von Biogas gewinnt in Europa zunehmend an Bedeutung. Als Substrat dienen dabei überwiegend Energiepflanzen, und zur Zeit werden etwa 2% der landwirtschaftlichen Fläche in Deutschland für den Anbau von Pflanzen zur Biogaserzeugung verwendet. Etwa 80 % davon ist Mais, eine ursprünglich subtropische Pflanze mit niedriger Kühletoleranz, die erst Ende April gesät werden kann. Zur Erzeugung hoher Biomasseerträge ist es daher sinnvoll, den Anbau von Mais oder anderer wärmeliebender Arten wie Hirse oder Sonnenblume zu kombinieren mit dem Anbau von Arten mit hoher Biomasseproduktion auch unter niedrigen Temperaturen wie Getreidearten, Gräsern, oder *Brassica*-Arten. So können zwei Kulturen in einer Saison angebaut werden: die erste wird im Herbst gesät und im Frühjahr geerntet, und die zweite wird anschließend im Frühjahr gesät und im Herbst geerntet.

Rübsen (*Brassica rapa*) ist eine traditionelle Ölfrucht in Mittel- und Nordeuropa, aber ihr Anbau ist heute nahezu erloschen. Allerdings gibt es ein erneutes Interesse am Anbau von *B. rapa* in Europa zur Erzeugung von Biomasse, da es kaum eine andere Fruchtart mit ähnlich hohen Wachstumsraten unter niedrigen Temperaturen im zeitigen Frühjahr gibt. Die Entwicklung von Zuchtprogrammen für *B. rapa* zur Erzeugung von Biomasse erfordert aber eine bessere Kenntnis der genetischen Variation im europäischen Genpool dieser Art.

Bisher hat sich die Züchtung von Rübsen vor allem auf die Verbesserung der Qualität für die menschliche Ernährung oder zur Verwendung als Futtermittel konzentriert, wofür die Erucasäurefreiheit und ein niedriger Glucosinolatgehalt von Bedeutung sind. Diese Zuchtziele unterscheiden sich stark von den Anforderungen an eine Sorte für die Biogaserzeugung. Die Qualitätszüchtung hat dazu geführt, dass das Zuchtmaterial für heutige Sorten zweimal durch eine genetischen „Flaschenhals“ gehen musste, wodurch möglicherweise die genetische Variation reduziert wurde. Daher ist es von Interesse, auch das Potential älterer Sorten zu untersuchen.

Diese Arbeit hatte die folgenden Zielsetzungen: (1) die Entwicklung einer Züchtungsstrategie für *B. rapa* zur Biogaserzeugung unter besonderer Berücksichtigung von Kombinationseignung und Heterosis; (2) die Bestimmung von Biomasseertrag und Heterosis in Kreuzungen innerhalb und zwischen Europäischen Rübsensorten.; (3) die Untersuchung des Einflusses der Qualitätszüchtung auf die genetische Diversität mit Hilfe von molekularen Markern.

Zur Untersuchung von Kombinationseignung und Heterosis wurden 15 europäische Winterrübsensorten verwendet. Durch diallele Durchkreuzung dieser Elternsorten wurden 105 Keuzungskombinationen erzeugt. Die Eltern und ihren Kreuzungen wurden in einer Gitteranlage mit zwei Wiederholungen in zwei Jahren an zwei Orten geprüft. Dabei wurden die Merkmale Tage bis Blühbeginn (DTF), Frischmasseertrag (FBY), Trockenmassegehalt (DMC) und Trockenmasseertrag (TBY) erfasst.

Die Kreuzungen hatten gegenüber ihren Eltern eine Mehrleistung von 7,6 % für FBY und 5,9 % für DBY. Die höchsten relativen Heterosiswerte waren 21,0 % FBY und 30,4 % für DBY. Die Varianzanalyse zeigte, dass vor allem die spezifische Kombinationsfähigkeit (SCA) von Bedeutung war. Die Korrelation zwischen allgemeiner Kombinationsfähigkeit (GCA) und Elternleistung betrug 0.42** für FBY und 0,53** für DBY. Durch eine Selektion von Elternkombinationen mit hoher SCA für die Herstellung synthetischer Sorten sollte eine schnelle Steigerung der Biomasseleistung von Rübsen möglich sein.

Die Leistung synthetischer Sorten im Vergleich zu Vollgeschwister-Nachkommenschaften innerhalb bzw. zwischen Sorten wurde an drei europäischen Winterrübsensorten näher untersucht. Die Vollgeschwister übertrafen die Leistung der Eltern in den meisten Merkmalen. Die Vollgeschwister aus Kreuzungen zwischen Sorten hatten einen signifikant höheren Frisch- und Trockenmasseertrag als die Vollgeschwister aus Kreuzungen innerhalb der Sorten.

Die Heterosis der Vollgeschwister zwischen Sorten im Vergleich zu den Vollgeschwistern innerhalb Sorten betrug 9,2 % für DBY, 4,4 % für FBY, und 3,0 % für DMC. Die Korrelation zwischen DBY betrug FBY war 0,61** und die zwischen DBY und DMC 0,86**. Insgesamt gesehen war die Heterosis relativ gering, was darauf hinweist, dass die untersuchten Elternsorten eine geringe Diversität hatten.

Um zu untersuchen, ob die Züchtung zu einer Einengung der genetischen Diversität geführt hat, wurden drei unterschiedlich alte Sorten verglichen, die verschiedene Züchtungsperioden repräsentieren. Ihre Diversität wurden an je 32 Pflanzen mit Hilfe von Mikrosatelliten (SSR) untersucht, die alle Kopplungsgruppen des Genoms abdecken. Es wurde nur eine sehr leichte, nicht signifikante Abnahme der Diversität beobachtet, gemessen an der Allelanzahl, dem Informationsindex, und der erwarteten Heterozygotie. Die Qualitätszüchtung hat daher bei Rübsen kaum zu einem Verlust an genetischer Variation geführt.

Eine Analyse der molekularen Variation (AMOVA) zeigte, dass 83 % der genetischen Variation innerhalb der Sorten und nur 17 % zwischen den Sorten auftrat. Einzelpflanzen der drei Sorten wurden durch Hauptkoordinatenanalyse sowie durch ein Dendrogram aua einer Clusteranalyse charakterisiert. Auch hier zeigte sich, dass die genetische Variation vor allem innerhalb der Sorten auftrat, wodurch sich der relativ geringe Heterosiszuwachs in Kreuzungen zwischen Sorten erklären lässt. Dennoch trat bei einigen Kombinationen eine deutliche Heterosis auf, die sich zur Steigerung der Biomasseleistung relativ einfach durch die Entwicklung von synthetischen Sorten nutzen lässt.

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9. Appendices

Appendix 9.1 Mean values for the four traits among 15 cultivars *B. rapa* over environments

PG no.	Parents cultivars	DTF (days)	DMC (%)	FBY (kg/m^2)	DBY (g/m^2)
1	Steinacher	237.81	14.1	3.55	499.73
2	Weibulls Storrybs	237	15.03	3.42	521.82
3	BRA 245	235.84	15.53	3.56	549.8
4	Lemkes Winter	236.66	15.65	3.5	550.22
5	Lemkes Malchower	236.09	14.41	3.54	490.34
6	Arktus	236.46	13.67	3.49	477.18
7	Schneiders Sprengel	235.01	14.42	3.81	554.16
8	Hege's Winter	238.32	15.6	3.85	576.1
9	Janetzki's	236.33	16.07	3.22	544.78
10	Opava	236.23	15.25	3.41	509.11
11	Grubes Winter	237.82	15.02	3.59	494.99
12	Wild accession	233.91	15.33	3.28	501.21
13	Orbit	235.13	17.32	3.56	605.19
14	Largo	233.74	15.6	3.48	541.91
15	Rex	232.95	16.99	3.14	541.69

Appendix 9.2 Mean values for general combining ability effects for four traits among 15 cultivars *B. rapa* over environments

Parents	DTF (days)	DMC (%)	FBY (kg/m^2)	DBY (g/m^2)
1	0.8**	-0.55**	0.07*	-24.52**
2	0.77**	-0.44**	0.04	-14.28*
3	0.11	0.13	-0.11**	-8.47
4	0.27*	0.25	-0.16**	-12.32+
5	0.38**	-0.09	0.09**	6.48
6	0.2+	-0.22	-0.02	-4.91
7	-0.16	0.05	0.01	6.06
8	0.37**	-0.17	0.11**	7.52
9	-0.29**	0.35*	-0.13**	0.37
10	0.12	-0.29+	0.03	-7.68
11	0.38**	-0.27	0.02	-2.69
12	-0.39**	0.03	-0.03	6.09
13	0.16	0.25	0.1**	30.66**
14	-0.68**	0.47**	-0.03	-0.37
15	-2.04**	0.50**	-0.01	18.04**

*, ** and + statically significantly different from zero at respectively P = 0.10, P = 0.05 and P = 0.01 respectively (LSD -test)

Appendix 9.3 Mean days to flowering of 105 full-sib crosses of 15 European winter *B. rapa* over environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	237.72	235.3	236.82	236.93	238.3	235.41	237.82	234.98	236.94	236.74	237.1	237.84	236.01	234.0
2		236.06	236.93	237.2	236.52	236.79	236.15	236.56	237.74	237.11	235.44	237.3	236.26	233.83
3			237.5	236.34	236.03	235.48	236.73	235.39	236.01	237.23	235.88	234.98	236.12	233.91
4				235.15	237.11	235.35	236.5	236.18	236.87	236.08	235.39	236.88	234.54	233.73
5					236.31	237.76	235.88	236.14	237.05	236.21	235.89	236.29	235.66	233.61
6						236.32	236.59	235.61	235.29	235.93	235.68	236.37	233.71	234.4
7							235.78	234.55	235.16	236.5	235.46	236.9	233.98	234.0
8								236.34	236.37	236.59	236.02	236.12	234.75	234.7
9									234.59	237.04	234.35	235.93	235.84	234.26
10										236.39	235.96	234.83	235.52	234.39
11											236.14	235.68	235.63	233.19
12												233.66	236.38	233.09
13													235.56	235.22
14														232.69

Appendix 9.4 Mean dry matter content of 105 full-sib crosses of 15 European winter *B. rapa* over environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	14.58	13.41	13.79	13.36	14.37	14.65	13.31	15.34	14.24	14.65	15.15	16.15	15.78	15.51
2		15.22	15.84	14.43	13.65	15.28	14.38	13.83	14.7	14.79	14.84	14.71	14.27	15.10
3			15.84	15.58	13.85	15.03	15.89	14.62	14.92	15.58	15.1	16.08	15.07	16.85
4				15.89	14.09	14.42	15.75	15.82	14.96	15.76	16.13	14.28	16.15	15.87
5					15.2	15.57	14.13	15.31	14.69	15.13	14.92	15.32	15.84	14.85
6						15.03	14.96	16.88	14.35	15.28	15.34	15.66	15.4	14.52
7							16.28	16.09	16.28	14.22	13.45	15.58	16.05	14.19
8								15.75	12.86	14.46	14.85	14.53	15.93	16.09
9									13.69	14.76	15.91	16.68	14.98	16.28
10										15.11	15.08	14.16	16.19	16.41
11											13.98	14.37	14.55	15.31
12												15.97	16.31	14.70
13													14.97	16.20
14														16.05

Appendix 9.5 Mean fresh biomass yield of 105 full-sib crosses of 15 European winter *B. rapa* over environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	3.96	3.69	3.59	4.29	3.4	4.04	3.59	3.56	3.86	3.81	4.12	3.99	3.77	3.8
2		3.97	3.89	3.61	4.05	3.87	3.94	3.34	3.66	3.96	3.55	3.61	3.83	3.82
3			3.26	3.70	3.58	3.79	3.65	3.79	3.48	3.53	3.84	3.85	3.43	3.55
4				3.63	3.44	3.46	3.73	3.59	3.94	3.26	3.54	3.76	3.71	3.63
5					3.82	3.64	3.97	3.69	3.93	3.87	3.85	4.2	3.69	3.87
6						3.56	3.58	3.7	3.79	3.65	3.91	3.99	3.93	3.92
7							3.91	3.57	3.86	3.64	3.81	3.83	3.71	3.99
8								4.11	3.91	4.19	3.61	3.69	4.26	3.87
9									3.51	3.67	3.68	3.82	3.28	3.58
10										3.79	3.84	4.01	3.7	3.6
11											3.75	3.92	3.93	3.89
12												3.74	3.46	3.52
13													3.77	3.69
14														3.69

Appendix 9.6 Mean dry biomass yield of 105 full-sib crosses of 15 European winter *B. rapa* across environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	514.42	484.86	489.68	549.18	481.76	589.14	470.16	442.66	536.68	571.66	641.68	620.91	583.17	563.86
2		620.61	618.76	523.47	551.84	560.09	561.48	463.57	519.2	570.17	521.32	536.59	538.62	572.69
3			513.09	572.27	493.2	553.71	567.16	547.46	523.15	556.62	587.39	635.68	496.83	596.43
4				573.14	471.59	503.45	588.01	563.44	613.37	511.9	578.28	523.26	573.43	576.99
5					576.38	560.63	498.55	577.8	583.9	585.59	567.7	633.87	570.2	570.13
6						558.39	526.92	666.35	544.9	549.02	604.13	593.43	612.16	564.66
7							623.2	571.11	614.05	516.75	512.9	599.62	603.21	571.08
8								657.93	499.32	634.87	579.83	531.95	615.54	601.35
9									499.26	538.02	603.14	665.31	495.04	572.28
10										577.52	573.68	562.83	532.41	578.37
11											518.32	560.26	551.86	580.99
12												601.42	521.11	526.84
13													567.39	624.52
14														592.78

Appendix 9.7 Estimates of specific combining ability effects for day to flowering among 105 full-sib crosses in winter *B. rapa* over environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.32	-1.43	-0.07	-0.07	1.48	-1.05	0.83	-1.35	0.19	-0.26	0.87	1.06	0.07	-0.58
2		-0.65	0.06	0.22	-0.28	0.36	-0.82	0.25	1.02	0.13	-0.77	0.54	0.34	-0.73
3			1.29	0.03	-0.11	-0.30	0.42	-0.25	-0.05	0.92	0.34	-1.11	0.87	0.01
4				-1.32	0.81	-0.58	0.03	0.38	0.66	-0.39	-0.31	0.63	-0.87	-0.32
5					-0.10	1.72	-0.69	0.23	0.73	-0.37	0.08	-0.06	0.14	-0.55
6						0.45	0.19	-0.13	-0.85	-0.48	0.04	0.19	-1.63	0.41
7							-0.25	-0.83	-0.62	0.46	0.19	1.08	-1.00	0.37
8								0.43	0.06	0.02	0.22	-0.23	-0.76	0.55
9									-1.07	1.13	-0.79	0.24	0.99	0.77
10										0.06	0.4	-1.27	0.26	0.48
11											0.32	-0.68	0.11	-0.97
12												-1.93	1.63	-0.30
13													0.26	1.28
14														-0.41

Appendix 9.8 Estimates of specific combining ability effects for dry matter content among 105 full-sib crosses in winter *B. rapa* across environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.47	-1.27	-1	-1.1	0.04	0.04	-1.07	0.43	-0.02	0.36	0.57	1.35	0.75	0.46
2		0.44	0.94	-0.14	-0.79	0.57	-0.11	-1.18	0.34	0.4	0.16	-0.2	-0.86	-0.06
3			0.37	0.44	-1.16	-0.25	0.84	-0.96	-0.02	0.62	-0.16	0.61	-0.62	1.12
4				0.63	-1.04	-0.98	0.58	0.12	-0.09	0.68	0.76	-1.32	0.33	0.03
5					0.41	0.51	-0.71	-0.05	-0.03	0.39	-0.11	0.06	0.36	-0.66
6						0.09	0.25	1.65	-0.24	0.66	0.43	0.53	0.05	-0.87
7							1.29	0.59	1.42	-0.67	-1.73	0.17	0.42	-1.46
8								0.48	-1.78	-0.2	-0.1	-0.65	0.53	0.66
9									-1.47	-0.42	0.44	0.98	-0.94	0.33
10										0.56	0.24	-0.90	0.90	1.10
11											-0.88	-0.71	-0.76	-0.03
12												0.59	0.71	-0.92
13													-0.85	0.34
14														-0.03

Appendix 9.9 Estimates of specific combining ability effects for fresh biomass yield among 105 full-sib crosses in winter *B. rapa* over environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.09	-0.02	-0.07	0.37	-0.41	0.21	-0.35	-0.14	0.01	-0.04	0.32	0.06	-0.03	-0.02
2		0.29	0.26	-0.28	0.27	0.06	0.04	-0.32	-0.16	0.14	-0.22	-0.28	0.06	0.04
3			-0.22	-0.04	-0.05	0.14	-0.11	0.28	-0.19	-0.14	0.22	0.11	-0.18	-0.08
4				-0.06	-0.13	-0.14	0.02	0.13	0.33	-0.35	-0.03	0.07	0.15	0.05
5					-0.01	-0.22	0.01	-0.03	0.05	0	0.03	0.25	-0.13	0.04
6						-0.19	-0.27	0.09	0.03	-0.11	0.2	0.16	0.22	0.19
7							0.03	-0.07	0.07	-0.15	0.08	-0.04	-0.03	0.23
8								0.37	0.02	0.31	-0.23	-0.28	0.43	0.01
9									-0.14	0.02	0.08	0.09	-0.32	-0.04
10										-0.02	0.09	0.13	-0.05	-0.17
11											0	0.04	0.18	0.12
12												-0.09	-0.24	-0.20
13													-0.06	-0.16
14														-0.02

Appendix 9.10 Estimates of specific combining ability effects for dry biomass yield among 105 full-sib crosses in winter *B. rapa* over environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-8.11	-51.59	-34.81	5.89	-50.14	46.27	-74.17	-94.52	7.55	37.54	98.78	53.45	46.73	9.02
2		82.04	84.04	-30.05	9.71	6.99	6.92	-83.84	-20.16	25.82	-31.81	-41.1	-8.05	7.62
3			-27.45	12.93	-54.75	-5.21	6.78	-5.77	-22.02	6.46	28.44	52.16	-55.66	25.53
4				17.65	-72.51	-51.62	31.49	14.06	72.05	-34.41	23.18	-56.4	24.79	9.95
5					13.48	-13.24	-76.78	9.62	23.78	20.47	-6.2	35.41	2.76	-15.71
6						-4.09	-37.01	109.56	-3.83	-4.7	41.62	6.35	56.11	-9.79
7							48.3	3.36	54.35	-47.95	-60.58	1.57	36.19	-14.34
8								88.71	-61.84	68.72	4.9	-67.55	47.07	14.47
9									-54.76	-20.99	35.35	72.96	-66.29	-7.45
10										26.57	13.94	-21.46	-20.86	6.69
11											-46.41	-29.03	-6.4	4.32
12												3.35	-45.94	-58.61
13													-24.22	14.51
14														13.79

Appendix 9.11 Mid parent heterosis for fresh biomass yield among 105 full-sib crosses of 15 European winter *B. rapa* across environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	4.54	-8.47	-2.39	13.28	-10.03	1.74	-8.18	-4.69	5.18	12.06	17.83	16.03	-4.34	3.52
2		16.33	16.96	10.78	10.54	12.02	6.77	-5.03	-1.31	4.77	2.29	-2.10	4.04	11.49
3			-8.01	6.54	2.86	2.45	-4.39	4.68	10.68	6.80	18.88	25.07	12.40	9.20
4				2.03	-4.47	-14.36	2.94	3.87	22.57	-5.49	8.67	9.86	-12.45	10.45
5					8.94	5.16	9.84	15.35	26.71	14.24	12.72	24.85	6.80	14.92
6						0.39	-0.77	6.55	8.01	12.36	25.09	19.91	13.35	17.20
7							2.67	-5.45	7.27	10.08	2.37	7.32	4.35	9.24
8								16.68	4.53	15.57	22.07	2.11	18.12	9.10
9									1.75	1.96	8.04	19.18	-5.27	2.62
10										21.49	18.63	21.55	0.23	7.19
11											13.22	16.50	13.90	14.15
12												25.66	-1.72	9.09
13													16.96	22.08
14														7.95

Appendix 9.12 Mid parent heterosis for dry biomass yield among 105 full-sib crosses of 15 European winter *B. rapa* across environments

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-7.48	-15.36	-7.60	-0.53	2.82	12.60	-20.72	-32.06	-3.03	21.18	40.57	15.80	11.06	-3.50
2		12.46	14.66	1.36	-6.42	7.99	-1.21	-29.18	-14.55	7.16	-2.80	-18.11	-10.01	-1.23
3			-13.35	10.29	-9.30	-1.67	-1.66	-7.88	0.38	3.39	9.01	19.07	-10.07	12.56
4				12.22	-18.27	-20.12	5.84	-2.57	19.36	-1.10	13.38	-18.42	-5.88	3.07
5					29.65	22.57	-12.74	12.20	29.60	29.74	17.25	24.62	8.40	6.91
6						13.28	-1.84	46.85	8.93	26.35	46.99	11.20	22.54	7.24
7							8.44	-0.21	26.52	9.59	-3.07	11.39	20.02	-1.89
8								18.90	-15.30	20.29	27.16	-11.05	7.02	5.89
9									-14.09	-7.02	9.27	23.81	-15.43	-0.75
10										29.76	16.58	-5.58	-15.14	10.93
11											0.55	4.13	3.08	11.31
12												21.19	-10.01	-4.73
13													-2.99	12.70
14														-1.87

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CURRICULUM VITAE

Personal data

Name: Atta Ofori

Date of birth: 07-12-1977

Place of birth: Kukurantumi

Nationality: Ghanaian

Address: Von - Siebold - Str. 8, 37075 Göttingen, Germany

Telephone: 00491797012105

E-mail: ofori77@yahoo.com

Academic History and Qualifications

April 2005 –January 2008: Doctor of philosophy in Plant Breeding and Genetics, Georg-August University Germany. Thesis: Genetic diversity based on SSR markers, heterosis and yield performance of *Brassica rapa* for biomass production

2002-2004: Master of Science in Natural Resources and Crop Production, Georg-August University, Germany. Thesis: Plant Genetic Resources Management in Ghana: A case study of Plant Genetic Resources Centre

1997-2001: Bachelor of Science in Agriculture with major in plant production, Kwame University of Science and Technology, Ghana

Research Experience and Working History

April 2005 –January 2008: PhD activities involves Monitoring of greenhouse plants, Field experimentation and evaluation, Genetic diversity investigation with molecular markers, data analysis with different software's, etc.

July 2003-October 2003: Visiting fellow, Plant Genetic Resources Centre for data collection for MSc. thesis

June 2004-March 2005: Technical Assistant in the Green house and Laboratory of Plant Breeding and Genetics

Sept. 2001-March 2002: National Service at New Nsutem Senior Secondary school, Ghana Integrated Science Tutor

July-Sept.1999: Industrial Attachment with Cocoa Research Institute Biotechnology and Soil Science Division, Ghana

July-Sept.1998: Vacation training at Horticultural Department, University of Science and Technology Kumasi, Ghana

Sept.1996-July 1997: National Service at Rapid International School, Eastern Region, Ghana, General Science Tutor

Informatics and statistical knowledge

- Excel, Word and Power Point
- Statistical software (SPSS and PLABSTAT)

Publications and presentations

Ofori A and H C Becker (2008) Breeding of *Brassica rapa* for biogas: Heterosis and combining ability of biomass yield. BioEnergy Research (**In press**)

Ofori A, Kopisch-Obuch F J and H C Becker (2008) Effect of crop improvement on genetic diversity in oilseed *Brassica rapa* cultivars detected by SSR markers. Journal of Applied Genetic (**Accepted**)

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Ofori A, Use of *Brassica rapa* resources for biomass production: Biomass yield and heterosis in crosses within and between populations crosses. **Presentation**, 18th EUCARPIA Meeting (May 2007) Piest'any, Slovak Republic

Associations

European Association for Research on Plant Breeding, EUCARPIA