

Breeding of *Brassica rapa* for Biogas Production: Heterosis and Combining Ability of Biomass Yield

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Abstract The use of plant biomass as substrate for biogas production has recently become of 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 15 European winter *B. rapa* cultivars for biomass yield at end of flowering. A half-diallel without reciprocals was conducted among 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) and dry biomass yield (DBY). The mean DBY was 5.3 t/ha for the parental cultivars and 5.6 t/ha for their crosses. The crosses surpassed on 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 parental combinations with high specific combining ability to produce synthetic cultivars can rapidly improve biomass yield.

Keywords Biogas · Diallel · Full-sibs · General combining ability · Synthetic cultivars · Specific combining ability

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Introduction

Different technologies to convert biomass to bioenergy have been developed and among them biogas production has gained major interest in the past years in Europe. The number of biogas plants operating in Germany at the end of 2006 was 3.500 [22], an increased 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 [22], 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 adapted to higher temperatures like maize, sorghum or sunflower [16]. Crops with high biomass production at low temperature are rye, some forage grasses, and also *Brassica* crops. Among the *Brassicacae*, *B. rapa* is of special interest, because it has a higher early biomass and flowers earlier than *B. napus* [9, 15].

Today, *B. rapa* is mainly grown as spring oilseed crop in Canada and some marginal regions in Northern Europe. Traditionally, winter *B. rapa* was also grown as winter oilseed crop in Northern and Central Europe, but the 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 at low temperature in early spring. Harvest of biomass could be at end of flowering when biomass yield has nearly reached its maximum [3].

For biomass production, also older cultivars of winter *B. rapa* can be used. They have sometimes low grain yield and

relatively poor seed quality which is of little relevance for biomass production. Also, this could contribute to increased biodiversity.

B. rapa is a cross-pollinated and self-incompatible crop with high genetic diversity within cultivars [25]. Different methods such as full-sib selection or the development of synthetic cultivars have been exploited in *Brassica* population improvement (for review see [2]). Among these methods, full sib selection makes direct use of combining ability and heterosis [11] and utilizes both within and between family genetic variation [1]. Lamkey and Edwards [12] 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 combining ability and heterosis for yield and yield components have been carried out. Singh and Murty [18] in *B. rapa* subspecies yellow sarson observed predominance of non additive genetic effects. Wang et al. [21] among different subspecies of *B. rapa* observed the importance of general combining ability and specific combining ability in controlling different traits. Yadav et al. [23] in nine inbred lines of brown sarson used as females and three other cultivars as males observed large specific combining ability for plant height, number of branches per plant, number of seed per pot, 1,000-seed weight and seed yield per plant.

Schuler et al. [17] in inter-cultivar F_1 s of *B. rapa* reported mid parent heterosis (MPH) of 18% for seed yield and Falk et al. [6] in inter-cultivar of spring *B. rapa* reported heterosis of 13% for seed yield. Kaur et al. [10] in *B. rapa* subspecies 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. [21] 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.

The aim of this study is to analyse the genetics of biomass yield of 15 winter *B. rapa* cultivars and F_1 s derived from crosses amongst them. The specific objectives were: (1) to evaluate the biomass yield during flowering, (2) to estimate the effects of general and specific combining ability (GCA and SCA), and (3) 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.

Materials and Methods

Plant Materials The plant materials used in this experiment consisted of 15 winter *B. rapa* cultivars of European origin. Seeds were obtained from either genebanks or breeding companies (Table 1). The cultivars are from different geographical regions within Europe and their seed quality differs with respect to erucic acid and seed glucosinolate content.

Full-sib Development The 15 winter *B. rapa* cultivars were crossed in half-diallel manner without reciprocals in May 2005 and 2006. In each year, three full sibs (FS) were produced from each of the 105 parental combinations by isolating two plants under one large bag before begin of flowering.

Field Evaluation For evaluation, equal amounts of seed from the three FS of each combination were bulked. The 15

Table 1 *Brassica rapa* cultivars used in this study with their country of origin

Cultivar name	Country of origin	Seed quality ^a	Sources/breeder	Accession number
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 collection	Germany	++	Bonn, Germany ^b	
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 Thomas Gladis, University of Kassel, Germany

Table 2 Minimum, maximum, mean, least significant difference (LSD) for 15 winter *B. rapa* cultivars and their 105 diallel crosses across four environments

Traits	Parents			Crosses			LSD (5%)
	Min	Max	Mean	Min	Max	Mean	
DTF (days)	233.0	238.3	236.0	232.7	238.3	235.8	2.1
DMC (%)	13.7	17.3	15.3	12.9	16.9	15.1	2.3
FBY(kg/m ²)	3.1	3.9	3.5	3.3	4.3	3.8	0.5
DBY (g/m ²)	477.2	605.2	530.6	442.7	666.4	561.3	105.5

DTF days to flowering, DMC dry matter content, FBY fresh biomass yield, DBY dry biomass yield

parental cultivars, their 105 crosses, and a 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 2005/2006, the FS produced in 2006 were tested in 2006/2007. The experimental design was an 11×11 lattice with two replicates.

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/m². Plot size in Göttingen was 11.25 m² and each plot consisted of six rows, 7.5 m long and 0.25 m between rows. In Einbeck, plot size was 9 m² and plots consisted of six rows, 5 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 (from the day of sowing until 50% of plants were flowering), fresh biomass yield (kg/m²), dry matter content (%) and dry biomass yield (g/m²). Plots were harvested on the 8th of

May at Einbeck and 15th of May at Göttingen in 2006, and 2nd of May at Einbeck and 21st of May at Göttingen in 2007. The growth stage at that time was end of flowering. Harvesting was done with a harvester that cut plants at 5 cm above ground and the total fresh biomass yield was measured. From each plot a sample of 300 g fresh weight was dried at 60°C for 6 days to determine the dry matter content and to calculate the dry biomass yield per plot.

Statistical Analysis Analyses of variance (ANOVA) were first run separately for each experiment using PLABSTAT software [19]. A combined analysis of variance of the adjusted means was then computed over the four environments. Random effects model was assumed. 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 GCA and SCA, according to analyses III of Gardner and Eberhart [8] using PZ14 software [20].

Table 3 Mean squares (MS) and variance components (Vc) from combined analysis of variance for 15 winter *B. rapa* cultivars and their 105 diallel crosses over four environments

Source	df	DTF (days)		DMC (%)		FBY (kg/m ²)		DBY (g/m ²)	
		MS	Vc	MS	Vc	MS	Vc	MS	Vc
Environment (E)	3	25,533.23**	212.8	1,460.75**	12.14	12.80**	0.106	2,356,116**	19,586.35
Genotypes	119	5.91**	0.934	3.05	0.05	0.20**	0.023	8,373.36**	654.78
Parents	14	9.79**	1.781	3.90	0.149	0.14	0.045	4,998.32	0 ^a
Parent vs crosses	1	0.87		2.79		3.57**		49,722.04**	
Crosses	104	5.44**	0.831	2.93	0.048	0.17**	0.017	8,430.11**	717.78
GCA	14	24.92**	0.358	5.48*	0.061	0.35*	0.004	9,496.28	9.209
SCA	90	2.41**	0.205	2.54	0	0.16*	0.011	8,264.27**	701.66
G×E	357	2.18**	1.500	2.83**	1.000	0.11**	0.053	5,754.21**	3,123.23
Parent×E	42	2.67**	1.999	3.30**	1.468	0.12**	0.070	6,302.84**	3,671.86
Parent vs crosses×E	3	5.61**		16.52**		0.45**		55,131.32**	
Crosses×E	312	2.11**	1.44	2.74**	0.910	0.10**	0.049	5,558.98**	2,927.00
GCA×E	42	5.48**	0.300	2.56	0	0.12	0.002	6,210.163	57.92
SCA×E	270	1.59**	0.915	2.77**	0.938	0.10**	0.047	5,457.61**	2,826
Error	390	0.67		1.83		0.05		2,630.98	

DTF Days to flowering, DMC dry matter content, FBY fresh biomass yield, DBY dry biomass yield

*, **Significantly different from 0 at $P=0.05$ and $P=0.01$, respectively

^a Negative estimates of variance component

Table 4 Minimum, maximum, mean and standard error (SE) for relative mid parent heterosis (MPH%) and relative high parent heterosis (HPH%) for 105 crosses of winter *B. rapa* cultivars over four environments

Traits	MPH%				HPH%			
	Min.	Max.	Mean	SE	Min.	Max.	Mean	SE
DTF	-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	-8.04	21.02	7.56	0.62	-9.19	20.85	4.37	0.62
DBY	-15.24	30.41	5.93	0.88	-18.75	28.03	2.10	0.89

DTF Days to flowering, DMC dry matter content, FBY fresh biomass yield, DBY dry biomass yield

Heterosis was calculated as follows: relative mid parent heterosis $MPH\% = (Crosses - MP) / MP \times 100$ and relative high parent heterosis $(HPH\%) = (Crosses - HP) / HP \times 100$, 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.

Results

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 0.3 kg/m² (7.5%) and for dry biomass yield (DBY) by 30.7 g/m² (5.8%). Dry matter content (DMC) was 0.2% higher in the parents. Days to flowering (DTF) were nearly the same in the crosses and the parents. The differences between maximum and minimum values were low for DTF but relatively higher for 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×Lembkes Winter (666 g/m²), Orbit×Opava (665 g/m²) and Orbit×Arktus (657 g/m²).

The combined ANOVA pooled over four environments showed highly significant variation among environments for all traits (Table 3). Significant differences among genotypes were observed for all traits except DMC and genotype×environments were significant for all traits.

Partitioning of genotypes into parents and crosses showed significant variation of the parents only for DTF whereas the variation of parents×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 parents vs crosses, indicating the presence of heterosis, was significant for FBY and DBY, and parent vs crosses×environments were highly significant for all traits.

Partitioning of the variance among crosses into GCA and SCA gave different results for different traits. 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 environment for all traits.

The MPH% and HPH% estimates varied for the different traits over environments (Table 4). Heterosis was observed for FBY and DBY but not for DMC. The small negative estimate for DTF means that flowering was earlier in the crosses compared to their parents. The effects of heterosis were generally low; for FBY the average mid parent heterosis (MPH) was 7.6%, ranging from -8.0 to 21.0, and for DBY the average MPH was 5.9%, ranging from -15.2 to 30.4. The average high parent heterosis (HPH) was 4.4% for FBY and 2.1% for DBY.

Analysis of variance for MPH% and HPH% (Table 5) showed significant variation among environments for both MPH% and HPH%. The variation among crosses was

Table 5 Mean squares of analysis of variance for relative mid parent heterosis (MPH%) and relative high parent heterosis (HPH%) for 105 crosses of winter *B. rapa* cultivars across four environments

Source	df	%MPH				%HPH			
		DTF	DMC	FBY	DBY	DTF	DMC	FBY	DBY
Environment (E)	3	2.65***	1,078***	852***	3,315***	3.51***	3,663***	323.7*	6,568.7***
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***	144.06**	138.83***	268.85***	0.44***	138.43	128.8***	238.6***
Error	390	0.18	116.89	67.24	140.36	0.24	146.48	84.85	174.28

DTF Days to flowering, DMC dry matter content, FBY fresh biomass yield, DBY dry biomass yield

*, **, ***Significantly different from 0 at P=0.10, P=0.05 and P=0.01, respectively

Table 6 Correlation coefficients between general combining ability (GCA) and parent value (PV), performance of crosses and mid parent value (MPV), relative mid parent heterosis (MPH%) and MPV, MPH% and performance of crosses of winter *B. rapa* over four environments

Traits	GCA vs PV	Crosses vs MPV	MPH% vs MPV	MPH% vs crosses
DTF	0.83**	0.65**	-0.29**	0.53**
DMC	0.69**	0.34**	-0.41**	0.72**
FBY	0.42**	0.22*	-0.37**	0.83**
DBY	0.53**	0.21*	-0.29**	0.87**

DTF Days to flowering, DMC dry matter content, FBY fresh biomass yield, DBY, dry biomass yield

*, **Significantly different from 0 at $P=0.05$ and $P=0.01$, respectively

significant only for DTF, and the crosses \times environment interactions were significant for all traits.

The correlations between GCA and parent were positive and highly significant for all traits (Table 6). The correlations between crosses and parents were also always positive and significant. However, correlations between mid parents value and MPH% were significantly 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 among traits were significantly positive between DBY and DMC and DBY and FBY over the environments (Table 7). The DMC was negatively correlated with both FBY and DTF.

Discussion

The crosses surpassed on average their parents for FBY and DBY indicating the presence of heterosis (Table 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 [4]. A much higher heterosis for *B. rapa* grain yield has been reported with 18 [17] and 13% [6]. 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 [24]. When analyzing the diversity of 32 plants each of three cultivars Rex, Largo and Steinacher with molecular markers, we observed most of the variation within populations and only a relatively small part of the variation between populations (unpublished results). This can explain

the relative low amount of heterosis observed in crosses between different cultivars, because the parental populations are already highly heterozygous. This may also explain the negative correlation between parental value and MPH. The higher the parental value is, the lesser is the amount of heterosis already utilized within the parental populations, and the smaller is the MPH observed in crosses between populations.

Variations in heterosis were low (Table 5) and significant only for DTF. The possible explanation may be the very high crosses \times environment interactions. A random model was used for the analyses due to which the different components were tested against their environment \times genotype interactions. If a fixed model would have been used, the genotypes would have been tested against the error. In that case, more sources of variation would have become significant. However, to get results that can be generalized, the more conservative tests using a random model were chosen.

It has to be mentioned that the genotype environment interactions are overestimated, because in the two different years three different FS of each cross were used. So each parental population was represented by only six plants. Therefore, a more detailed analysis of the most promising populations with a larger number of FS tested at a larger number of environments is desirable.

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 \times environment variance compared to the parents \times environment variance for most traits (Table 3)

Table 7 Correlation coefficient between four traits of winter *B. rapa* across four environments

	DMC	FBY	DBY
DTF	-0.31**	0.04	-0.22*
DMC		-0.16	0.59**
FBY			0.57**

DTF Days to flowering, DMC dry matter content, FBY fresh biomass yield, DBY dry biomass yield

*, **Significantly different from 0 $P=0.05$ and $P=0.01$, respectively

agrees with the philosophy, that hybrids are more stable than parents. However, Singh and Murty [18] in *B. rapa* observed a higher crosses \times environment variance compared to the parents \times environment and concluded, that stability of a cultivar is influenced not only by heterozygosity.

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 will not necessarily improve biomass yield. The correlation between FBY and DBY over environments was only 0.57**, which is much lower than the value of 0.95** reported in intersepecific crosses of *B. rapa* \times *B. napus* by Liu et al. [13]. When analyzing the environments separately (data not shown), we observed low and non significant in one of the environments. Probably there was a technical problem with taking a representative sample of leaves and stem for DMC determination. This problem may partly explain the positive correlation between DMC and DBY.

B. rapa is a diploid species, but tetraploid cultivars have also been developed. For comparison, we included the tetraploid German 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.

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, as suggested by Falk et al. [7] large quantities of seed can be produced by the approach of synthetic cultivars when different parents are mixed and propagated under open pollination.

When starting with two populations, the first generation after random mating 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. Synthetic cultivars of *B. rapa* can have nearly the same yield as hybrids [7]. The first synthetic cultivars for oilseed *B. rapa* were Hysyn 100 and Hysyn 110 released in Canada in 1994 [5]. Also Niemelä et al. [14] observed a yield advantage of up to 18% in synthetics compared to open pollinated cultivars. Similar approaches could also be successful for breeding *B. rapa* cultivars for biomass production.

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