

Biomass yield and heterosis of crosses within and between European winter cultivars of turnip rape (*Brassica rapa* L.)

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Abstract Because of its high growth rate at low temperatures in early spring, there is renewed interest in *Brassica rapa* as a winter crop for biomass production in Europe. The available cultivars are not developed for this purpose however. An approach for breeding bioenergy cultivars of *B. rapa* could be to establish populations from two or more different cultivars with high combining ability. The objective of this study was to evaluate the heterosis for biomass yield in the European winter *B. rapa* gene pool. The genetic variation and heterosis of the biomass parameters: dry matter content, fresh and dry biomass yields were investigated in three cultivars representing different eras of breeding by comparing full-sibs-within and full-sibs-between the cultivars. Field trials were performed at two locations in Germany in 2005–2006. Mean mid-parent heterosis was low with 2.5% in fresh and 3.0% in dry biomass yield in full-sibs-between cultivars. Mean values of individual crosses revealed a higher variation in mid-parent heterosis ranging from 14.6% to –7.5% in fresh biomass yield and from 19.7% to –12.7% in dry biomass yield. The low heterosis observed in hybrids between European winter cultivars can be explained by the low genetic variation between these cultivars as shown earlier with molecular markers. In conclusion, a *B. rapa* breeding

program for biomass production in Europe should not only use European genetic resources, but should also utilize the much wider worldwide variation in this species.

Keywords Genetic variation · Heterosis

Today, *Brassica rapa* is primarily grown as a spring oilseed crop in Canada and in some northern European regions. Whereas its cultivation as winter oilseed crop in Central Europe has nearly ceased, there is renewed interest in *B. rapa* as a winter crop for biomass production because of its high growth rate at low temperatures in early spring (Ofori and Becker 2008). If cultivated as a pre-crop, it can be harvested early in the year before planting the major crop, such as maize, sorghum or sunflower. *B. rapa* is of particular interest among *Brassica* crops as it has a higher early biomass than *B. napus* (unpublished results). For biomass substrate storage and biogas processing, a high dry matter content is important, therefore, the major selection criteria for biomass yield are fresh biomass and dry biomass yields.

The *B. rapa* species includes highly diverse morphotypes with a respective varying utilization as oilseed crop, root and leaf vegetables or forage plants. Zhao et al. (2005) suggested two major areas of domestication and respective gene pools in Asia and Europe. *B. rapa* cultivars exhibit a high genetic diversity within cultivars (Zhao et al. 2005; Zhao et al. 2009), whereas the genetic variance of crosses between *B. rapa* cultivars mainly originated from specific combining ability (SCA) effects (Ofori and Becker 2008). Cultivars of *B. rapa* are self-incompatible (Franklin-Tong and Franklin 2000) and completely cross-pollinated. Sakamoto and Nishio (2001) discussed the use of the self-incompatibility in F_1 hybrid breeding. However, an F_1 hybrid production, based

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on parental inbred lines, requires a high input into parental line development, combination testing and F_1 seed production, and a breeding method with a lower input, nevertheless exploiting heterosis, would be of interest. A simple method of hybrid production in cross-pollinated species is the crossing of two populations (Falconer and Mackay 1996). Considering two parental breeding populations of a diploid outcrossing species with completely random mating and in linkage equilibrium, the resulting hybrid shows a higher degree of heterozygosity compared to the mean of parental populations and an increase in heterosis. Schuler et al. (1992) found an average of 18% mid-parent heterosis in seed yield, in 19 intervarietal and interpopulation crosses in *B. rapa* with Canola quality. Niemelä et al. (2006) reported 18% and 23% heterosis in seed yield for synthetics and composite hybrids, respectively. Ofori and Becker (2008) showed that heterosis for biomass yield in *B. rapa* crosses was low in general, but up to 30% in the best crossing combinations.

Breeding intensity and investment in the development of populations from full-sib progenies between different cultivars are much lower than in F_1 hybrid breeding. For a 'neglected' crop such as winter *B. rapa*, this might be a cost-reduced and effective alternative in the breeding of cultivars for biomass production. In addition to an earlier study by Ofori and Becker (2008), in which crosses of a half diallel were tested for GCA and SCA with respect to the possible use in F_1 hybrid breeding, in this experiment, we compare the biomass yield of three groups of genotypes: of full-sibs from crosses within three *B. rapa* winter cultivars, of full-sibs between these cultivars, and mixtures of these, to evaluate the genetic variation and heterosis and to draw conclusions for breeding strategies of *B. rapa* for biomass production.

The three *B. rapa* cultivars in this study were selected as genetically different in their breeding history and representative for various eras of European winter *B. rapa* breeding. The diploid cultivars Steinacher (S), Rex (R), and Largo (L) were released in 1954, 1984, and 2002, respectively. Steinacher, the eldest cultivar, is high in glucosinolates and erucic acid in the seed oil. Rex, a forage type of *B. rapa*, is high in glucosinolates but has zero erucic acid and was released after the first obvious bottleneck in breeding history, the introduction of zero erucic acid genetic material into the *B. rapa* gene pool. Largo has "00" quality, zero erucic acid combined with low seed glucosinolate content, and was thus released after the second bottleneck in quality breeding. Seeds of Steinacher were obtained from the genebank at IPK Gatersleben, Germany, and were multiplied under isolation (Ofori et al. 2008), while seeds of Rex and Largo were obtained from the plant breeders Norddeutsche Pflanzenzucht Hans Georg Lembke KG and SW Seeds, respectively.

Crosses between plants of the three cultivars ($R \times R$, $S \times S$, $L \times L$) were performed by isolation of two parental plants with a bag. Nine crosses of each crossing combination were harvested at Reinshof experimental station in 2005. The resulting 27 crossing combinations ($R \times R$, $S \times S$, and $L \times L$) are referred to as full-sibs-between (FS_{between}). Three plants of each cultivar were selfed to prove their self-incompatibility and, as expected, showed hardly any seed set (data not shown).

The seed of full-sibs-within (FS_{within}) cultivar crosses was produced by isolation of two plants each from the same cultivar. Equal quantities of seed from ten FS_{within} crosses per cultivar ($R \times R$, $S \times S$, and $L \times L$) were bulked for the field evaluation of FS_{within} .

Three mixed bulks (FS_{mix}) of the combinations $R \times L$, $R \times S$, and $L \times S$ were composed of FS_{within} and FS_{between} , which are genetically equivalent to a synthetic population (syn-1 generation) developed by random mating of a mixture of two cultivars each. Equal quantities of seed from the nine crosses of each FS_{between} combination were bulked. The three FS_{mix} bulks were produced by mixing 50% FS_{between} bulked seed and 25% of the two corresponding parental FS_{within} lots. The proportions of the lots were based on their 1,000 seed weight.

The plant material, including the three parental cultivars, was tested at Göttingen and Einbeck in central Germany in the growth season 2005–2006. At both locations, a lattice field design with two replications was used. Plot size differed with 11.25 m² in Göttingen and 9 m² in Einbeck, with a sowing rate of 90 and 110 seeds per m⁻², respectively. Fertilization and weed control were performed in accordance with standard crop management protocols. Days to flowering (DTF) were recorded as number of days from sowing till 50% of the plants in a plot started flowering. At the end of flowering, fresh biomass yield (FBY kg m⁻²) was determined by cutting all plants of a plot 5 cm above the ground with a harvester and weighing the biomass immediately. Biomass yield has nearly reached its maximum at this stage, as demonstrated for *Brassica napus* by Diepenbrock (2000). Dry matter content (DMC, %) was determined after drying a sub-sample of 300 g fresh biomass per plot for 6 days at 60°C in a Memmert ULM 800 drying oven. Dry biomass yield (DBY g m⁻²) was calculated on the basis of DMC and FBY.

The analysis of variance (ANOVA) was performed for each location as lattice using PLABSTAT software (Utz 2001) and the model: $Y_{ijk} = \mu + r_i + g_j + \beta_k + e_{ijk}$. Y_{ijk} was defined as the observation of genotype j in block k and replication i . μ is the overall mean; r_i is the effect of replication i (for $i=1, 2$); g_j is the effect of genotype j (for j =number of genotypes); β_k is the effect of block k (for $k=1, \dots, B$); and e_{ijk} is the error. A combined analysis of variance using the adjusted means of

Table 1 Mean squares from combined analysis of variance of parental cultivars, full-sibs-within cultivars (FS_{within}), full-sibs-between cultivars ($FS_{between}$), and their mixture (FS_{mix}), evaluated in two environments

Source of variation	Df	DTF (days)	DMC (%)	FBY (kg m ⁻²)	DBY (g m ⁻²)
Environment (E)	1	1163.95**	180.60**	2.97**	49783.79**
Genotypes (G)	35	3.23**	1.00	0.09	1548.45
Parental cultivar	2	3.50	2.82	0.22	1533.57
FS_{within}	2	3.36	3.89	0.02	2891.70
$FS_{between}$	26	3.45**	0.78	0.09	1082.43
Between crosses	2	10.44**	1.56	0.28*	1946.39
Within crosses	24	2.87**	0.72	0.07	1010.44
$FS_{between}$ vs. FS_{within}	1	0.57	0.01	0.04	541.10
FS_{mix}	2	4.15+	0.40	0.03	259.01
G×E	35	0.55+	0.71	0.07**	1453.67*
Error	50	0.36	0.48	0.03	829.36

For *DTF*, days to flowering; *DMC*, dry matter content; *FBY*, fresh biomass yield; *DBY*, dry biomass yield. +, *, ** significant at $P=0.10$, $P=0.05$ and $P=0.01$, respectively

environments was performed for separate groups of genotypes (parental cultivars, FS_{within} , $FS_{between}$, FS_{within} plus $FS_{between}$, and FS_{mix}) based on the model: $Y_{ij} = \mu + l_i +$

$g_j + lg_{ij} + e$. Y_{ij} is defined as the observation of means of genotype j in environment i ; l_i defined as the environmental effect (l) at location i (for $i=1, 2$); lg_{ij} is the corresponding

Table 2 Mean values of parental cultivars, full-sibs-within cultivars (FS_{within}), full-sibs-between cultivars ($FS_{between}$), and their mixture (FS_{mix}), as well as the heterosis of the latter two, evaluated at two environments

Groups	n	DTF (days)	DMC (%)	FBY (kg m ⁻²)	DBY (g m ⁻²)	
Cultivars	Parent R	1	246.76	12.38	3.00	369.05
	Parent L	1	249.03	11.58	3.07	344.46
	Parent S	1	246.71	13.92	3.62	399.73
	<i>Parental mean</i>		247.50	12.63	3.23	371.08
FS_{within}	R×R	1	248.75	11.72	3.44	407.96
	L×L	1	248.43	11.59	3.41	383.38
	S×S	1	246.36	14.06	3.24	457.99
	<i>FS_{within} mean</i>		247.85	12.46	3.36	416.45
$FS_{between}$	R×L	9	248.40	12.16	3.59	431.05
	R×S	9	247.17	12.69	3.39	433.76
	L×S	9	247.00	12.64	3.36	414.55
	<i>$FS_{between}$ mean</i>		247.52	12.50	3.45	426.46
FS_{mix}	R×L	1	248.85	12.79	3.51	433.57
	R×S	1	246.37	11.97	3.29	410.82
	L×S	1	246.32	12.05	3.49	422.29
	<i>FS_{mix} mean</i>		247.18	12.27	3.43	422.23
	<i>Overall SE</i>	36	0.60	0.70	0.18	28.80
Heterosis	R×L	9	-0.08	4.09	4.84	9.33
$FS_{between}$ (%)	R×S	9	-0.16	-1.65	1.49	0.82
	L×S	9	-0.16	-1.57	1.09	-1.21
	<i>$FS_{between}$ mean</i>		-0.13	0.29	2.47	2.98
Heterosis	R×L	1	0.10	9.17	2.47	9.67
FS_{mix} (%)	R×S	1	-0.49	-7.10	-1.52	-5.31
	L×S	1	-0.44	-6.27	5.06	0.43
	<i>FS_{mix} mean</i>		-0.27	-1.40	2.01	1.60

For n , number of genotypes per trial; *DTF*, days to flowering; *DMC*, dry matter content; *FBY*, fresh biomass yield; *DBY*, dry biomass yield; *SE*, standard error

interaction effect; and e is the pooled error from lattice experimental analyses at the two locations. The environment was considered as fixed. In the ANOVA, FS_{between} effects were partitioned into ‘between crosses’ with $(3-1)$ degrees of freedom and ‘within crosses’ with $3 \times (9-1)$ degrees of freedom. The FS_{between} vs. FS_{within} effects (Table 1) were calculated based on the sum of squares (SS): $SS_{FS_{\text{between}} \text{ vs } FS_{\text{within}}} = SS_{FS_{\text{within}}} \text{ plus } FS_{\text{between}} - SS_{FS_{\text{within}}} - SS_{FS_{\text{between}}}$.

Heterosis was estimated as mid-parent heterosis and FS_{within} was used as parental value.

For all biomass parameters DMC, FBY and DBY, the combined ANOVA (Table 1) revealed the environment as largest effect and significant genotype \times environment-interactions for beginning of flowering (days to flowering; DTF), DBY and FBY. For the interpretation of the latter, it has to be considered, that the experimental basis is rather small with only two environments.

The parental cultivars Rex, Largo, and Steinacher varied in fresh and dry biomass parameters (Table 2). The eldest cultivar Steinacher showed the highest DMC with 13.92% as parent and 14.06% for FS_{within} $S \times S$, respectively, whereas the more recently released cultivar Largo revealed the lowest DMC and DBY. Significant differences were detected in yield parameter FBY and in flowering time (Table 1) between the three FS_{between} crossing combinations.

The mean performance of FS_{within} , which was developed by randomly crossing individual plants in a population, is expected to be the same as the parental cultivar mean (Falconer and Mackay 1996). FS_{within} produced higher biomass yields than the parental cultivars. This may have been the result of unconscious positive selection for better plants for the production of FS_{within} . Moreover, the seeds of parental lines were not produced in the same environment as FS_{between} , FS_{within} and FS_{mix} . For these reasons, FS_{within} was used instead of the parental cultivars for the estimation of heterosis; otherwise, heterosis might have been overestimated.

The mean values of biomass yield parameters (FBY and DBY) increased in general as expected due to heterosis: $FS_{\text{within}} < FS_{\text{mix}} < FS_{\text{between}}$.

A small and positive mid-parent heterosis was observed for the mean values of the yield traits FBY and DBY for FS_{between} (Table 2). The average mean heterosis effects of FS_{between} were small and the largest effect was observed in FS_{between} crossing combination $R \times L$ with 4.84% for fresh and 9.33% for dry biomass yield (Table 2). Heterosis estimations of individual FS_{between} revealed a maximum of 14.58% for FBY and 19.65% for DBY and a minimum of -7.46% and -12.68% , respectively.

The mean mid-parent heterosis effects of FS_{between} exceeded FS_{mix} in both yield parameters fresh and dry matter yield. The maximum heterosis for fresh biomass

yield was observed in FS_{mix} crossing combination $L \times S$ with 5.06% and for dry matter yield in FS_{mix} crossing combination $R \times L$ with 9.67% (Table 2). Minimum heterosis was negative with -1.52 for fresh and -5.31 for dry matter yield. The small negative heterosis for flowering time (DTF) showed that FS_{between} flowered slightly earlier than the corresponding parental FS_{within} .

The low amount of heterosis in crosses between the three cultivars Rex, Steinacher and Largo, supports earlier results with molecular markers, revealing that most of the genetic diversity is within cultivars (83%) compared to only 17% between cultivars (Ofori et al. 2008). In this experiment, only three cultivars were used, but they represent nearly 50 years of European winter *B. rapa* breeding. They differ completely in seed quality, which is of no importance for biomass use, and show the maximum diversity which can be expected in the European genepool. Other studies with six (Zhao et al. 2009) or ten (Zhao and Becker 1998) cultivars corroborate the low diversity in European winter cultivars and suggest to broaden the diversity by utilizing Chinese genetic resources.

Self-incompatibility (SI) supports cross pollination by rejection of self-pollen. In *B. rapa*, a multiallelic gene complex at the S-locus controls the SI recognition and a number of different S-alleles differing in intensity have been described (Sakamoto and Nishio 2001). In this study, FS_{between} crosses were produced without emasculation by isolation of two plants, assuming that the plants were self-incompatible. Hybridity was confirmed by using seed erucic acid content as a marker. The selection for different S-alleles in parental cultivars could be a basis for future inter-population or inter-varietal crosses, which would increase the level of hybridity and reduce the amount of within cultivar pollinations.

It could be assumed that heterosis in crosses between cultivars (FS_{between} and FS_{mix}) was low because of the low genetic variation between and the high genetic variation within the cultivars. Therefore, it is not very promising to develop populations of synthetic cultivars by combining lines from different European winter cultivars. Increasing the amount of heterosis by including less closely related cultivars or genotypes of the *B. rapa* genepool, such as Chinese genotypes (Zhao and Becker 1998; Zhao et al. 2009), could be an option.

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