

# Heterosis and combining ability in *Brassica rapa* populations for biomass production

Atta Ofori, Heiko C. Becker

Department of Crop Sciences, Georg-August-University Göttingen,  
Von - Siebold - Str. 8, 37075 Göttingen, Germany Email: hbecker1@gwdg.de

## Abstract

Presently there is an increasing interest in Europe to use plant biomass for biogas production. Winter *B. rapa* is a potential biomass crop, mainly because of its high early biomass production and could be used as a pre-crop harvested early in the year for biogas and followed by a second crop. This study was therefore conducted to estimate heterosis and combining ability for biomass yield and other related traits among inter-population full-sibs of winter *B. rapa*. A half-diallel without reciprocals was conducted among 15 populations of winter *B. rapa* after which the parents and their 105 full-sibs (FS) produced were evaluated at two locations for days to flowering (DTF), fresh biomass yield (FBY), dry matter content (DMC), and dry biomass yield (DBY). Analysis of variance (ANOVA) showed significant differences among locations, entries and crosses for all traits except DTF. The expression of the various traits studied seems to be controlled by both general combining ability (GCA) and specific combining ability (SCA). Percent mid parent heterosis (MPH %) and percent high parent heterosis (HPH %) for DBY ranged from -14% to 50% with a mean of 13% and -22% to 45% with a mean of 3%, respectively. From all 105 FS, 81% showed positive MPH% and 56% showed positive HPH% for DBY. Significant variation in heterosis over locations and crosses was observed for MPH% and HPH% for most traits.

**Key words:** *Brassica rapa*, heterosis, biomass, biogas, general combining ability, specific combining ability

## Introduction

*Brassica rapa* is being cultivated for centuries in different parts of the world as oilseed, leafy vegetables or fodder based on its morphological characteristics. In northern Spain, it is used as winter fresh fodders where it is left either to be grazed *in situ* by foddred sheep or harvested to feed cattle in winter fold yards (Padilla et al. 2005). Other characteristics of *B. rapa* are the wide genetic variation between and within cultivars (Zhao and Becker 1998; Qian et al. 2003), fast growth and early maturity with less nutrient demand (Bradshaw et al. 2002). Recently, biomass production has received much attention as energy source in most parts of the world for biogas production and other applications. Breeding of high yielding, pest and disease resistant biomass crops has therefore been accelerated and in Europe. Biomass from poplar, miscanthus, willow, reed canary grass and some energy grains are highly used for energy production (Venendaal 1998). In developing countries and to be specifically Africa, it has been estimated that biomass constituted about 60% of total energy used in 1995 (IEA 1998). Winter *B. rapa* develops high early biomass which can be harvested earlier in the year for biogas production and then followed by a second crop. As at present, no breeding investigation has been conducted on the early biomass yield potential of *B. rapa*. The need to generate information on genetic variation, biomass yield, combining ability and heterosis of *B. rapa* is necessary for its application as biomass crop. This study therefore determines the general combining ability (GCA) and specific combining ability (SCA) and heterosis in a 15×15 diallel set in *B. rapa* populations under two locations to identify best parental combinations for biomass yield.

## Materials and methods

The plant materials used in this experiment consisted of 15 *B. rapa* populations. They are of European origin and obtained from different genebanks in Germany, Sweden and Czechoslovakia (Table 1). The populations represent a large range of materials from different geographical regions. Seed samples from each population were analysed with flow cytometer to confirm their diploid level.

The 15 parental populations were crossed during April 2005 in a 15×15 half-diallel without reciprocals by producing full-sibs (FS). Three FS were produced from each parental combination. Crossings were done by isolating two plants between parental populations with one pollination bags, at the beginning of flowering. After harvesting and threshing, equal amount of seeds from each of the three FS seeds were bulked to form 105 cross combinations. In August 2005, the 105 FS bulks plus the 15 parental populations and a control 'Perko', totalling 121 entries were sown. Sowing was done at a rate of 1000 seeds plot<sup>-1</sup> and plot sizes were 11.25 m<sup>2</sup>. The experimental design was an 11×11 lattice with two replications at two locations, Göttingen and Einbeck in central Germany. Each plot consisted of 6 rows plots, at a row length of 7.5 m and 0.25 m row distance. Data were recorded on days to flowering (from the day of sowing until 50% of plants were flowering), fresh biomass yield (kg/m<sup>2</sup>), dry matter content (%), and dry biomass yield (g/m<sup>2</sup>). Plots were harvested on 10th May 2006, 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°C for 6 days to determine the dry matter content. Based on this, total dry biomass yield per plot was calculated.

**Table 1. *Brassica rapa* populations used in this study and the country of origin**

PG No.	Populations	Country	PG No.	Populations	Country
1	Rex	Germany	9	Orbit	Sweden
2	Largo	Sweden	10	Schneiders Sprengel	Germany
3	Steinacher	Germany	11	Hege's Winter	Germany
4	Weibulls Storybys	Sweden	12	Janetzki's	Germany
5	BRA 245	Bulgaria	13	Opava	Czechoslovakia
6	Lemkes Winter	Germany	14	Grubers Winter	Germany
7	Lemkes Malchow	Germany	15	Rübsen (T. Gladis Bonn)	Germany
8	Arktus	Germany			

### Statistical analysis

Separate variance analyses for all four traits were run for each location using PLABSTAT software (Utz 2001). A combined analysis of variance using adjusted means obtained from each location was then computed. A random effects model was assumed for the analysis. The sum of squares for entry effects were then partitioned into parents, FS and parents vs. FS effects. The sum of squares for parents vs FS effects were calculated as follows;  $SS_{pvfs} = SSE - SSp - SSfs$ ; where  $SS_{pvfs}$  is the sum of squares for parents vs FS,  $SSE$  is the sum of squares for entries,  $SSp$  is the sum of squares for parents and  $SSfs$  is the sum of squares for FS. Effects of FS were further divided into GCA and SCA, according to analyses III of Gardner and Eberhart (1966). Heterosis increase as suggested for populations Frisch (2006), were estimated for percent mid-parent heterosis (MPH %) and percent high-parent heterosis (HPH %). The following formulae were used for the estimation in each location for all traits,  $MPH\% = (FS - MP)/MP100$  and  $HPH\% = (FS - HP)/HP100$ , where MP is mid-parent and HP is high parent.

### Results

Generally, the FS performed better than the parents for all traits as shown by their mean values over locations (Table 2). Full-sibs were higher than the parent by 11%, 0.9% and 11% for DBY, DMC and FBY, respectively. Variation across locations was also higher in the FS than in the parents depicted by the differences between minimum and maximum trait values. Flowering was however late in the parents in comparison with FS. The mean values at the individual locations differed for both parents and FS for all traits. Means of DBY, FBY and DTF were higher in Einbeck (448.82 g/m<sup>2</sup>, 3.99 kg/m<sup>2</sup> and 252.79 days respectively) for FS while DMC and DBY were higher in Göttingen (13.91 % and 445.05 g/m<sup>2</sup> respectively) for parents.

**Table 2. Minimum, maximum, mean over locations, least significant difference (LSD) and mean at individual locations for 4 traits of the 15 parental populations and their 105 diallel crosses grown in Germany.**

Traits	Parents					Mean at Einbeck	Crosses					
	Min.	Max.	Mean over locations	LSD %	Mean at Göttingen		Min	Max	Mean over locations	LSD %	Mean at Göttingen	Mean at Einbeck
DTF (days)	246.39	250.41	248.29	2.45	243.57	252.00	244.74	251.09	247.86	2.76	242.95	252.79
DMC (%)	11.48	13.82	12.48	1.62	13.91	11.05	10.76	12.59	12.59	1.33	14.14	11.04
FBY Kg/m <sup>2</sup>	2.49	3.95	3.31	1.17	3.19	3.43	2.66	3.68	3.68	0.69	3.36	3.99
DBY g/m <sup>2</sup>	332.54	491.19	414.90	142.50	445.04	384.76	364.04	461.34	461.34	92.14	473.85	448.82

The combined ANOVA showed highly significant variance for all traits between the two locations ( $P = 0.01$ ) (Table 3). Significant differences among entries were also observed for all traits except DTF. Separation of entries into parents, parents vs. FS and FS indicated no significant variation for the parents and the parents vs. FS effects. Nevertheless, DMC and FBY were highly significant for FS effect. Partition of FS into GCA and SCA showed significant GCA for only DMC while SCA showed significant differences for DBY ( $P = 0.1$ ), DMC ( $P = 0.05$ ) and FBY ( $P = 0.01$ ). With the exception of SCA, all the different sources (entries, parents, FS and GCA) significantly interacted with the environment for DBY. However, no environment $\times$ interaction effects was observed for DMC, indicating the stability of the trait across locations.

Both MPH% and HPH% varied among the FS and for the different traits (Table 4). Maximum MPH% and HPH% for DBY, FBY, DMC and DTF over mid-parent and high parent were 49.93%, 44.44%, 19.375 and 1.22%, and 45.13%, 38.74%, 14.73% and 1.01% respectively. The average heterosis was however, negative for DTF in both MPH% and HPH%, and for DMC in HPH%. Negative mean value for DTF is desirable as it expresses the earliness of FS over parental populations. The average effects of heterosis for DTF and DMC were however very low when compared with FBY and DBY which were about 12% and 3%, respectively for MPH% and HPH%.

**Table 3. Mean squares from combined analysis of variance of parents and diallel FS among 15 *B. rapa* accessions**

Source	df	DTF	DMC	FBY	DBY
Location (L)	1	5741.05**	565.52**	20.16**	51976.02**
Entries	119	2.23	11.1**	0.29**	350.72*
Parents	14	2.39	0.84	0.40	5324.62
Parent vs Crosses	1	4.69	0.33	3.55	56612.80
Crosses	104	2.18	1.15**	0.25**	2754.80
GCA	14	4.39	3.91**	0.39	3498.53
SCA	90	1.81	0.72*	0.23**	2638.83+
Entries×L	119	1.86**	0.46	0.14	2474.82**
Parent×L	14	1.30	0.57	0.29**	4414.45**
Parent vs Crosses×L	1	1.01	0.38	1.04**	8156.35*
FS×L	104	1.94**	0.44	0.12	2159.35*
GCA×L	14	3.30*	0.45	0.13	3777.12*
SCA×L	90	1.72**	0.45	0.12	1907.18
Error	198	1.05	0.42	0.13	1816.53

\*, \*\* and + significantly different from zero at  $P = 0.01$ ,  $P = 0.05$  and  $P = 0.1$  probability levels, respectively.

**Table 4. Minimum, maximum, mean and number of Crosses showing positive heterosis for MPH% and HPH%.**

Traits	MPH%				Min	HPH%			
	Min	Max	Mean	NH		Max	Mean	NH	
DTF	-1.31	1.22	-0.14	38	-1.59	1.01	-0.41	14	
DMC	-16.54	19.37	0.92	54	-18.04	14.73	-2.64	33	
FBY	-16.64	44.44	12.31	85	-30.45	38.74	2.71	60	
DBY	-14.21	49.93	12.96	85	-21.92	45.13	3.04	60	

NH - Number of FS showing positive heterosis

Analysis of variance for MPH% and HPH% showed the level of variation for location, crosses and their interaction (Table 5). Significant variation in heterosis over locations was observed for MPH% and HPH% for all traits except FBY and DBY. Full-sibs also recorded significance for all levels of heterosis for all other traits except DTF.

**Table 5. Mean squares of analysis of variance for MPH% and HPH%.**

Source	df	MPH%				HPH%			
		DTF	DMC	FBY	DBY	DTF	DMC	FBY	DBY
Location (L)	1	1.63*	140.53+	9453**	7816**	3.23**	462**	907*	772+
Crosses (C)	104	0.41	69.22*	408**	362+	0.49	71*	333*	286+
C×L	104	0.38*	48.43	250*	263**	0.45+	49	212	218
Error	198	0.25	40.45	164.8	158.9	0.34	55.9	187.3	181.6

\*, \*\* and + significantly different from zero at  $P = 0.01$ ,  $P = 0.05$  and  $P = 0.1$  probability levels, respectively.

## Discussions

The results from our studies showed that mean of the inter-population FS performed better than the mean of parental populations for all traits except DTF indicating the presence of heterosis (Table 2). Individual locations for parents as well as FS for all traits gave different mean values explaining the effects of environment location interaction and ANOVA was highly significance for all traits over locations. Significant GCA variances were lower than SCA variances indicating the predominance of non-additive gene action. However, the magnitudes of GCA mean squares were higher than SCA for all traits explaining the importance of additive effects. According to Melchinger and Gumber (1998), SCA largely contributes to variance of traits for parents derived from diverse background and this is because their gene pools consist of different heterotic groups. The mean MPH% of 13% for DBY that we observed in our study is in the range 25% observed in *B. rapa* biomass yield after six generations of population improvement by half-sib family selection (Bradshaw 2002). However, this is lower than MPH% of about 30% in trigonomic hybrids of *B. napus* and *B. rapa* observed by Liu et al. (2002) that were derived from crossing inbred lines. After observing 50% less MPH% for seed yield in inter-population  $F_1$ s when compared with inbred parent derived  $F_1$ s in *B. carinata*, Teklewold and Becker (2005) concluded that populations used as parents already utilize a considerable level of heterozygosity. Schuler et al. (1992) also reported 18% MPH% for seed yield in inter-cultivar  $F_1$ s of *B. rapa*. In our study there were significant ( $P = 0.01$ ) correlations of 0.73 between MPH% and FS and -0.57 between MPH% and parents. Also in other studies it was observed that the higher the parental value due to heterozygosity of their genetic makeup the lower the heterosis (Becker et al. 1998; Teklewold and Becker 2005). In summary, the 13% mean MPH% for DBY is quite considerable. Nevertheless, with the identification of best parental combinations we expect to develop FS with higher level of biomass in the coming years.

## Acknowledgements

This project is being financially supported by KWS, Einbeck, Germany. Many thank also goes to Gerald Miotke and colleagues for taking care of the field activities and Friedrich Kopisch-Obuch for his diverse suggestions and corrections.

## References

- Becker HC, Svensk H and Engqvist GM (1998) Chances and limitations for the use of heterosis and synthetic cultivars of rapeseed. GCIRC Bulletin 15: 51-57
- Bradshaw JE, Gemmel DJ Gowers S and Wilson RN (2002) Turnip (*Brassica rapa* L. ssp. *Rapifera* Metzg.) population improvement and cultivar production. Plant Breeding 121: 301-306
- Frisch M (2006) Experimental designs for plant breeding trials. <http://www.uni-hohenheim.de/frisch>
- Gardner CO and Eberhart SA (1966) Analysis and interpretation of the variety cross diallel and related populations. Biometrics 22: 439-4452
- IEA (1998) The environmental implications of renewables. Organization of Economic Co-operation and Development. International Energy Agency. Paris 1-45
- Liu R, Qian W and Meng J (2002) Association of RFLP markers and biomass heterosis in trigenomic hybrids of oilseed rape (*Brassica napus* × *B. campestris*) Theor Appl Genet 105:1050-1057
- Melchinger AE and Gumber RK (1998) Overview of heterosis and heterotic groups in agronomic crops. In: Lamkey KR and Staub JE (ed) Concepts and breeding of heterosis in crop plants. CSSA Special publication, vol. 25 CSSA, Madison, pp 29-44
- Padilla G, Cartea ME, Rodriguez VM and Ordas A (2005) Genetic diversity in a germplasm collection of *Brassica rapa* subsp *rapa* L. from northern Spain. Euphytica 145: 171-180
- Qian W, Liu R and Meng J (2003) Genetic effects on biomass yield in interspecific hybrids between *Brassica napus* and *B. rapa*. Euphytica 134: 9-15
- Schuler TJ, Hutcheson DS and Downey RK (1992) Heterosis in inter-varietal hybrids of summer turnip rape in Western Canada. Can J Plant Sci 72: 127-136
- Teklewold A and Becker HC (2005) Heterosis and combining ability in diallel cross of Ethiopian mustard inbred lines. Crop Science 45: 2629-2635
- Utz HF (2001) PLABSTAT: A computer program for the statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science and Population genetics, University of Hohenheim, Stuttgart, Germany
- Venendaal R (1998) European energy crops overview. Utilisation for power and heat generation and for the production of transport fuels. In James and James (ed) Sustainable agriculture for food, energy and industry, pp737-742
- Zhao J and Becker HC (1998) Genetic variation in Chinese and European oilseed rape (*B. napus*) and turnip rape (*B. campestris*) analysed with isozymes. Acta Agron Sinica 24:213-220