Estimation of heterosis for yield-related traits for single cross and three-way cross hybrids of oilseed rape (*Brassica napus* L.)

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Abstract Rapeseed breeding programs are focused mainly on improving plant potential and seed yield. One of the ways to improve seed yield in oilseed rape is heterosis, which is hybrid vigor that results in a greater biomass, increased seed yield, and faster development. The purpose of this study was to estimate yield-related trait heterosis for single cross and three-way cross hybrids of winter oilseed rape (*Brassica napus* L.). A population of 60 doubled-haploid (DH) lines and two generations of hybrids were evaluated in field trials to assess six yield-related traits: plant height, number of branches per plant, number of siliques per plant, silique length, number of seeds per silique, and thousand seed weight. Heterosis effects for each trait were estimated by a comparison of the particular hybrid with: (1) the trait mean over both parents—mid-parent heterosis and (2) to the value of better parent—best-parent heterosis. Traits with clear positive heterosis and traits with varied heterosis were observed in this study. For the first group, we include plant height, silique length, and the number of seeds per silique—a large number of hybrids expressed significant positive heterosis for these traits, independent from the year of observations and the type of hybrid. For the second group, with varied heterosis, we can include the number of branches and siliques per plant and thousand seed weight. For these traits, hybrids exhibited both positive and negative significant heterosis, without a clear pattern for the years and types of hybrids.

Keywords Heterosis · Yield-related traits · Oilseed rape · Doubled haploids · Hybrids

Abbreviations
CMS   Cytoplasmic male sterility
CV   Coefficient of variation
GCA   General combining ability
GD   Genetic distance
DH   Doubled haploid
SCA   Specific combining ability
TSW   Thousand seed weight

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Introduction

Over the last 30 years, due to quality breeding, oilseed rape (*Brassica napus* L.) has become a very important food crop of increasing significance in the international market. Currently, the production of rapeseed

ranks second place among oilseeds in the world, after soybean. The European Union countries produce the most rapeseed, with about one-third of the global production of rapeseed. Poland, depending on the year, ranks third or fourth place among the largest producers of rapeseed in Europe (in the 2017/2018 season—2.2 million tons). Nowadays in Poland, only double low (00-quality) varieties of oilseed rape are cultivated (zero-erucic and low glucosinolate content) (Bocianowski et al. 2019). Rapeseed oil is used in the production of margarine and cooking oil, due to its superior nutritional qualities, which include a low saturated fat content and a high level of monosaturated fat and omega-3 fatty acids. Rapeseed is also a source of oil extraction meal (Friedt and Snowdon 2009), which is used as animal feed, due to its high level of protein. Oilseed rape seeds are processed not only for food and feed, but they are also used for the production of biofuels. Therefore, the demand for them is still growing, and breeding programs are focused mainly on improving plant potential and seed yield.

One of the ways to improve seed yield in oilseed rape is heterosis (Wolko 2012). Heterosis or hybrid vigor is a phenomenon in which the offspring (F1) of genetically diverse individuals of a given species or resulting from interspecific cross-breeding exhibit greater biomass, increased seed yield, faster development, better tolerance to stress conditions, and a higher resistance to disease than both parents (Fujimoto et al. 2018). A study on heterosis was initiated by Darwin (1876), who observed that the offspring resulting from the cross-pollination of maize were 25% taller than the offspring of inbred maze lines. At the beginning of the 20th century, this phenomenon was independently rediscovered in maize by Shull (1908) and East (1908). In the last 50 years, the use of heterosis in the breeding of many plants has significantly increased, and thus, this has increased the production and yield of hybrid varieties. For example, in maize and sugar beet, 100% of varieties registered in The Polish National List are hybrids. Among the winter oilseed rape varieties on this list, hybrids represent a significant majority. Heterosis has also a large degree of participation in the cultivation of crops such as rye, sunflower, and sorghum. The highest level of heterosis is observed for the most complex trait, seed yield. Positive mid-parent heterosis for seed yield in *Brassica napus* was reported by many researchers (Azizinia 2011; Radoev et al. 2008; Qian et al. 2007; Shen

et al. 2005). In winter oilseed rape hybrids, seed yield is about 30% higher, compared with open-pollinated cultivars (Brandt et al. 2007). McVetty (1995) reviewed that for winter rapeseed hybrids, high parent heterosis was on average 50%, with a range of 20–80%.

Oilseed rape is self-fertile, partially wind pollinated, and it produces large amounts of pollen, transferred by a wide range of insects. There are several systems for controlling cross-pollination in oilseed rape. The most widespread is based on cytoplasmic male sterility (CMS) *ogura* hybridization system, which is characterized by the complete stability of sterile male cytoplasm and the *Rfo* restorer gene from *Raphanus sativus* L. (Bartkowiak-Broda et al. 1979; Brown et al. 2003; Szała et al. 2016).

Although heterosis increases the yield of seeds in many crops and vegetables, and it has been intensively used in plant breeding, the molecular and genetic mechanisms underlying this phenomenon are not well understood (Birchler et al. 2010). Many studies have been conducted to broaden the knowledge on this effect, so that heterosis can be used more effectively and more consciously. Researchers have suggested three main hypotheses in order to explain the genetic basis of heterosis: dominance, overdominance, and epistasis. In the dominance model (Jones 1917), the interaction and complementation of superior dominant alleles of both parental inbred lines takes place. The opposite hypothesis is overdominance (Crow 1948), where heterozygous individuals have more strongly expressed phenotype traits than any of the homozygous individuals. This is one of the mechanisms that maintains polymorphism in the population. Epistasis (Goodnight 1999; Bocianowski 2012; Bocianowski and Nowosad 2015) is the interaction of non-allelic genes. One gene masks the expression of another, and several pairs of alleles determine a single feature in hybrids. These classical hypotheses were studied, and their connection with heterosis was observed by many researchers with different results. The diversity of these findings indicates that the heterosis phenomenon has complex causes. For example, Liu et al. (2017), on the basis of phenotypic analyses, quantitative trait loci mapping, and genome-wide predictions, stated that a mix of dominance, overdominance, and epistasis affects the heterosis effect. These classical hypotheses for heterosis have provided guidance for experimentation, but they were formed before the molecular

concept, and therefore there are limits in describing the genetic parameters that explain heterosis (Birchler et al. 2003).

The identification of parental combinations that provide high heterosis for yield is the most important factor in hybrid development (Zhao et al. 2015). This is possible thanks to genome-wide prediction, where many molecular markers are used. Their effects are estimated in populations that have been genotyped and phenotyped. These identified markers can be applied to predict the performance of hybrids that have not yet been phenotyped, based on their molecular marker profiles (Bocianowski et al. 2011; Kozak et al. 2011).

The results of polymorphism studies can be useful in defining the genetic distance (GD) and its correlation with heterosis. According to the theory of heterosis in *Brassica napus* (Ali et al. 1995; Teklewold and Becker 2006), an increase in heterosis was observed with increasing parental distance (Ahmad et al. 2011). Therefore, genetic distance can be helpful for predicting the performance and heterosis of hybrids in oilseed rape (Yu et al. 2005). In contrast, Diers et al. (1996) reported weak correlations between hybrid productivity and genetic distance in *B. napus* L. They suggested that GD estimated alone does not identify high-yielding combinations, but in connection with general and specific combining ability effects (GCA and SCA), it creates a tool for the identification the potential of inbred lines in hybrid combinations. Researchers such as Rameeh (2012) or Shehzad et al. (2015) also indicated GCA and SCA to be useful in rapeseed breeding programs for hybrid- and open-pollinated varieties.

The purpose of this study was to estimate yield-related traits heterosis for single cross and three-way cross hybrids of winter oilseed rape (*Brassica napus* L.). The plant material is constructed in such way that the observation of the heterosis effect in two generations of hybrids is possible.

Materials and methods

A population of 60 doubled haploid (DH) lines and two generations of hybrids (single cross and three-way cross) were evaluated in field trials. DH lines were derived from single cross between recombinant inbred lines RIL 324/2 (high oleic acid content: 77.9%), as a female parent, and RIL 622/3 (high oil content: 51.9% and high seed yield), as a male parent. DH lines were developed from F1 hybrids using isolated microspore cultures method, according to the procedure described by Cegielska-Taras et al. (2002). Single cross hybrids were created by crossing these 60 DH lines with the CMS *ogura* line, and three-way cross hybrids—by crossing the obtained male-sterile single hybrids with the restorer (*Rfo*) line. CMS and *Rfo* lines were selected based on large genetic distance in relation to DH lines. Therefore, the whole plant material used in the study included 182 objects: 60 DH lines, 60 single cross hybrids (CMS × DH), 60 three-way cross hybrids (CMS/DH × *Rfo*), the CMS *ogura* line, and the *Rfo* line.

Field experiments including all tested objects were placed in Borowo (52°70′N, 16°46′E), Plant Breeding Strzelce Ltd., Co. – PBAI-NRI Group, and they were conducted in two growing seasons, 2014/15 and 2015/16. The field experiment was conducted on grey-brown podzolic soil and the soil texture was sandy loam. Borowo had an average daily temperature of 9.8 °C in first growing season (08.2014–07.2015) and 10.3 °C in second growing season (08.2015–07.2016), when a many years background was 8.4 °C. The sum of precipitation was 399.2 mm in the first growing season and 627.7 mm in the second, while in a many years background it was 533.7 mm. The experiment had a randomized block design with three replications and two randomly distributed standards: Monolit (open pollinated) and Arsenal (hybrid) varieties. These varieties are routinely used in Poland as a standards by Research Centre for Cultivar Testing (COBORU). The entire experiment consisted of 594 plots (182 objects + standards), each of them containing four rows with a length of 2 m. The distance between the rows was 30 cm. The field management followed standard agricultural practice. Yield-related traits evaluated in the field were: plant height, number of branches per plant, number of siliques per plant, silique length, number of seeds per silique, and thousand seed weight. Plant height was measured on three randomly selected plants from each plot after the end of flowering time. The number of branches per plant and the number of siliques per plant were estimated on three well-developed, randomly selected plants from each plot at the green siliques stage. Silique length and the number of seeds per silique were estimated from 25 siliques from each plot; siliques were collected at the mature seeds stage from

the main branch, and then dried. The thousand seed weight was estimated from the average of three measurements from the mixed seeds of plants in a plot. The phenotypic variation of yield-related traits in the first year of field trials was described by Dobrzycka et al. (2016), and the genetic parameters of DH lines by Bocianowski et al. (2017).

The results of the phenotypic observations have been subjected to statistical analysis. Firstly, the normality of the distribution of the observed traits was tested, using the Shapiro–Wilk normality test. A two-way analysis of variance (ANOVA) was carried out to determine the effects of years and genotypes, as well as the years-by-genotypes interaction on the variability of the plant height, the number of branches per plant, the number of siliques per plant, silique length, number of seeds per silique, and thousand seed weight. The mean values and coefficients of variation for each observed trait were calculated in particular years. The relationships between the observed traits were assessed on the basis of the correlation coefficients. Heterosis effects for each trait were estimated by a comparison of the particular hybrid with: (1) the trait mean over both parents for single cross hybrids, and over two direct (CMS × DH and Rfo line) or three indirect (DH, CMS and Rfo lines) parents for three-way cross hybrids—mid-parent heterosis and (2) to the value of better parent—best-parent heterosis. Data analysis was carried out by using the statistical package GenStat 18.

In this article terms "positive" and "negative" heterosis are used, meaning that the trait value is increased/decreased compared to the mean of the parents or to the better parent. The use of the term "cheterosis" is not synonymous with the assessment of the suitability of a given trait in breeding.

Results

Variability of the tested genotypes

An analysis of variance indicated that the main effects of year and genotype, as well as the year-by-genotype interaction, were significant for all of the traits of study (Table 1).

Since heterosis was expressed as a value, not as a percentage, Table 2 presents the means and coefficients of variation (CVs) for the observed traits in the

particular years. Plant height was a fairly stable trait, with CVs ranging from 5.03 to 8.37% in the first year, and from 6.58 to 9.83% in the second year of field trials. For this feature, the difference in the values between the parental lines and the hybrids was clearly visible. In 2015, the average values for CMS, DH, and Rfo lines ranged from 144 to 146.8 cm, and for single cross and three-way cross hybrids, from 153.8 to 163.3 cm. In 2016, it was 140.2–151.4 and 160–161.6 cm, respectively. For standard varieties (Arsenal and Monolit) mean plant height oscillated around 156 cm, regardless from year of observations.

A more variable trait was the number of branches per plant, where in the first year, the CV in three parental lines ranged from 19.45 to 29.26%, in hybrids from 25.28 to 31.22%, and in standards from 23.24 to 28.93%. In the second year of observations, it ranged from 24.52 to 26.51% and from 23.36 to 26.22%, respectively. The means for this trait were similar for parental lines and hybrids (9.80–10.67 in 2015, and 8.04–10.30 in 2016) (Table 2). For Arsenal variety, mean values for number of branches per plant were almost the same in both years (9.32 and 9.35, respectively). For Monolit, these values were 9.56 and 8.5, dependently on year.

The most diverse among the studied traits was the number of siliques per plant. The coefficients of variation were high—from 33.50 to 53.68% in the first year, and in the second year, they reached even higher values, from 48.60 to 64.87%. The mean values for this trait in 2015 were at a similar level in the CMS line (485.1), DH lines (434.8), and single cross hybrids (465.3). The means for the Rfo line (574.0) and the three-way cross hybrids (507.7) were significantly higher. This regularity was not noticed in 2016, when high diversity between the parental lines (342.0–656.8) resulted in average values of this trait in hybrids (approximately 450) (Table 2). The average number of siliques per plant in standard varieties was slightly lower and ranged from 328.2 to 448 in both years.

For silique length, the next observed trait, CVs were at a medium level, and slightly lower in the first year, ranging from 12.38 to 15.93%, and in the second year, from 16.25 to 20.66%. The standards had a bit lower variability, from 11.22 to 13.08% in 2015 and from 15.05 to 15.17% in 2016. In both years of trials, the average values for CMS and DH lines and for CMS × DH hybrids were at similar levels

Table 1 Mean squares (m.s.) from two-way analysis of variance for observed phenotypic traits

Source of variation	Plant height (cm)		No. of branches per plant		No. of siliques per plant		Silique length (mm)		No. of seeds per silique		Thousand seed weight (g)	
	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.
Year	1	2649.6***	1	430.0***	1	389,976**	1	4,290,545***	1	1115***	1	527.9***
Genotype	183	1383.5***	183	19.3***	183	139,220***	203	1879.84***	203	520***	183	1.899***
Year × genotype	183	264.93***	183	14.3***	183	106,431***	203	435.68***	203	222***	183	0.563***
Residual	3196	98.79	3196	5.829	3196	53,959	23,352	70.38	23,352	30.9	3196	0.111

$**P < 0.01$; $***P < 0.001$

(59.29–62.21 mm in 2015 and 51.37–52.97 mm in 2016). The mean for the *Rfo* line in both years was much lower than for the other genotypes (43.25 and 37.05 mm in 2015 and 2016, respectively), but it did not cause a significant decrease of this trait value in the CMS/DH × *Rfo* hybrids (58.37 and 49.95 mm). Both standard varieties had longer siliques in the first year of trials (Arsenal 68.7, Monolit 64.14 mm), and shorter in the second year (55.67 and 59.58 mm, respectively) (Table 2).

The number of seeds per silique is a trait with quite a high degree of variation in the studied objects (the CVs ranged from 31.60 to 42.84% over 2 years of studies), and slightly lower in standard varieties (from 24.47 to 32.41% in both years). In both 2015 and 2016, the following relation was observed—the means for the CMS and DH lines were at a similar level (14.80–15.84), and for the *Rfo* line, they were much lower (11.47 and 13.82), while for the CMS × DH and CMS/DH × *Rfo* hybrids, they reached the highest values (16.33–17.84). The mean values for this trait in Arsenal and Monolit varieties were higher (20.36–24.18 and 18.91–21.72, respectively) (Table 2).

The final discussed trait, the thousand seed weight, had rather low coefficients of variation. The lowest values were calculated in both years for the CMS line (4.50 and 4.29%, respectively), and the highest values were calculated for the DH and *Rfo* lines (from 9.06 to 12.11%). Both groups of hybrids had average CVs (6.14–7.67%). Arsenal variety had lower coefficient of variation than Monolit (4.73–6.97% and 7–7.55%, respectively). The mean values for this trait did not differ significantly between the studied genotypes, and they ranged from 4.11–5.41 g in 2015, and from 3.64 to 4.76 g in 2016. Both standard varieties had higher values in the first year of observations (5.02–5.12 g) than in the second year (4.19–4.41 g) (Table 2).

The correlations between six observed traits in both growing seasons shows Table 3. Highly significant positive correlations repeated in both years occurred between: plant height and number of siliques per plant, plant height and number of seeds per silique, number of branches and number of siliques per plant, silique length and number of seeds per silique, silique length and thousand seed weight. Additionally, in 2014/2015 two more negative correlations were found: between plant height and thousand seed weight, and between number of seed per silique and thousand seed weight.

Table 2 Mean values and coefficients of variation (CV) for six traits in 2 years of observations

Trait	Year		Genotype						
			CMS	DH	*Rfo*	CMS × DH	CMS/DH × *Rfo*	Arsenal	Monolit
Plant height (cm)	2015	Mean	144.9	146.8	144.0	153.8	163.3	156.1	155.1
		CV	5.03	8.37	5.68	6.74	6.74	7.23	5.86
	2016	Mean	151.4	147.4	140.2	160.0	161.6	157.5	157.8
		CV	8.24	8.07	9.83	7.09	7.28	9.05	6.58
No. of branches per plant	2015	Mean	10.41	10.17	10.67	9.80	10.06	9.32	9.56
		CV	19.45	29.26	24.25	25.28	31.22	27.33	28.93
	2016	Mean	8.04	9.96	10.30	9.06	8.95	9.35	8.50
		CV	24.52	26.11	26.51	23.36	26.22	23.24	26.44
No. of siliques per plant	2015	Mean	485.1	434.8	574.0	465.3	507.7	390.3	385.3
		CV	33.50	49.86	41.31	44.10	53.14	53.68	47.94
	2016	Mean	342.0	444.5	656.8	445.3	451.9	448.00	328.2
		CV	48.60	59.98	57.69	55.38	58.13	64.87	58.62
Silique length (mm)	2015	Mean	60.80	59.29	43.25	62.21	58.37	68.7	64.14
		CV	15.85	15.93	12.38	15.22	13.08	11.22	13.08
	2016	Mean	52.89	51.37	37.05	52.97	49.95	55.67	59.58
		CV	17.23	17.91	20.66	19.22	16.25	15.17	15.05
No. of seeds per silique	2015	Mean	15.73	14.80	13.82	16.59	17.84	24.18	18.91
		CV	35.29	42.84	38.02	32.96	31.60	24.47	32.41
	2016	Mean	15.84	15.30	11.47	16.33	16.38	20.36	21.72
		CV	32.70	39.47	41.65	36.95	32.90	28.82	30.03
Thousand seed weight (g)	2015	Mean	5.41	5.28	4.11	5.40	4.86	5.02	5.12
		CV	4.50	10.25	9.06	7.67	6.93	6.97	7.55
	2016	Mean	4.76	4.45	3.64	4.68	4.07	4.19	4.41
		CV	4.29	11.03	12.11	6.47	6.14	4.73	7.00

Table 3 Matrix of correlations for investigated traits in 2 years of observations

Trait	Plant height	No. of branches per plant	No. of siliques per plant	Silique length	No. of seeds per silique	Thousand seed weight
Plant height	1	− 0.015	0.094***	0.019	0.105***	− 0.124***
No. of branches per plant	0.080***	1	0.765***	0.015	0.006	0.016
No. of siliques per plant	0.209***	0.696***	1	0.035	0.018	− 0.040
Silique length	0.080***	− 0.021	− 0.012	1	0.211***	0.134***
No. of seeds per silique	0.151***	− 0.046	− 0.003	0.818***	1	− 0.170***
Thousand seed weight	− 0.014	− 0.092***	− 0.112***	0.089***	− 0.025	1

Above diagonal—season 2014/15, below—season 2015/16

***$P < 0.001$

In 2015/16 only, following correlations were noted: positive between plant height and number of branches per plant, and between plant height and silique length; negative between number of branches per plant and thousand seed weight, and between number of seeds per silique and thousand seed weight.

Heterosis

For plant height, the positive best- and mid-parent heterosis effects were the highest for three-way cross hybrids, regardless of how they were calculated. Best-parent heterosis for these hybrids oscillated between 0.2 to 28.4 cm, while mid-parent—between 5.6 and 28.6 cm (in both years). For single cross hybrids, it ranged from 0.1 to 18.9 and from 5 to 18.6 cm, respectively (Tables 4, 5). Also histograms show, that three-way cross hybrids demonstrated higher values of mid-parent heterosis than single cross hybrids (Fig. 1a). Additionally, for CMS/DH × *Rfo* hybrids, a greater number of observations were concentrated in the area of high heterosis values. Almost all of the hybrids displayed a significant positive mid-parent heterosis effect for this trait, and most of them repeated this effect in both growing seasons (Fig. 2a). Negative best-parent heterosis values were between − 0.1 and − 19.4 cm for single cross hybrids and between − 0.1 and − 7.9 cm for three-way cross hybrids (in both years). The negative mid-parent heterosis occurred only for one CMS × DH hybrid (− 5.7 cm in the first year, − 8.5 cm in the second year) (Table 5). Interestingly, a lot of hybrids showed a positive best-parent heterosis effect for this trait (but slightly fewer than a mid-parent, data not shown).

Considering the best-parent heterosis of the number of branches per plant, a greater positive effect was found in the first year of observations for the CMS/DH × *Rfo* hybrids (0.11–5.18), and in the second—for CMS × DH hybrids (0.51–2.1) (Table 4). Similar relationship between years and groups of hybrids was observed for mid-parent heterosis—it was higher in the first year for three-way cross hybrids (1.37–6.21), while in the second year—for the CMS × DH hybrids (from 1.08 to 3.29) (Table 5). Negative heterosis reached similar values for all hybrids, ranged from − 0.04 to − 3.09 (best-parent heterosis) and from − 1.04 to − 3.52 (mid-parent heterosis) (Tables 4, 5). For this trait, the vast majority of mid-parent heterosis values were close to zero, which means that they did not show significant (either positive or negative) effects (Fig. 1b). If heterosis was statistically significant, more hybrids showed a negative effect, but only a few of them repeated this effect in both years of observations (Fig. 2b). More genotypes demonstrated negative heterosis, when calculated to the best-parent than to the mid-parent (data not shown).

For the number of siliques per plant, best-parent heterosis for CMS × DH hybrids was higher in the second growing season and reached from 21 to 332, while in three-way cross hybrids it was higher in the first season (from 5 to 303) (Table 4). The positive effect of mid-parent heterosis ranged from 116 to 386 for the CMS × DH hybrids in both years. For the CMS/DH × *Rfo* hybrids, it reached values from 108 to 436, and it was slightly higher in the first year of observations and when calculated in reference to the mean of three indirect parents. Negative heterosis (both best- and mid-parent) was higher for all hybrids in the second year of field trials. The values of either the positive or negative effects were very diverse over both years of observations (Tables 4, 5). Similarly, as for the number of branches per plant, the majority of the noted mid-parent heterosis values oscillated around zero, which means that they were not statistically significant (Fig. 1c). The number of hybrids that showed significant positive or negative mid- and best-parent heterosis effects was small and differentiated in both years of field trials (Fig. 2c, data not shown).

The positive effect of best-parent heterosis for silique length was higher in the first growing season and ranged from 0.3 to 7.2 mm for CMS × DH hybrids and from 0.1 to 13 mm for CMS/DH × *Rfo* hybrids (Table 4). Mid-parent heterosis for this trait was also higher in the first year and oscillated from 4.2 to 10.6 mm for single cross hybrids, and from 3.7 to 11.9 mm for three-way cross hybrids (Table 5). Negative best-parent heterosis had similar values regardless of the year, and it was slightly higher in three-way cross hybrids (− 0.1 to − 11.7 mm) (Table 4). A lot of hybrids showed positive mid-parent heterosis, while negative occurred only for four CMS × DH hybrids and for one CMS/DH × *Rfo* hybrid when calculated with regard to the mean of the indirect parents (Table 5, Fig. 2d). Opposite situation was when considering best-parent heterosis—more hybrids exhibited negative than positive effect (data not shown). According to the histograms, the majority of the single cross hybrids had lower values of mid-parent heterosis than the three-way cross hybrids, for which it mostly ranged between 2 and 8 mm (Fig. 1d). This was approximately two-fold more than the CMS/DH × *Rfo* hybrids, especially when calculated with reference to the means of the two direct parents, and it

Table 4 Maximal and minimal values of the best-parent heterosis effect for the studied hybrids

Hybrid	Heterosis effect	Plant height (cm)		No. of branches per plant		No. of siliques per plant		Silique length (mm)		No. of seeds per silique		Thousand seed weight (g)	
		I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr
CMS × DH	Max. (+)	18.9	17.2	1.79	2.10	164	332	7.2	4.9	3.00	3.60	0.44	0.26
	Min. (+)	0.4	0.1	0.03	0.51	2	21	0.3	1.5	0.08	0.14	0.06	0.02
	Max. (−)	− 14	− 19.4	− 2.94	− 2.02	− 148	− 350	− 7.2	− 4.2	− 4.13	− 3.54	− 0.41	− 0.51
	Min. (−)	− 0.9	− 0.1	− 0.14	− 0.07	− 15	− 3	− 0.2	− 0.2	− 0.03	− 0.39	− 0.01	− 0.01
CMS/DH × *Rfo* (2)	Max. (+)	23.8	22.6	5.06	0.55	208	–	13	3.1	4.67	3.50	0.27	0.42
	Min. (+)	0.3	0.2	0.11	0.04	5	–	0.1	0.1	0.70	0.40	0.01	0.04
	Max. (−)	− 4.9	− 7.9	− 2.42	− 2.43	− 219	− 249	− 10.2	− 9.9	− 1.60	− 1.50	− 0.77	− 0.65
	Min. (−)	− 0.1	− 0.2	− 0.11	− 0.07	− 18	− 13	− 0.1	− 0.2	− 0.60	− 0.10	− 0.03	− 0.03
CMS/DH × *Rfo* (3)	Max. (+)	28.4	26.3	5.18	0.37	303	85	7.7	2.4	6.04	4.28	–	0.17
	Min. (+)	2.7	5.8	1.54	0.22	5	85	0.1	0.1	0.40	0.05	–	0.02
	Max. (−)	− 0.3	− 2.6	− 3.09	− 2.33	− 143	− 142	− 11.7	− 8.5	− 1.43	− 2.75	− 0.82	− 0.59
	Min. (−)	− 0.3	− 0.6	− 0.11	− 0.04	− 4	− 2	− 0.1	− 0.1	− 0.40	− 0.09	− 0.02	− 0.01

The table shows only highly statistically significant values ($P < 0.001$). Max. (+), maximal positive heterosis effect; Min. (+), minimal positive heterosis effect; Max. (−), maximal negative heterosis effect; Min. (−), minimal negative heterosis effect. I yr, II yr, first and second year of observations. CMS/DH × *Rfo* (2), heterosis calculated in reference to the two direct parents, CMS/DH × *Rfo* (3), heterosis calculated in reference to the three indirect parents

Table 5 Maximal and minimal values of the mid-parent heterosis effect for the studied hybrids

Hybrid	Heterosis effect	Plant height (cm)		No. of branches per plant		No. of siliques per plant		Silique length (mm)		No. of seeds per silique		Thousand seed weight (g)	
		I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr
CMS × DH	Max. (+)	17.6	18.6	4.54	3.29	282	386	10.6	5.9	4.56	4.27	0.59	0.49
	Min. (+)	5.0	5.1	2.00	1.08	116	126	4.2	4.4	2.77	2.86	0.21	0.12
	Max. (−)	− 5.7	− 8.5	− 3.34	− 2.11	− 223	− 289	− 8.5	− 5.8	–	− 5.69	− 0.54	− 0.41
	Min. (−)	− 5.7	− 8.5	− 1.36	− 1.34	− 117	− 289	− 6.0	− 5.8	–	− 4.02	− 0.25	− 0.14
CMS/ DH × *Rfo* (2)	Max. (+)	26.1	25.4	5.45	1.51	309	138	11.9	9.9	5.92	5.79	0.49	0.15
	Min. (+)	6.1	5.6	1.54	1.12	108	138	4.1	4.3	2.74	2.74	0.21	0.13
	Max. (−)	–	–	− 2.75	− 3.29	− 225	− 316	–	–	–	–	− 0.30	− 0.47
	Min. (−)	–	–	− 1.76	− 1.14	− 111	− 127	–	–	–	–	− 0.25	− 0.12
CMS/ DH × *Rfo* (3)	Max. (+)	28.6	23.1	6.21	1.65	436	233	11.1	8.2	6.45	5.39	0.25	0.21
	Min. (+)	6.2	6.1	1.37	1.13	114	127	3.7	4.2	1.71	1.04	0.19	0.16
	Max. (−)	–	–	− 3.52	− 3.50	− 211	− 247	− 3.8	–	–	− 3.35	− 0.34	− 0.61
	Min. (−)	–	–	− 1.24	− 1.04	− 108	− 120	− 3.8	–	–	− 3.35	− 0.19	− 0.11

The table shows only highly statistically significant values ($P < 0.001$). Max. (+), maximal positive heterosis effect; Min. (+), minimal positive heterosis effect; Max. (−), maximal negative heterosis effect; Min. (−), minimal negative heterosis effect. I yr, II yr, first and second year of observations. CMS/DH × *Rfo* (2), heterosis calculated in reference to the two direct parents, CMS/DH × *Rfo* (3), heterosis calculated in reference to the three indirect parents

demonstrated a significant positive effect of heterosis for this trait (Fig. 2d).

Similar to the previously described feature, the positive best- and mid-parent heterosis effects for the number of seeds per silique were higher for the three-way cross hybrids (0.4–6.04 and 1.04–6.45, respectively) than for the single cross hybrids (0.08–3.6 and 2.77–4.56, respectively) (Tables 4, 5). This dependence for mid-parent heterosis is also confirmed by histograms (Fig. 1e). Also, more of three-way cross hybrids showed significant positive heterosis, regardless of the method of calculation (Fig. 2e). A significant negative effect of best-parent heterosis for this trait ranged from − 0.03 to − 4.13, and was higher in CMS × DH hybrids, regardless from year (Table 4). Negative mid-parent heterosis occurred only in the second year of field trials for two single cross hybrids and for one three-way cross hybrid (Table 5, Fig. 2e). Much more hybrids showed a negative heterosis effect when calculated in relation to the best-parent, but they were still fewer than those with a positive effect (data not shown).

In contrast to the aforementioned traits, the positive effect of best- and mid-parent heterosis for the thousand seed weight was higher for the single cross hybrids, and it ranged from 0.02 to 0.44 g and from 0.12 to 0.59 g, respectively (Tables 4, 5). For the three-way cross hybrids, the positive effect ranged from 0.01 to 0.42 g for best-parent and from 0.13 to 0.49 g for mid-parent heterosis, and it was usually higher when it was calculated with regard to the mean of the two direct parents (Tables 4, 5). Histograms show that there is a large difference in the mid-parent heterosis values distribution between 2 years of observations for the CMS/DH × *Rfo* hybrids (Fig. 1f). Among the CMS × DH hybrids, more of them revealed positive rather than negative significant heterosis effects. The opposite situation was noticed for three-way cross hybrids, especially in the second year of the trials, and when mid-parent heterosis was calculated in reference to the three indirect parents (Fig. 2f). In the case of best-parent heterosis, the vast majority of hybrids showed a significant negative effect (data not shown).

Discussion

Complex traits such as seed yield and other yield-related traits are controlled by multiple genetic factors.
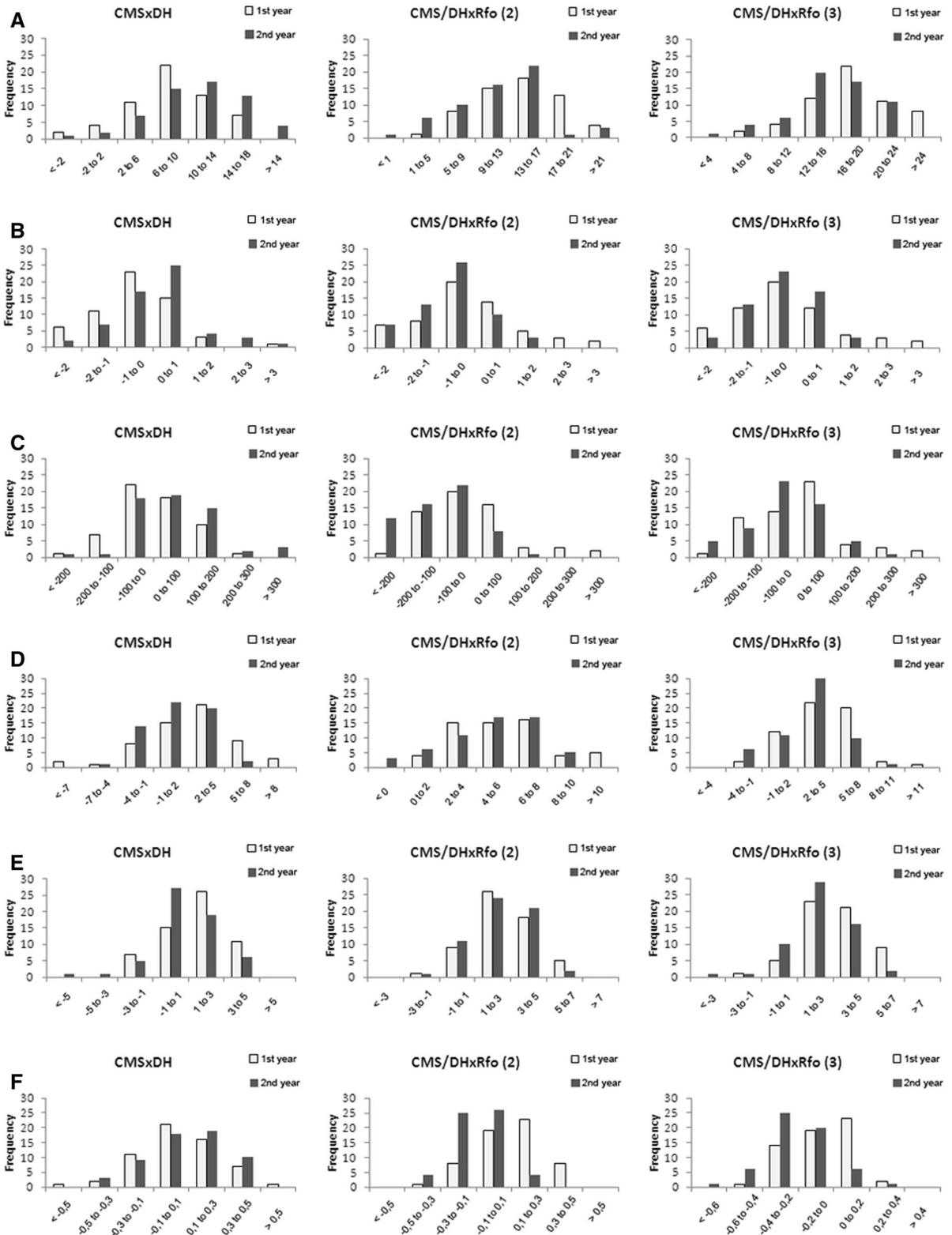
◄**Fig. 1** Distribution of heterosis effect values for six studied traits: **a** plant height, **b** number of branches per plant, **c** number of siliques per plant, **d** silique length, **e** number of seeds per silique, **f** thousand seed weight. The histograms do not take into account the statistical significance of the heterosis effect

The genetic complexity of the quantitative traits and the interaction between the genotype and the environment make it difficult to select the parental forms that provide high yielding hybrids. In all of the traits observed in this study, a combined analysis of variance for 2 years of field trials showed a highly significant ($P \leq 0.01$) impact of year and genotype, and the interaction between them. Also, the analysis of variance performed by Shi et al. (2011) revealed that genotype, environment, and the environment × genotype interaction had significant effects on the performances of 15 yield-correlated traits. Strong genotype effects ($P \leq 0.01$) for 14 phenotypic traits among the nine parental lines and the F1 hybrids observed in three locations were noted by Teklewold and Becker (2006). The plant material used in this study allowed for the estimation of the heterosis effect for two generations of rapeseed hybrids. Different values of heterosis were noticed for the six yield-related traits in this study.

All yield components have a close interaction with each other and with seed yield. A lot of authors report, that genetic correlations differ considerably in different environments, what suggest that they depend strongly on the environmental conditions. In our study, slight differences in correlations between investigated traits were noticed in both years of observation. Highly significant correlation was repeated for plant height and number of siliques per plant and for plant height and number of seeds per silique in 2014/15 and 2015/16. Similar results were obtained by Shi et al. (2011), who also observed significant positive correlations for these traits. Additionally, in the second growing season a correlation between plant height and silique length was found, what was also noticed by Chen et al. (2007) in their study. Among the yield components, number of siliques per plant and seeds per silique are most highly correlated with seed yield, what suggests, that these traits are the major contributors to the yield in rapeseed (Shi et al. 2015). Rameeh (2012) came to similar conclusions after determining significant positive correlation between pods per plant

and seed yield, and stated that this trait can be suitable for selection to improve seed yield. In our study, a significant positive correlation of number of siliques and number of branches per plant was observed in both years of trials. In addition, in the second growing season a negative correlations of number of branches and siliques per plant with thousand seed weight were noticed. Comparable dependence was detected by Shi et al. (2009), who observed in his research that pod number, seed number, and seed weight were significantly negatively correlated, what he explained by competition for assimilates. Further positive correlations repeated in 2 years of our field trials were between silique length and number of seeds per silique and between silique length and thousand seed weight. The same correlation between SL and TSW in doubled haploids and RC-F2 populations was observed by Fu et al. (2015). The authors concluded, that long siliques has the potential to increase seed weight. A positive correlation between TSW and SL was also observed with moderate coefficients in Li et al. (2014) research. A negative correlation between TSW and SS were found by Xing et al. (2014). The same correlation was also noticed in our research in 2014/15 growing season. In general, positive correlations between yield traits are desirable for breeders because they result in a yield increase, and negative dependence between traits can make difficult to improve them simultaneously through traditional breeding. For example, correlations observed in our study suggest, that increasing silique length would also increase the number of seeds per silique, which is one of the main features affecting the yield of rapeseed. On the other hand, positive correlations can also be undesirable, for example between plant height and number of siliques per plant. The second trait is major contributor to the yield, but simultaneously high plants cannot tolerate heavy winds and are vulnerable to lodging. In rapeseed, short plants with strong structure containing more number of branches (and thus—siliques) per plant provide better opportunity for increased yield.

Most of the tested hybrids showed a significant positive mid- and best-parent heterosis effect for plant height, which proves their increased vigor. The mean values for the single cross and three-way cross hybrids in 2 years were 156.9 and 162.5 cm, respectively, while the mean value for all parental forms was 145.8 cm. Comparable values for hybrids were

Fig. 2 The number of
hybrids demonstrating a
statistically significant
effect of heterosis for six
observed traits (**a–f**). H(−),
negative heterosis effect;
H(+), positive heterosis
effect; I yr, II yr, the number
of hybrids with a statistically
significant heterosis effect in
the first or second year of
observations; I + II, the
number of hybrids with the
same significant effect of
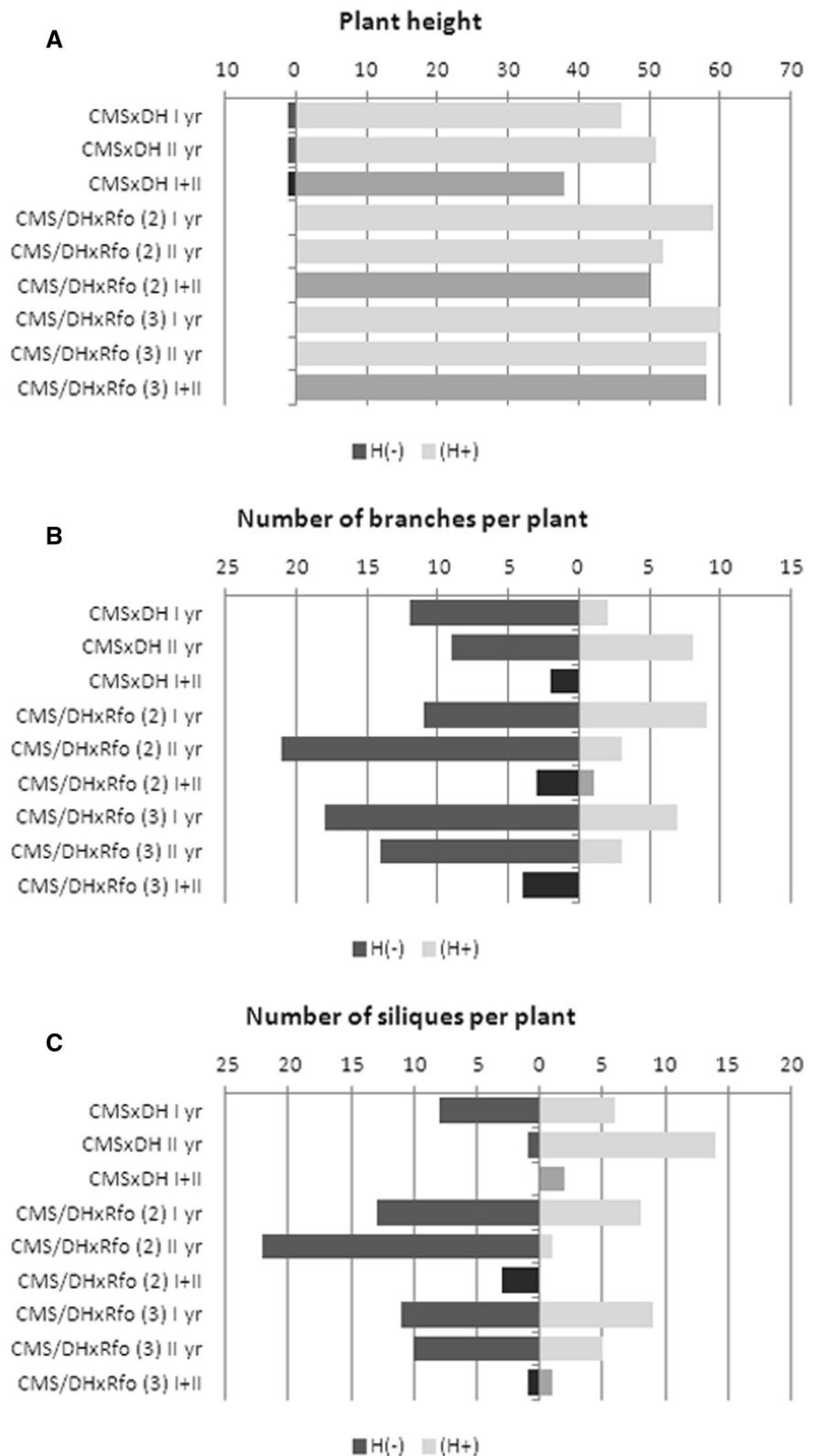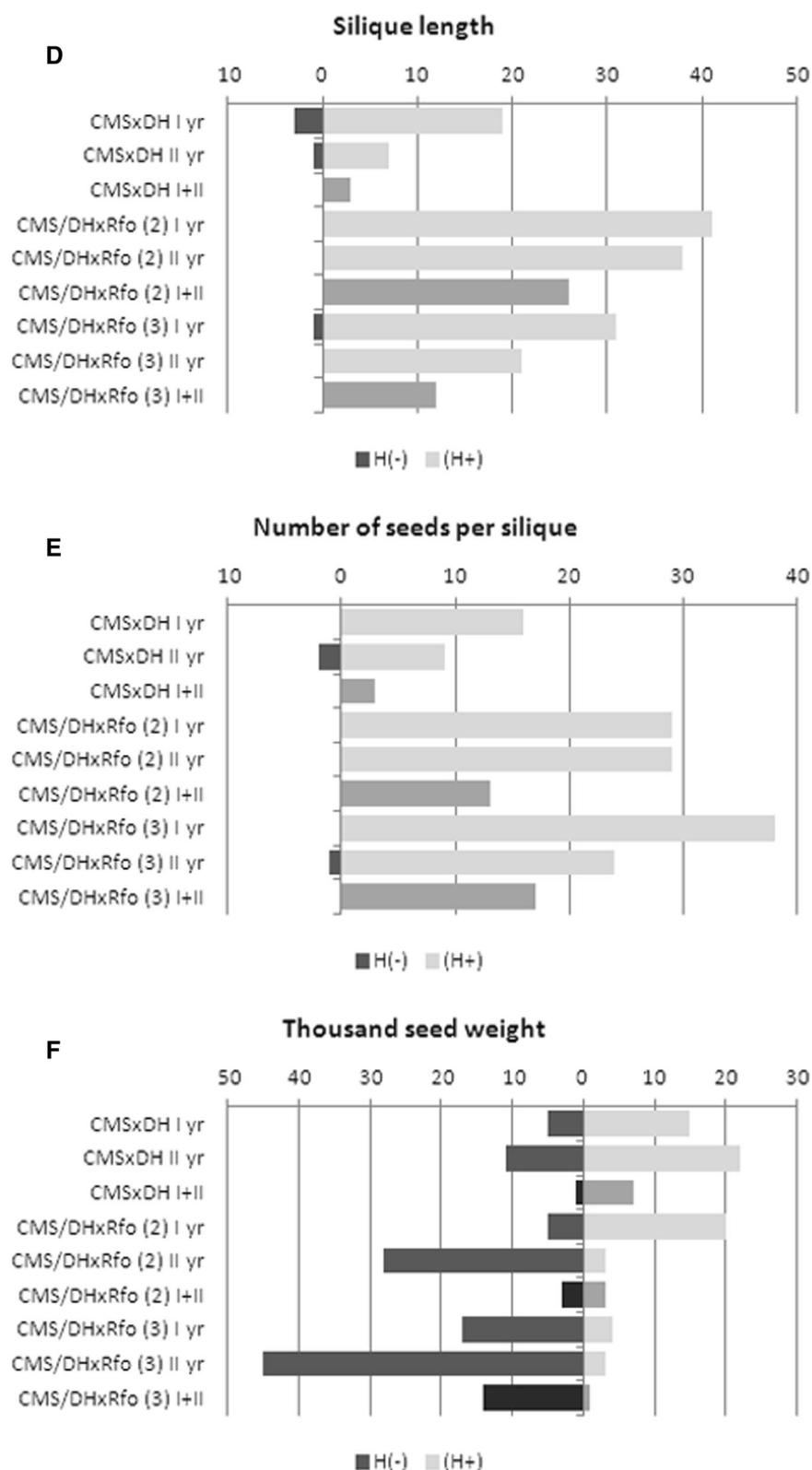heterosis (positive or
negative) in both years of
observations

Fig. 2 continued

Silique length

D

Number of seeds per silique

E

Thousand seed weight

F

observed by Shehzad et al. (2015), who noted that the plant height for 15 crosses was on the level of 157.83 cm. Shi et al. (2011) noticed significant but low mid-parent heterosis for this trait in the F1 and F2 generations, and the performance of F1 was significantly higher than the mean of the F2 population. In our study, mid- and best-parent heterosis values for plant height were significantly higher for three-way cross hybrids than for the single cross hybrids in both years of observations. The higher heterosis for these hybrids is not surprising, considering that this is not a typical F2 generation, but new population formed by crossing single cross hybrids with *Rfo* line. Some authors observed non-significant differences for plant height between parental inbred lines and hybrids of spring canola types (Ahmad et al. 2011). Likewise, the analysis of variance in Sincik et al. (2011) research indicated significant differences in plant height among parents and their hybrids in the F1 generation. Positive best-parent heterosis in the F1 offspring for this feature was also noticed by Basunanda et al. (2010).

The number of siliques per plant is generally considered to be the most important trait affecting seed yield in *Brassica napus* (Li et al. 2012). In our research, the average number of siliques per plant for the CMS × DH hybrids was 455.3, while for CMS/DH × *Rfo* hybrids, it was 479.8. Higher mean values of this trait were observed by Shehzad et al. (2015), in whose research the mean value for the hybrids was 639.41. Li et al. (2012) noted that among the yield-related traits, the number of siliques per plant displayed the highest heterosis, followed by seeds per silique. Shi et al. (2011) concluded in their study that genotype, environment, and the interaction between them had significant influences on branch number and pod number. They observed a positive heterosis for these traits on the F1 and F2 generations with regard to the mean of the parents and the DH population. For the F1 hybrids, the pod number per plant showed moderate mid-parent heterosis, while the branch number showed low mid-parent heterosis. In the F2 generation, the values of heterosis varied widely for these traits, from highly negative to highly positive. Similarly, in our study for these two traits, mid-parent heterosis was varied (both negative and positive), but there was no evident regularity in its occurrence in single cross and three-way cross hybrids. At the same time, a number of the hybrids did not exhibit any heterosis. The lack of significant

heterosis effect for pods per plant was also noted in the research of Rameeh (2011) in a line × tester analysis of two testers of spring type and six lines of winter type oilseed rape.

Silique length is directly correlated with the number of seeds, and thus, seed yield, and so a positive heterosis effect for this trait is desirable. The mean silique length in the studied genotypes ranged from 50.66 mm in parental forms to 55.88 mm in hybrids (including 2 years). Slightly higher values for this trait in hybrids were noted in the studies of Shehzad et al. (2015)—6.44 cm, and Noor-Ul-Abideen et al. (2013)—7 cm. In our research, the majority of the three-way cross hybrids demonstrated highly significant positive mid-parent heterosis effects. Similarly, a comparison of the parents with the F1 hybrids, which indicates the presence of heterosis for this trait, was also highly significant ($P \leq 0.01$) in the study by Teklewold and Becker (2006). Sabaghnia et al. (2010) noted that 21 (58%) of all observed crosses exhibited positive mid-parent heterosis effects for pod length (from -7.1 to 20.3%). Comparable results were obtained by Satwinder et al. (2000), who reported a significant degree of heterosis in the F1 generations for all studied yield components, including the length of pods.

The number of seeds per silique is one of the most important traits affecting seed yield in rapeseed. The average number observed in the studied genotypes was 14.49 for parental forms, and 16.76 for hybrids. A much higher mean value of this trait in hybrids (25.99) was noted by Shehzad et al. (2015). The highest mid-parent heterosis effect observed in our study was clearly visible in the three-way cross hybrids, which not only had higher values of heterosis than single cross hybrids, but also more of them exhibited highly significant positive effects for this trait. Similarly, Radoev et al. (2008) observed that the number of seeds per silique showed a positive mid-parent heterosis trend (11.2%) in the F1 hybrids, and an even higher trend (12.7%) in the testcross hybrids. They indicated that this was the only yield component with a positive average high parent heterosis, and that this also contributed to the average testcross mid-parent heterosis. Also in our research it was one of two features (including plant height), in which more hybrids showed positive than negative best-parent heterosis. A high positive heterosis for the number of seeds per silique, together with residual heterosis in the F3

generation, was also noticed in the study by Li et al. (2012). Significant low mid-parent heterosis in the F1 and F2 generations compared with the mean of the parents for the number of seeds per pod was observed by Shi et al. (2011). The authors noted that the average mid-parent heterosis in the F2 population was generally lower than in F1, but in each environment, some combinations were higher in the F2 population. In contrast to the previous results, Sincik et al. (2011) observed that the heterosis for the number of seeds per pod calculated in relation to the better-parent had low or negative values in many of the crosses, over 2 years of studies.

The thousand seed weight (TSW) is a trait with quite a low degree of variability, but an analysis of variance has indicated a strong influence of year, genotype, and interactions between them with regard to this trait value. On the other hand, Sincik et al. (2011) noticed that differences between the F1 hybrids and their parents were non-significant for TSW. Shehzad et al. (2015) also observed that genotypes and crosses demonstrated significant differences ($P \leq 0.05$), while the interactions between them showed highly significant ($P \leq 0.01$) influences on this trait. The authors observed in 15 *Brassica* genotypes that the mean value of TSW was 3.51 g, while Akbar et al. (2008) calculated the mean TSW to be 3.16 g. These values are quite low compared to that noted in our study (4.609 g for parental forms and 4.751 g for hybrids). For this trait, there is a large difference in the mid-parent heterosis values distribution between 2 years of observations for the CMS/DH × *Rfo* hybrids. At the same time, a large difference between the numbers of lines with positive or negative mid-parent heterosis in both groups of hybrids is visible (which proves the strong year/genotype effect). Very low, but significant heterosis for TSW (the average testcross mid-parent heterosis reached 1.2%) was observed by Radoev et al. (2008). Sincik et al. (2011) stated that the highest positive heterosis for TSW was 7.65% and 6.13% (for the two study years, respectively), and none of the crosses exhibited highly positive heterosis results for this trait for either year. Similarly, average significant heterosis was observed by Rameeh (2011), who remarked on the impact of additive genetic effects on this trait. An opposite trend of results was obtained by Shi et al. (2011), who did not note heterosis for seed weight, and by Li et al.

(2012), who estimated negative heterosis in their studied crosses.

The heterosis of complex traits, like seed yield, can be estimated on the basis of yield-related traits heterosis, because even weak positive effect of the component traits may result in high heterosis of the complex one (Shi et al. 2011). Concluding our study, traits with clear positive and traits with varied mid-parent heterosis were observed. For the first group, we can include plant height, silique length, and the number of seeds per silique—a large number of hybrids expressed significant positive mid-parent heterosis for these traits, independent to the year of observations and the type of hybrid. Negative heterosis was practically absent. Additionally, it can be stated that CMS/DH × *Rfo* hybrids usually expressed higher positive heterosis than CMS × DH hybrids. To the second group with varied mid-parent heterosis, we can include the number of branches and siliques per plant, and the thousand seed weight. For these traits, hybrids exhibited both positive and negative significant heterosis trends without a clear pattern for the years and types of hybrids. On this basis, a general conclusion can be drawn, that the greatest impact on the seed yield heterosis would result from the silique length and the number of seeds per silique, as these are the traits with the largest and the most stable positive mid-parent heterosis. This partially overlaps with the observations of Radoev et al. (2008), who indicated that yield heterosis was mainly dependent on the heterosis levels of seeds per silique, and the number of siliques per square decimeter. Li et al. (2012) put forward a slightly different hypothesis, that the F1 heterosis for seed yield was mainly a result of the heterosis for the number of siliques per plant, and less for the number of seeds per silique and seed weight. They noted that the yield heterosis was large because of higher number of siliques per plant, even when the seed weight was decreased in the progeny. However, it should be remembered that positive heterosis is not desirable in all traits, for example in case of plant height. Small and medium plants with greater number of branches are preferred in rapeseed varieties, because they can tolerate heavy winds and can be prevented from lodging (Turi et al. 2006). Therefore, a positive correlation between plant height and number of branches per plant can be an obstacle in rapeseed breeding.

It should be noticed that the main goal in hybrid breeding is not to maximize heterosis itself, but to improve the hybrid yield, as a result of parental performance and heterosis. As can be concluded from heterosis research conducted worldwide, the use of conventional breeding is still helpful for producing high-yielding rapeseed hybrids with good seed quality. Genomic methods are increasingly being used, but they cannot completely replace phenotypic evaluations. They should complement each other, especially in species like oilseed rape, where the development of sterile CMS lines and fertile *Rfo* lines can be expensive and laborious.

Author contributions The first two authors contributed equally to this work, and should be regarded as joint First Authors. Wolko and Dobrzycka conceived and designed the project, developed the hybrids, carried out the field experiments, collected phenotypic data, and wrote the manuscript. Bocianowski performed the statistical analyses, and edited and revised the manuscript. Bartkowiak-Broda supervised the experiment, edited and revised the manuscript.

Compliance with ethical standard

Conflict of interest   The authors declare that there is no conflict of interest.

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