Agronomic Performance of Doubled Haploid Lines and Pedigree-Derived Lines of Winter Oilseed Rape

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Abstract: In four not overlapping sets of cultivars, including doubled haploid (DH) lines and pedigree derived (PD) lines of winter oilseed rape, both types of lines were compared in basic agronomic traits. The tested lines originated from breeding programmes of different breeders. Each set of cultivars was tested in a different year from 2001 to 2004 in multi-location trials with four replicates. There was no consistency in yield superiority of DH or PD lines and in all four sets the differences between the means of DH and PD groups were insignificant. On average, the differences between the groups of DH and PD lines were negligible in all evaluated agronomic traits. The statistical analyses of yield performance however revealed a very low predictive value of the multi-location trials. It can be concluded that with the given selection techniques and the actual system of cultivar trials nearly random samples of possible DH and PD lines were obtained in which no superiority of DH or PD lines in the evaluated agronomic traits could be detected.

Keywords: winter oilseed rape; doubled haploid; seed yield; disease resistance

Since Lichter (1982) succeeded to produce haploids of *Brassica napus* from microspores, doubled haploids (DH) have increasingly been used in oilseed rape breeding. There are advantages and also disadvantages of DH lines. Homozygosity is reached within one generation. This saves to the breeder about 4 years of pedigree selection and simplifies cultivar maintenance. DH lines are often surprisingly vigorous since there is strong selection against recessive lethal and subvital alleles in the haploid phase (Kuckuck *et al.* 1985). The meiotic crossovers during the heterozygous generations of pedigree-derived (PD) lines do not occur in DH lines. Recombinations between linked loci are therefore less frequent in DH lines than in PD lines and genetic variation among DH lines is thus smaller than among unselected PD lines. Genetic gain from selection in heterozygous generations of PD lines is impossible in DH lines. The production of sufficiently large DH populations was reported nearly twice more costly than those of PD lines (Frauen 1994). But continuous improvements of methods reduce the costs.

The production of DH lines from microspore cultures is routinely used in many *Brassica napus* line and hybrid breeding programmes (Stringam *et al.* 1999). Attempts were made to fix hybrid performance in DH lines and to circumvent the problems with hybrid seed production in this way (Maluszynski *et al.* 2001). In the Czech official cultivar trials foreign DH lines of winter oilseed rape were often highly competitive in comparison with other cultivars, even hybrids. However, there are also doubts concerning the effectiveness of DH technology in practical breeding (Niemirowicz-Szczytt 1997). Dewan *et al.* (1998) noted strong inbreeding depression of seed yield in some *Brassica rapa* DH lines. In an earlier paper (Kučera *et al.* 1996) high

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variability, especially in glucosinolate content, was found among DH lines derived from one donor plant of oilseed rape.

In the Czech Republic breeders lines are traditionally tested in breeders joint multi-location trials (JML-trials) before entering official trials. Every year a different, not overlapping set of lines from different breeding programmes and breeders is tested in the JML-trials. Since the trials include both DH and PD lines, the JML-trials offer a possibility to compare the performance of DH and PD lines produced in practical breeding. In the present study we used data of the Czech JML winter oilseed rape trials from 2001 to 2004 for this purpose.

MATERIAL AND METHODS

The tested winter oilseed rape lines were selected in practical breeding programmes of the Research Institute of Oilseed Crops Opava of OSEVA PRO s. r. o., of SEMPRA a. s. and of SELGEN a. s. The lines originated from various parent combinations, mostly not disclosed by the breeders, and were preselected for agronomic performance and disease resistance at the particular breeding sites and in on-station trials. The DH plants were produced for the breeders in the Research Institute of Crop Production Prague from microspore cultures, as described by Vyvadilová and Zelenková (1992). The tested entries, summarised as "cultivars", included each year from two to four cultivars registered in the National Cultivar List. In total 27 DH lines, 93 PD lines and 9 registered cultivars were tested in the JML-trials from 2001 to 2004. On average 35 entries were tested each year.

Each trial consisted of four complete randomised blocks with 10 m^2 plots. The trial locations were in the regions of Opava, Kujavy, Chlumec n. C., Humpolec, Slapy u Tábora and Šumperk. Each year the same set of cultivars was tested at all locations. After one year in JML-trials the breeding lines either proceeded to official trials or were discarded. Seed yield of individual plots was corrected to 12% moisture content. Resistance to *Phoma lingam*, *Sclerotinia sclerotiorum* and *Alternaria brassicae* and lodging resistance were scored using a 1–9 scale, with 9 indicating the most favourable score. The thousand seed weight (TSW) was usually determined from two 500-seed samples. The specified year is always the harvest year.

Statistical procedures. A complete statistical analysis could be performed only for yield data since other agronomic traits were evaluated only in some trials and often just in one of the four replicates. Therefore only cultivar averages over all trials within a year and the variance of the obtained averages were calculated for these traits.

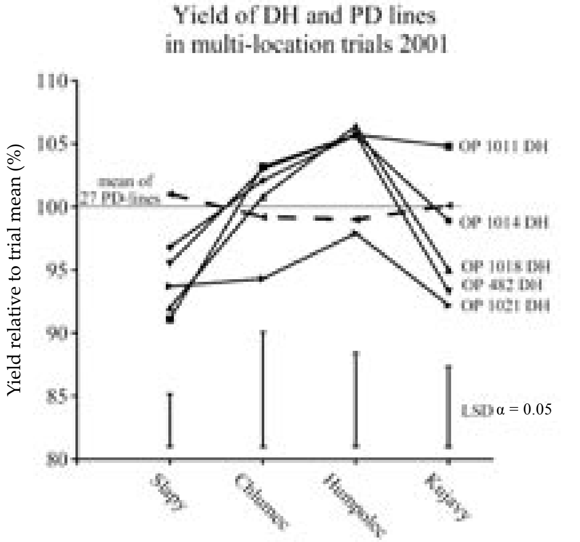
The tested sets of breeding lines changed almost completely from year to year. Therefore the trial series of each year was statistically evaluated independently. A survey of the tested DH lines and registered cultivars is summarised in Table 2. Within each year a two-stage statistical analysis was performed, as suggested by Patterson (1997) for series of cultivar trials. The two-stage analysis offers a better insight into the behaviour of cultivars and is justified by variance non-homogeneity of the individual trials. Each trial was first analysed individually by ANOVA. Least significant differences (LSD) for pairwise comparisons were derived from the residual variance. The local cultivar means of each year were assembled in a summary table classified by locations and cultivars. In the second stage the summary tables were analysed separately using ANOVA. The trial at Šumperk in 2004 was excluded from evaluation because of bad differentiation and missing correlation with the other trials. To visualise the combined analysis, the local means of DH-lines and the local averages of PD lines were converted to percentages of the local trial mean and presented in charts. Since the aim of the paper was to compare DH lines and PD lines, the simultaneously tested registered cultivars are not included in the charts. Genetic variance was estimated from the variance of averages of all tested cultivars in a trial or year, after subtraction of residual variance, divided by replicates or sites, respectively.

RESULTS AND DISCUSSION

The performance of DH lines at different locations and the means of PD lines, relative to the local trial means, are summarised in the four charts in Table 1.

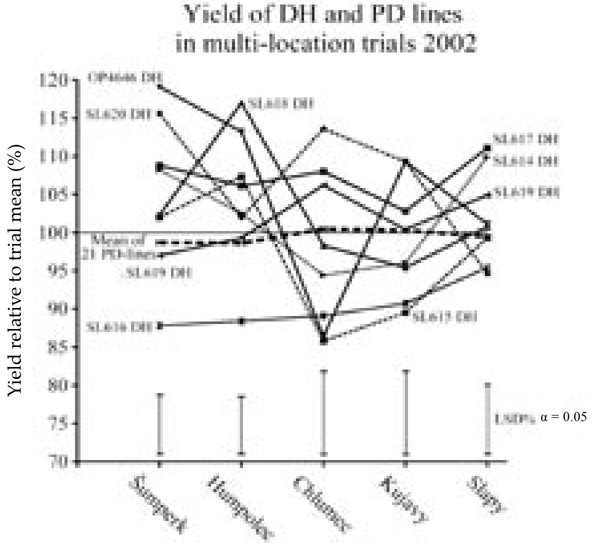
The ranking of DH lines differs between the trials considerably. The correlation coefficients between trials within a year are rather low, in average $r = 0.36$, indicating a low predictive power of individual trials. In contrast, the trials themselves were quite good since the genetic variance within trials was on average about twice larger than the residual variance (Figure 1), and the cultivars were seemingly well differentiated. But the individual

Table 1. Relative performance of DH lines and PD lines during four years of JML trials and correlations between trials within the same year



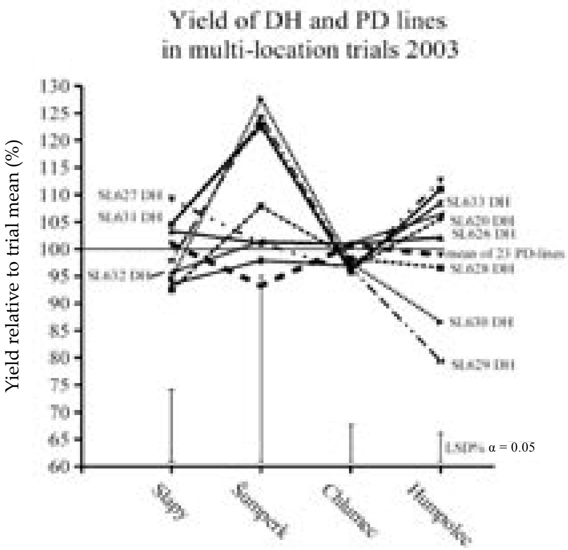
Correlations (34 cultivars):

	Chlumec	Humpolec	Kujavy
Slapy	0.47**	0.32*	0.37*
Chlumec		0.64**	0.22
Humpolec			0.39*



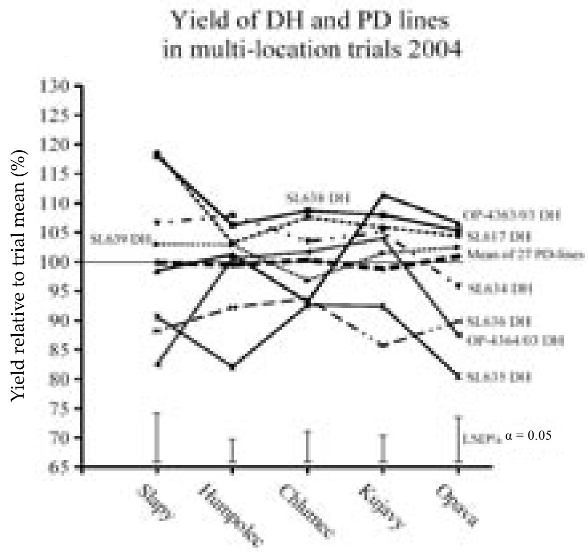
Correlations (32 cultivars):

	Humpolec	Chlumec	Kujavy	Slapy
Šumperk	0.34*	0.25	0.28	0.20
Humpolec		0.22	0.35*	0.58**
Chlumec			0.43**	0.41**
Kujavy				0.23



Correlations (35 cultivars):

	Chlumec	Humpolec	Kujavy
Slapy	0.02	0.24	0.38*
Šumperk		0.09	0.41**
Chlumec			0.38**



Correlations (38 cultivars):

	Humpolec	Chlumec	Kujavy	Slapy
Šumperk	0.48**	0.41**	0.49**	0.51**
Humpolec		0.29*	0.50**	0.46**
Chlumec			0.33*	0.41**
Kujavy				0.38**

trials express just the interactions of cultivars with the unique and unrepeatable local combination of soil conditions, climate, disease situation and treatment. The local LSD are therefore of no use for the prediction of cultivar behaviour outside the given trial. This is well reflected in Figure 2

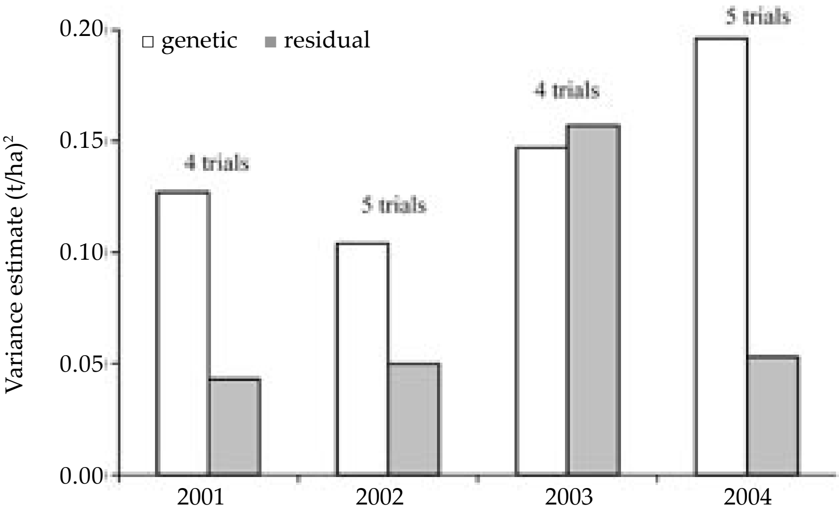


Figure 1. Average genetic and residual variance within trials

by the approx. twice higher residual than genetic variance in the analyses of trial series.

Table 2 shows average yields of the tested DH lines, relative to the general mean of the corresponding series. To enable pairwise comparisons of cultivar means, they are presented together with the interval of ± half the LSD, calculated from the residual variance within the given series. Therefore, two means can be regarded as significantly different in the given year if the indicated intervals do not overlap. For the comparison with PD lines the average performance of PD lines and of registered cultivars is also included. For easier comparison the DH lines and the line group means are ranked in each year.

The LSD calculated from trial series within one year are valid for the mentioned reasons just for the given year since in Central Europe the climate of a year is largely unpredictable and the rankings of cultivars differ between years. An example is the converse ranking of the cultivars Navajo and Rasmus in 2002 and 2003 in Table 2. In the Czech official trials, which use the same trial design as JML-trials, but considerably more locations, the average correlation of oilseed rape yield between years is approx. 0.6 (Czech Variety Office, pers. commun.), indicating a low predictive power of results from just one year. Breeders lines, before reaching JML-trials, are tested just on small plots at the breeding site and necessarily are subject to larger errors than in the JML-trials. The lines tested in JML-trials can therefore be regarded as four nearly random samples of possible HD and PD lines from random crosses. This is supported by results from Monte Carlo simulation experiments in which the outcome of selection for a polygenic

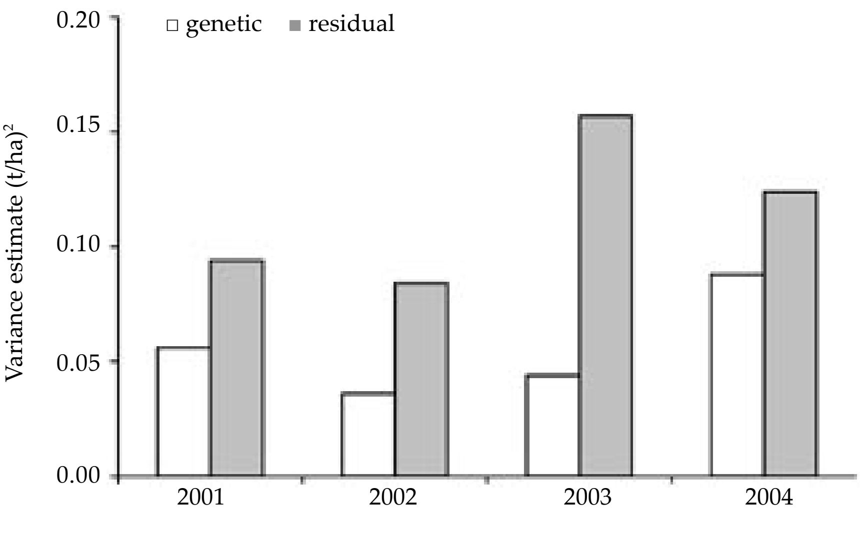


Figure 2. Genetic and residual variance in series of trials

Table 2. Relative yield* and intervals of ± LSD/2 of tested DH lines, registered cultivars and groups of DH and PD cultivars

Tested cultivars	2001	2002	2003	2004
OP 1011 DH	101.2 ± 4.7			
OP 1014 DH	100.9 ± 4.7			
Mean of 27 PD-lines	**99.8 ± 0.9**			
OP 482 DH	99.3 ± 4.7			
Mean of 5 DH-lines	**98.9 ± 2.1**			
OP 1018 DH	98.5 ± 4.7			
OP 1021 DH	94.5 ± 4.7			
SL 617 DH		107.4 ± 5.4		108.0 ± 3.6
SL 620 DH		107.0 ± 5.4	107.2 ± 7.8	
OP 4646 DH		105.8 ± 5.4		
SL 618 DH		102.7 ± 5.4		
SL 614 DH		102.2 ± 5.4		
Mean of 8 DH-lines		**101.8 ± 1.9**		
SL 619 DH		101.6 ± 5.4		
Mean of 21 PD-lines		**99.5 ± 1.2**		
SL 615 DH		96.8 ± 5.4		
SL 616 DH		90.3 ± 5.4		
SL 631 DH			108.5 ± 7.8	
SL 627 DH			104.7 ± 7.8	
SL 626 DH			101.8 ± 7.8	
Mean of 8 DH-lines			**101.8 ± 2.6**	
SL 630 DH			101.5 ± 7.8	
SL 632 DH			101.0 ± 7.8	
SL 629 DH			99.5 ± 7.8	
SL 633 DH			99.1 ± 7.8	
SL 628 DH			98.7 ± 7.8	
Mean of 23 PD-lines			**98.4 ± 1.6**	
SL 638 DH				109.3 ± 3.6
SL 634 DH				103.8 ± 3.6
OP-4363/03 DH				102.2 ± 3.6
SL 639 DH				101.4 ± 3.6
Mean of 27 PD-lines				**99.9 ± 0.7**
Mean of 7 DH-lines				**98.5 ± 1.4**
OP-4364/03 DH				95.3 ± 3.6
SL 636 DH				89.9 ± 3.6
SL 635 DH				87.6 ± 3.6
Aviso			106.9 ± 7.8	
Catonic				96.0 ± 3.6
Laser			95.7 ± 7.8	100.8 ± 3.6
Navajo		96.4 ± 5.4	111.3 ± 7.8	104.9 ± 3.6
Odila	98.7 ± 4.7			
Orkan		91.5 ± 5.4		
Rasmus	111.9 ± 4.7	108.7 ± 5.4	101.5 ± 7.8	104.5 ± 3.6

*relative to the general mean of the corresponding series

Table 3. Average values and standard deviations of agronomic traits of DH and PD lines

Trait	2001		2002		2003		2004	
	5 DH	27 PD	8 DH	21 PD	9 DH	23 PD	8 DH	27 PD
Yield (t/ha)	4.56 ± 0.14*	4.60 ± 0.30	3.44 ± 0.20	3.37 ± 0.24	3.80 ± 0.13	3.66 ± 0.32	6.12 ± 0.49	6.13 ± 0.32
Phoma score	6.25 ± 0.26	6.26 ± 0.30	5.32 ± 0.39	5.44 ± 0.41	6.13 ± 0.37	6.43 ± 0.32	5.85 ± 0.55	6.02 ± 0.48
Sclerotinia score	6.23 ± 0.40	6.66 ± 0.40	5.36 ± 0.14	5.50 ± 0.41	7.27 ± 0.17	7.50 ± 0.26	6.51 ± 0.48	6.25 ± 0.36
Alternaria score	6.46 ± 0.22	6.51 ± 0.24	7.33 ± 0.20	7.37 ± 0.26	6.15 ± 0.25	6.21 ± 0.17	8.00 ± 0.14	8.02 ± 0.20
Lodging score	7.41 ± 0.20	6.96 ± 0.63	7.69 ± 0.44	7.32 ± 0.37	6.89 ± 0.48	6.45 ± 0.68	7.28 ± 0.32	7.10 ± 0.54
TSW	4.58 ± 0.24	4.36 ± 0.33	4.60 ± 0.89	4.58 ± 0.37	4.38 ± 0.42	4.83 ± 0.34	5.53 ± 0.73	5.37 ± 0.69

*± standard deviation of cultivar averages within the groups of lines

trait with low heritability is almost the same or worse than without selection, see Figure 1.8 in Fischbeck (1985). This justifies the comparison of DH and PD lines although they do not proceed from the same crosses.

The comparison of group means of DH lines and PD lines in Table 2 reveals no consistency in ranking and no significant difference between the group means in all four years. It can therefore be concluded that there is no statistical indication of superiority of DH or PD lines in yield over each other.

Since the quality of data of the other agronomic traits did not permit a comprehensive statistical analysis, just the average values of the evaluated traits in the groups of DH and PD lines were calculated. The averages are summarised in Table 3. To provide information about the variability among the lines, the standard deviations of cultivar averages within the groups are also included in Table 3.

In the table there is no indication of superiority of either DH or PD lines in any of the traits. Each year the distributions of DH and PD lines largely overlap in all traits and also the variation range within both groups of lines is very similar in all the traits.

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Abstrakt

Kučera V., Schwarzbach E., Klíma M., Vyvadilová M. (2004): **Srovnání výkonnosti dihaploidních linií a konvenčně vyšlechtěných linií řepky ozimé**. Czech J. Genet. Plant Breed., **40**: 127–133.

Základní agronomické znaky dihaploidních (DH) a konvenčně šlechtěných (PD) linií řepky ozimé byly srovnávány ve čtyřech nepřekrývajících se souborech. Zkoušené linie pocházely ze šlechtitelských programů různých šlechtitelů. Každý soubor linií byl zkoušen v jiném roce letech 2001–2004 v pokusech na několika lokalitách ve čtyřech opakováních ve znáhodněných blocích. Ve výnosu semen byly mezi průměry skupin DH a PD linií jen velmi malé neprůkazné rozdíly. Rozdíly mezi skupinami DH a PD linií byly zanedbatelné i ve všech ostatních hodnocených agronomických znacích. Statistická analýza výnosu však odhalila velmi nízkou vypovídací schopnost jednoletých pokusů na více lokalitách. Závěrem lze konstatovat, že s použitím současných výběrových metod v praktickém šlechtění a současným systémem odrůdových pokusů byly získány téměř náhodné vzorky z DH a PD linií, které se v hodnocených znacích významně nelišily.

Klíčová slova: řepka ozimá; dihaploidy; výnos semen; odolnost k chorobám

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