COMPARISONS OF AGRONOMIC AND COMPOSITIONAL TRAITS IN MICROSPORE-DERIVED AND CONVENTIONAL POPULATIONS OF SPRING BRASSICA NAPUS

D.G. Charne(1), W.D. Beversdorf(2)

(1) Pioneer Hi-Bred Production Ltd., RR#4, Georgetown, Canada L7G 4S7
(2) University of Guelph, Guelph, Canada N1G 2W1

INTRODUCTION

The development of a reliable and efficient microspore culture system in Brassica napus has provided rapeseed breeders with a potential alternative to traditional methods of inbred line production. The efficiency of microspore culture relative to conventional approaches, and hence its value to breeders, can be measured in terms of: (1) the time required to obtain finished inbreds, and hence, the length of the breeding cycle, and (2) the genetic gain attained per cycle for economically-important traits. In this paper, the results of comparisons of microspore-derived and single seed descent populations of spring B. napus are presented, and the implications for the use of microspore culture as a routine breeding approach are discussed.

MATERIALS AND METHODS

Production and Evaluation of Plant Material:

The microspore-derived and SSD populations used in this study were produced from two spring B. napus crosses, Westar/Topas (W/T) and Regent/Westar (R/W). F1 plants for microspore culture were grown to flowering in a controlled-environment growth room (22/18 C day/night temperatures, 16 h photoperiod). The microspore culture and plant regeneration procedures used were similar to those described by Chuong and Beversdorf (1985).

Regenerated plants from each cross were divided into haploid and diploid groups on the basis of flower morphology and fertility (Keller and Armstrong, 1978). The chromosomes of haploid plants were doubled by immersing the roots for five hours in a 0.1% (w/v) colchicine solution. Seed was harvested from diploid plants (SDH lines), and from fertile sectors on colchicine-doubled haploid plants (CDH lines) at maturity.

Following microspore isolation, axillary racemes on F1 plants were self-pollinated to produce F2 seed for SSD line development. SSD populations from both crosses were advanced from F3 to F6 in the growth room. All CDH, SDH, and F6-derived SSD lines were grown in a field nursery in 1986 to increase seed for replicated trials.

The microspore-derived (SDH and CDH) and SSD lines from each cross were evaluated in replicated field trials at Elora, Ont., Canada during the summers of 1987 and 1988. Data were collected on days to flowering and maturity, seed yield, and seed oil content in both years. Lodging was scored on all lines in 1987 only, while plant height, seed protein content, and thousand seed weight were measured in 1988 only. All data was subjected to appropriate ANOVA procedures. For each trait in each year, the mean of each line (over blocks), and the means and variances of SDH, CDH, MD (SDH and CDH combined), and SSD populations from each cross were estimated, and used in the following comparisons.

SDH and CDH Population Comparisons:

For each trait within each year, the means and variances of SDH and CDH populations were compared by a t-test and an F-test, respectively (Snedecor and Cochran, 1980). The distributions of SDH and CDH line means were also compared, using a non-parametric, Mann-Whitney U-test (Siegel,

1988). SDH lines from both crosses were screened for heterozygosity, using glucosephosphate isomerase (GPI) isozyme markers for which the parents differed.

MD and SSD Population Comparisons:

Within each cross, the means, variances, and distributions of MD (combined SDH and CDH) and SSD line means were compared on a trait-by-trait basis, using the same approaches as were used for the SDH and CDH comparisons. The normality of each distribution was also assessed by calculating co-efficients of skewness and kurtosis (Snedecor and Cochran, 1980).

Predicted gains from 10% selection (G_s) for days to maturity, seed yield, and seed oil content were estimated for MD and SSD populations, using the relationship: $G_s = iH^2s_p$ where i is the standardized selection differential for 10% selection intensity (1.755), H^2 is the broad-sense heritability (s_g^2/s_p^2), and s_p is the phenotypic standard deviation, in the units in which the trait is measured. For each trait, G_s was compared for MD and SSD populations by calculating a 95% confidence interval in phenotypic s.d. units (Wricke and Weber, 1986).

RESULTS

SDH and CDH Population Comparisons:

The comparisons of SDH and CDH populations with respect to means, variances, and shapes of distributions detected no significant differences for any important traits in either cross (results not presented). Heterozygosity for the GPI marker was detected in one of 94 SDH lines screened. The ratios of GPI isozyme types in the SDH populations did not differ significantly (p=.05) from the expected 1:1 ratio, based on a Chi square test.

MD and SSD Population Comparisons:

The results of MD and SSD population comparisons are summarized in Table 1. For conciseness, population variances are expressed as standard deviations associated with their respective means.

The only trait for which MD and SSD means or variances differed significantly was for lodging response in the W/T cross. The MD population had a lower mean lodging score and a larger variance than the SSD population. These differences are also reflected in the highly significant (p<.01) z-value from the U-test comparison of MD and SSD line distributions. The comparisons of line distributions also detected significant (p<.05) differences in the shapes of MD and SSD populations for days to maturity and seed protein content in the W/T cross, and for seed oil content in the R/W cross.

Non-normality was detected for a few traits in both crosses. In most cases, the non-normality was present, and was of a similar nature, in both MD and SSD distributions (results not shown).

Overall, there were few differences between MD and SSD populations from either cross with respect to important traits. The similarities are evident in the examples shown in Figs. I and II, for seed yield (1988) and seed oil content (1987) in the R/W cross.

Predicted gains from 10% selection (G_s) for days to maturity, seed yield, and seed oil content are presented in Table 2. The G_s estimates for all traits were larger in both MD and SSD populations in 1987 than in 1988, due to larger phenotypic variances, and hence, s_p values. The 95% confidence intervals (CI) for iH^2 in MD and SSD populations overlapped, both within and across years, for all traits in the W/T cross, and for days to maturity and seed oil content in the R/W cross (Table 3). For seed yield in the R/W cross, MD and SSD CIs overlapped within each year, but not between years.

Table 1. Summary of comparisons of MD and SSD populations from the Westar/Topas (W/T) and Regent/Westar (R/W) crosses.

	MD population			SSD population					
Trait	Mean	sd	Normal	Mean	sd	Normal	t[1]	F	z
W/T Cross 1987:									
Flowering[1]	46.3	5.6	Yes	46.7	5.1	Yes	0.43	1.22	-.59
Maturity	90.0	8.4	Yes	89.5	9.9	Yes	0.30	1.41	0.51
Lodging	2.30	1.20	No	2.50	.90	Yes	2.56*	1.78*	-4.67**
Seed Yield	1,452	620	Yes	1,490	526	Yes	0.38	1.38	-1.03
% Oil	42.5	3.6	Yes	42.6	3.2	Yes	0.17	1.29	-0.34
W/T Cross 1988:									
Flowering	49.0	5.2	Yes	49.5	4.9	Yes	0.56	1.13	-1.53
Maturity	88.9	4.9	Yes	89.8	4.9	Yes	1.02	1.02	-2.13
Plant Height	87.3	9.2	Yes	85.7	11.2	Yes	0.85	1.48	1.93
Seed Yield	913	229	Yes	859	202	Yes	1.47	1.29	1.66
1000 Seed Wt	3.32	.47	Yes	3.31	.58	Yes	0.10	1.54	0.04
% Oil	46.7	1.5	Yes	46.6	1.7	No	0.34	1.20	-0.16
% Protein	25.7	1.5	Yes	26.0	1.3	Yes	1.25	1.35	-2.55*
R/W Cross 1987:									
Flowering[1]	44.2	7.2	No	44.6	6.6	No	0.33	1.20	-1.28
Maturity	90.0	12.8	No	91.0	12.9	No	0.44	1.01	-1.19
Lodging	2.35	.79	No	2.50	.81	No	1.20	1.03	0.32
Seed Yield	1,472	717	Yes	1,464	593	Yes	0.07	1.46	0.15
% Oil	42.5	3.5	Yes	42.4	3.4	Yes	0.16	1.03	0.22
R/W Cross 1988:									
Flowering	49.9	5.8	Yes	49.7	5.5	Yes	0.20	1.09	0.11
Maturity	91.1	8.3	No	90.9	7.9	No	0.14	1.08	0.03
Plant Height	85.4	9.1	Yes	83.4	9.7	Yes	1.18	1.12	1.73
Seed Yield	1,058	359	Yes	1,047	362	No	0.17	1.01	0.31
1000 Seed Wt	3.40	.44	Yes	3.36	.44	Yes	0.51	1.00	1.06
% Oil	46.0	1.2	Yes	46.4	1.2	Yes	1.90	1.12	-2.12*
% Protein	26.4	1.1	Yes	26.3	1.2	Yes	0.48	1.35	0.78

[1]t-values for comparisons of means, F-values for comparisons of variances, z-values for comparisons of distributions of line means (by Mann-Whitney U-test)
[2]Flowering and maturity measured in days from planting, lodging on scale of 1 (upright) to 5 (prostrate), height in cm, seed yield in kg/ha (10% moisture), 1000 seed weight in gm, and oil and protein as a percentage of moisture-free, whole seed
[3]*,** = significant at the 5% level and the 1% level, respectively

Table 2. Predicted gains from selection (G_s) and 95% confidence intervals for (CI) for iH^2 in MD and SSD populations from the W/T and R/W crosses. All values based on 10% selection intensity (i = 1.755).

Cross	Trait	Year	Population	Predicted G_s	95% CI for iH^2
W/T	Maturity	1987	MD	6.7 days	$1.53 \leq iH^2 \leq 1.63$
			SSD	8.1 days	$1.59 \leq iH^2 \leq 1.66$
		1988	MD	3.8 days	$1.51 \leq iH^2 \leq 1.63$
			SSD	3.9 days	$1.49 \leq iH^2 \leq 1.64$
	Seed Yield	1987	MD	464 kg/ha	$1.40 \leq iH^2 \leq 1.56$
			SSD	371 kg/ha	$1.27 \leq iH^2 \leq 1.50$
		1988	MD	144 kg/ha	$1.08 \leq iH^2 \leq 1.41$
			SSD	114 kg/ha	$1.02 \leq iH^2 \leq 1.44$
	% Oil	1987	MD	2.75 %	$1.40 \leq iH^2 \leq 1.58$
			SSD	2.31 %	$1.21 \leq iH^2 \leq 1.53$
		1988	MD	1.10 %	$1.30 \leq iH^2 \leq 1.52$
			SSD	1.23 %	$1.33 \leq iH^2 \leq 1.58$
R/W	Maturity	1987	MD	10.8 days	$1.65 \leq iH^2 \leq 1.70$
			SSD	10.8 days	$1.65 \leq iH^2 \leq 1.70$
		1988	MD	7.0 days	$1.67 \leq iH^2 \leq 1.71$
			SSD	6.7 days	$1.65 \leq iH^2 \leq 1.71$
	Seed Yield	1987	MD	579 kg/ha	$1.55 \leq iH^2 \leq 1.65$
			SSD	445 kg/ha	$1.43 \leq iH^2 \leq 1.60$
		1988	MD	246 kg/ha	$1.21 \leq iH^2 \leq 1.48$
			SSD	248 kg/ha	$1.16 \leq iH^2 \leq 1.50$
	% Oil	1987	MD	2.61 %	$1.42 \leq iH^2 \leq 1.59$
			SSD	2.58 %	$1.41 \leq iH^2 \leq 1.59$
		1988	MD	0.91 %	$1.32 \leq iH^2 \leq 1.54$
			SSD	0.85 %	$1.22 \leq iH^2 \leq 1.53$

DISCUSSION

The results of the SDH and CDH population comparisons in this study indicate that SDH lines produced from the F_1 are homozygous, and include a similar array of recombinant genotypes as do CDH lines. Thus, where SDHs can be produced in high enough frequency to satisfy breeding needs, colchicine-doubling can be eliminated, reducing the time required to obtain microspore-derived lines by three to four months.

The comparisons of MD and SSD populations detected no substantial differences for economically-important traits, with the sole exception of lodging response in progeny populations from the W/T cross. For that trait, the MD population had a lower mean lodging score and a significantly greater variance than the SSD population. These differences are consistent with the simultaneous presence of additive x additive epistasis and coupling phase linkage between factors controlling the trait (Riggs and Snape, 1977; Jinks and Pooni, 1981). In this particular case, there is greater opportunity for selecting lines with lower lodging scores in the MD population, since such lines are present in higher frequency.

The comparisons of G_s in MD and SSD populations for days to maturity, seed yield, and seed oil content detected no differences in predicted gain for any of the traits. This indicates that similar levels of genetic improvement should be realized from a single cycle of selection using microspore culture and conventional methods, as exemplified by SSD.

Further comparisons of MD and conventional lines are necessary, to

ascertain that the results obtained here have broader relevance. The similarities in gain from a single cycle of selection in MD and SSD populations obtained in this study indicate that the key factor influencing the rate of genetic improvement (gain/cycle) will be cycle length. It follows, then, that the advantage of microspore culture will: (1) be greater in winter-type B. napus, where any method based on self-pollination requires a lengthy vernalization with each generation of selfing, and (2) be enhanced in both spring- and winter-types if SDH lines only are used.

One very important factor which has not been addressed in this study is the resource requirement and relative cost of microspore culture and conventional methods. Genetic comparisons such as those made in this study must ultimately be expressed in terms of the cost of attaining equivalent levels of genetic gain through different breeding approaches. This will allow breeders to identify the specific materials and situations in which microspore culture can be used to greatest advantage.

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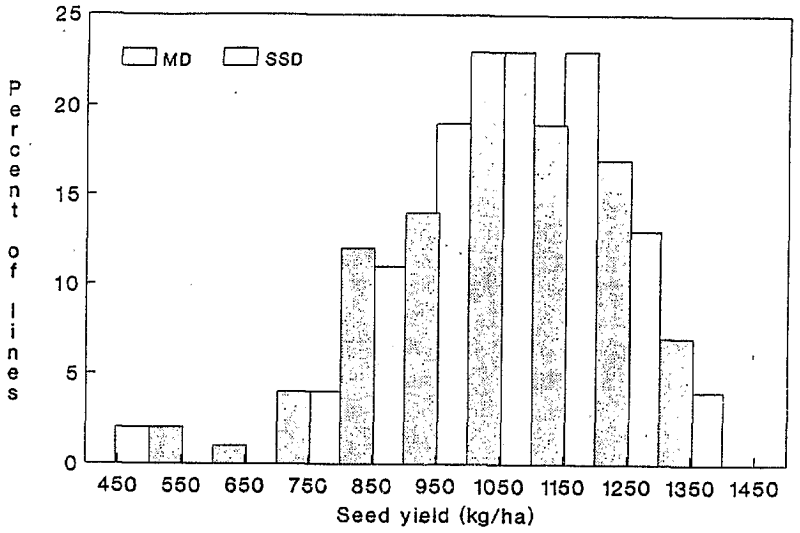

Fig. I. Frequency distributions for seed yield of MD and SD lines from the Regent/Westar cross (1988).

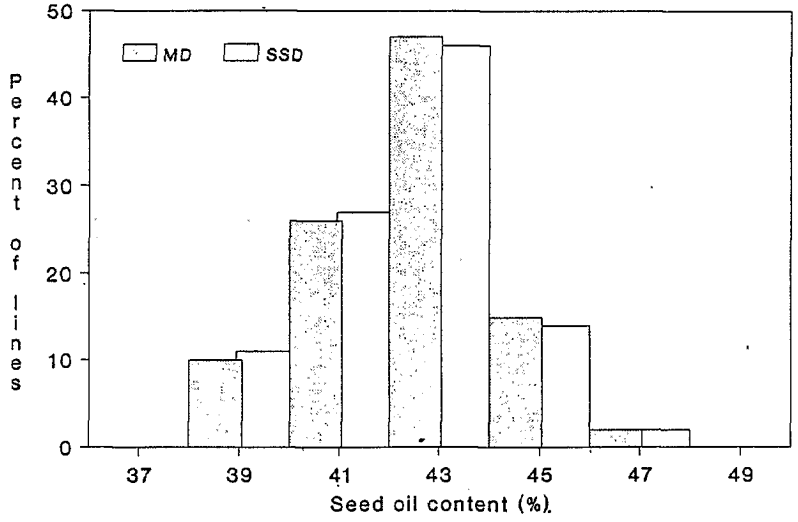

Fig. II. Frequency distributions for seed oil content in MD and SSD lines from the Regent/Westar cross (1987).