EFFECTIVENESS OF SELECTION FOR EARLY FLOWERING IN F-2 POPULATIONS OF BRASSICA NAPUS L. - A COMPARISON OF DOUBLED HAPLOID AND SINGLE SEED DESCENT METHODS

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INTRODUCTION

Recent improvements in the isolated microspore culture procedure in B.napus (Lichter 1985; Coventry et al. 1988) have stimulated interest in using this technique in Brassica breeding programs. Although several reports suggest genetic segregation of qualitative traits is similar in both doubled haploid and conventionally-derived genetic populations (Chen and Beversdorf 1990, Lichter et al. 1988), there are limited data on the response of quantitative traits studied under the two approaches. A recent report by Charne (1990) suggested there is no apparent difference in genetic variability present in Microspore-derived and Single Seed Descent-derived populations for characters such as oil and protein contents, yield, and maturity in B.napus. These results require confirmation using diverse germplasm sources before the Doubled Haploid (DH) system can be utilized with full confidence by Brassica breeders.

This presentation describes a study, undertaken in part, to compare the effectiveness of the Doubled Haploid vs Single Seed Descent methods in selecting for the quantitatively inherited early flowering trait in F-2 populations of B.napus

MATERIALS AND METHODS

Two F-2 populations, derived from crosses between genetically diverse germplasm sources, were selected for the study. A common canola parent, cv Westar, was used in each case as the female parent in a cross with a weak winter-type Chinese cultivar, Ganyou-5, and a Pakistani spring cultivar, DGL. The two populations were grown in growth cabinets maintained at 20 C constant temperature and 14h photoperiod. The photoperiod was selected to approximate the photoperiod experienced outdoors at Edmonton for a sowing in mid May. Photosynthetic photon flux density at plant level was 425-450 uE m^{-2} s^{-2}. The growth medium used was soil free (Stringam 1971), and the plants were fertilized once a week with a 20-20-20 nutrient solution. Four growth cabinet runs were completed for each cross with 50 plants/cross grown in each run. The first 10% of the plants to flower (first open flower) were selected as donor plants for isolated microspore culture. The flowering behaviour of the two populations was estimated by recording the days to flower on all plants grown to a maximum of 100 days.

The microspore culture and colchicine treatment of microspore derived plantlets to obtain doubled haploid plants were accomplished as described by Coventry et al. (1988). Seed from the DH plants was increased in the green house by self-pollination to produce DH-1 seed. The early flowering donor plants were also self-pollinated and carried forward in a modified 5-generation Single Seed Descent (SSD) inbreeding program with 15 seeds representing each F-2 plant.

A total of 19 Single Seed Descent (SSD) lines and 20 Doubled Haploid (DH) lines derived from three F-2 donor plants of the Ganyou-5 cross, and 7 SSD lines and 9 DH lines from two donor plants of the DGL cross, were grown in an RCB design under the same growth conditions as described for the F-2 populations. The DH-1 and SSD F-5 derived lines were chosen at random from each of the selected F-2 donor parents. Two plants/line were grown in 12 cm plastic pots to the first-flower stage, and the experiment was repeated three times. Days to first flower was recorded as described earlier.

A statistical analysis was performed using procedures of SAS Institute,Inc.(1985). The flowering data from the F-2 plants of the two crosses were analysed as an RCB design and the mean square of the cross x run interaction was used as the error mean square to compute the F value for comparing the two populations. The flowering data from the SSD and DH lines were analysed separately for each cross as an RCB design with breeding method (SSD vs DH) being fixed and donor plant being random. The lines nested within donor plant by breeding method was also considered as random. The F value for testing the breeding method was derived using the mean square of the mother plant x breeding method interaction as the error term. A non-parametric test, Wilcoxon rank test (Mann-Whitney U test) was used to compare the frequency distribution of the SSD and DH lines from each cross for the flowering behaviour.

RESULTS AND DICUSSION

A comparison of the flowering behaviour between the two F-2 populations indicated the difference was highly significant ($P<0.01$). The F-2 progeny from the Westar x Ganyou-5 cross had a lower mean value for this characteristic than did the equivalent population from the Westar x DGL cross (Table 1). The range of flowering dates, however, was greater in the Westar x Ganyou-5 cross. This result was expected since Ganyou-5 is a weak winter type cultivar, and the F-2 progeny should segregate for winter type plants requiring vernalization to induce flowering. Indeed, there were several plants in this population which failed to flower and were eventually discarded.

Table 1. Days to flower of two B.napus F-2 populations

Cross	Mean	CV(%)	Range
Westar x Ganyou-5	54.0	17.9	39-96
Westar x DGL	63.3	19.2	38-94

Flowering date comparisons between SSD and DH lines indicated that there were no differences ($P<0.87$ and $P<0.33$) between the two breeding methods. The ranges and means of the two methods within each cross were also similar indicating that each breeding method produced a similar range of variability (Table 2).

Table 2. Days to flower of F-2 derived SSD and DH lines

Cross	SSD			DH		
	Mean	CV(%)	Range*	Mean	CV(%)	Range*
Westar x Ganyou-5	49.7	5.3	45.8-53.4	49.5	5.6	45.0-53.8
Westar x DGL	51.1	8.5	45.5-59.7	51.5	12.1	41.3-61.5

* means of three experiments

The distributions of flowering date for both SSD and DH populations for each cross are presented in Fig.1a,b. Since the population sizes in the Westar x DGL cross were small, the frequency data were inconclusive. Frequency data for the Westar x Ganyou-5 cross suggested a multi-modal pattern with approximately the same distribution for both SSD and DH lines. The non-parametric test performed to compare the frequency distributions of the SSD and DH lines also indicated that the distributions from either cross were similar (Table 3). There was a tendency for DH lines to appear more frequently in the distributions at the end of the flowering spectrum, especially in the Westar x DGL cross. This result is not unexpected since similar observations have been reported for glucosinolates in B.napus (Siebel and Pauls 1989).

Table 3.Results of the non-parametric test on the frequency distribution in the flowering trait in SSD and DH lines

Cross	Wilcoxon rank sum test		
	S	Z-value	P
Westar x Ganyou-5	389.5	0.25	0.80*
Westar x DGL	56.0	-0.32	0.75*

* non-significant

The results of this study are in agreement with the findings of others (Charne 1990; Chen and Beversdorf 1990; Siebel and Pauls 1989) in that microspore-derived and SSD lines in B.napus produce a similar array of recombinant genotypes. While the F-1 generation is usually the one of choice for microspore culture, in the present study, selected F-2 donor plants were used. One consequence of using the F-2 generation for deriving haploid lines, is the avoidance of screening a high frequency of agronomically undesirable genotypes for traits normally selected under field conditions (Snape and Simpson 1981). By choosing selected F-2 donor plants in the present study, it was anticipated that a rapid shift of the early flowering mean could be realized. Since significant differences existed between growth cabinet runs of the F-2 populations, statistical comparisons could not be made between the flowering dates of the F-2 donor plants and the DH and SSD-derived lines. It is of interest to note, however, that mean days to flower in lines derived from F-2 donor plants were substantially less than the means of their parent F-2 populations, indicating that a shift of the population means occurred in the direction of selection. This shift has important implications for B.napus breeding programs as early flowering is positively correlated with early maturity in B.napus (Campbell and Kondra 1977; 1978). On the Canadian Prairies at least, selection for early maturity is a major objective in Brassica breeding programs.

Although the present study was conducted using a limited number of SSD and DH lines from two B.napus crosses, the results suggest that both the Single Seed Descent and the Doubled Haploid breeding methods could be equally effective for deriving early flowering lines. The main advantage of the DH method is that its use should result in more rapid gains per unit time (Chen and Beversdorf 1990). The DH method may also enhance selection for extremes in genotypes at either end of the selection curve (Siebel and Pauls 1989). This

aspect will be the subject of further field studies where other important agronomic traits can be compared using larger numbers of SSD and DH lines from the Ganyou-5 and DGL-derived breeding populations.

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Fig.1 (a)

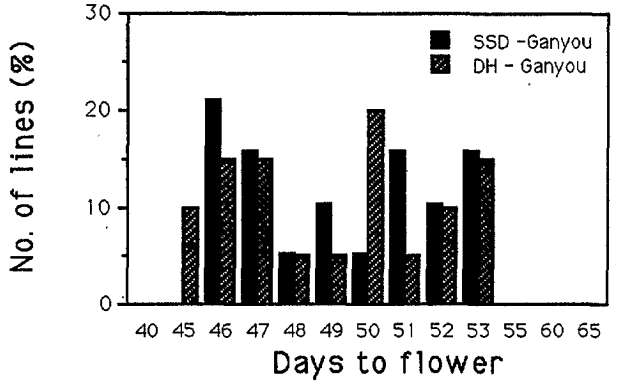

Fig.1(b)

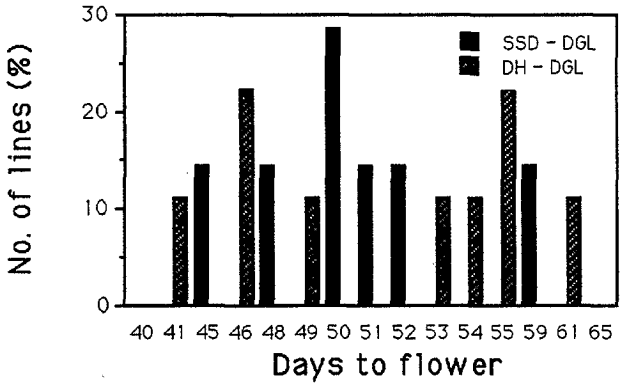

Fig.1. Frequency distribution for flowering in SSD and DH lines from (a) Westar x Ganyou-5, and (b) Westar x DGL crosses.