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Comparison of Frequency Distributions of Doubled Haploid and Single Seed Descent Lines in Barley*

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Summary. Both doubled haploid (DH) and single seed descent (SSD) methods were used to derive homozygous lines from two crosses of barley. The frequency distributions of grain yield, heading date, and plant height of the DH and SSD lines were compared by the Mann-Whitney U test, Kolmogorov-Smirnov two-sample test and Wald-Wolfowitz runs test. It was found that the DH lines distributed in the same manner as the SSD lines with respect to the three characters. The results indicated that although the SSD method had more opportunity for recombination than the DH method, it did not produce a sample of recombinants which differed significantly from the DH sample; thus both methods were equally efficient for use in deriving homozygous lines from F_1 hybrids in a relatively short time.

Key words: Doubled haploid method – Single seed descent method – Linkage – Barley

Introduction

Both doubled haploid (DH) and single seed descent (SSD) methods can be employed to derive homozygous lines from a segregating generation in a relatively short time. In theory, the means and variances for a quantitative character of the two populations of lines derived by the two methods are expected to be the same in the absence of linkage; when linkage and/or additive epistasis are present, however, they differ due to a higher frequency of recombinant lines in the SSD population (Snape 1976). These theoretical findings have been confirmed by the results of computer simulation studies (Riggs and Snape 1977). Likewise, the skewness and kurtosis are also expected to be different between the DH and SSD populations in the presence of linkage and additive epistasis. Consequently, a comparison of the frequency distributions of DH and SSD lines provides us a test for linkage. The two distributions should be different in the presence of linkage; if linkage is absent, they should be similar. Information on the linkage of quantitative characters is helpful for designing effective breeding strategies and procedures. Such information is particularly useful for the DH method since there may be only one chance for recombination. Choo (1981) suggested that in case of tight linkage and high additive epistasis, a large number of doubled haploids should be produced from F_1 plants in the hope of obtaining the best recombinant; alternatively, haploids should be produced from F_2 instead of F_1 plants. Snape (1976) stated that since plant breeder is generally interested in maximizing the variation from a cross of dispersed parents, SSD would appear to be a more efficient method than haploid production due to the opportunity for recombination over more than one generation. This paper reports the results of a comparative study on the frequency distributions of DH and SSD lines in two crosses of barley.

Materials and Methods

Two spring barley crosses, R 1 ('Paragon'×'Zephyr') and R 2 (OB 73–18×'Champlain') were used to derive homozygous lines by SSD and DH methods. SSD lines were developed to the F_6 generation using the modification of growing two seeds from each plant in the preceding generation and, upon occasion when one plant headed much later, saving the one which headed first. Generation time was shortened by dissecting embryos from immature seeds for embryo culture. DH lines were obtained by using colchicine to double the chromosomes of haploids cultured from the embryos of interspecific hybrids between the F_1 plants and *Hordeum bulbosum* (see Subrahmanyan and Kasha 1975; Jensen 1975 for the haploid-production technique).

About 100 lines derived by each method from the two crosses were increased for seed at Brawley, California during the

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winter of 1972–1973. The materials were evaluated for grain yield, heading date, and plant height at Elora, Ontario in 1973. In the evaluation trial, single hill plots spaced 30 cm × 45 cm with 25 seeds per hill were used in a split plot design with 10 replications. Cross-method combinations were arranged as the main plot units with lines within each combination as subplot units. Data of the DH lines obtained from this trial were originally used to study the minimum population size for identifying superior crosses (Reinbergs et al. 1976). However, results of the SSD lines have not been reported previously.

Three nonparametric tests, i.e. the Mann-Whitney U test, Kolmogorov-Smirnov two-sample test, and Wald-Wolfowitz runs test (Siegel 1956) were used to determine if the frequency distributions of the DH and SSD lines differ in any way: in mean, in variance, in skewness, etc. In using the Mann-Whitney test, we first combined the observations from the DH and SSD samples, ranked them in order of increasing size and calculated the U-statistics by using the following equation,

$$U = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1$$

where n_1, n_2 are the number of the DH and SSD lines, respectively, and R_1 is the sum of the ranks assigned to the DH sample.

To apply the Kolmogorov-Smirnov test, we made a cumulative frequency distribution for each sample of observations, using the same intervals for both distributions. For each interval, we subtracted one step function from the other and found the maximum absolute value of the difference (D). Symbolically,

$$D = \text{maximum}\ |Sn_1(X) - Sn_2(X)|$$

where $Sn_1(X)$, $Sn_2(X)$ are the observed cumulative step functions of the DH and SSD samples, respectively; they were obtained by dividing the number of observations equal to or less than the maximum value of an interval, X, by the total number of observations in the sample.

The Wald-Wolfowitz test was conducted by ranking the $n_1 + n_2$ observations in order of increasing size and then determining the number of runs, r, in the ordered series. A run is defined as any sequence of observations from the same sample (either DH or SSD sample).

Tests of significance of an observed value of U, D, and r were conducted according to the procedures outlined in Siegel (1956).

Results and Discussion

The frequency distributions of grain yield, heading date, and plant height of the DH and SSD lines derived from the R1 and R2 crosses are illustrated in Figs. 1, 2 and 3. All three nonparametric tests showed that the DH and SSD lines had the same frequency distribution of grain yield or plant height in either of the two crosses (Table 1). For heading date, however, the three tests exhibited different results. Siegel (1956) reported that whereas for very small samples the Kolmogorov-Smirnov test is slightly more efficient than the Mann-Whitney test, for large samples the converse holds. In

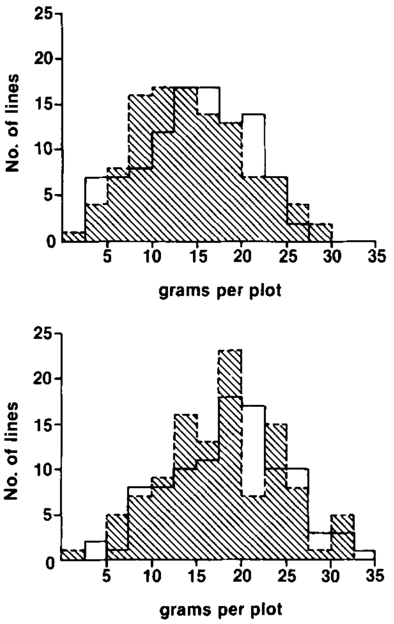

Fig. 1

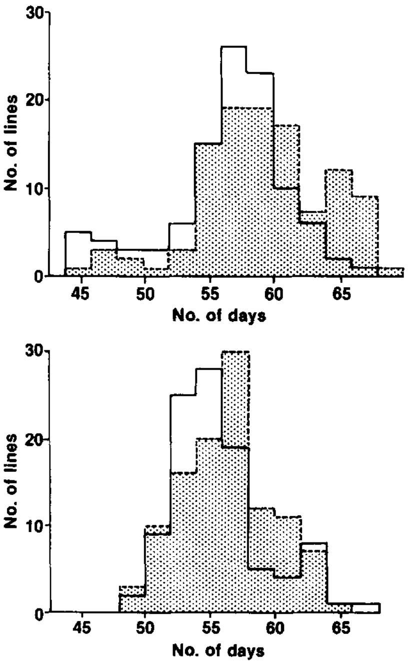

Fig. 2

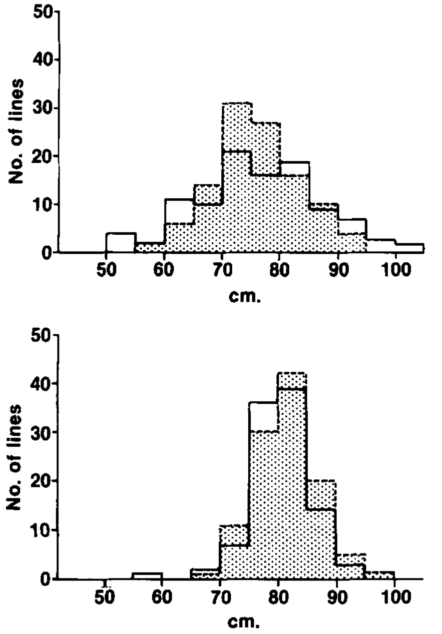

Fig. 3

Figs. 1–3. 1. Frequency distributions of grain yield of doubled haploid (shaded distribution) and single seed descent (unshaded distribution) lines produced from the R 1 (upper figure) and the R 2 (lower figure) crosses of barley. **2.** Frequency distributions of heading date of doubled haploid (shaded distribution) and single seed descent (unshaded distribution) lines produced from the R 1 (upper figure) and the R 2 (lower figure) crosses of barley. **3.** Frequency distributions of plant height of doubled haploid (shaded distribution) and single seed descent (unshaded distribution) lines produced from the R 1 (upper figure) and the R 2 (lower figure) crosses of barley

Table 1. The results of the Mann-Whitney U test, Kolmogorov-Smirnov two sample test (D) and Wald-Wolfowitz runs test (r) on the frequency distributions of DH and SSD lines derived from two crosses of barley[a]

Cross	U	D	r
	Grain yield		
R 1	6081	0.13	111
R 2	6148	0.12	112
	Heading date		
R 1	4032**	0.24**	114
R 2	4860	0.19*	105
	Plant height		
R 1	6115	0.14	111
R 2	4997	0.10	104

[a] The DH sample consisted of 110 lines in both crosses and the SSD sample consisted of 104 and 102 lines in the R 1 and R 2 crosses, respectively

* Significant at 0.05 level

** Significant at 0.01 level

view of the Siegel's report and the fact that the observed D value (=0.1911) for heading date in the R 2 cross was just slightly over the critical D value at 0.05 level (=0.1869), thus we did not reject the null hypothesis in the Kolmogorov-Smirnov test for the R 2 cross and believed that the two frequency distributions in the R 2 cross were similar. It appears that the Wald-Wolfowitz test is not as powerful as the other two tests (Siegel 1956). Consequently, it did not detect the difference between the two distributions of heading date of the DH and SSD lines in the R 1 cross when in fact the SSD sample did contain more early heading lines than the DH sample in that cross (Fig. 2). As mentioned earlier, during the SSD process, we grew two seeds from each plant in the preceding generation and upon occasion, saved the plant which headed earlier. It seems that this procedure caused the increase of the frequency of early heading genotypes in the R 1 cross. The two frequency distributions of heading date in the R 1 cross might not be different had the selection pressure not been used in the propagation generation in the SSD method. Therefore, the results of this study indicated that the DH lines distributed in the same manner as the SSD lines in both crosses with regard to grain yield, heading date, and plant height, if no selection has been applied during the SSD process.

Although the SSD method had more opportunity for recombination than the DH method, it did not produce a sample of recombinants which is significantly different from the DH sample. This phenomenon can be explained by one or more of the following reasons: (1) genes controlling these characters in both crosses are loosely, or not linked (Riggs and Snape 1977), (2) genes in the F_1 plants are in mixed coupling and repulsion phases (Riggs and Snape 1977), and (3) the difference between the two distributions, if any, is too small for detection. Whatever the causes, the results of this study do not support the viewpoint that SSD is a more efficient method than haploid production due to the opportunity for recombination over more than one generation (Snape 1976; Riggs and Snape 1977). Our results suggest that both DH and SSD methods are equally efficient for use in deriving homozygous lines from F_1 hybrids in a relatively short time.

Choo (1981) showed that when linkage is present, a doubled haploid population derived from F_2 plants may contain almost 50% more of the best recombinant than the one derived from F_1 plants since the former population gets one more chance of recombination. Therefore, the author suggested that in case of tight linkage and high additive epistasis, the production of haploids should be delayed until the F_2 generation; if not, a large number of doubled haploids should be produced from F_1 plants in the hope of obtaining the best recombinant. However, the findings of this study may imply that the frequency distributions of the F_1- and F_2-derived doubled haploid lines would likely be the same. If so, then there is no need to delay the production of haploids until the F_2 generation. More field experiments are needed in order to determine the relative efficiency of the DH and SSD methods and the optimum filial generation for producing doubled haploids.

It has been shown that doubled haploids obtained by the Bulbosum method are a random sample of gametes from F_1 plants (Johns 1974; Pickering 1980). Our results indicated that both DH and SSD samples had the same mean, variance, skewness, etc. for the quantitative characters that had not been subjected to selection pressures. This appears to suggest that the SSD lines are a random sample of genotypes also. As far as practical plant breeding and quantitative genetic studies are concerned, the property of randomness is a highly desirable feature of the DH and SSD methods.

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