Doubled Haploids for Estimating Genetic Variances and a Scheme for Population Improvement in Self-pollinating Crops

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Summary. An analysis is derived for a diallel experiment in which each cross is represented by a number of homozygous lines developed by the doubled haploid method. Both additive and additive × additive genetic variances can be estimated with this analysis. A population-improvement scheme involving the doubled haploid or single seed descent methods is also proposed.

Key words: Diallel analysis – Recurrent selection – Barley

Introduction

Diallel cross analyses have been used extensively to study the combining ability and inheritance of quantitative characters in self-pollinating crops. Data for the analyses are usually obtained from the F_1 or F_2 families of each cross (Griffing 1956; Mather and Jinks 1971).

In many species, haploid plants can be derived from a normal diploid plant. If the chromosome number of the haploid plant is doubled, a homozygous diploid plant is obtained. These techniques are very useful in breeding self-pollinating crops, as pure lines can be derived from F_1 plants by the above procedures, thus eliminating several generations of self-pollination. There are no reports in the literature of analytical methods for a diallel cross in which doubled haploid lines are evaluated.

It has been shown that the population improvement method is a highly effective breeding approach toward accumulating desirable genes and to produce superior germ plasm in cross-pollinating crops, particularly in corn. For example, a corn survey in the U.S.A. indicates that in 1975, 33.5% of the inbred lines used in hybrid seed production were derived either directly or indirectly from the Iowa stiff stalk synthetic (Zuber 1976). In self-pollinating crops, however, relatively few population improvement programs have been established.

This paper presents a diallel cross analysis in which each cross is represented by doubled haploid progenies and then proposes a population-improvement scheme with doubled haploid lines for self-pollinating crops.

Estimation of Genetic Variances

Assume a set of diploid inbred lines in which U_a of the lines are AA and V_a lines ($V_a = 1 - U_a$) are aa. The genotypic values, measured as deviations from the midparent, are $+d_a$, h_a, and $-d_a$ for AA, Aa, and aa respectively. The variance among the lines is

$$\sigma_p^2 = U_a d_a^2 + V_a d_a^2 - (U_a d_a - V_a d_a)^2 = 4 U_a V_a d_a^2$$

If these lines are crossed in a diallel series, the frequencies and genotypic values of the F_1 families can be calculated (Table 1). Doubled haploid lines can be produced from each F_1 family. Johns (1974) concluded that for simply inherited traits, the doubled haploid lines represented a random sample of gametes from the F_1 hybrid. On the basis of that assumption, the variance among the doubled haploid lines is:

$$\sigma_{DH}^2 = U_a^2 d_a^2 + U_a V_a d_a^2 + U_a V_a d_a^2 + V_a^2 d_a^2$$
$$- [(U_a - V_a) d_a]^2 = 4 U_a V_a d_a^2$$

This variance can be subdivided into that among crosses or families ($\sigma_c^2 = 2 U_a V_a d_a^2$) and that within crosses ($\sigma_w^2 = 2 U_a V_a d_a^2$).

Let us extend the derivation of these equations to a two-locus system. The frequencies and means of the doubled haploid progenies, in terms of two unlinked loci, derived from different F_1 families are given in Table 2 in which i is the interaction between the two-loci when both are homozygous. The variance of the parents becomes (see Mather and Jinks (1971))

$$\sigma_p^2 = 4\, U_a V_a\, [d_a + (U_b - V_b)\, i]^2$$
$$+ 4\, U_b V_b\, [d_b + (U_a - V_a)\, i]^2 + 16\, U_a V_a U_b V_b\, i^2$$

It again is equal to the variance of the doubled haploid population whereas the variances among crosses and within crosses are

$$\sigma_c^2 = 2\, U_a V_a\, [d_a + (U_b - V_b)\, i]^2 +$$
$$2\, U_b V_b\, [d_b + (U_a - V_a) i]^2 + 4\, U_a V_a U_b V_b\, i^2$$

and $\sigma_w^2 = 2\, U_a V_a\, [d_a + (U_b - V_b)\, i]^2$
$$+ 2\, U_b V_b\, [d_b + (U_a - V_a) i]^2 + 12\, U_a V_a U_b V_b\, i^2$$

Generalizing for any number of genes and assuming that the higher order gene interactions are negligible, the variances can be written as:

$$\sigma_p^2 = \sigma_{DH}^2 = \sigma_A^2 + \sigma_{AA}^2$$
$$\sigma_c^2 = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_{AA}^2$$

and $\sigma_w^2 = \tfrac{1}{2}\sigma_A^2 + \tfrac{3}{4}\sigma_{AA}^2$

Table 1. Genotypes and means of parents, F_1 families, and doubled haploid progenies of a diallel cross involving one gene pair

	Genotype frequency mean	Maternal parents AA U_a d_a		aa V_a $-d_a$			Paternal array means
Paternal parent	AA U_a d_a	$\begin{bmatrix} AA \\ U_a^2 \\ d_a \end{bmatrix}$ *	AA U_a^2 d_a	$\begin{bmatrix} Aa \\ U_a V_a \\ h_a \end{bmatrix}$	AA $\tfrac{1}{2}U_a V_a$ d_a	aa $\tfrac{1}{2}U_a V_a$ $-d_a$	$U_a d_a$
	aa V_a $-d_a$	$\begin{bmatrix} Aa \\ U_a V_a \\ h_a \end{bmatrix}$	AA $\tfrac{1}{2}U_a V_a$ d_a	aa $\tfrac{1}{2}U_a V_a$ $-d_a$	$\begin{bmatrix} aa \\ V_a^2 \\ -d_a \end{bmatrix}$	aa V_a^2 $-d_a$	$-V_a d_a$
Maternal array means			$U_a d_a$			$-V_a d_a$	Overall mean $(U_a - V_a)d_a$

* Genotypes, frequencies, and means of F_1 families are given in parentheses

Table 2. Phenotypes and means of parents and doubled haploid progenies of a diallel cross involving two gene pairs with interaction

Parents phenotype frequency	$AABB$ $d_a + d_b + i$ $U_a U_b$	$AAbb$ $d_a - d_b - i$ $U_a V_b$	$aaBB$ $-d_a + d_b - i$ $V_a U_b$	$aabb$ $-d_a - d_b + i$ $V_a V_b$
$AABB$ ($U_a U_b$)	$d_a + d_b + i$ $U_a^2 U_b^2$	d_a $U_a^2 U_b V_b$	d_b $U_a V_a U_b^2$	0 $U_a V_a U_b V_b$
$AAbb$ ($U_a V_b$)	d_a $U_a^2 U_b V_b$	$d_a - d_b - i$ $U_a^2 V_b^2$	0 $U_a V_a U_b V_b$	$-d_b$ $U_a V_a V_b^2$
$aaBB$ ($V_a U_b$)	d_b $U_a V_a U_b^2$	0 $U_a V_a U_b V_b$	$-d_a + d_b - i$ $V_a^2 U_b^2$	$-d_a$ $V_a^2 U_b V_b$
$aabb$ ($V_a V_b$)	0 $U_a V_a U_b V_b$	$-d_b$ $U_a V_a V_b^2$	$-d_a$ $V_a^2 U_b V_b$	$-d_a - d_b + i$ $V_a^2 V_b^2$

Mean of parents = mean of crosses = $(U_a - V_a)\, d_a + (U_b - V_b)\, d_b + (U_a - V_a)(U_b - V_b)i$

where σ_A^2 and σ_{AA}^2 are respectively the additive and additive × additive genetic variances of the inbred parents. It becomes apparent that the additive and additive × additive genetic variances, which are of most concern to plant breeders in self-pollinating crops, can be obtained by solving any two of the three equations, such as $\sigma_{AA}^2 = 2$ $(\sigma_p^2 - 2\sigma_c^2)$. It should be noted that additive × additive genetic variance cannot be estimated when F_1 or F_2 bulk materials are used in a diallel experiment (Mather and Jinks 1971).

To obtain these genetic variances, both parents and all possible crosses represented by doubled haploid lines are required for evaluation. Reciprocal crosses may not be necessary. Materials can be evaluated in a split-plot layout with parents and crosses as main plot units, and the doubled haploid lines within a cross as subplot units. The appropriate analysis of variance is given in Table 3. Differences among parents and crosses can be tested against the experimental error. Genetic variances of parents and among crosses are obtained by equating the appropriate mean square to its expected mean square, and the additive and additive × additive genetic variances can be estimated accordingly. Theoretically, the means of the parents and the means of the crosses should be equal. Thus, if the parents vs crosses mean square is significantly greater than the experimental error, considerable sampling error may be present if the number of doubled haploid lines derived from crosses is small. The combining ability analysis, as proposed by Griffing (1956), should be included to acquire further information about the parents. When a bulk sample of doubled haploid lines is derived from each cross, variation among doubled haploid lines within crosses may also serve well for estimating both the additive and additive × additive genetic variances.

Population-Improvement Scheme

A diallel selective mating system for cereal breeding has been proposed by Jensen (1970). The author indicates that the system provides for broad use of germplasm, simultaneous input of parents, creation of persistent gene pools, breaking of linkage blocks, freeing of genetic variability and general fostering of genetic recombination. We are proposing a doubled haploid scheme that provides similar distinguished features and requires only 2 to 3 years to complete a selection cycle if a winter nursery is available.

In this scheme several parental cultivars are crossed in all possible combinations. However, a partial diallel cross may be used when the number of parents is large. Twenty doubled haploid lines, which has been reported to be the minimum number for predicting the relative yield of a cross (Reinbergs et al. 1976) are derived from the F_1 hybrid of each cross. To eliminate the effect of seed sources and to increase the amount of seeds, the doubled haploid lines should be advanced one generation (Walsh et al. 1973). Since the number of entries is large and the amount of seeds may be limited, it may be more appropriate to evaluate the materials in hill plots (Walsh et al. 1976). If a complete diallel with no reciprocals is used, the data obtained from the evaluation trial can be analyzed by the method given in the previous section.

Table 3. Analysis of variance for the data obtained from a diallel experiment in which each cross is represented by a number of doubled haploid lines (no reciprocal crosses)

Source	df	M.S.	Expected mean square	
			Random model	Fixed model
Replicates	$r - 1$			
Entries	$\dfrac{p(p + 3)}{2}$			
Parents vs crosses	1			
Parents	$p - 1$	M_1	$\sigma_e^2 + r\sigma_p^2$	$\sigma_e^2 + \dfrac{r}{p - 1}\Sigma p_i^2$
Crosses	$\dfrac{(p - 1)(p + 2)}{2}$	M_2	$\sigma_e^2 + r\sigma_c^2$	$\sigma_e^2 + \dfrac{2r}{(p - 1)(p + 2)}\Sigma c_j^2$
General combining ability	$p - 1$	M_3	$\sigma_e^2 + r\sigma_s^2 + r(p + 2)\sigma_g^2$	$\sigma_e^2 + \dfrac{r(p + 2)}{(p - 1)}\Sigma g_i^2$
Specific combining ability	$p(p - 1)/2$	M_4	$\sigma_e^2 + r\sigma_s^2$	$\sigma_e^2 + \dfrac{2r}{p(p - 1)}\Sigma\Sigma s_{ij}^2$
Error	$(r - 1)[½p(p + 3)]$	M_5	σ_e^2	σ_e^2

Lines may be chosen on the basis of their performance alone or in combination with their cross performance. The genetic gains expected from these two selection methods are as follows:

$$\Delta G_{line} = \frac{K\,\sigma_A^2}{\sqrt{\sigma_A^2 + \sigma_{AA}^2 + \dfrac{\sigma_e^2}{r}}}$$

$$\Delta G_{combined} = \frac{K(\tfrac{1}{2})\,\sigma_A^2}{\sqrt{\tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_{AA}^2 + \dfrac{\sigma_e^2}{nxr}}}$$

$$+ \frac{K(\tfrac{1}{2})\,\sigma_A^2}{\sqrt{\tfrac{1}{2}\sigma_A^2 + \tfrac{3}{4}\sigma_{AA}^2 + \dfrac{\sigma_e^2}{r}}}$$

where K is the standardized selection differential, n is the number of doubled haploid lines per cross, r is the number of replications, σ_A^2, σ_{AA}^2 are additive and additive × additive genetic variances, respectively, and σ_e^2 is the experimental error. From the above two equations, it is clear that combined selection is more effective than selection based on line performance alone, particularly when n is large and heritability is low. Combined selection can be performed by selecting the highest yielding doubled haploid lines from the superior crosses. However, with this approach, it is possible that the highest yielding lines may not be selected due to a mediocre cross performance. The more appropriate approach, therefore, may be using index selection. The selection index suggested by Lerner (1958) may serve well in this situation. Its expression is: $I = P_i + W\overline{P}$ where I is the selection index, P_i and $\overline{P}$ are the yield performance of the ith line and the average cross performance associated with the ith line, respectively, and W is the cross weighting coefficient and is chosen so as to maximize the regression of the true genotype of the line in question on the index. The weighting coefficient W is given as follows:

$$W = \frac{n}{n + (n-1)\,r^P} \cdot \frac{r^G - r^P}{1 - r^G}$$

where r^G and r^P are the genotypic and phenotypic correlation respectively between the doubled haploid lines within a cross. The value of r^G is taken as 0.5 for full sibs, and that of r^P is $r^G h^2$ (h^2 is the heritability). Lines with the highest index are selected and used as parents for another diallel cross, production of doubled haploids and selection cycle. At any stage, additional genotypes from other sources can be used as entries in the diallel cross. Also, additional genetic variability should be added whenever the additive variance is small.

It would be desirable to produce more doubled haploid lines from the most promising crosses, with the hope that more doubled haploid lines would enhance the chances of obtaining the best recombinants. Further, a preliminary study indicates that fixation of the genes involved in heterosis may be possible in barley by using the doubled haploid technique (Kasha et al. 1977). Thus, it may be worthwhile to produce more doubled haploid lines in the crosses with parents of high general combining ability and with parents of highest specific combining ability when a significant additive × additive variance is detected. Alternately, the progenies of the F_1 hybrids of these crosses may be handled by conventional breeding methods.

Discussion

The population improvement scheme presented in this paper permits the development of an improved gene pool and the release of superior doubled haploid lines at any time. It makes use of all the genetic information obtained from the diallel analysis, so that the breeding materials can be reasonably handled. It also maximizes the efficiency of the doubled haploid technique by identifying the most promising crosses in which the production of doubled haploids can be increased. Two population-improvement programs with different breeding objectives are being initiated using doubled haploid techniques for barley that were developed at this institution (Kasha and Kao 1970). The results will be reported in due course.

Similar population-improvement schemes can be developed by the single seed descent method in any self-pollinating crop. In fact, a recurrent selection program being conducted in oats uses the single seed descent method. The first cycle of recurrent selection was found to be effective in producing a 12% gain in yield, or 4% per year in oats (Stuthman, personal communication). The diallel analysis given in this paper can be applied also to materials derived by the single seed descent method assuming that the frequency of heterozygotes is negligible at the advanced generation.

Acknowledgement

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