

THE USE OF DOUBLED HAPLOID LINES FOR ESTIMATION OF GENETIC PARAMETERS¹

MARIA SURMA, TADEUSZ ADAMSKI, ZYGMUNT KACZMAREK²

Institute of Plant Genetics, Polish Academy of Sciences, Poznań

Summary. This paper presents the method of estimation of genetic parameters associated with additive gene action, domination and non-allelic interaction on the basis of double haploid lines and F_1 and F_2 hybrids. Theoretical considerations were illustrated by an example concerning the 1000-kernel weight in barley.

Basic genetic information in studies concerning the inheritance of quantitative traits may be obtained on the basis of estimates of genetic parameters associated with some effects of gene action. The so-far methods of estimation of genetic parameters dealing with additive gene action, domination and non-allelic interaction were based on the analysis of variation of F_1 , F_2 , F_3 , B_1 , B_2 hybrids.

This paper presents a new method of estimation of genetic parameters using doubled haploid lines and F_1 and F_2 hybrids.

ESTIMATION OF GENETIC PARAMETERS m , $[d]$, $[i]$

Four types of doubled haploid lines — $AABB$, $AAbb$, $aaBB$ and $aabb$ may be obtained from F_1 hybrids ($AaBb$) as a result of crossing two homozygous forms P_1 and P_2 differing by two pairs of genes $A-a$ and $B-b$ and by applying afterwards the double haploid methods. The ratio of the frequency of particular lines corresponds to that of the respective gametes produced by the F_1 hybrid and amounts to 1 : 1 : 1 : 1. The expected phenotypic values of the lines presented as functions of genetic parameters are given in Table 1 (F_∞ -metric). As seen from the table, the phenotypic values of doubled haploid lines are functions of the mean m and parameters associated with additive gene action d and with homozygote \times homozygote interaction i .

The expected mean phenotypic value for a set of lines covering all recombination types (compare Table 1) is equal to:

$$\bar{L}_m^e = m. \quad (1)$$

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² First author: Dr.; second: Dr.; third: Dr. Present address: ul. Strzeszyńska 30-36, 60-479 Poznań, Poland.

Table 1. Expected phenotypic values of doubled haploid lines in the case of segregation of two genes

Doubled haploid lines	Frequency	Phenotypic value
<i>AABB</i>	$\frac{1}{4}$	$m + d_a + d_b + i_{ab}$
<i>AAbb</i>	$\frac{1}{4}$	$m + d_a - d_b - i_{ab}$
<i>aaBB</i>	$\frac{1}{4}$	$m - d_a + d_b - i_{ab}$
<i>aabb</i>	$\frac{1}{4}$	$m - d_a - d_b + i_{ab}$

Each set of doubled haploid lines has a line distinguishing by a maximal and minimal expression of a given trait (lines *AABB* and *aabb*). As seen from Table 1, the expected phenotypic values for the line L_1 with the maximal value of a trait and for the line L_2 with the minimal value of the trait are equal to:

$$\begin{aligned}\bar{L}_1^e &= m + d_a + d_b + i_{ab}, \\ \bar{L}_2^e &= m - d_a - d_b + i_{ab}.\end{aligned}\quad (2)$$

Generalizing the discussed phenotypic values of the lines in the case of segregation of two pairs of genes for any number of gene pairs, the above formulas (2) will be:

$$\begin{aligned}\bar{L}_1^e &= m + [d] + [i], \\ \bar{L}_2^e &= m - [d] + [i].\end{aligned}\quad (3)$$

Having the expected values of the lines L_m , L_1 and L_2 , one can find estimators of the parameters m , $[d]$, $[i]$. They are as follows:

$$\begin{aligned}\hat{m} &= \bar{L}_m, \\ [\hat{d}] &= \frac{1}{2} (\bar{L}_1 - \bar{L}_2), \\ [\hat{i}] &= \frac{1}{2} (\bar{L}_1 + \bar{L}_2) - \bar{L}_m.\end{aligned}\quad (4)$$

ESTIMATION OF THE PARAMETERS m , $[d]$, $[h]$, $[i]$, $[l]$

Having only doubled haploid lines it is possible to find estimates of the $[d]$ and $[i]$ parameters. The both parameters are important in the studies of genetic transgression, since they concern effects of action and interaction of homozygous genes. If we want to obtain information about effects of domination ($[h]$ parameter) and heterozygote \times heterozygote interaction ($[l]$ parameter), it is necessary to lay out an experiment, which beside doubled haploid lines would also contain F_1 and F_2 plants. Estimates of the parameters m , $[d]$, $[h]$, $[i]$ and $[l]$ may be obtained in a way similar to that described in Kaczmarek, Surma and Adamski (1982). The expected values of means of F_1 and F_2 generations are equal to (Mather and Jinks 1971):

$$\begin{aligned}\bar{F}_1^e &= m + [h] + [l], \\ \bar{F}_2^e &= m + \frac{1}{2} [h] + \frac{1}{4} [l].\end{aligned}\quad (5)$$

Knowing the expected means for doubled haploid lines and F_1 and F_2 hybrids, it is possible to find estimators of the parameters $m, [d], [h], [i], [l]$ solving the equation:

$$\mathbf{b} = \mathbf{A}\mathbf{g}, \quad (6)$$

where

$$\mathbf{b} = [\bar{L}_m, \bar{L}_1, \bar{L}_2, \bar{F}_1, \bar{F}_2]',$$

is a vector of values of experimental means,

$$\mathbf{g} = [m, [d], [h], [i], [l]]'$$

is a vector of the corresponding genetic parameters, whereas

$$\mathbf{A} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 \\ 1 & -1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 1 \\ 1 & 0 & \frac{1}{2} & 0 & \frac{1}{4} \end{bmatrix}$$

is the matrix of the coefficients of these parameters.

As a result of equation (6) the following estimators of the parameters are obtained:

$$\begin{aligned} \hat{m} &= \bar{L}_m, \\ [\hat{d}] &= \frac{1}{2} (\bar{L}_1 - \bar{L}_2), \\ [\hat{h}] &= 4\bar{F}_2 - \bar{F}_1 - 3\bar{L}_m, \\ [\hat{i}] &= \frac{1}{2} (\bar{L}_1 + \bar{L}_2) - \bar{L}_m, \\ [\hat{l}] &= 2\bar{F}_1 - 4\bar{F}_2 + 2\bar{L}_m. \end{aligned} \quad (7)$$

The above theoretical considerations are true when the following conditions are fulfilled: homozygosity of the parental forms, diploid segregation, no linkage. In addition to that, it is assumed that a set of doubled haploid lines represents all the types of gametes produced by F_1 hybrids and that the probability of obtaining individual lines is similar.

TESTING OF HYPOTHESES

The significance of particular parameters may be tested by the t statistic, which is a ratio of a parameter estimate and standard error corresponding to it. The roots of variance of individual parameters estimated in the following way are taken as standard errors:

$$\begin{aligned} V_m &= V_{\bar{L}_m}, \\ V_{[d]} &= \frac{1}{4} (V_{\bar{L}_1} + V_{\bar{L}_2}), \\ V_{[h]} &= 9V_{\bar{L}_m} + V_{\bar{F}_1} + 16V_{\bar{F}_2}, \\ V_{[i]} &= V_{\bar{L}_m} + \frac{1}{4} (V_{\bar{L}_1} + V_{\bar{L}_2}), \\ V_{[l]} &= 4V_{\bar{L}_m} + 4V_{\bar{F}_1} + 16V_{\bar{F}_2}, \end{aligned} \quad (8)$$

where $V_{\bar{L}_m}$, $V_{\bar{L}_1}$, $V_{\bar{L}_2}$, $V_{\bar{F}_1}$, $V_{\bar{F}_2}$ are estimates of variance of the means of doubled haploid lines and F_1 and F_2 generations obtained in the experiment.

Using the above estimates of the parameter variance, one can test the hypotheses:

$H_{[d]} : [d] = 0$, of no effects of additive gene action,

$H_{[h]} : [h] = 0$, of no domination effects,

$H_{[i]} : [i] = 0$, of no homozygote \times homozygote interaction effects;

$H_{[l]} : [l] = 0$, of no heterozygote \times heterozygote interaction effects.

EXAMPLE

Determination of estimates of genetic parameters described in this paper is illustrated by an example from studies conducted on doubled haploid lines of barley at the Institute of Plant Genetics, Polish Academy of Sciences in Poznań. Dihaploid lines were obtained by bulbos method (Kasha and Kao 1970; Adamski 1979).

The experiment containing 43 lines derived from F_1 hybrids of the cross Emir \times Himalaya, as well as F_1 and F_2 hybrids was laid out at a completely random block design. Traits of the grain yield structure were studied. In the example, data concerning the 1000-kernel weight were used. Means and variances of means for the doubled haploid lines L_1 and L_2 , i.e. lines with the highest and lowest 1000-kernel weight, as well as the mean and variances of the mean for the whole set of L_m lines

Table 2. Means and mean variances of the 1000-kernel weight for doubled haploid lines and F_1 and F_2 hybrids of barley

Lines and hybrids	Mean	Variance of mean
L_1	51.84	0.317
L_2	30.82	0.355
L_m	42.93	0.249
F_1	53.85	0.205
F_2	40.40	0.305

are presented in Table 2. This table contains also means and mean variances for F_1 and F_2 hybrids. Using data from Table 2, we can find estimates of the genetic parameters m , $[d]$, $[h]$, $[i]$, $[l]$ and their standard errors. They are equal to:

$$\hat{m} = 42.93 \pm 0.50,$$

$$[\hat{d}] = 10.51 \pm 0.41,$$

$$[\hat{h}] = -21.04 \pm 2.71,$$

$$[\hat{i}] = -1.60 \pm 0.65,$$

$$[\hat{l}] = 31.96 \pm 1.29.$$

DISCUSSION

As mentioned before, having only doubled haploid lines, one obtains estimates of the parameters $[d]$ and $[i]$ and, therefore, information about effects of additive gene action and about homozygote \times homozygote interaction effects.

In the discussed example effects of additive gene action and homo \times homo interaction appeared to be significant. The significant parameter $[i]$ indicates that non-allelic interaction occurs in the studied set of doubled haploid lines. This information is of fundamental importance regarding the use of these lines for further genetic studies.

Estimates of the parameters $[d]$ and $[i]$ may be sufficient with respect to genetic analysis of doubled haploid lines, however, they do not give a full answer to the question concerning the inheritance of the considered trait in the studied cross. This fact is particularly important in the case of studies on the occurrence of heterosis. In this respect it is necessary to conduct observations of hybrids of F_1 and F_2 generations. In the case of using the method of double haploids, hybrids of these generations constitute an integral part of the process of obtaining doubled haploid lines, and for that reason their incorporation in the experiment does not make difficulties.

In the presented example the joint analysis of lines and F_1 and F_2 generations permitted to obtain, beside estimates of the parameters $[d]$ and $[i]$, also estimates of $[h]$ and $[l]$. Estimates of the both parameters associated with domination effects appeared to be significant, attention is being drawn by their relatively high absolute values in comparison with the mean m and the value of the parameter concerning additive gene action $[d]$. These results show that the effect of heterosis in the 1000-kernel weight, which was found in earlier studies (Surma 1978), was caused by domination and interaction between loci in a heterozygous state.

REFERENCES

1. Adamski, T., 1979: The obtaining of autodiploid barley lines using haploids from the cross *Hordeum vulgare* L. \times *Hordeum bulbosum* L. *Genetica Polonica*, 20, 31 - 42.
2. Kaczmarek, Z., Surma, M., Adamski T., 1982: Genetyczna i matematyczna interpretacja ważniejszych parametrów genetycznych oraz ich wykorzystanie w analizie pojedynczych kombinacji krzyżówkowych. Dwunaste Colloquium Metodologiczne z Agro-Biometrii, PAN, 285 - 305.
3. Kasha, K. J., Kao, K. N., 1970: High frequency haploid production in barley (*Hordeum vulgare* L.). *Nature* (Lond.), 225, 874 - 875.
4. Mather, K., Jinks, J., 1971: *Biometrical Genetics*. London.
5. Surma, M. A., 1978: Diallel analysis of the number of spikes, number of spikelets per spike, 1000-kernel weight and protein content in spring barley (*Hordeum vulgare* L.). *Genetica Polonica*, 19, 377 - 402.

WYKORZYSTANIE LINII AUTODIPLOIDALNYCH DO WYZNACZANIA OCEN PARAMETRÓW GENETYCZNYCH

Streszczenie

W pracy przedstawiono metody estymacji parametrów genetycznych, związanych z addytywnym działaniem genów, dominacją i niealleliczną interakcją, na podstawie linii autodiploidalnych oraz mieszańców pokolenia F_1 i F_2 . Rozważania teoretyczne zostały zilustrowane przykładem dotyczącym masy 1000 ziarn u jęczmienia.

ИСПОЛЬЗОВАНИЕ УДВОЕННЫХ ГАПЛОИДНЫХ ЛИНИЙ ДЛЯ ОПРЕДЕЛЕНИЯ ОЦЕНОК ГЕНЕТИЧЕСКИХ ПАРАМЕТРОВ

Резюме

В настоящей работе представлен метод определения генетических параметров, связанных аддитивным действием генов, доминацией и неаллелическим взаимодействием на основании удвоенных гаплоидных линий и гибридов поколения F_1 и F_2 . Теоретические рассуждения иллюстрируются примером относительно массы 1000 зерен у ячменя.