Article

# Use of Opstat to validate results in a partial diallelic with eight corn lines evaluated in an environment

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## Abstract

Sampling procedures in a partial diallelic experiment applying the method of Kempthorne and Curnow (1961) to eight corn lines ($S_7$) derived from the same variety, each used in five crosses (s= 5), are described. For the 20 single-cross hybrids, the calculations to obtain the analysis of variance for an environment with a randomized complete block experimental design with four repetitions are indicated. The effects between crosses are divided into general and specific combining ability using matrix algebra, the effects of $g_i$ and $S_{ij}$ are estimated, for each parent and in each cross, respectively. In addition, the components of variance and heritability are calculated in a broad and narrow sense. At all stages, it is indicated how to verify the calculations using a desktop calculator and, finally, the results are validated using the Opstat statistical package.

**Keywords:** circulant matrix, combining ability, heritability, incomplete diallelic.

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# Introduction

In a complete diallelic cross experiment, there are $p^2$ genetic combinations that include p parents (i= j), p (p-1)/2 direct crosses (DC; i< j)) and p (p-1)/2 reciprocal crosses (RC; i> j) (Griffing, 1956). This design has been widely used in plant breeding (Saavedra *et al*., 2021) but is impractical when p increases because DC and RC also increase, a similar situation arose when $2^H$ experiments were designed, with H varying from 2 to 8, to be analyzed with InfoStat and InfoGen, if H= 8, there will be 256 treatments and 247 interactions. To save time and resources, a fractional factorial that includes only a subset of these could be designed (Pérez *et al*., 2021). If 13 parents, 78 DCs and 78 RCs will be studied, even for rectangular lattice designs there would be serious limitations (González *et al*., 2007).

Cochran and Cox (1958); Gomez and Gomez (1984); Martínez (1998) showed the basic plans for balanced and partially balanced lattice; up to 144 treatments could be randomized in a 12 x 12 triple lattice. If in an environment corn (*Zea mays* L.) is evaluated in three repetitions with plots of three furrows of 5 m in length, each spaced at 0.8 m, to evaluate these 144 treatments, almost 6 400 $m^2$ would be required (repetitions separated by 2 m and incomplete blocks spaced at 1.5 m). This problem will be greater when considering several years, localities or combinations of these. At the other extreme, with p= 4, the estimates of general combining ability will be biased due to the small sample size used.

Both limitations have led to the sampling of the crosses including more parents without affecting the efficiency of the diallelic technique. Hinkelmann and Stern (1960); Kempthorne and Curnow (1961); Fyfe and Gilbert (1963); Rojas (1973) designed methodologies for the sampling and analysis of a partial diallelic. Kempthorne and Curnow (1961) invented an incomplete diallelic technique from Griffing's (1956) method 4, using a sample of size ps/2. Where: s is the number of times that each parent (p) is used in the crosses, p and s cannot be simultaneously odd numbers but the precision with which the parameters of interest to the plant breeder are estimated depends on both values. Murthy *et al*. (1966); Chaudhary *et al*. (1977) stated that the bias is greater when s is less than p/2. With s= p-1, one will have the method 4 of Griffing (1956).

However, it should be considered that the randomized complete block experimental design could lose efficiency if p and s are increased significantly because the size of the block will increase and there will be less uniformity within it, so the experimental error will be greater, thus, a very good option. desirable would be to divide each repetition into balanced incomplete blocks. This topic has been studied by several authors, such as Shunmugathai and Srinivasan (2012), who have discussed the decrease in efficiency of an incomplete diallelic, even with the loss of one or more observations.

The partial diallel cross has been used mainly to estimate genetic parameters in single cross hybrids formed with inbred lines derived from the same population evaluated in a randomized complete block experimental design, under consideration of a fixed effects genetic model (Christie and Shattuck, 1992; Mumtaz *et al*., 2015; Awata *et al*., 2018), but Miranda and Vencousky (1999) and Silva *et al*. (2017) among others, have developed another methodology to analyze a partial diallel formed with two groups of genetically contrasting lines or parents; they made crosses between exotic material and locally adapted genotypes.

## Design of the crosses

K must first be calculated, which must be an integer. Thus: k= $\frac{(p + 1 - s)}{2}$. The parents, randomized and numbered consecutively, will generate the crosses: Parent 1 x parent k + 1, k + 2, k + 3, ..., k + s. Parent 2 x parent k + 2, k + 3, k + 4, ..., k + 1 + s. Parent p x parent k + p, k + p + 1, ..., k + p – 1 + s. With eight corn lines, s= 5 and k= 2, the 20 crosses that will be sampled are: Line 1 x lines 2+1, 2+2, 2+3, 2+4, 2+5 = 1x3, 1x4, 1x5, 1x6, 1x7. Line 2 x lines 2+2, 2+3, 2+4, 2+5, 2+6 = 2x4, 2x5, 2x6, 2x7, 2x8. Line 3 x lines 2+3, 2+4, 2+5, 2+6, 2+7 = 3x5, 3x6, 3x7, 3x8. Line 4 x lines 2+4, 2+5, 2+6, 2+7, 2+8 = 4x6, 4x7, 4x8. Line 5 x lines 2+5, 2+6, 2+7, 2+8, 2+9 = 5x7, 5x8. Line 6 x lines 2+6, 2+7, 2+8, 2+9, 2+10 = 6x8. Line 7 would cross with males 2+7, 2+8, 2+9, 2+10 and 2+11, as they are greater than p, multiples of 8 are applied: 7 x (9-8) = 7 x 1; 7 x (10 -8) = 7 x 2; 7 x (11-8) = 7 x 3; 7 x (12-8) = 7 x 4; 7 x (13-8) = 7 x 5. These are eliminated because they are RCs, and the crosses originated with line 8 (Table 1).

**Table 1. 20 crosses sampled with the method of Kempthorne and Curnow (1961).**

| Line i (♀) | Line j (♂) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
| P1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| P2 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| P3 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| P4 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| P5 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| P6 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| P7 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| P8 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |

Aij= 1 or aij= 0 if the ixj cross is sampled or not, respectively. RCs are also indicated.

## General analysis of variance (Anova)

The statistical model for a randomized complete block experimental design is: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$. Where: $\mu$ is the arithmetic mean of the tr data, $\tau_i$ is the effect of the i-th treatment, $\beta_j$ is the contribution of the j-th repetition, and $\varepsilon_{ij}$ is the experimental error or residual of the model.

## Stages to obtain a general Anava

E1. Concentrate the data in a table: the rows (subscripts ij) will represent crosses and the columns (subscript k) repetitions, calculate totals and arithmetic means (Table 2).

**Table 2. Grain yield (t ha$^{-1}$) of 20 single-cross corn hybrids.**

| Cross (ij) | Repetitions (k) | | | | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | Total | Mean |
| 1x3 | 7.56 | 4.5 | 8.68 | 8.43 | 29.17 | 7.292 |
| 1x4 | 6.62 | 7.33 | 6.87 | 7.16 | 27.98 | 6.995 |
| 1x5 | 7.1 | 6.87 | 6.7 | 5.83 | 26.5 | 6.625 |

| Cross (ij) | Repetitions (k) | | | | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | Total | Mean |
| 1x6 | 7.36 | 4.81 | 7.64 | 7.64 | 27.45 | 6.862 |
| 1x7 | 6.06 | 6.68 | 5.20 | 6.37 | 24.31 | 6.077 |
| 2x4 | 6.35 | 6.16 | 6.56 | 6.66 | 25.73 | 6.432 |
| 2x5 | 7.58 | 5.33 | 7.85 | 7.41 | 28.17 | 7.042 |
| 2x6 | 8.66 | 7.29 | 8.35 | 6.75 | 31.05 | 7.762 |
| 2x7 | 6.91 | 6.62 | 7.02 | 7.12 | 27.67 | 6.917 |
| 2x8 | 7.62 | 9.41 | 6.99 | 7.41 | 31.43 | 7.857 |
| 3x5 | 6.99 | 5.7 | 6.27 | 6.75 | 25.71 | 6.427 |
| 3x6 | 6.62 | 5.23 | 9.16 | 7.95 | 28.96 | 7.24 |
| 3x7 | 6.27 | 5.83 | 6.62 | 6.6 | 25.32 | 6.33 |
| 3x8 | 6.87 | 5.12 | 7.56 | 7.73 | 27.28 | 6.82 |
| 4x6 | 5.73 | 4.37 | 5.2 | 8.08 | 23.38 | 5.845 |
| 4x7 | 6.95 | 6.66 | 6.45 | 6.08 | 26.14 | 6.535 |
| 4x8 | 7.7 | 5.31 | 7.08 | 8.33 | 28.42 | 7.105 |
| 5x7 | 6.18 | 5.62 | 6.14 | 5.83 | 23.77 | 5.942 |
| 5x8 | 6.89 | 5.75 | 7.89 | 8.08 | 28.61 | 7.152 |
| 6x8 | 5.77 | 5.68 | 8.2 | 6.83 | 26.48 | 6.62 |
| Total | 137.79 | 120.27 | 142.43 | 143.04 | 543.53 | 135.877 |

E2. Define the format of the general Anova (Table 3). Concentrate the above calculations in the format of the general Anova.

**Table 3. General anova for grain yield.**

| Source of variation | Degrees of freedom | Sum of squares | Mean squares | F values | | |
|---|---|---|---|---|---|---|
| | | | | Calculated | Tables | |
| | | | | | 5% | 1% |
| Repetitions | 3 | 17.07 | 5.69 | 6.84$^{**}$ | 2.78 | 4.16 |
| Crosses | 19 | 22.43 | 1.18 | 1.42 ns | 1.75 | 2.22 |
| Error | 57 | 47.38 | 0.83 | | | |
| Total | 79 | 86.89 | | | | |

$^{**}$= highly significant ($p$= 0.01); ns= not significant ($p$= 0.05). CV= 13.4%.

## Calculate degrees of freedom (DF)

In this section, t is the number of crosses sampled, so t=ps/2. DF of the total = (ps/2) r-1= 20(4)-1= 79; DF Rep= r - 1= 4 - 1= 3; DF crosses= (ps/2) - 1= 20 - 1= 19; DF of the error= [(ps/2)- 1] (r-1)= DF total - DF Rep - DF crosses = 57.

## Estimate sums of squares (SS)

Here the restriction i< j is introduced. Additionally, ij ≠ 12, 18, 23, 34, 45, 56, 67 and 78, which are the crosses not sampled in the partial diallelic, in the order of female and male. Total SS = $\sum_{i=1}^{p}\sum_{j=1}^{p}\sum_{k=1}^{r} Y_{ijk}^{2} - \frac{Y_{...}^{2}}{\frac{psr}{2}}$= $(7.56^2+6.62^2+, +6.83^2)-[(543.53)^2/80]$ = 86.9. SS Rep = $\frac{\sum_{k=1}^{r} Y_{..k}^{2}}{(\frac{ps}{2})} - \frac{Y_{...}^{2}}{(\frac{psr}{2})} = \frac{137.79^2+,\ldots,143.04^2}{20} - \frac{543.53^2}{20(4)}$= 17.07. SS crosses = $\frac{\sum_{i=1}^{p}\sum_{j=1}^{p} Y_{ij}^{2}}{r} - \frac{Y_{...}^{2}}{(\frac{psr}{2})} = \frac{29.17^2+27.98^2+26.50^2+,\ldots,+26.48^2}{4} - \frac{543.53^2}{20(4)}$= 22.439. SS error = SS total - SS Rep - SS crosses = 86.898 - 17.07 - 22.439 = 47.384.

## Determine mean squares (MS)

MS Rep= SS Rep/r -1= 17.07/3= 5.691; MS crosses = SS crosses /[(ps/2)-1]= 22.439/19 = 1.181; MS error = SS error / (r-1) [(ps/2)-1] = 47.384/57 = 0.8312

## Get calculated F values

F Rep = MS Rep/MS error = 5.691/0.8312 = 6.846. F Treat = MS crosses/MS error = 1.181/ 0.8312 = 1.42.

## Definition of the circulant matrix $\underline{A}$ = [sI + N]

$$\underline{A}_{8x8}=\begin{bmatrix} 5 & 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 5 & 0 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 5 & 0 & 1 & 1 & 1 & 1 \\ 1 & 1 & 0 & 5 & 0 & 1 & 1 & 1 \\ 1 & 1 & 1 & 0 & 5 & 0 & 1 & 1 \\ 1 & 1 & 1 & 1 & 0 & 5 & 0 & 1 \\ 1 & 1 & 1 & 1 & 1 & 0 & 5 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 & 5 \end{bmatrix}$$

. The sum in each row (r) or column (c) is:

$\sum_{r=0}^{p-1} a_r = \sum_{c=0}^{p-1} a_c = 2s = 2(5) = 10.$

The inverse of $\underline{A}$ was obtained with a matrix calculator (https://matrixcalc.org.es). As follows:

$$\underline{A}^{-1}_{8x8}=\begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix}$$

The sum in each row (r) or column (c) is: $\sum_{r=0}^{p-1} a^r = \sum_{c=0}^{p-1} a^c = \frac{1}{2s} = \frac{1}{2(5)} = 0.1$

When only a desktop calculator is available, the methodologies of Kempthorne and Curnow (1961; Singh and Chaudhary (1985); Martinez (1991) could be applied. To obtain the elements of the first row of its inverse, Martínez (1991) calculated its characteristic roots as: $\lambda j = \sum_{l=1}^{p} b_{11}\mathrm{Cos}\left\{j\left[\frac{2\pi(l\text{-}1)}{p}\right]\right\}$, j= 1, 2, 3, …, p. In this, $\lambda p = \sum_{l=1}^{p} b_{11}=2s$. $\lambda 1= b_{11}\cos\left(\frac{2\pi(1\text{-}1)}{8}\right)+b_{12}\cos\left(\frac{2\pi(2\text{-}1)}{8}\right)+b_{13}\cos\left(\frac{2\pi(3\text{-}1)}{8}\right)+ b_{14}\cos\left(\frac{2\pi(4\text{-}1)}{8}\right)+b_{15}\cos\left(\frac{2\pi(5\text{-}1)}{8}\right)+b_{16}\cos\left(\frac{2\pi(6\text{-}1)}{8}\right)+b_{17}\cos\left(\frac{2\pi(7\text{-}1)}{8}\right)+b_{18}\cos\left(\frac{2\pi(8\text{-}1)}{8}\right)$

The crosses 1x2 and 1x8 were not sampled ($b_{12}$ and $b_{18} = 0$) and will be eliminated from this formula; in $b_{11}$, its coefficient is s=5 and in the remaining bs is 1, after regrouping its components: $\lambda_1 = 5\cos(0)+\cos(\frac{\pi}{2})+\cos(\frac{3\pi}{4})+\cos(\pi)+\cos(\frac{5\pi}{4})+ . = 5 - 0.00000367 - 0.7071 - 1 - 0.7071 + 0.000011 = 2.5858$. $\lambda_2 = 5\cos(0)+\cos(\pi)+\cos(\frac{3\pi}{2})+\cos(2\pi)+\cos(\frac{5\pi}{2})+\cos(3\pi) . = 5 - 1 + 0.000011 + 1 - 0.0000183 - 1 = 4$. Similarly: $\lambda_3= 5.4141$; $\lambda_4= 6$; $\lambda_5= 5.414$; $\lambda_6= 4$; $\lambda_7= 2.5857$; $\lambda_8= 2s =10$.

Kempthorne and Curnow (1961) estimated the λs as: $\lambda_j = s\text{-}\ \frac{\mathrm{Sin}\ (n\text{-}\ 2)\frac{j\pi}{n}}{\mathrm{Sin}\frac{j\pi}{n}} = \frac{s\left[\mathrm{Sin}\left(\frac{j\pi}{n}\right)\right]\text{-}\ \mathrm{Sin}(n\text{-}\ s)(\frac{j\pi}{n})}{\mathrm{Sin}(\frac{j\pi}{n})}$. Where j=1, 2, …, n-1; $\lambda_n = 2s$, and s is the number of crosses sampled per parent. $\Lambda_1= \frac{5\left[\mathrm{Sin}\left(\frac{\pi}{8}\right)\right]\text{-}\mathrm{Sin}(8\text{-}5)(\frac{\pi}{8})}{\mathrm{Sin}(\frac{\pi}{8})} = \frac{5[\mathrm{Sin}(0.3927)]\text{-}\mathrm{Sin}(1.1781)}{\mathrm{Sin}(0.3927)} =2.5857$. $\Lambda_2 =\frac{5\left[\mathrm{Sin}\left(\frac{2\pi}{8}\right)\right]\text{-}\mathrm{Sin}(8\text{-}5)\ (\frac{2\pi}{8})}{\mathrm{Sin}\ (\frac{2\pi}{8})} = \frac{5[\mathrm{Sin}\ (0.7854)]\text{-}\mathrm{Sin}\ (2.3562)}{\mathrm{Sin}\ (0.7854)} = 4$.

The remaining λs are identical to those calculated with the method of Martínez (1991). With these, the elements of the first row of the inverse of matrix <u>A</u> are obtained. $a^o= \left(\frac{1}{n}\right)\left[\!\!\left[\frac{1}{\lambda_1}+\frac{1}{\lambda_2}+,\ldots,+\frac{1}{\lambda_8}\right)\right.= (\frac{1}{8})\left[\!\!\left[\frac{1}{2.5857}+\frac{1}{4.0}+\frac{1}{5.4142}+\frac{1}{6.0}+\frac{1}{5.4142}+\frac{1}{4}+\frac{1}{2.5857}+\frac{1}{10.0}\right)\right.= 0.2387$. The other elements of the first row are calculated as follows: $a^j = (\frac{1}{n})\sum_{l=1}^{n}\frac{1}{\lambda_l}\ \mathrm{Cos}\frac{j(n\text{-}l)}{n}(2\pi)$. In this, j=1, 2, …, n-1. $a^1=\left(\frac{1}{8}\right)[\ \frac{1}{\lambda_1}\mathrm{Cos}\ \frac{1(8\text{-}1)(2\pi)}{8}+\frac{1}{\lambda_2}\mathrm{Cos}\ \frac{1(8\text{-}2)(2\pi)}{8}+\frac{1}{\lambda_3}\mathrm{Cos}\ \frac{1(8\text{-}3)(2\pi)}{8}+\frac{1}{\lambda_4}\mathrm{Cos}\ \frac{1(8\text{-}4)(2\pi)}{8}+\frac{1}{\lambda_5}\mathrm{Cos}\ \frac{1(8\text{-}5)(2\pi)}{8}+\frac{1}{\lambda_6}\mathrm{Cos}\ \frac{1(8\text{-}6)(2\pi)}{8}+\frac{1}{\lambda_7}\mathrm{Cos}\ \frac{1(8\text{-}7)(2\pi)}{8}+\frac{1}{\lambda_8}\mathrm{Cos}\ \frac{1(8\text{-}8)(2\pi)}{8}= \left(\frac{1}{8}\right)[\ \frac{1}{\lambda_1}\mathrm{Cos}\ \frac{7\pi}{4}+\frac{1}{\lambda_2}\mathrm{Cos}\ \frac{(3\pi)}{2}+\frac{1}{\lambda_3}\mathrm{Cos}\ \frac{(5\pi)}{4}+\frac{1}{\lambda_4}\mathrm{Cos}\ (\pi)+\frac{1}{\lambda_5}\mathrm{Cos}\ \frac{(3\pi)}{4}+\frac{1}{\lambda_6}\mathrm{Cos}\ \frac{(\pi)}{2}+\frac{1}{\lambda_7}\mathrm{Cos}\ \frac{(\pi)}{4}+\frac{1}{\lambda_8}\mathrm{Cos}\ (0)= \left(\frac{1}{8}\right)(0.2734+0.00000275\text{-}0.1306\text{-}0.1666666\text{-}0.1306\text{-}0.00000092+0.27347+0.1= \frac{0.219}{8}= 0.027$.

**This is the value of row 1, column 2**

The other elements are calculated similarly, as the matrix is symmetric and circulant, to obtain those of the next row, those of the previous one simply moves one column to the right. This procedure is repeated until all the rows in the inverse of the matrix are completed.

## Calculation of the corrected sum of the sampled crosses ($Q_i$)

The correction factor (CFQ) is calculated with the same restrictions as for SS crosses: $\mathrm{CFQ} = \frac{2\sum_{i=1}^{p}\sum_{j=1}^{p}\bar{Y}_{ij.}}{ps} = \frac{2(135.877)}{8(5)} = \frac{\frac{2\sum_{i=1}^{t}\sum_{j=1}^{p} Y_{ij.}}{r}}{ps} = \frac{\frac{2Y\ldots}{r}}{ps} = \frac{\frac{2(543.53)}{4}}{8(5)} = 6.794\ \mathrm{t\ ha}^{-1}$. The corrected means of the 20 crosses are calculated as the difference between the arithmetic mean of each of them and CFQ. For the cross 1x3 = 7.292 - 6.794 = 0.498. The remaining values are shown in Table 4.

**Table 4. Uncorrected or corrected arithmetic means (above or below the diagonal).**

| Line i (♀) | Line j (♂) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
| P1 | - | - | 7.292 | 6.995 | 6.625 | 6.862 | 6.077 | - |
| P2 | - | - | - | 6.432 | 7.042 | 7.762 | 6.917 | 7.857 |
| P3 | 0.498 | - | - | - | 6.427 | 7.24 | 6.33 | 6.82 |
| P4 | 0.201 | -0.361 | - | - | - | 5.845 | 6.535 | 7.105 |
| P5 | -0.168 | 0.248 | -0.366 | - | - | - | 5.942 | 7.152 |
| P6 | 0.068 | 0.968 | 0.446 | -0.948 | - | - | - | 6.62 |
| P7 | -0.716 | 0.123 | -0.463 | -0.258 | -0.851 | - | - | - |
| P8 | - | 1.063 | 0.026 | 0.311 | 0.358 | -0.173 | - | - |

The sum of $Q_i$s is zero and these are calculated as: $Q_1$= 1x3 + 1x4 + 1x5 + 1x6 + 1x7= 0.498 + 0.201 - 0.168 + 0.068 - 0.716= - 0.118. $Q_2$= 2x4 + 2x5 + 2x6 + 2x7 + 2x8 = - 0.361 + 0.248 + 0.968 + 0.123 + 1.063= 2.041. $Q_3$= 0.140; $Q_4$= -1.057; $Q_5$= -0.781; $Q_6$= 0.36 ; $Q_7$= -2.168; $Q_8$= 1.585.

## Estimation of the effects of general combining ability (GCA)

The estimation of the GCA for each parent ($g_i$) is done with matrix algebra. $\underline{A}\ \underline{G} = \underline{H}$, its solution is: $G = A^{-1} H$. Where: $A^{-1}$ is the inverse of matrix A; H is a column vector formed by the values of the corrected sums of the sampled crosses ($Q_i$), and G is another column vector composed of the estimates of $g_i$.

Thus: $G_{8x1} = A^{-1}{}_{8x8}\ H_{8x1}$

$$-\begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix} \begin{bmatrix} -0.118 \\ 2.041 \\ 0.140 \\ -1.057 \\ -0.781 \\ 0.360 \\ -2.168 \\ 1.585 \end{bmatrix} = \begin{bmatrix} 0.1977 \\ 0.5853 \\ 0.1028 \\ -0.3137 \\ -0.3005 \\ -0.1071 \\ -0.4879 \\ 0.3236 \end{bmatrix}$$

The value of $g_1$ is obtained as the sum of the products of the first row of the inverse of matrix $\underline{A}$ with the only column of matrix $\underline{H}$; that is: $g_1$ = [(0.238) (-0.118) + (0.027) (2.041) + (-0.029) (0.114) +, ..., + (0.027) (1.585)] = 0.1977. Also: $g_2$= 0.5853; $g_3$= 0.1028; $g_4$= -0.3137; $g_5$= -0.3005; $g_6$=-0.1071; $g_7$= -0.4879; $g_8$= 0.3236.

## Estimation of $S_{ij}$ values

The effects of the specific combining ability ($S_{ij}$) for each single cross are estimated as: $S_{ij} = \bar{Y}_{ij} - \mu - g_i – g_j$. Where: µ is the arithmetic mean of the (ps/2) r data, $\bar{Y}_{ij}$ is the average value of the cross between the parents i, j, $g_i$, $g_j$ are the estimates of the general combining ability of lines i, j. The restrictions are: $\sum_{i=1}^{p} g_i = 0$ and $\sum_{j=1}^{p-1} S_{ij} = 0$. For crosses that involve parent 1, one will have: $S_{13} = \bar{Y}_{13} - \mu - g_1 - g_3$= 7.292 - 6.794 - 0.1977 - 0.1028 = 0.1974. Similarly: $S_{14}$= 0.3172; $S_{15}$= -0.066; $S_{16}$= -0.0223; $S_{17}$= -0.4265.

## Calculation of SS GCA

The sum of squares between treatments is divided into GCA and SCA; if the value of two of these three sources of variation is known in the diallelic table, the third is calculated by difference. SS GCA = $r\Sigma\underline{G_i}'\ \underline{H_i}$. Where: r is the number of repetitions, $G_i$' is the transpose of the column vector 8x1 formed by the values of $g_i$ and $H_i$ is the column vector integrated with the values of $Q_i$., it is multiplied by r because arithmetic means were used in the calculations. SS GCA = 4[(0.1977) (-0.118) + (0.5853) (2.041) +, + (0.3236) (1.585) = 13.137. SS SCA = SS Crosses - SS GCA = 22.439 - 13.1370 = 9.3019.

From Table 5, it can be concluded that only between repetitions and between effects of the general combining ability, there were significant differences (*p*= 0.05 and *p*= 0.01, respectively). These results indicate that the blocking in the experimental area, established perpendicular to the gradient of environmental heterogeneity, was efficient. This helped to decrease the experimental error. Regarding the grain yield of the 20 crosses sampled, in these, the additive genetic effects were of greater importance than the non-additive ones (dominance and epistasis), so that this group of eight corn parents could be used efficiently in the formation of synthetic varieties. In the segregating generations ($F_2$ or higher), from random mating between this group of inbred lines, plants could be selected to obtain new and better varieties of free pollination.

**Table 5. Anova with partition of effects between crosses in combining ability.**

| Source of variation | Degrees of freedom | Sum of squares | Mean squares | F values | | |
|---|---|---|---|---|---|---|
| | | | | Calculated | Tables | |
| | | | | | 5% | 1% |
| Repetitions | 3 | 17.07 | 5.69 | 6.84$^{**}$ | 2.78 | 4.16 |
| Crosses | (19) | (22.43) | 1.18 | 1.42 ns | 1.75 | 2.22 |
| GCA | 7 | 13.137 | 1.876 | 2.25$^{*}$ | 2.18 | 2.98 |
| SCA | 12 | 9.302 | 0.775 | 0.93 ns | 1.93 | 2.53 |
| Error | 57 | 47.38 | 0.83 | | | |
| Total | 79 | 86.89 | | | | |

DF GCA= p - 1= 8 - 1= 7; DF SCA= DF crosses - DF GCA= 19 - 7= 12. Also, DF SCA= [p(s-2)/2]= [8(5-2)/2]= 12.

Estimation of components of variance and heritability. $\sigma^2_e$ = mean square of the error = 0.8312. $\sigma^2_s$ = (MS of SCA - MS of the error)/r = (0.7751 - 0.8312)/4 = - 0.014. $\sigma^2_g$ = (p-1) (MS GCA - MS SCA)/rs(p-2) = 7(1.8767 - 0.7751)/4(5)(6)= 0.0642. The average variance for calculating the differences between two estimated $g_i$ values is calculated as: $AV\ (g_i - g_j) = 2\left\{\frac{pa^\circ}{p-1} - \frac{1}{2s(p-1)}\right\}\left(\sigma^2_s + \frac{\sigma^2_e}{r}\right) = 2\left\{\frac{8(0.238)}{7} - \frac{1}{2(5)(7)}\right\}\left(-0.014 + \frac{0.8312}{4}\right)$ = 2(0.272 - 0.014286) (0.1938)= 0.0998. And its standard error is: $EE\ (g_i - g_j) = \sqrt{AV\ (g_i - g_j)} = \sqrt{0.0998} = 0.3159$.

The equivalence between the previously calculated variances and the additive and dominance variances is established when it is assumed that the inbreeding coefficient is equal to one, because the lines used in the partial diallelic are inbred ($S_7$). So: $\sigma^2_A = 2\ \sigma^2_g = 2(0.0642) = 0.1284$; $\sigma^2_D = \sigma^2_s$

= -0.014. The total genetic variance, $\sigma^2_G$, is estimated as: $\sigma^2_G = \sigma^2_A + \sigma^2_D = 0.1284 – 0.014 = 0.1144$. Broad-sense heritability, $H^2$, is estimated as: $H^2 = 100\ (\sigma^2_G / \sigma^2_F) = 100\ (0.1144/0.3222) = 35.5\%$, where $\sigma^2_F$ is the phenotypic variance. $\sigma^2_F = 2\ \sigma^2_g + \sigma^2_s + (\sigma^2_e /r) = 0.1284 – 0.014 + 0.2078 = 0.3222$. Narrow-sense heritability, $h^2$, is estimated as: $h^2 = 100\ (\sigma^2_A / \sigma^2_F) = 100\ (0.1284/0.3222) = 39.85\%$.

The negative estimate of the variance of the specific combining ability (non-additive genetic effects) suggests that there was an underestimation in broad-sense heritability (35.5%) compared to the estimate of narrow-sense heritability (39.85%). The value of $h^2$ suggests that the total phenotypic variability measured in the 20 single crosses of corn is related to additive genes that determine grain yield. In this context, it is assumed that 39.8% of the total phenotypic variability estimated in this quantitative variable is attributed to the differences that exist between the inbred parents, whose predominant effects are additive.

**Results obtained with OPSTAT**

Character 1. Analysis of variance table for RBD.

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replications | 3 | 17.074 | | | |
| Treatments | 19 | 22.438 | 1.181 | 1.421 | 0.15415 |
| Error | 57 | 47.385 | 0.831 | | |
| Total | 79 | 86.897 | | | |

Mean and standard error table.

| Cross | Mean | Standard error |
|---|---|---|
| 1 X 3 | 7.293 | 1.665 |
| 1 X 4 | 6.995 | 0.272 |
| 1 X 5 | 6.625 | 0.48 |
| 1 X 6 | 6.863 | 1.191 |
| 1 X 7 | 6.077 | 0.552 |
| 2 X 4 | 6.433 | 0.193 |
| 2 X 5 | 7.043 | 1.001 |
| 2 X 6 | 7.763 | 0.774 |
| 2 X 7 | 6.918 | 0.187 |
| 2 X 8 | 7.858 | 0.925 |
| 3 X 5 | 6.428 | 0.494 |
| 3 X 6 | 7.24 | 1.468 |
| 3 X 7 | 6.33 | 0.32 |
| 3 X 8 | 6.82 | 1.033 |
| 4 X 6 | 5.845 | 1.378 |
| 4 X 7 | 6.535 | 0.317 |
| 4 X 8 | 7.105 | 1.127 |

| Cross | Mean | Standard error |
|---|---|---|
| 5 X 7 | 5.943 | 0.23 |
| 5 X 8 | 7.153 | 0.927 |
| 6 X 8 | 6.62 | 1.018 |

The standard errors in the table above correspond to $\sqrt{\sigma_{ij}^2}$

GCA effect are.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.198 | 0.586 | 0.103 | -0.314 | -0.3 | -0.107 | -0.488 | 0.324 |

Combining ability analysis.

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replications | 3 | 17.074 | 5.691 | | |
| Crosses | 19 | 22.438 | 1.181 | 1.421 | 0.15415 |
| Due to GCA | 7 | 13.142 | 1.877 | 2.258* | 0.0423 |
| Due to SCA | 12 | 9.296 | 0.775 | 0.932 | 0.52237 |
| Error | 57 | 47.385 | 0.831 | | |
| Total | 79 | 86.897 | | | |

Estimation of component of variances.

| SIGMASQ-G | 0.064 |
|---|---|
| SIGMASQ-S | -0.014 |
| SIGMASQ-A | 0.129 |
| SIGMASQ-D | -0.014 |
| Average variance(gi-gj) | 0.1 |
| SE (gi-gj) (EE) | 0.316 |

The equivalence between the variances reported in the table above with those in the heritability estimation section is $\sigma^2_g$, $\sigma^2_s$, $\sigma^2_A$, $\sigma^2_D$, AV, respectively. Heritability (Narrow Sense)= 1.12.

## Conclusions

Obtaining the inverse of matrix, A is very laborious when using a desktop calculator, but the estimation of its characteristic roots, λs, is simplified when applying the method of Kempthorne and Curnow (1961), compared to that of Martínez (1991) and both lead to similar results; with these, it is also easier to estimate the elements of its first row. As the matrix $A^{-1}$ is symmetric and circulant, the elements of the second row are obtained by moving those of the first to the next column and so on. If the use of software is not possible, both methodologies will be very useful. Additionally, it was observed that there are few statistical packages for the analysis of an incomplete diallelic that are freely available.

The Opstat software analyzes experiments in a single environment, it easily, quickly, and reliably verified the analyses obtained with the desktop calculator, including the average variance and standard errors for any contrast of $g_i$ with $g_j$. If $S_{ij}$ is ignored in the genetic model, the production potential of single crosses can be estimated using the two methods of Kempthorne and Curnow (1961). As the dominance variance was negative, the software estimated $h^2$ incorrectly.

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