

Use of artifices in Opstat to analyze series of experiments in a partial diallel

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Abstract

The use of specialized software saves time and resources when designing, randomizing and analyzing diallel cross experiments in a plant breeding program. In this study, the components of an analysis of variance (Anova) are calculated for a series of experiments in a randomized complete block design applied to the grain yield of a partial diallel cross generated with eight lines of corn (*Zea mays* L.), each sampled five times ($p=8$; $s=5$), evaluated in three environments and in four repetitions per environment ($n=240$ data). The main objective was to validate these results with the Opstat statistical package, which produces the Anova outputs for each trial and some indirect calculations to complete this in the series of experiments. The most critical part of the statistical genetic analysis was to propose an artifice to decompose the effects of GCA and SCA in the crosses and those corresponding to GCA x A and SCA x A in the interaction crosses x environments. In these calculations, the inverse of the matrix A is common in all operations performed with matrix algebra. In the above context, it was observed that Opstat is very friendly and reliable to generate the Anovas and g_i estimators for the evaluated parents, but it incorrectly calculates narrow-sense heritability when some negative variance is estimated.

Keywords: circulating matrices, free software, randomized complete block design, symmetrical incomplete diallel.

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Introduction

The design and analysis of a partial diallel cross necessarily leads to a series of experiments in time, space, or both (Dhillon, 1978; Martínez, 1983, 1991; González *et al.*, 2007). In agricultural sciences, it is common to evaluate genetic material in several years, different localities or multiple environments formed by them (Gomez and Gomez, 1984; González *et al.*, 2019). The generation, validation, application and transfer of technology also depends on this type of planning when, in factorial trials, it is desired to evaluate a set of genotypes on planting dates, population densities, fertilization formulas, pesticide doses or tillage methods, among others (Torres *et al.*, 2017; Padilla *et al.*, 2019; González *et al.*, 2019; Pérez *et al.*, 2021).

The partial diallel cross was created as an option to Griffing's (1956) method 4, because it presents serious limitations when the number of parents that will be used in the crosses increases (Kempthorne and Curnow, 1961; Mastache *et al.*, 1998; Saavedra *et al.*, 2021), which would make their evaluation unmanageable and impractical, particularly in multiple environments (Dhillon, 1978; Martínez, 1983, 1991; Jasso *et al.*, 2022). In a partial diallel experiment, circulating matrices with special mathematical properties are used, but when using a desktop calculator is used, it is very laborious to obtain its inverse and the estimates that involve it (Martínez, 1991; Jasso *et al.*, 2022).

The use of a matrix calculator or a specialized statistical package, preferably free, is very desirable and necessary to analyze this type of trials; some options are Opstat (Jasso *et al.*, 2022), Statistical Analysis System (SAS; Martínez, 1991; Mastache and Martínez, 1998) or Genes (Cruz, 2013), although with these it is not possible to generate an analysis of variance (Anova) for the series of experiments (Jasso *et al.*, 2022).

In the present study, calculations are performed to obtain the Anova of a partial diallel evaluated in three environments under a randomized complete block design using grain yield data from corn crosses formed with eight inbred lines, sampled five times each; in each environment (A) four repetitions were used. To complete the Anova table, the effects of general and specific combining ability (GCA and SCA) for parents and crosses, respectively, as well as those of GCA x A and SCA x A, were obtained from the genetic analysis generated by Opstat applying an artifice to the data concentrated in a table of values crosses x environments (CxA), averaged over repetitions; the inverse of the matrix A is common in all calculations involving matrix algebra. Based on the above, the objective of the present study was to design some artifices to obtain and validate with Opstat the results presented in this study.

Algebraic procedures

The sums of squares of crosses will be split into GCA and SCA; then, the interaction crosses x environments (C x A) will be fractionated into GCA x A and SCA x A. It will be considered $t = (ps/2) = 20$ crosses ($p=8$, $s=5$), where p and s are the number of parents and times each of these participates in the partial diallel, respectively. Totals or arithmetic means can be used in calculations (Kempthorne and Curnow, 1961; Dhillon, 1978; Martínez, 1983; Mastache *et al.*, 1998).

Statistical models

One environment: $Y_{ijk} = \mu + C_{ij} + R_k + E_{ijk}$, multiple environments: $Y_{ijkl} = \mu + A_k + C_{ij} + (AC)_{ijk} + R_{l(k)} + E_{ijkl}$.

Genetic models

One environment: $Y_{ijk} = \mu + [g_i + g_j + S_{ij}] + R_k + E_{ijk}$, Multiple environments: $Y_{ijkl} = \mu + A_k + [g_i + g_j + S_{ij}] + [(gl)_{ik} + (gl)_{jk} + (SI)_{ijk}] + R_{l(k)} + E_{ijkl}$. Where: μ is the population arithmetic mean; g_i, g_j are the effects of GCA in parents; S_{ij} are the effects of SCA in crosses; A_k is the contribution of the k -th environment; $(gl)_{ik}, (gl)_{jk}$ are the interaction effects GCA x A; $(SI)_{ijk}$ is the interaction SCA x A; $R_i, R_{l(k)}$ are the effects of repeats and repeats nested within environments; E_{ijk}, E_{ijkl} are the residuals of the models (Dhillon, 1978; Jasso *et al.*, 2022), in which Martínez (1983) and Mastache *et al.* (1998) imposed the restriction $n_{ij} = 1$ or $n_{ij} = 0$ whether or not the cross $i \times j$ is included, respectively. Jasso *et al.* (2022) also introduced the restriction $i < j$, as well as $i \times j \neq$ of crosses that complete, along with the partial diallel, Griffing's (1956) method 4.

General variance analysis

The design of the crosses and the Anova for one trial was shown by Jasso *et al.* (2022). For the Anova of the series of experiments, the following stages will be applied: Stage 1. Construct Table 1; generate the Anova for each trial and obtain the sums of squares of repetitions within environments and the combined error (Gomez and Gomez, 1984).

Table 1. Grain yield (t ha⁻¹) of 20 corn crosses evaluated in three environments.

Crosses (ij)	Environments (k)											
	A ₁				A ₂				A ₃			
	R ₁	R ₂	R ₃	R ₄	R ₁	R ₂	R ₃	R ₄	R ₁	R ₂	R ₃	R ₄
1) 1x3	9.25	5.46	5.62	7.14	3.64	7.64	6.46	6.19	10.96	10.52	8.48	9.14
2) 1x4	9.56	8.4	6.27	5.56	6.31	6.91	8.02	7.43	10.37	11	9.52	11.04
3) 1x5	6.87	7.71	5.56	6.73	5.81	6.89	8.02	7.12	8.44	8.7	10.06	7.50
4) 1x6	8.87	7.75	6.75	6.46	7.54	5.48	6.98	7.96	9.73	7.85	9.27	11.29
5) 1x7	8.46	8.23	6.1	6.54	5.41	6.73	6.83	7.31	9.33	11.08	8.41	8.66
6) 2x4	9.77	8.77	7.35	6.77	5.35	7.29	7.81	7.21	8.64	9.14	10.5	8.43
7) 2x5	8.31	8.46	7.23	5.79	5.64	8.29	7.02	6.37	9.75	8.25	10.46	10.08
8) 2x6	6.98	5.85	6.68	5.08	5.68	6.52	6.87	6.79	7.83	5.3	9.16	8.66
9) 2x7	7.75	8.18	6.45	6.87	7.35	6.25	7.43	8.02	9.93	7.54	9.23	10.31
10) 2x8	7.25	6.18	6.14	5.75	6.06	8.23	7.12	6.91	11.2	9.39	9.06	10.38
11) 3x5	7.6	6.12	6.91	8.16	5	6.1	5.14	4.96	10.43	8.77	10.52	8.58
12) 3x6	7.44	6.58	5.7	7.79	5.93	6.25	7.25	8.12	8.77	11.12	7.83	8.33
13) 3x7	7.85	7.1	4.79	6.71	7.41	6.6	6.46	3.79	7.71	8.31	6.81	8.35

Crosses (ij)	Environments (k)											
	A ₁				A ₂				A ₃			
	R ₁	R ₂	R ₃	R ₄	R ₁	R ₂	R ₃	R ₄	R ₁	R ₂	R ₃	R ₄
14) 3x8	8.6	6.93	6.16	6.91	7.41	5.89	6.98	6.08	7.52	9.81	8.23	7.7
15) 4x6	8.04	7.73	5.23	4.62	5.54	7.02	6.27	6.46	9.06	8.98	7.46	8.89
16) 4x7	6.21	7.12	6.16	5.5	6.93	7.91	6.5	7.23	7.7	10.12	8.1	7.43
17) 4x8	8.64	5.5	7.04	7.68	6.39	5.62	5.14	6.79	11.2	11.66	8.33	8.12
18) 5x7	7.37	4.18	4.46	4.99	6.58	9.64	4.37	6.56	7.2	7.89	7.79	6.85
19) 5x8	5.62	6.46	6.46	4.58	5	7.12	6.32	6.29	7.52	9.41	8.62	7
20) 6x8	7.62	6.43	6.23	6.66	7.14	4.37	6.66	6.5	7.87	6.77	8.18	7.81

Environments= A₁, A₂, A₃; repetitions = R₁, R₂, R₃, R₄.

Stage 2. Calculate degrees of freedom (DF)

DF of the Total= [ar(ps/2)] - 1= 3(4)(20) - 1= 239; DF Environments (A)= a - 1= 2; DF Rep(A)= a(r-1)= 3(3)= 9. For verification: DF Trat 1= DF A + DF Rep(A)= ar - 1, then: DF Rep(A)= DF Trat 1 - DF A= (ar - 1) - (a - 1)= ar - 1 - a + 1= a(r-1)= 9; DF Crosses (C)= (ps/2) - 1= [(8) (5)/2] - 1= 19; DF C x A= [(ps/2) - 1] (a-1)= 19(2)= 38; DF Error= DF Total - DF A - DF Rep (A) - DF C - DF C x A= 239 - 2 - 9 - 19 - 38= 171. Also: DF Error= a(r-1) [(ps/2) - 1]= 3(3) (19)= 171. For verification: DF Error= {[a(ps/2)r] - 1} - (a - 1) - a(r-1) - [(ps/2) - 1] - {[(ps/2) - 1] (a-1)} = [a(ps/2)r] - 1 - a + 1 - ar + a - (ps/2) + 1 - (aps/2) + a + (ps/2) - 1= (apsr/2) - ar - a(ps/2) + a= a[(psr/2) - r - (ps/2) + 1] = a(r-1) [(ps/2) - 1].

Stage 3. Obtain sums of squares (SS)

$$SS A_{(k=3)} = \frac{\sum_{k=1}^a Y_{..k}^2}{\left(\frac{psr}{2}\right)} - \frac{2 \left(\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl} \right)^2}{arps} = \left(\frac{546.8^2 + 526.48^2 + 712.72^2}{20(4)} \right) - \frac{2(1786)^2}{3(4)(8)(5)} = 260.94$$

$$SS C_{(ij=20)} = \frac{\sum_{i=1}^p \sum_{j=1}^p Y_{ij..}^2}{(ar)} - \frac{2 \left(\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl} \right)^2}{arps} = \left(\frac{90.48^2 + 100.4^2 + \dots + 82.2^2}{3(4)} \right) - \frac{2(1786)^2}{3(4)(8)(5)} = 62.35$$

To calculate SS C x A, one first defines SS Trat 1 = SS A + SS C + SS C x A, where: SS Trat

$$1_{(ijk=60)} = \frac{\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a Y_{ijk.}^2}{r} - \frac{2 \left(\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl} \right)^2}{arps} = \left(\frac{27.48^2 + 29.8^2 + \dots + 30.64^2}{4} \right) - \frac{2(1786)^2}{3(4)(8)(5)} = 374.38$$

$$SS C x A_{(ijk=60)} = SS Trat 1 - SS A - SS C = 374.38 - 260.94 - 62.35 = 51.09$$

$$SS Rep (A) = \sum_{k=1}^a (SS Rep)_k = SS RepA_1 + SS RepA_2 + SS RepA_3 = 1.765 + 6.34 + 37.52 = 45.625$$

Table A x Rep allows verifying the previous calculation (Table 2) and the procedure shown below is very useful to calculate the contribution of any interaction, in this and in other factorial experiments (Padilla *et al.*, 2019; González *et al.*, 2019; Pérez *et al.*, 2021).

Table 2. Data to calculate SS Rep (A).

Environments (.k)	Repetitions (i)				Total
	R ₁	R ₂	R ₃	R ₄	Y _{..k}
A ₁	158.06	139.14	123.29	126.29	546.78
A ₂	122.12	136.75	133.65	134.09	526.61
A ₃	181.16	181.61	176.02	174.55	713.34
Total	461.34	457.5	432.96	434.93	1786.73

$$SS \text{ TRAT } 2_{(kl=12)} = SS \text{ A} + SS \text{ Rep (A)} = \frac{\sum_{k=1}^a \sum_{l=1}^r Y_{..kl}^2}{\left(\frac{ps}{2}\right)} - \frac{2 \left(\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl}\right)^2}{arps} = \frac{(158.06^2 + 122.12^2 + 181.16^2 + \dots + 174.55^2)}{20} - \frac{2 (1786.73)^2}{3(4)(8)(5)} = 308.35$$

$$SS \text{ A} = \frac{\sum_{k=1}^a Y_{..k}^2}{\left(\frac{ps}{2}\right)} - \frac{2 \left(\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl}\right)^2}{arps} = \left(\frac{546.78^2 + 526.6^2 + 713.34^2}{20(4)}\right) - \frac{2 (1786.73)^2}{3(4)(8)(5)} = 262.57$$

$$SS \text{ Rep (A)} = SS \text{ Trat } 2 - SS \text{ A} = 308.35 - 262.57 = 45.78$$

$$SS \text{ Error} = \sum_{k=1}^a (SS \text{ Error})_k = SS \text{ ErrorA}_1 + SS \text{ ErrorA}_2 + SS \text{ ErrorA}_3 = 46.32 + 61.53 + 73.88 = 181.73$$

$$SS \text{ Total} = SS \text{ A} + SS \text{ Rep (A)} + SS \text{ C} + SS \text{ CxA} + SS \text{ Error} = 601.75$$

$$\text{Also: } SS \text{ Total}_{(ijkl=240)} = \sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl}^2 - \frac{2 \left(\sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^a \sum_{l=1}^r Y_{ijkl}\right)^2}{arps} = (9.25^2 + 9.56^2 + 6.87^2 + \dots + 6.66^2 + 3.64^2 + 6.31^2 + 5.81^2 + \dots + 6.50^2 + 10.96^2 + 10.37^2 + 8.44^2 + \dots + 7.81^2) - \frac{2 (1786)^2}{3(4)(8)(5)} = 601.75$$

Stage 4. Break down DF and SS of crosses into GCA and SCA

DF GCA = p - 1 = 7; DF SCA = DF Crosses - DF GCA = 19 - 7 = 12. Also: DF SCA = [(ps/2) - 1] - (p - 1) = (ps/2) - 1 - p + 1 = (ps - 2p)/2 = p (s-2)/2 = 8(3)/2 = 12

Similarly to the case of individual trials, in the series of experiments: $SS \text{ GCA} = \sum_{i=1}^p \hat{g}_i' Q_i$; where: $Q_i = \sum_{j=1}^p (Y_{ij..} - \frac{2Y_{i...}}{ps}) y \hat{g}_i = \left(\frac{1}{r}\right) \sum_{j=1}^p a^{ij} Q_j$

The correction factor for crosses is $CFQ = \frac{2Y_{i...}}{ps}$; a^{ij} or A^{-1} is the inverse of the matrix $A_{p \times p} = [sI + N] = [a_{ij}]$, Q_j forms a column vector (\underline{H}) and \hat{g}_i generates another column vector (\underline{G}), in the latter its solution is: $\underline{G} = A^{-1} \underline{H}$ (Jasso *et al.*, 2022). Q_i values are calculated using the data in Table 3.

Table 3. Data to calculate the interaction CxA.

No.	Crosses (ij)	Environments (k)						Total (Y _{ij..})	Means Ȳ _{ij..}
		A ₁		A ₂		A ₃			
		Y _{ij1.}	Ȳ _{ij1.}	Y _{ij2.}	Ȳ _{ij2.}	Y _{ij3.}	Ȳ _{ij3.}		
1	1 x 3	27.48	6.87	23.92	5.98	39.08	9.77	90.48	7.54
2	1 x 4	29.8	7.45	28.68	7.17	41.92	10.48	100.4	8.36
3	1 x 5	26.88	6.72	27.84	6.96	34.68	8.67	89.4	7.45
4	1 x 6	29.84	7.46	27.96	6.99	38.12	9.53	95.92	7.99
5	1 x 7	29.32	7.33	26.28	6.57	37.48	9.37	93.08	7.76
6	2 x 4	32.64	8.16	27.64	6.91	36.72	9.18	97	8.08
7	2 x 5	29.84	7.46	27.32	6.83	38.52	9.63	95.68	7.97
8	2 x 6	24.6	6.15	25.84	6.46	30.96	7.74	81.4	6.78
9	2 x 7	29.24	7.31	29.04	7.26	37	9.25	95.28	7.94
10	2 x 8	25.32	6.33	28.32	7.08	40.04	10.01	93.68	7.8
11	3 x 5	28.8	7.2	21.2	5.3	38.28	9.57	88.28	7.36
12	3 x 6	27.52	6.88	27.56	6.89	36.04	9.01	91.12	7.59
13	3 x 7	26.44	6.61	24.24	6.06	31.16	7.79	81.84	6.82
14	3 x 8	28.6	7.15	26.36	6.59	33.24	8.31	88.2	7.35
15	4 x 6	25.6	6.4	25.28	6.32	34.4	8.6	85.28	7.1
16	4 x 7	25	6.25	28.56	7.14	33.36	8.34	86.92	7.24
17	4 x 8	28.84	7.21	23.92	5.98	38.8	9.7	91.56	7.63
18	5 x 7	21	5.25	27.16	6.79	29.72	7.43	77.88	6.48
19	5 x 8	23.12	5.78	24.72	6.18	32.56	8.14	80.4	6.7
20	6 x 8	26.92	6.73	24.64	6.16	30.64	7.66	82.2	6.85
		546.8		526.48		712.72		1786	

Q₁= (Y_{13..}- CFQ) + (Y_{14..}- CFQ) + (Y_{15..}- CFQ) + (Y_{16..}- CFQ) + (Y_{17..}- CFQ); CFQ= 2(1786)/40= 89.3. Q₁= (90.48 - 89.3) + (100.4 - 89.3) + (89.4 - 89.3) + (95.92 - 89.3) + (93.08 - 89.3)= 22.78. Likewise: Q₂= 16.54; Q₃= -6.58; Q₄= 14.66; Q₅= -14.86; Q₆= -10.58; Q₇= -11.5; Q₈= -10.46. The sum over Q_i is zero and the GCA estimators for parents (\hat{g}_i) are calculated

as: $\hat{g}_i = \left(\frac{1}{r}\right) \sum_{j=1}^p a^{ij} Q_j =$

$$\left(\frac{1}{3(4)}\right) \begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix} \begin{bmatrix} 22.78 \\ 16.54 \\ -6.58 \\ 14.66 \\ -14.86 \\ -10.58 \\ -11.5 \\ -10.46 \end{bmatrix}$$

$$= \begin{bmatrix} 0.552 \\ 0.492 \\ 0.042 \\ 0.2277 \\ -0.3533 \\ -0.4034 \\ -0.383 \\ -0.1752 \end{bmatrix} = \begin{bmatrix} \widehat{g}_1 \\ \widehat{g}_2 \\ \widehat{g}_3 \\ \widehat{g}_4 \\ \widehat{g}_5 \\ \widehat{g}_6 \\ \widehat{g}_7 \\ \widehat{g}_8 \end{bmatrix}$$

With this information, one carries out the breakdown of the SS of crosses into

$$SS\ GCA = \sum_{i=1}^p \widehat{g}_i' Q_i = (\widehat{g}_1 Q_1 + \widehat{g}_2 Q_2 + \dots + \widehat{g}_8 Q_8) = [0.552 (22.78) + 0.492(16.54) + \dots + (-0.1752) (-10.46)] = 39.529$$

$$SS\ SCA = SS\ Crosses - SS\ GCA = 62.36 - 39.529 = 22.831$$

Stage 5. Split DF and SS of interaction crosses x environments (Cx A)

DF GCA x A = (p-1)(a-1) = 7(2) = 14; DF SCA x A = DF C x A - DF GCA x A = 38 - 14 = 24. Also: DF SCA x A = [(ps/2) - 1] (a-1) - (p-1)(a-1) = (aps/2) - a - (ps/2) + 1 - pa + p + a - 1 = (aps/2) - (ps/2) - pa + p = p(as - s - 2a + 2p) / 2 = p(a - 1)(s - 2) / 2 = 8(2)(3) / 2 = 24. To split SS (C x A), Q_{ik} and $(\widehat{gl})_{ik}$ must first be calculated as: $Q_{ik} = \sum_{j=1}^p (Y_{ijk} - \frac{2Y_{.k}}{ps})$; $(\widehat{gl})_{ik} = (\frac{1}{r}) \sum_{j=1}^p a^{ij} Q_{jk} - (\frac{1}{lr}) \sum_{j=1}^p a^{ij} Q_j$.

For environment k=1, the correction factor, $CFA_1 = 2(Y_{..1})/ps = 2(546.8)/40 = 27.34$. $Q_{11} = (Y_{131} - CFA_1) + (Y_{141} - CFA_1) + (Y_{151} - CFA_1) + (Y_{161} - CFA_1) + (Y_{171} - CFA_1) = (27.48 - 27.34) + (29.8 - 27.34) + (26.88 - 27.34) + (29.84 - 27.34) + (29.32 - 27.34) = 6.62$, $Q_{21} = 4.94$; $Q_{31} = 2.14$; $Q_{41} = 5.18$; $Q_{51} = -7.06$; $Q_{61} = -2.22$; $Q_{71} = -5.7$; $Q_{81} = -3.9$; $\sum_{i=1}^p Q_{i1} = 0$.

For environment k=2, the correction factor, $CFA_2 = 2(Y_{..2})/ps = 2(526.48)/40 = 26.324$. $Q_{12} = (Y_{132} - CFA_2) + (Y_{142} - CFA_2) + (Y_{152} - CFA_2) + (Y_{162} - CFA_2) + (Y_{172} - CFA_2) = (23.92 - 26.324) + (28.68 - 26.324) + (27.84 - 26.324) + (27.96 - 26.324) + (26.28 - 26.324) = 3.06$, $Q_{22} = 6.54$; $Q_{32} = -8.34$; $Q_{42} = 2.46$; $Q_{52} = -3.38$; $Q_{62} = -0.34$; $Q_{72} = 3.66$; $Q_{82} = -3.66$; $\sum_{i=1}^p Q_{i2} = 0$.

For environment k=3, the correction factor, $CFA_3 = 2(Y_{..3})/ps = 2(712.72)/40 = 35.636$. $Q_{13} = (Y_{133} - CFA_3) + (Y_{143} - CFA_3) + (Y_{153} - CFA_3) + (Y_{163} - CFA_3) + (Y_{173} - CFA_3) = (39.08 - 35.636) + (41.92 - 35.636) + (34.68 - 35.636) + (38.12 - 35.636) + (37.48 - 35.636) = 13.1$, $Q_{23} = 5.06$; $Q_{33} = -0.38$; $Q_{43} = 7.02$; $Q_{53} = -4.42$; $Q_{63} = -8.02$; $Q_{73} = -9.46$; $Q_{83} = -2.9$; $\sum_{i=1}^p Q_{i3} = 0$, As: $(\widehat{gl})_{ik} = (\frac{1}{r}) \sum_{j=1}^p a^{ij} Q_{jk} - (\frac{1}{lr}) \sum_{j=1}^p a^{ij} Q_j$, then:

For trial 1

$$\begin{aligned}
 (\widehat{\mathbf{gl}})_{i1} &= \left(\frac{1}{r}\right)[\mathbf{A}^{-1}] \begin{bmatrix} Q_{11} \\ Q_{21} \\ Q_{31} \\ Q_{41} \\ Q_{51} \\ Q_{61} \\ Q_{71} \\ Q_{81} \end{bmatrix} - \left(\frac{1}{lr}\right)[\mathbf{A}^{-1}] \begin{bmatrix} Q_1 \\ Q_2 \\ Q_3 \\ Q_4 \\ Q_5 \\ Q_6 \\ Q_7 \\ Q_8 \end{bmatrix} \\
 &= \left(\frac{1}{4}\right) \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} 6.62 \\ 4.94 \\ 2.14 \\ 5.18 \\ -7.06 \\ -2.22 \\ -5.7 \\ -3.9 \end{bmatrix} \\
 &= \left(\frac{1}{12}\right) \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} 22.78 \\ 16.54 \\ -6.58 \\ 14.66 \\ -14.86 \\ -10.58 \\ -11.5 \\ -10.46 \end{bmatrix} \\
 &= \begin{bmatrix} 0.4771 \\ 0.5102 \\ 0.333 \\ 0.291 \\ -0.4635 \\ -0.3817 \\ -0.5137 \\ -0.2522 \end{bmatrix} - \begin{bmatrix} 0.5516 \\ 0.4893 \\ 0.042 \\ 0.2356 \\ -0.357 \\ -0.4086 \\ -0.385 \\ -0.1676 \end{bmatrix} = \begin{bmatrix} -0.0745 \\ 0.0209 \\ -0.291 \\ 0.0554 \\ -0.1065 \\ 0.0269 \\ -0.1287 \\ -0.0846 \end{bmatrix}
 \end{aligned}$$

For trial 2

$$(\widehat{\mathbf{gl}})_{i2} = \left(\frac{1}{r}\right)[\mathbf{A}^{-1}] \begin{bmatrix} Q_{12} \\ Q_{22} \\ Q_{32} \\ Q_{42} \\ Q_{52} \\ Q_{62} \\ Q_{72} \\ Q_{82} \end{bmatrix} - \left(\frac{1}{lr}\right)[\mathbf{A}^{-1}] \begin{bmatrix} Q_1 \\ Q_2 \\ Q_3 \\ Q_4 \\ Q_5 \\ Q_6 \\ Q_7 \\ Q_8 \end{bmatrix}$$

$$= \left(\frac{1}{4}\right) \begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix} \begin{bmatrix} 3.06 \\ 6.54 \\ -8.34 \\ 2.46 \\ -3.38 \\ -0.34 \\ 3.66 \\ -3.66 \end{bmatrix}$$

$$- \left(\frac{1}{12}\right) \begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix} \begin{bmatrix} 22.78 \\ 16.54 \\ -6.58 \\ 14.66 \\ -14.86 \\ -10.58 \\ -11.5 \\ -10.46 \end{bmatrix}$$

$$= \begin{bmatrix} 0.2517 \\ 0.363 \\ -0.432 \\ -0.008 \\ -0.220 \\ -0.028 \\ 0.192 \\ -0.117 \end{bmatrix} - \begin{bmatrix} 0.5516 \\ 0.4893 \\ 0.042 \\ 0.2356 \\ -0.357 \\ -0.4086 \\ -0.385 \\ -0.1676 \end{bmatrix} = \begin{bmatrix} -0.2999 \\ -0.1263 \\ -0.474 \\ -0.2436 \\ 0.137 \\ 0.3806 \\ 0.577 \\ 0.0506 \end{bmatrix}$$

For trial 3

$$(\widehat{gl})_{i3} = \left(\frac{1}{r}\right)[A^{-1}] \begin{bmatrix} Q_{13} \\ Q_{23} \\ Q_{33} \\ Q_{43} \\ Q_{53} \\ Q_{63} \\ Q_{73} \\ Q_{83} \end{bmatrix} - \left(\frac{1}{lr}\right)[A^{-1}] \begin{bmatrix} Q_1 \\ Q_2 \\ Q_3 \\ Q_4 \\ Q_5 \\ Q_6 \\ Q_7 \\ Q_8 \end{bmatrix}$$

$$= \left(\frac{1}{4}\right) \begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix} \begin{bmatrix} 13.10 \\ 5.06 \\ -0.38 \\ 7.02 \\ -4.42 \\ -8.02 \\ -9.46 \\ -2.9 \end{bmatrix}$$

$$-\left(\frac{1}{12}\right) \begin{bmatrix} 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 \\ 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 \\ -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 & -0.044 \\ -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 & -0.047 \\ -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 & -0.044 \\ -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 & -0.029 \\ -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 & 0.027 \\ 0.027 & -0.029 & -0.044 & -0.047 & -0.044 & -0.029 & 0.027 & 0.238 \end{bmatrix} \begin{bmatrix} 22.78 \\ 16.54 \\ -6.58 \\ 14.66 \\ -14.86 \\ -10.58 \\ -11.5 \\ -10.46 \end{bmatrix}$$

$$= \begin{bmatrix} 0.928 \\ 0.604 \\ 0.227 \\ 0.401 \\ -0.376 \\ -0.800 \\ -0.827 \\ -0.156 \end{bmatrix} - \begin{bmatrix} 0.5516 \\ 0.4893 \\ 0.042 \\ 0.2356 \\ -0.357 \\ -0.4086 \\ -0.385 \\ -0.1676 \end{bmatrix} = \begin{bmatrix} 0.3764 \\ 0.1147 \\ 0.185 \\ 0.1654 \\ -0.019 \\ -0.3914 \\ -0.442 \\ 0.0116 \end{bmatrix}$$

Considering the above estimators, the following is calculated

$$\begin{aligned} \text{SS GCA}_{xA} &= \sum_{k=1}^a \sum_{i=1}^p \widehat{g}_{ik} Q_{ik} - \sum_{i=1}^p \widehat{g}_i Q_i = [(\widehat{g}_{11}Q_{11} + \widehat{g}_{21}Q_{21} + \dots + \widehat{g}_{81}Q_{81}) + (\widehat{g}_{12}Q_{12} + \widehat{g}_{22}Q_{22} + \dots + \widehat{g}_{82}Q_{82}) + (\widehat{g}_{13}Q_{13} + \widehat{g}_{23}Q_{23} + \dots + \widehat{g}_{83}Q_{83})] - (\widehat{g}_1Q_1 + \widehat{g}_2Q_2 + \dots + \widehat{g}_8Q_8) = \\ &= [0.4771(6.62) + 0.5102(4.94) + 0.333(2.14) + \dots - 0.2522(-3.9) + 0.2517(3.06) + 0.363(6.54) - 0.432(-8.34) + \dots - 0.117(-3.63) + 0.928(13.1) + 0.604(5.06) + 0.227(-0.38) + \dots - 0.156(-2.9)] - [0.5516(22.78) + 0.4893(16.54) + 0.042(-6.58) + \dots - 0.1676(-10.46)] = [SC ACG A1 + SC ACG A2 + SC ACG A3] - SC ACG = (15.9301 + 8.6115 + 34.2955) - 39.6446 = 19.1925. \end{aligned}$$

Dhillon (1978) developed the formulas for estimating

$$\text{SS SCA}_{xA} = \left[\sum_{k=1}^a \left(\frac{\sum_{ij}^p Y_{ijk}^2}{r} - \frac{2Y_{..k}^2}{psr} \right) \right] - \left(\frac{\sum_{ij}^p Y_{ij..}^2}{lr} - \frac{2Y_{....}^2}{pslr} \right) - \left(\sum_{k=1}^a \sum_{i=1}^p \widehat{g}_{ik} Q_{ik} - \sum_{i=1}^p \widehat{g}_i Q_i \right)$$

Also, by statistical inference from the previous calculations, it deduced that: $\text{SS SCA}_{xA} = \sum_{k=1}^a (\text{SS C})_k - \text{SS C} - \text{SS GCA}_{xA} = (\text{SS CA}_1 + \text{SS CA}_2 + \text{SS CA}_3) - \text{SS C} - \text{SS GCA}_{xA} = (\text{SS Trat 1} - \text{SS A} - \text{SS C}) - \text{SS GCA}_{xA} = \text{SS C}_{xA} - \text{SS GCA}_{xA}$.

Thus, by approximation of the results that the above will generate, it must be obtained that: $\text{SS SCA}_{xA} = 31.897$.

Stage 6. Obtain mean squares (MS)

$\text{MS A} = \text{SS A} / (a-1) = 260.94/2 = 130.47$; $\text{MS Rep(A)} = \text{SS Rep(A)} / a(r-1) = 45.625/9 = 5.07$; $\text{MS C} = \text{SS C} / [(ps/2) - 1] = 62.35/19 = 3.28$; $\text{MS GCA} = \text{SS GCA} / (p-1) = 39.529/7 = 5.647$; $\text{MS SCA} = \text{SS SCA} / [p(s-2)/2] = 22.831/12 = 1.90$; $\text{MS C} \times \text{A} = \text{SS C} \times \text{A} / (a-1)[(ps/2) - 1] = 51.09/38 = 1.34$; $\text{MS GCA} \times \text{A} = \text{SS GCA} \times \text{A} / (p-1)(a-1) = 19.925/14 = 1.37$; $\text{MS SCA} \times \text{A} = \text{SS SCA} \times \text{A} / [p(a-1)(s-2)]/2 = 31.89/24 = 1.32$; $\text{MS Error} = \text{SS Error} / \{a(r-1)[(ps/2) - 1]\} = 181.746/171 = 1.06$.

Stage 7. Calculate F values

In this section, reference should be made to the type of model selected (fixed, random, mixed) and based on this define the correct GLs and MSs to test the statistical hypotheses of interest (Sahagún, 1998). The F tests (Table 4) were proposed by Martínez (1983). $F(GCA) = [MS GCA / (MS SCA + MS GCA \times A - MS SCA \times A)] = 2.94$; $F(SCA) = MS SCA / MS SCA \times A = 1.44$; $F(GCA \times A) = MS GCA \times W / MS SCA \times A = 1.01$; $F(SCA \times A) = MS SCA \times A / MS Error = 1.24$.

Stage 8. Concentrate the calculations previously obtained in the Anova format

Table 4. Anova for grain yield for a partial diallel in a series of experiments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares (MS)	F value	Expectation of mean squares (EMS)
Environments(A)	2	260.94	130.47		
Rep(A)	9	45.62	5.07		
Crosses (C)	(19)	62.35	3.28		
GCA	7	39.529	5.647	2.94	$\sigma_e^2 + r\sigma_{sp}^2 + [rs(p-2)/(p-1)] \sigma_{gp}^2 + ar\sigma_s^2 + [ars(p-2)/(p-1)] \sigma_g^2$
SCA	12	22.831	1.9	1.44	$\sigma_e^2 + r\sigma_{sp}^2 + ar\sigma_s^2$
C x A	(38)	51.09	1.34		
GCA x A	14	19.192	1.37	1.01	$\sigma_e^2 + r\sigma_{sp}^2 + [rs(p-2)/(p-1)] \sigma_{gp}^2$
SCA x A	24	31.89	1.32	1.24	$\sigma_e^2 + r\sigma_{sp}^2$
Error	171	181.746	1.06		σ_e^2
Total	239	601.75			

Application of the Opstat Software

The software is freely available at <https://14.139.232.166/opstat>. This analyzes a partial diallel cross based on a circulating sample (Kempthorne and Curnow, 1961). To create the database on its web page, the crosses are ordered in the rows and the repetitions in the columns, the latter delimited by spaces (*.prn) or by tabs (*.txt); between rows there are no additional spaces. If this is done from Microsoft Excel, the file will have an extension *.prn or *.txt. The labels on the first row will correspond to crosses, R1, R2, ..., Rn, the latter are repetitions. Each cross could be identified as 1x3, 1x4, 1x5, ..., 6x8. When pasting the data on the web page, the labels are not included. If the parents are included, they are captured below the last cross, as 1x1, 2x2, p x p (Sheoran *et al.*, 1998).

To send the data to the Opstat platform, submit is selected; the software will request number of parents, number of repetitions, and sample size. Press Analyse. The output, for a single trial, includes (Sheoran *et al.*, 1998; Jasso *et al.*, 2022): a) Anova for a randomized complete block design, b) arithmetic mean and standard error for each cross, c) g_i estimators for each parent, d) Anova with the breakdown of the effects between crosses in GCA and SCA, e) estimates of variances of GCA, SCA, additive, dominant and average and, f) standard error to compare g_i values, and g) estimation of narrow-sense heritability.

Analyses of variance obtained with Opstat

To generate the series of experiments, the analysis of variance for each environment and the corresponding to the table crosses x environments must be performed.

To split the SS (C x A), the following is calculated

$SS_{SCA \times A} = \sum_{k=1}^a (SS_{SCA})_k - SS_{SCA} = (SS_{SCAA_1} + SS_{SCAA_2} + SS_{SCAA_3}) - SS_{SCA} = (18.892 + 11.458 + 24.599) - 22.696 = 32.253$. By subtraction, one obtains: $SS_{GCA \times A} = SS_{C \times A} - SS_{SCA \times A} = 51.09 - 32.253 = 18.837$. Both calculations are approximate to those obtained with other methods.

The first three sums of squares for SCA are obtained from individual analyses of variance (A_1 , A_2 , A_3 ; Tables 5, 6, 7); when considering the fourth sum of squares (Table 8), it must be corrected by multiplying it by $r=4$, because the table of values crosses x environments was built by adding over the four repetitions that are in each environment.

Table 5. Analysis of variance for environment 1.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	$p > F$
Repetitions	3	37.605	12.535		
Crosses	(19)	34.819	1.833	2.259	0.00938
GCA	7	15.927	2.275	2.805	0.014
SCA	12	18.892	1.574	1.941	0.0481
Error	57	46.241	0.811		
Total	79	118.665			

Table 6. Analysis of variance for environment 2.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	$p > F$
Repetitions	3	6.338	2.113		
Crosses	(19)	20.098	1.058	0.98	0.496
GCA	7	8.64	1.234	1.143	0.349
SCA	12	11.458	0.955	0.884	0.567
Error	57	61.536	1.08		
Total	79	87.972			

Table 7. Analysis of variance for environment 3.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	$p > F$
Repetitions	3	1.922	0.641		
Crosses	(19)	59.351	3.124	2.362	0.00653
GCA	7	34.752	4.965	3.754	0.00207
SCA	12	24.599	2.05	1.550	0.13339
Error	57	75.372	1.322		
Total	79	136.644			

Table 8. Analysis of variance to break down SS (CxA).

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	$p > F$
Repetitions	2	65.236	32.618		
Crosses	(19)	(15.587)	0.82	2.441	0.00945
GCA	7	9.914	1.416	4.214	0.00159
SCA	12	5.674	0.473	1.407	0.20534
Error	38	12.771	0.336		
Total	59	93.594			

In this last table, repetitions=environments, error= C x A, Total= Trat 1= SS A + SS C + SS C x A; each source of variation must be multiplied by 4 (number of repetitions) and F values must be obtained by applying the procedures described by Martínez (1991) or Dhillon (1978), among others (Table 4).

The procedures described for a single environment were also referenced by Singh and Chauhdary (1985); the artifices implemented in the present study to be analyzed with Opstat can also be applied to the analysis of a partial diallel cross corresponding to the methodologies proposed by Fyfe and Gilbert (1963); Federer (1967); Narain *et al.* (1974), as suggested by Dhillon (1978); Martínez (1991).

Conclusions

The analysis of a partial diallel cross is very laborious when using a desktop calculator; this situation will be more critical when increasing p and s in the trial or when several agronomic characteristics and more years, localities or both are considered in the series of experiments. To save time and resources, a matrix calculator or Opstat, available for free on their websites, should be used. To complete the Anova table in the series of experiments, the data contained in a table of values crossed x environments, averaged over repetitions, or averaged over environments, must be entered as if analyzing a single environment; for the second option, the output will correspond to a partition of effects $\text{Trat 1} = \text{SS A} + \text{SS C} + \text{SS (Cx A)}$; in this, Opstat produces the SS of GCA and the SS of SCA in an easy and reliable way, information that, additionally, allows calculating the sum of squares of the interactions GCA x A and SCA x A, using some of the recommendations suggested in this study.

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