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Article

Example for the analysis of a series of experiments arranged in balanced complete blocks

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Abstract

There is little published information in some series of experiments, such as in the randomized complete block design in a balanced complete block arrangement (RCBD-BCBA). This study presents a hypothetical example to apply the methodology published in González *et al* . (2024b), which is an extension of a case formulated by Gomez and Gomez (1984). The analysis of variance and the comparison of means between treatments using Fisher's least significant difference test correspond to the analysis of the data combining the information of two trials, based on the statistical model that was chosen for the present research, the validation of the results generated can be done with other statistical packages, such as Info-Gen, SAS, OPSTAT, STAR, PBTools, or Agrobase, among others.

Keywords:

application of InfoStat, randomized complete block design, two-factor experiments.

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Introduction

In the High Valleys of Mexico, precipitation, solar radiation, the incidence of hail or frost, as well as the heterogeneity in the soils of cooperating farmers are the primary sources of random variability that mask the evaluation and identification of better cultivars; these also affect the validation, generation, application, or transfer of technology (González *et al* ., 2008 ; González *et al* ., 2010; González *et al* ., 2011).

In Mexico, the statistical genetic analysis of the series of experiments has been theoretically addressed in Sahagún (1993 ; 1994 ; 1998 ; 2007a) for the completely randomized experimental design (CRD) and the randomized complete block design (RCBD). For a series of trials in a Latin square design (LSD), De la Loma (1982) presented the results of the analysis of variance of three years, as well as the comparison of treatment means with Student's t-test. Rodríguez *et al* . (2025) presented a methodology for analyzing a split-plot arrangement in an LSD, without and with balanced subsampling.

Studies such as those by Gomez and Gomez (1984) ; Shikari *et al* . (2015) ; Maranna *et al* . (2021) presented the analysis of data from a trial conducted in an experimental design of randomized complete blocks in a balanced complete block arrangement (RCBD-BCBA); González *et al* . (2024a) built the statistical model, presented the formulas for calculating degrees of freedom and sum of squares, and developed a code for Info-Gen to analyze the data published by Gomez and Gomez (1984).

In an RCBD in a BCBA, each replication is divided into g groups, and each of these receives a different subset of treatments, similar to Lattices, to control for heterogeneity caused by two gradients of random variability (Gomez and Gomez, 1984 ; Martínez, 1988 ; Cochran and Cox, 2004). González *et al* . (2024a , 2024b) divided the experimental area into a main unit (MU) and subunits (SUB), as in a split-plot arrangement, and extended it to a series of experiments. Thus, the primary objective of the present study was to show how to analyze data from a series of experiments across environments in an RCBD-BCBA, validating the results with InfoStat.

Materials and methods

Model, symbology and software used

The statistical model and symbology employed in this study were reported in González *et al* . (2024b) . The artificial data were analyzed using InfoStat (https://www.InfoStat.com.ar), but SAS (https://www.sas.com) and Info-Gen (http://www.info-Gen.com.ar) could also be used; to validate the comparison of means of treatments within groups, the OPSTAT package (http://14.139.232.166/opstat/default.asp) could also be applied (Sheoran *et al* ., 1998)

Results

Calculating degrees of freedom (DF

DF total = art-1= 2(3) (45)-1 = 269; DF environments (A)= a - 1= 2-1= 1; DF groups (G)= g -1= 3-1= 2. DF replications within A= a(r-1)= 2(2)= 4; DF A x G= (a -1)(g -1)= 2; DF error a= a(g-1)(r-1)= 2(2)(2) = 8; DF treatments within groups {T(G)}= t -g= 45 - 3= 42; DF AxT(G)= (a-1) (t-g)= 42; DF error b= a(r-1)(t-g)= 2(2)(42)= 168.

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Calculating the sum of squares (SS)

SS total= $\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}^{2}$-$\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^{2}}{art}$ = $\{4.21^2+4.01^2+3.93^2+,\ldots,+4.31^2+[3.41^2+3.06^2+2.76^2+,\ldots,+3.86^2\}$- $\frac{(1084.067)^2}{2(3)(45)}$ = 4516.517261 - 4352.597261.

SS environments (A)= $\frac{1}{rt}\sum_{i=1}^{a}Y_{i\ldots}^{2}$-$\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^{2}}{art}$ = $\frac{462.357^2+621.71^2}{3(45)}$ - $\frac{(1084.067)^2}{2(3)(45)}$ = 4446.646811 - 4352.597261 = 94.049.

SS groups= $\frac{g}{art}\sum_{j=1}^{g}Y_{.j..}^{2}$-$\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^{2}}{art}$ = $\frac{3(346.935^2+348.656^2+388.476^2)}{2(3)(45)}$ - $\frac{(1084.067)^2}{2(3)(45)}$ = 4364.872257 - 4352.597261= 12.27499.

With the information in Table 1 , the following is calculated: SS AxG= { $\frac{g}{rt}\sum_{i=1}^{a}\sum_{j=1}^{g}Y_{ij..}^{2}$ - $\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^{2}}{art}$ }-SS A-SS G = $\frac{(147.825^2+150.496^2+\ldots+224.44^2)}{(3)(45)}$ - $\frac{(1084.067)^2}{2(3)(45)}$ } - 94.049 - 12.27499= 0.9582.

Table 1. Values for calculating the AxG interaction.

Environments (i)	Groups (j)			
	1	2	3	Total
1	147.825	150.496	164.036	462.357
2	199.11	198.16	224.44	621.71
Sum	346.935	348.656	388.476	1084.067

With the data in Table 2 , the following is calculated: SS replications within environments A= { $\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^{2}}{art}$ } - SS A= { $\frac{(166.969^2+148.441^2+\ldots+203.87^2)}{(45)}$ - $\frac{(1084.067)^2}{2(3)(45)}$ } - 94.049= (4453.4655 - 4352.597261) - 94.049= 6.819.

Table 2. Data to calculate SS replications within environments {R(A)}.

Environments (i)	Replications (k)			
	1	2	3	Total
1	166.969	148.441	146.947	462.357
2	213.45	204.39	203.87	621.71
Sum	380.419	352.831	350.817	1084.067

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The data in Table 3 are used to indirectly obtain the SS of error a: SS MU= SS A + SS G + SS AxG + SS R(A) + SS error a.

Therefore: SS error a= SS MU - SS A - SS G - SS AxG - SS R(A)= { $\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^2}{art}$ } - SS A - SS G - SS AxG - SS R(A)= [$\frac{3(53.53^2+48.77^2+\ldots+75.06^2)}{45}$ - $\frac{(1084.067)^2}{2(3)(45)}$] - 94.049 - 0.9582 - 12.27499 - 6.819= 1.3336.

Table 3. Data to calculate SS error a.

Environment	Combination for Groups and Replications (jk)								
	11	12	13	21	22	23	31	32	33	Sum
1	53.5	48.77	45.51	54.82	48.31	47.36	58.6	51.35	54	462.35
2	67.1	66.66	65.28	69.36	65.27	63.53	76.92	72.46	75	621.71
Total	120.7	115.43	110.79	124.18	113.58	110.88	135.52	123.81	129.1	1084.1

Also: SS error a: $\frac{1}{rt}\sum_{i=1}^{a}\sum_{k=1}^{r}Y_{i.k.}^2$- $\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^2}{art}$ + $\frac{1}{rt}\sum_{i=1}^{a}Y_{i...}^2$ = 4468.032093 - 4459.879494 - 4453.46555 + 4446.646811= 1.33386.

The SS of treatments (TREATs) nested within each group (Gs) will be calculated as follows.

SS TREAT(G1)= $\frac{1}{ar}\sum_{l=1}^{t/g}Y_{.1l}^2$- $\frac{\left(\frac{g}{art}\right)\left(\sum_{l=1}^{t/g}Y_{.1l}\right)^2}{2(3)}$ = $\frac{(23.064^2+23.602^2+\ldots+22.862^2)}{2(3)}$ - $\frac{3(346.935^2)}{2(3)(45)}$ = 3.679.

SS TREAT(G2)= $\frac{1}{ar}\sum_{l=1}^{t/g}Y_{.2l}^2$- $\frac{\left(\frac{g}{art}\right)\left(\sum_{l=1}^{t/g}Y_{.2l}\right)^2}{2(3)}$ = $\frac{(22.865^2+25.185^2+\ldots+25.72^2)}{2(3)}$ - $\frac{3(348.656^2)}{2(3)(45)}$ = 8.2.

SS TREAT(G3)= $\frac{1}{ar}\sum_{l=1}^{t/g}Y_{.3l}^2$ - $\frac{1}{ar}\sum_{l=1}^{t/g}Y_{.3l}^2$- $\frac{\left(\frac{g}{art}\right)\left(\sum_{l=1}^{t/g}Y_{.3l}\right)^2}{2(3)}$ = $\frac{(25.533^2+21.639^2+\ldots+28.758^2)}{2(3)}$ - $\frac{3(388.476^2)}{2(3)(45)}$ = 14.548.

To verify: SS T(G) : $\frac{1}{ar}\sum_{l=1}^{t}Y_{...l}^2$ - $\frac{\left(\frac{g}{art}\right)\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^2}{art}$ = 26.427.

SS Trea 5= SS A + SS G + SS AxG + SS T(G) + SS AxT(G).

Where: SS Treat 5= $\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^2}{art}$.

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From Table 4 , SS A x T(G)= $\frac{1}{3}$ {(10.914^2+,…, +9.942^2)+ (9.985^2+,…,+11.12^2) + (10.433^2+,…, +12.478^2) + (12.15^2+,…,+12.92^2) + (12.88^2+,…,+14.58^2)+ (15.10^2+,…,+ 16.282)}- 94.049 - 12.27 - 0.9582 - 26.43= (4491.873261 - 4352.597261) - 94.049 - 12.27 - 0.9582 - 26.43= 5.568.

Table 4. Data to calculate SS A xT(G).

Environment 1			Environment 2		
Group 1 (T1-T15)	Group 2 (T16-T30)	Group 3 (T31-T45)	Group 1 (T1-T15)	Group 2 (T16-T30)	Group 3 (T31-T45)
10.914	9.985	10.433	12.15	12.88	15.1
8.972	10.335	8.729	14.63	14.85	12.91
8.885	9.983	10.892	13.67	13.18	13.7
11.563	9.55	9.889	13.18	13.16	13.74
9.241	9.057	9.815	12.05	10.38	13.57
9.076	9.09	11.81	13.86	12.2	16.11
9.85	9.56	11.82	13.35	12.46	16.03
9.286	11.26	9.358	13.4	14.45	11.99
9.048	9.439	11.139	13.32	12.77	15.9
10.982	9.314	11.107	12.82	12.45	15.64
11.084	10.158	12.096	14.55	13.11	16.28
9.852	11.361	12.222	12.75	14.94	16.8
8.619	10.046	11.336	12.25	13.87	15.62
10.511	10.238	10.921	14.21	12.88	14.77
9.942	11.12	12.478	12.92	14.58	16.28
147.825	150.496	164.036	199.11	198.16	224.44

Additionally, SS total= SS A + SS G + SS R(A) + SS AxG + SS error a + [SS TREAT (G1) + SS TREAT (G2) + SS TREAT (G3) + ,..., + SS TREAT (Gg)] + SS AxT(G) + SS error b.

Thus: SS error b= SS total - (SS A + SS G + SS R(A) + SS AxG + SS error a) - [SS TREAT (G1) + SS TREAT (G2) + SS TREAT (G3) + ,..., + SS TREAT (Gg)] - SS AxT(G).

With the previous information, the following is calculated: SS error b= 163.92 - 94.049 -12.27499 - 6.819 - 0.9582 - 1.33361 - 26.42816 - 5.56644= 16.4906.

Another alternative is presented below: SS error b= $\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}^2$ - $\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}Y_{ijk.}^2$ - $\frac{1}{r}\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{l=1}^{t}Y_{ij.l}^2$ + $\frac{g}{rt}\sum_{i=1}^{a}\sum_{j=1}^{g}Y_{ij..}^2$ = 4516.517261- 4468.032093 - 4491.873261 + 4459.879494= 16.4914.

If the experimental area is divided into main unit (MU) and subunit (SU) and as proposed by González *et al* . (2024a , 2024b), SS total is defined as follows= SS MU + SS SU, then the following expression will also be valid: SS MU= SS A + SS G + SS R(A) + SS AxG + SS Error a= 94.049 + 12.27499 + 6.819 + 0.9582 + 1.33361= 115.4348.

Also, SS MU= SS Treat 1 = $\frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t}Y_{ijkl}\right)^2}{art}$ = 4468.032093 - 4352.597261= 115.434832.

By difference: SS SU= SS total - SS MU= 163.91 - 115.434832= 48.471568.

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For verification, based on previous calculations, SS SU= $\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r}\sum_{l=1}^{t} Y_{ijkl}^{2}$ - $\frac{g}{t}\sum_{i=1}^{a}\sum_{j=1}^{g}\sum_{k=1}^{r} Y_{ijk.}^{2}$ = 4516.517261- 4468.032093= 48.485168.

Using InfoStat

The labels for the columns will be environments, groups, replications, treatments, and response variable, identified as A, G, R, T, X, respectively. The art= 270 data are entered in that order (Balzarini *et al* ., 2008 ; Di Rienzo *et al* ., 2008 ; Balzarini and Di Rienzo, 2016) 246]. First, a general analysis of variance will be obtained, and then the comparison of treatment means within groups will be carried out with Fisher’s least significant difference (LSD) test; Figures 1 and 2 show the procedures for applying this software and Tables 5 , 6 , 7 , 8 and 9 present the outputs with the results of the reference statistical analysis 247, 248].

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Figure 1. Successive stages to obtain the general analysis of variance 249].

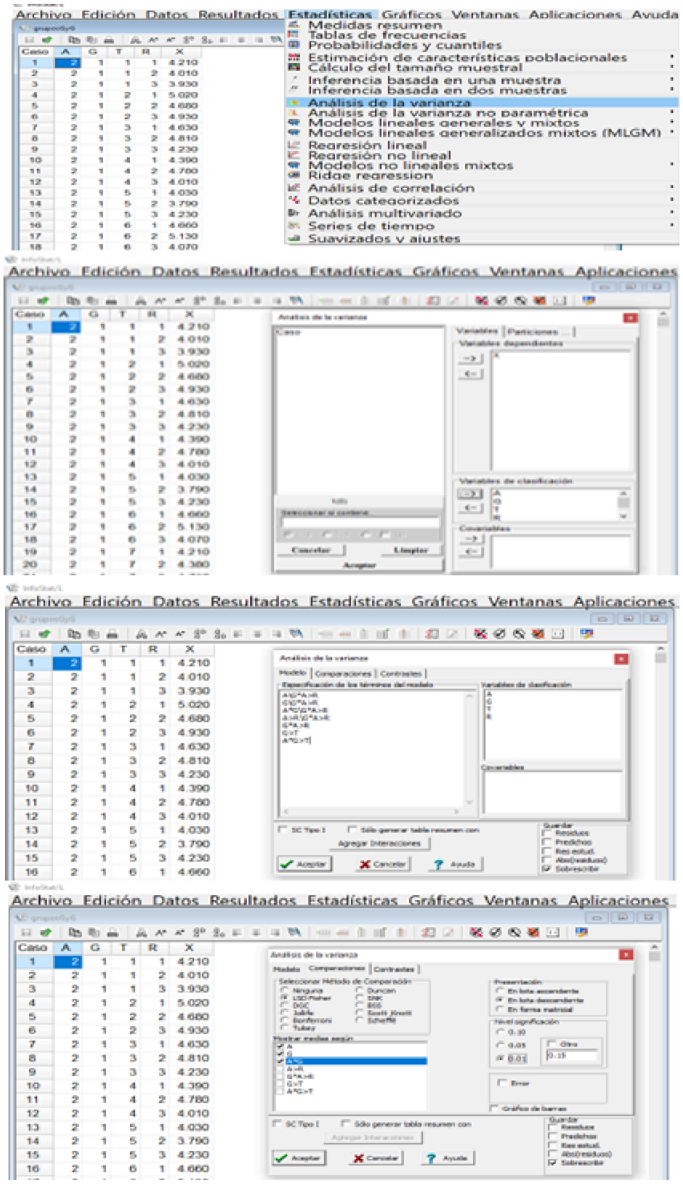

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Figure 2. Successive stages to obtain the comparison of means for treatments nested within groups 250].

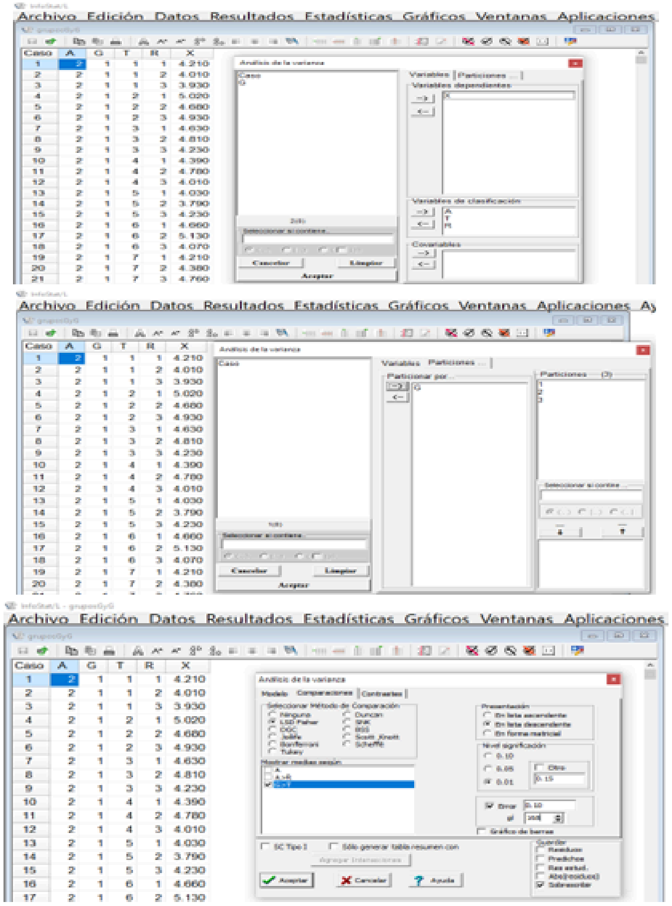

Table 5. Combined Anova generated by InfoStat 251].

SV	DF	SS	MS	F	p	Error
A	1	94.05	94.05	564.1	< 0.01	GxA> R
G	2	12.27	6.14	36.81	< 0.01	GxA> R
AxG	2	0.96	0.48	2.87	0.11	GxA> R
R	4	6.82	1.7	10.22	< 0.01	GxA> R
GxA> R	8	1.33	0.17	1.7		
G> T	42	26.43	0.63	6.41	< 0.01	
AxG> T	42	5.57	0.13	1.35	0.09	
Error	168	16.49	0.1			
Total	269	163.91				

Table 6. Comparison of means for factor A (*p* = 0.01) 253].

Levels	Means	n	SE	Symbology
2	4.61	135	0.04	a
1	3.42	135	0.04	b

Means with the same letter are statistically the same (LSD= 0.16676) 255].

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Table 7. Comparison of means for factor G (*p* = 0.01) 256].

Levels	Means	n	SE	Symbology
3	4.32	90	0.04	a
2	3.87	90	0.04	b
1	3.85	90	0.04	b

Means with the same letter are statistically the same (LSD= 0.20424) 258].

Table 8. Comparison of means for A with G (*p* = 0.01).

A	G	Means	n	SE	Symbology
2	3	4.99	45	0.06	a
2	1	4.42	45	0.06	b
2	2	4.4	45	0.06	b
1	3	3.65	45	0.06	c
1	2	3.34	45	0.06	d
1	1	3.29	45	0.06	d

Means with the same letter are statistically the same (LSD= 0.2888).

Table 9. Comparison of means within groups (InfoStat, *p* = 0.01).

Treatment	G1	Treatment	G2	Treatment	G3
11	4.27 a	27	4.38 a	42	4.84 a
4	4.12 ab	23	4.29 ab	45	4.79 a
14	4.12 ab	30	4.28 ab	41	4.73 ab
10	3.97 abc	17	4.2 abc	36	4.65 ab
2	3.93 abcd	28	3.99 abcd	37	4.64 ab
7	3.87 abcd	26	3.88 bcd	39	4.51 abc
1	3.84 abcd	18	3.86 bcd	43	4.49 abc
6	3.82 abcd	29	3.85 bcd	40	4.46 abc
15	3.81 abcd	16	3.81 bcd	44	4.28 bcd
8	3.78 bcd	19	3.79 cd	31	4.26 bcd
12	3.77 bcd	24	3.7 de	33	4.1 cd
3	3.76 bcd	22	3.67 de	34	3.94 de
9	3.73 bcd	25	3.63 de	35	3.9 de
5	3.55 cd	21	3.55 de	32	3.61 e
13	3.48 d	20	3.24 e	38	3.56 e

Means with equal letters, within each group, are statistically the same.

Discussion

The use of a statistical model leads to the generation of an analysis of variance (Figure 1 , Table 5); De la Loma (1982) ; Mendenhall (1987) ; Martínez (1988) , Sahagún (2007b) ; Montgomery (2009) pointed out that this is important to face the problem of the design and analysis of a trial where the calculation of degrees of freedom, sum of squares, and the construction of appropriate statistical tests considering mean squares and mathematical expectations are involved, especially when considering random or mixed models for more complex situations.

The model built by González *et al* . (2024 a) is linked to an example provided by Gomez and Gomez (1984) ; Shikari *et al* . (2015) ; Maranna *et al* . (2021) ; they suggested that the groups of treatments

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could be formed by important differences between them, with minimal variation within them, or by their geographical and/or genetic origin (González *et al* ., 2008 ; González *et al* ., 2010 ; González *et al* ., 2011).

This study complements the research carried out by Sahagún (1993 ; 1994 ; 1998 ; 2007a); Martínez (1988) ; Gomez and Gomez (1984) ; González *et al* . (2024b) , the BCBA-RCBD is recommended when the experimental area is very heterogeneous and when the number of treatments is greater than 30; with two replications, the statistical hypotheses could be tested, and it would be feasible to extend their analysis to a series of experiments, particularly when completely randomized designs, RCBD, LSD or some Lattice present some disadvantages (Martínez, 1988 ; Cochran and Cox, 2004 ; Montgomery, 2009).

In González *et al* . (2019 ; 2023 ; 2024a ; 2024b); Rodríguez *et al* . (2025) , free versions of InfoStat, Info-Gen, and SAS were used; in these, they employed CRD, RCBD and LSD or LSD in split plots. This situation was generalized to a BCBA-RCBD, but if the experiments are extensive, the student versions will slow down or will not generate results; commercial versions of both would have a lower cost to the user, but SAS is the better software.

InfoStat and InfoGen are very flexible in a series of experiments as the data are automatically sorted, and the degrees of freedom and mean square of Error b are manually entered (Figure 2 ; González *et al* ., 2024 b); both are also very reliable and easy to use for analyzing data from each trial (Shikari *et al* ., 2015 ; Maranna *et al* ., 2021 ; González *et al* ., 2024a).

The Anovas prior to the comparison of means within groups (not included) are not correct, but they allow us to verify that the addition of SS T(G) in each group (3.68 + 8.2 + 14.55) is equal to SS T(G) [26.43; Table 5]; if differences between groups and between treatments withing groups are not significant, InfoGen or InfoStat can generate an Anova and a comparison of means with various methodologies, for a series of RCBD experiments, using the RCBA-RCBD database (González *et al* ., 2024b).

If Tukey's test is applied, its validation could be carried out with the OPSTAT software, available free of charge on its website; in this, only the arithmetic means of each treatment within each group are entered, along with the degrees of freedom and mean square of error b (Table 5 , Figure 2).

Conclusions

With InfoStat, it is easy and reliable to generate an analysis of variance (Anova) for the series of experiments and when applying the LSD test to treatments nested within groups, because it allows us to manually enter the degrees of freedom and mean square of Error b; the Anovas that are generated in conjunction with the comparison of means between treatments within groups are not correct, but can be used to verify that their addition is equal to SS T(G) of the Anova in the series of experiments. If the statistical model used in the present study is not chosen, the experiment series database can be used directly to obtain an Anova and a comparison of means of treatments nested within groups for each trial. If the groups of treatments in the BCBA-RCBD are statistically equal, the data could be analyzed as a series of experiments in an RCBD using the information contained in the same file.

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Example for the analysis of a series of experiments arranged in balanced complete blocks

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