#### Article

# InfoStat, InfoGen and SAS for mutually orthogonal contrasts in randomized complete block experiments in subdivided plots

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# Abstract

The series of experiments (SE) in time and space in arrangements of divided plots have been used daily in various disciplines of science and technology, but for subdivided plots (PS) there is little information. With mutually orthogonal contrasts (CMO's), the variability of means or total treatments in groups is divided into an analysis of variance in experiments of one or more factors. This study presents the programs to analyze data of the pod weight in green registered in fava beans in an SE in complete blocks at random in arrangement of PS to run in the System for Statistical Analysis, InfoStat or InfoGen, prepared by the author for correspondence. The procedures for determining how many and what the coefficients of each contrast are and how to calculate some CMO's are indicated. A combined analysis of variance and CMO's are presented for main factors and for their interactions, but the three statistical packages can generate the analyzes for each trial. With the platform that has been developed in the present study, it will be easier to obtain the decomposition of the effects of main factors and their interactions in an SE under PS based on the construction of an appropriate set of orthogonal polynomials or a combination between these and the CMO's.

**Keywords:** *Vicia faba* L., factorial experiments, fixed effects model, High Valleys of central Mexico.

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# Introduction

When designing and analyzing experiments, analysis of variance (Anava) has been conventionally used to minimize experimental error and reliably estimate the effects between treatments (Juarez and Corona, 1990; Sahagún and Frey, 1990; Sahagún, 1997; Meneses *et al.*, 2004). The assumptions that must be satisfied is that the chosen linear model adequately describes the observations, and that the errors follow a normal and independent distribution, with zero mean and constant variance, although unknown (Sahagún, 1990; Sahagún *et al.*, 2008).

The analysis of quantitative variables in fixed, random or mixed effects models can refer to balanced or unbalanced cases (Matzinger *et al.*, 1959; Sahagun, 1998; Montgomery, 2010) with qualitative or quantitative factors such as fertilization, planting density or population, insecticides, fungicides, plant hormones, cultivars, localities, years or their combinations, among others (Sahagún and Frey, 1990; Sahagún, 1997; Meneses *et al.*, 2004; González *et al.*, 2007).

Factorial experiments save resources, increase the accuracy of estimates of mean effects and make possible the study of their interactions (Sahagún *et al.*, 2008). The use of crossing plans and experimental designs is a common procedure in plant and animal genetic improvement (Matzinger *et al.*, 1959; Sahagun, 1997), as well as in seed production, generation, application, transfer and validation of technology (Sahagun and Frey, 1990; Meneses *et al.*, 2004; González *et al.*, 2007; Torres *et al.*, 2017).

Anava is a prerequisite in the comparison of treatment means, but its effects can also be divided into mutually orthogonal contrasts (CMO's) when one of the four basic experimental designs is chosen or when the series of experiments in time and space are applied with arrangements in strips and divided plots (Gomez and Gomez, 1984; Martínez, 1988; Sahagún, 1998; Rebolledo, 2002). The formation of CMO's has these advantages: a) each hypothesis test provides new, independent information; b) the interpretation of results is simpler; and c) the maximum estimated number is limited.

In its construction the basic guide is its congruence with the objectives of the research, no matter if they are mutually orthogonal or not, or how many treatments are evaluated (Sahagún *et al.*, 2008). In incomplete factories the interactions of greater degree have been used as experimental error, although it is not common to experiment with more than four factors and it is not frequent that all their interactions are significant (Sahagún *et al.*, 2008; Montgomery, 2010; Walpole *et al.*, 2012). Genotype x environment interaction, orthogonal polynomials, different regression techniques and various multivariate methods also use ANAVA (Gomez and Gomez, 1984; Sahagún, 1990; Sahagún *et al.*, 2008; Torres *et al.*, 2017).

The randomized complete block SE (DBCA) in divided plots is described in many publications, such as Martinez (1988); Rebolledo (2002) who also developed several programs for the SAS to generate Anava, the comparison of treatment means with the test of Tukey and the CMO's. The linear model of an SE in DBCA under PS was described by Herrera (2011) and Padilla *et al.* (2019) but there is still little information for random and mixed models and particularly when two factors are housed in a large, medium or small plot (Villa *et al.*, 2010).

The analysis of an SE in subdivided plots is subject to more errors when many variables have been recorded, but the development of programs for InfoStat, InfoGen and SAS, among others, will save time and effort if the linear model chosen is correct (Sahagún, 1998; Herrera, 2011; Padilla *et al.*, 2019). The objective of this study was to present programs to analyze an SE in PS in DBCA with CMO's using three statistical packages of common use.

# Framework

#### Linear model

It is established that: i = 1, 2, 3, ..., and experiments; j = 1, 2, 3, ..., r repetitions; k = 1, 2, 3, ... at levels on a large plot; l = 1, 2, 3, ..., b levels in medium plot; m = 1, 2, 3, ..., c levels in small plot. Thus:

$$\begin{split} Y_{ijklm} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + (\alpha\gamma)_{ik} + \epsilon_{ijk} + \delta_l + (\gamma\delta)_{kl} + (\alpha\delta)_{il} + (\alpha\gamma\delta)_{ikl} + \epsilon_{ijkl} + \theta_m + (\gamma\theta)_{km} + (\delta\theta)_{lm} + (\gamma\delta\theta)_{klm} + (\alpha\delta\theta)_{ilm} + (\alpha\gamma\theta)_{ikm} + (\alpha\delta\theta)_{ilm} + \epsilon_{ijklm} \end{split}$$

Where:  $\mu$  is the great arithmetic mean;  $\alpha i$  is the ith experiment;  $\beta_{j(i)}$  is the j-th repetition nested in the ith experiment;  $\gamma_k$  is the k-th fertilization;  $\delta_l$  is 1-th population density;  $\theta_m$  is the m-th cultivar;  $\varepsilon_{ijk}$ ,  $\varepsilon_{ijkl}$  and  $\varepsilon_{ijklm}$  are the errors a, b and c of large, medium and small plot, the remaining ten components are viable interactions (Herrera, 2011; Padilla *et al.*, 2019).

#### **Obtaining CMO coefficients**

Gomez and Gomez (1984); Sahagún *et al.* (2008) define an orthogonal contrast as a linear combination of treatment effects. If  $T_1$ ,  $T_2$ ,  $T_3$ , Tt are unknown parameters related to the effects of t treatments and  $C_1$ ,  $C_2$ ,  $C_3$ ,  $C_t$  are known constants, called coefficients of contrasts, in monofactorial experiments, each contrast (L<sub>i</sub>) is calculated as:

$$L_i = C_1T_1 + C_2T_2 + C_3T_3 + \dots$$
,  $C_tT_t = \sum_{i=1}^t C_iT_i$  with restriction  $\sum_{i=1}^t C_i = 0$ 

The sum of squares (SC) of L<sub>i</sub> with a degree of freedom (gl) is calculated as:

SC L<sub>i</sub> = 
$$\frac{\left[\sum_{i=1}^{t} \text{CiTi}\right]^2}{r\sum_{i=1}^{t} C_i^2}$$

With t treatments there are t-1 orthogonal contrasts. To test its statistical significance in a fixed effects model, the ratio that results from dividing SC L<sub>i</sub> by SC of the error is compared. The F test is used (1, degrees of freedom of error), at the level of significance chosen (p= 0.05 or p= 0.01).

Two contrasts,  $L_1$  and  $L_2$ , with a degree of freedom (GL), are mutually orthogonal if the sum of the cross products of their coefficients is equal to zero; that is to say:

$$\begin{split} L_1 &= C_{11}T_1 + C_{12}T_2 + C_{13}T_3 +, \dots, + C_{1t}T_t \\ L_2 &= C_{21}T_1 + C_{22}T_2 + C_{23}T_3 +, \dots, + C_{2t}T_t \\ &\sum_{i=1}^t C1iC2i = C11C21 + C12C22 + C13C23 +, \dots, + C1tC2t = 0 \end{split}$$

p contrasts with a GL (p>2) are mutually orthogonal if each pair and all pairs in the group are orthogonal. Since the maximum number of mutually orthogonal contrasts with a GL is equal to the number of treatments GL then:

 $SCL_1 + SCL_2 + SCL_3 +$ , +  $SCL_{t-1} = SC$  of treatments

To calculate the coefficients of mutually orthogonal contrasts (CMO) in the interactions of any order in factorial experiments, proceed as follows: 1. To determine how many coefficients there will be, multiply the CMO number of each factor; 2. The coefficients for each interaction are obtained as the product of their values and signs; and 3. All the coefficients of each contrast are captured in the SAS editor program or in the InfoStat or InfoGen dialog. 4. The program is run, the outputs are verified, a receipt is made or the outputs are printed.

With 5 and 2 CMO for factors A and B there will be 10 possibilities. Since there are 6 or 3 coefficients in factors A or B, any CMO will have 18 coefficients. If the coefficients of the first contrast for factors A and B are (1 - 1 1 - 1 1 - 1) and [-1 2 -1], the first combination with values: (1 - 1 1 - 1 1 - 1)[-1 2 - 1] will produce -1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 - 1 1 - 2 - 1 1 - 2 - 1 1 - 2 - 1 1 - 2 - 1 1 - 2 - 1 1 - 2 - 1 - 2

Note: multiply 1(-1), 1(2), 1(-1), -1(-1), -1(2), -1(-1), ..., -1(-1), -1(2), -1(-1).

For AxC there will also be 10 cases, with 18 coefficients each; its values and signs are equal to those of A1B1. Thus: (1 - 1 1 - 1 1 - 1) [-1 2 - 1] = -1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 1 - 1 2 - 1 1 - 2 -

Note: each value is obtained as -1(-1), -1(2), -1(-1), 2(-1), 2(2), 2(-1), -1(-1), -1(2), -1(-1).

In AxBxC, there are 6x3x3= 54 coefficients for each of the 5x2x2= 20 mutually orthogonal contrasts. For SAS its values and signs are obtained by multiplying the coefficients of each AxB interaction with those of C, but for InfoStat these are generated by multiplying the values of C by those of AxB. For SAS:

#### **Statistical analysis**

The pod weights in green were subjected to a combined analysis of variance. Algebraic procedures are described in Herrera (2011); Padilla *et al.* (2019). The outputs were obtained with the versions described in SAS Institute (1989); InfoStat (Balzarini *et al.*, 2008; Di Rienzo *et al.*, 2008) and InfoGen (Balzarini and Di Rienzo, 2016). Additionally, a subdivision of the three main effects and their four interactions with mutually orthogonal contrasts was made (Gomez and Gomez, 1984).

#### **Calculation of some CMO**

In Padilla *et al.* (2019) shows the performance data in green pod used to perform the following calculations. With the totals of AxB, AxC and AxBxC, the CMOs for the three factors and for the BxC interaction can be calculated.

Contrast 1 of factor A (FER)

Contrast 1 of factor B (DEN)

$$SCL1 = \frac{(\sum_{i=1}^{c} CiTi)^2}{eacr\sum_{i=1}^{c} Ci^2} = \frac{[(496.23 - 2(783.38) + 1032.39]^2}{2(6)(3)(3)(6)} = \frac{(1528.62 - 1566.76)^2}{648} = 2.245$$

Contrast 1 of factor C (CUL)

$$SCL1 = \frac{(\sum_{i=1}^{c} CiTi)^2}{eabr\sum_{i=1}^{c} Ci^2} = \frac{[(819.77 - 2(741.83) + 750.37]^2}{2(6)(3)(3)(6)} = \frac{(1570.14 - 1483.66)^2}{648} = 11.54$$

Contrast 1 of the interaction FER x DEN (AxB)

 $SCL1 = \frac{(\sum_{i=1}^{c} ciTi)^2}{ecr\sum_{i=1}^{c} ci^2}$ , where: e, c and r are localities, cultivars and repetitions, respectively.

= [81.81 - 70.94 + 108.36 - 79.72 + 82.8 - 72.57 - 2(128.61) + 2(137.40) - 2(147.09) + 2(113.16) - 2(143.56) + 2(113.56) + 154.69 - 116.72 + 208.03 - 151.22 + 184.50 - 167.23]2 / [2(3)(3)(36)] = (1548.43 - 1546.92)2 / 648 = 0.0035.

Contrast 1 of the FER x CUL (AxC) interaction.

 $SCL1 = \frac{(\sum_{i=1}^{C} ciTi)^{2}}{ebr \sum_{i=1}^{C} ci^{2}}, \text{ where: b are the density levels of population.}$  $= \frac{\{126.19 - 144.06 + 170.27 - 121.85 + 144.81 - 112.59 - 2(120.35) + 2(111.17) - 2(155.01) + 2(107.96) - 2(135.61) + 2(111.73) + 118.57 - 119.83 + 138.20 - 114.29 + 130.44 - 129.04)\}^{2}}{2(3)(3)(36)} = 8.31$ 

Contrast 1 of the DEN x CUL interaction (B x C).

 $SCL1 = \frac{(\sum_{i=1}^{c} CiTi)^2}{ear \sum_{i=1}^{c} Ci^2}, \text{ where: a are the levels of fertilization.}$  $= \frac{[188.54 - 2(269,27) + 361.96 - 2(152.38) + 4(260.93) - 2(328.52) + 155.28 - 2(253.18) + 341.91]^2}{2(6)(3)(36)} = 5.53.$ 

Contrast 1 of the interaction FER x DEN x CUL (AxBxC).

 $\text{SCL1} = \frac{(\sum_{i=1}^{c} CiTi)^2}{er\sum_{i=1}^{c} Ci^2}$ 

 $\begin{array}{l} [30.31-2(41.9)+53.98-29.29+2(52.3)-62.47+47.78-2(51.3)+71.19-30.49+2(37.81)-53.55\\+28.84-2(50.23)+65.74-21.83+2(35.73)-55.03-2(26.82)+4(45.81)-2(47.72)+2(15.05)-4(43.92)+2(52.2)\\-2(31.55)+4(53.34)-2(70.12)+2(22.7)-4(37.81)+2(47.45)-2(29.74)+4(42.67)-2(63.15)+2(26.47)-4(37.88)\\+2(47.88)+24.68-2(40.90)+52.99-26.6+2(41.18)-51.95+29.03-2(42.45)+66.72-26.53+2(37.54)-50.22\\+24.17-2(50.66)+55.61-24.27+2(40.45)-65.32]^2 \\ \hline 2(3)(216) \end{array}$ 

= 0.91.

The above calculations and the rest of the CMOs that are possible to estimate can be obtained with the following routine:

#### **Programs for InfoStat and InfoGen**

#### Stage 1

The data is ordered as experiments (EXP), repetitions (REP), fertilization (FER), population density (DEN), cultivars (CUL) and variables (s).

## Stage 2

In the main menu choose statistics\analysis of variance; In the define dialog box: Dependent variables: RVV. Classification variables: EXP REP FER DEN CUL, Choose accept.

## Stage 3

In specifications of the terms of the model write:

EXP\EXP>REP\*FER; EXP>REP\EXP>REP\*FER; FER\EXP>REP\*FER; EXP\*FER\EXP>REP\*FER; EXP>REP\*FER;DEN\EXP>FER>REP\*DEN;DEN\*EXP\EXP>FER>REP\*DEN;DEN\*FER\EX P>FER>REP\*DEN;DEN\*EXP\*FER\EXP>FER>REP\*DEN;EXP>FER>REP\*DEN;CUL;CUL\* EXP;CUL\*FER; CUL\*DEN;CUL\*EXP\*FER;CUL\*EXP\*DEN;CUL\*FER\*DEN; choose accept. Note: in the dialog box the instructions above must be written separately on each line and without the semicolon.

## Step 4

In the dialog box that shows Analysis of variance choose: comparisons\contrasts\treatments\choose effects\matrix of contrasts\accept.

Before clicking on accept, you must choose to control orthogonality. After defining whether a main effect or an interaction will be estimated, enter the coefficients in the contrast matrix.

#### **SAS Program**

In the database Exp= localities, A, B and C are fertilization, population density and cultivars and the variables are identified as X1, X2, X16.

**Data** fava bean; Input Exp rep A B C X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15 X16; Cards;

Here the data is captured in the order of the input.

PROC SORT; BY EXP; PROC GLM; BY EXP; CLASS REP A B C; MODEL X1-X16= REP A REP\*A B A\*B REP\*B(A) C A\*C B\*C A\*B\*C; TEST H=REP A E=REP\*A; TEST H=B A\*B E=REP\*B(A);

An analysis of individual variance with glm is generated and mutually orthogonal contrasts are calculated for main effects, large and medium plot errors are used for f tests.

CONTRAST 'A1, A3,A5 vs A2,A4,A6' A 1 -1 1 -1 /E=REP\*A; CONTRAST 'A1 vs A3, A5' A 2 0 -1 0 -1 0/E=REP\*A; CONTRAST 'A3 vs A5' A 0 0 1 0 -1 0/E=REP\*A; CONTRAST 'A2 vs A4, A6' A 0 2 0 -1 0 -1/E= REP \*A; CONTRAST 'A4 vs A6' A 0 0 0 1 0 -1/E=REP\*A; CONTRAST 'B2 vs B1, B3' B -1 2 -1/E=REP\*B(A); CONTRAST 'B1 vs B3' B 1 0 -1/E=REP\*B(A);

The contrasts of factor c are tested with the residual of the model, which is the small plot error; they do not need to be specified at the end.

CONTRAST 'C2 vs C1, C3' C -1 2 -1; CONTRAST 'C1 vs C3' C 1 0 -1;

The contrasts of the axb interaction are tested with the median plot error.

CONTRAST 'A1B1' A\*B -1 2 -1 1 -2 1 -1 2 -1 1 -2 1 -1 2 -1 1 -2 1/E=REP\*B(A); CONTRAST 'A2B1' A\*B -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1 0 0 0/E=REP\*B(A); CONTRAST 'A3B1' A\*B 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1 0 0 0/E=REP\*B(A); CONTRAST 'A4B1' A\*B 0 0 0 -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1/E=REP\*B(A); CONTRAST 'A5B1' A\*B 0 0 0 0 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1/E=REP\*B(A); CONTRAST 'A5B1' A\*B 0 0 0 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1/E=REP\*B(A); CONTRAST 'A1B2' A\*B 1 0 -1 -1 0 1 1 0 -1 -1 0 1 1 0 -1 -1 0 1/E=REP\*B(A); CONTRAST 'A2B2' A\*B 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1 0 0 0/E=REP\*B(A); CONTRAST 'A3B2' A\*B 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1 0 0 0/E=REP\*B(A); CONTRAST 'A4B2' A\*B 0 0 0 0 0 0 0 1 0 -1 0 1 0 0 0 -1 0 1 /E=REP\*B(A); CONTRAST 'A4B2' A\*B 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 -1 0 1 /E=REP\*B(A);

The errors of the axc interaction are tested with the residual of the model, so they do not need to be indicated at the end.

CONTRAST 'A1C1' A\*C -1 2 -1 1 -2 1 -1 2 -1 1 -2 1 -1 2 -1 1 -2 1; CONTRAST 'A2C1' A\*C -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1 0 0 0; CONTRAST 'A3C1' A\*C 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1 0 0 0; CONTRAST 'A4C1' A\*C 0 0 0 -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1; CONTRAST 'A4C1' A\*C 0 0 0 0 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1; CONTRAST 'A5C1' A\*C 0 0 0 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1; CONTRAST 'A1C2' A\*C 1 0 -1 -1 0 1 1 0 -1 -1 0 1 1 0 -1 -1 0 1; CONTRAST 'A2C2' A\*C 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1 0 0 0; CONTRAST 'A3C2' A\*C 0 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1 0 0 0; CONTRAST 'A4C2' A\*C 0 0 0 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1; CONTRAST 'A5C2' A\*C 0 0 0 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1; CONTRAST 'B1C1' B\*C 1 -2 1 -2 4 -2 1 -2 1; CONTRAST 'B1C2' B\*C -1 0 1 2 0 -2 -1 0 1; CONTRAST 'B2C1' B\*C -1 2 -1 0 0 0 1 -2 1; CONTRAST 'B2C2' B\*C 1 0 -1 0 0 0 -1 0 1;

The axbxc interaction coefficients are tested with the small or residual plot error of the model and are not indicated at the end.

000-10100010-100000000; 000-12-12-42-12-100000000; 00010-1-20210-1000000000; 0001-21000-12-1000000000; 00-10100010-100000000; CONTRAST 'A4B1C1' A\*B\*C 0 0 0 0 0 0 0 0 0 2 -4 2 -4 8 -4 2 -4 2 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1 0 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1; CONTRAST 'A4B1C2' A\*B\*C 0 0 0 0 0 0 0 0 0 -2 0 2 4 0 -4 -2 0 2 0 0 0 0 0 0 0 0 0 1 0 -1 -2 0 2 10-10000000010-1-20210-1; CONTRAST 'A4B2C1' A\*B\*C 0 0 0 0 0 0 0 0 -2 4 -2 0 0 0 2 -4 2 0 0 0 0 0 0 0 0 0 1 -2 1 0 0 0 -12 - 10000000001 - 21000 - 12 - 1;10-100000000-10100010-1; 1 -2 1 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1; 1010000000010-1-20210-1; -21000000001-21000-12-1; 0100000000-10100010-1;

PROC GLM; CLASS EXP REP A B C;

MODEL X1-X16= EXP REP(EXP) A A\*EXP REP\*A(EXP) B A\*B B\*EXP A\*B\*EXP REP\*B(A EXP) C A\*C B\*C A\*B\*C C\*EXP A\*C\*EXP B\*C\*EXP; TEST H= EXP A REP(EXP) EXP\*A E=REP\*A(EXP); TEST H= B A\*B B\*EXP A\*B\*EXP E=REP\*B(A EXP);

A combined analysis of variance is obtained with partition of main effects with mutually orthogonal contrasts.

CONTRAST 'A1, A3, A5 VS A2, A4, A6' A 1 -1 1 -1 1 -1/E=REP\*A(EXP); CONTRAST 'A1 VS A3, A5' A 2 0 -1 0 -1 0/E=REP\*A(EXP); CONTRAST 'A3 VS A5' A 0 0 1 0 -1 0/E=REP\*A(EXP); CONTRAST 'A2 VS A4, A6' A 0 2 0 -1 0 -1/E=REP\*A(EXP); CONTRAST 'A4 VS A6' A 0 0 0 1 0 -1/E=REP\*A(EXP); CONTRAST 'B2 VS B1, B3' B -1 2 -1/E=REP\*B(A EXP); CONTRAST 'B1 VS B3' B 1 0 -1/E=REP\*B(A EXP); CONTRAST 'B1 VS B3' B 1 0 -1/E=REP\*B(A EXP); CONTRAST 'C2 VS C1, C3' C -1 2 -1; CONTRAST 'C1 VS C3' C 1 0 -1; CONTRAST 'A1B1' A\*B -1 2 -1 1 -2 1 -1 2 -1 1 -2 1 /E=REP\*B(A EXP);

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CONTRAST 'A2B1' A*B -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1 0 0 0/E=REP*B(A EXP);
CONTRAST 'A3B1' A*B 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1 0 0 0/E=REP*B(A EXP);
CONTRAST 'A4B1' A*B 0 0 0 -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1/E=REP*B(A EXP);
CONTRAST 'A5B1' A*B 0 0 0 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1/E=REP*B(A EXP);
CONTRAST 'A1B2' A*B 1 0 -1 -1 0 1 1 0 -1 -1 0 1 1 0 -1 -1 0 1/E=REP*B(A EXP);
CONTRAST 'A2B2' A*B 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1 0 0 0/E=REP*B(A EXP);
CONTRAST 'A3B2' A*B 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1 0 0 0/E=REP*B(A EXP):
CONTRAST 'A4B2' A*B 0 0 0 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1/E=REP*B(A EXP);
CONTRAST 'A5B2' A*B 0 0 0 0 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1/E=REP*B(A EXP);
CONTRAST 'A1C1' A*C -1 2 -1 1 -2 1 -1 2 -1 1 -2 1 -1 2 -1 1 -2 1;
CONTRAST 'A2C1' A*C -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1 0 0 0;
CONTRAST 'A3C1' A*C 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1 0 0 0;
CONTRAST 'A4C1' A*C 0 0 0 -2 4 -2 0 0 0 1 -2 1 0 0 0 1 -2 1;
CONTRAST 'A5C1' A*C 0 0 0 0 0 0 0 0 0 -1 2 -1 0 0 0 1 -2 1;
CONTRAST 'A1C2' A*C 1 0 -1 -1 0 1 1 0 -1 -1 0 1 1 0 -1 -1 0 1:
CONTRAST 'A2C2' A*C 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1 0 0 0;
CONTRAST 'A3C2' A*C 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1 0 0 0:
CONTRAST 'A4C2' A*C 0 0 0 2 0 -2 0 0 0 -1 0 1 0 0 0 -1 0 1;
CONTRAST 'A5C2' A*C 0 0 0 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1;
CONTRAST 'B1C1' B*C 1 -2 1 -2 4 -2 1 -2 1;
CONTRAST 'BIC2' B*C -1 0 1 2 0 -2 -1 0 1;
CONTRAST 'B2C1' B*C -1 2 -1 0 0 0 1 -2 1;
CONTRAST 'B2C2' B*C 1 0 -1 0 0 0 -1 0 1;
CONTRAST 'A1B1C2' A*B*C -1 0 1 2 0 -2 -1 0 1 1 0 -1 -2 0 2 1 0 -1 -1 0 1 2 0 -2 -1 0 1 1 0 -1 -
20210-1-10120-2-10110-1-20210-1;
CONTRAST 'A1B2C1' A*B*C -1 2 -1 0 0 0 1 -2 1 1 -2 1 0 0 0 -1 2 -1 -1 2 -1 0 0 0 1 -2 1 1 -2 1
000-12-1-12-10001-211-21000-12-1;
010-110-1000-101-10100010-1;
CONTRAST 'A2B1C1' A*B*C 2 -4 2 -4 8 -4 2 -4 2 0 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1 0 0 0 0
00000-12-12-42-12-100000000;
CONTRAST 'A2B1C2' A*B*C -2 0 2 4 0 -4 -2 0 2 0 0 0 0 0 0 0 0 0 1 0 -1 -2 0 2 1 0 -1 0 0 0 0 0
000010-1-20210-100000000;
CONTRAST 'A2B2C1' A*B*C -2 4 -2 0 0 0 2 -4 2 0 0 0 0 0 0 0 0 0 1 -2 1 0 0 0 -1 2 -1 0 0 0 0 0
00001 - 21000 - 12 - 10000000000
000 - 10100010 - 100000000;
000-12-12-42-12-100000000;
00010-1-20210-100000000;
0001-21000-12-100000000;
00-10100010-100000000;
```

CONTRAST 'A4B1C1' A\*B\*C 0 0 0 0 0 0 0 0 2 -4 2 -4 8 -4 2 -4 2 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1 0 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1; CONTRAST 'A4B1C2' A\*B\*C 0 0 0 0 0 0 0 0 0 -2 0 2 4 0 -4 -2 0 2 0 0 0 0 0 0 0 0 0 1 0 -1 -2 0 2 10-10000000010-1-20210-1; CONTRAST 'A4B2C1' A\*B\*C 0 0 0 0 0 0 0 0 -2 4 -2 0 0 0 2 -4 2 0 0 0 0 0 0 0 0 1 -2 1 0 0 0 -12 - 10000000001 - 21000 - 12 - 1;10-100000000-10100010-1; 1 -2 1 0 0 0 0 0 0 0 0 -1 2 -1 2 -4 2 -1 2 -1; 10100000000010-1-20210-1;-21000000001-21000-12-1; 010000000-10100010-1;

RUN;

Some results obtained with the three statistical packages.

Source of	Degrees of	Sum of	Squares	E 1	1
variation	freedom	squares	means	F value	<i>p</i> value
Model	159	4 571.71	28.75	6.58	0.0001
Experiments (Exp)	1	37.13	37.13	3.7	0.0686
Rep (Exp)	4	1 650.68	412.67	41.16	0.0001
Fertilization (Fer)	5	185.09	37.02	3.69	0.0157
Exp x Fer	5	150.73	30.15	3.01	0.0349
Error a	20	200.5	10.02	2.29	
Density (Den)	2	1 333.63	666.63	61.46	0.0001
Fer x Den	10	49.90	4.99	0.46	0.9073
Exp x Den	2	67.69	33.84	3.12	0.0532
Exp x Fer x Den	10	106.91	10.69	0.99	0.4684
Error b	48	520.61	10.85	2.48	
Cultivars (Cul)	2	33.85	16.92	3.87	0.0228
Cul x Fer	10	50.63	5.06	1.16	0.3228
Cul x Den	4	7.92	1.98	0.45	0.7703
Cul x Fer x Den	20	59.49	2.97	0.68	0.8416
Cul x Exp	2	56.25	28.13	6.43	0.002
Cul x Exp x Fer	10	53.98	5.40	1.23	0.2725
Cul x Exp x Den	4	7.08	1.77	0.41	0.8048
Error c	164	716.96	4.37		
Total	323	5 288.67			

Fer (A)	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Large plot error
A1	1	86.01	8.58	0.0083	Exp>Rep>Fer
A2	1	64.11	6.39	0.02	Exp>Rep>Fer
A3	1	25.64	2.56	0.1255	Exp>Rep>Fer
A4	1	8.56	0.85	0.3665	Exp>Rep>Fer
A5	1	0.79	0.08	0.7813	Exp>Rep>Fer
Total	5	185.1	3.69	0.0157	Exp>Rep>Fer

Mutually orthogonal contrasts for fertilization (large plot).

Mutually orthogonal contrasts for population density (medium plot).

Den (B)	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Medium plot error
B1	1	2.25	0.21	0.65	Exp>Fer>Rep*Den
B2	1	1 331.02	122.72	0.001	Exp>Fer>Rep*Den
Total	2	1 333.27	61.46	0.0001	Exp>Fer>Rep*Den

Mutually orthogonal contrasts for fava bean cultivars (small plot).

Cul (C)	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Small plot error
C1	1	11.54	2.64	0.1061	Model Residual
C2	1	22.3	5.0	0.0252	Model Residual
Total	2	33.84	3.87	0.0228	Model Residual

Mutually orthogonal contrasts for the fertilization x density interaction (AxB).

DenxFer or AxB	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Interaction error
C1	1	0.003	0.0003	0.98	Exp>Fer>Rep*Den
C2	1	2.96	0.27	0.6	Exp>Fer>Rep*Den
C3	1	8.18	0.75	0.38	Exp>Fer>Rep*Den
C4	1	12.94	1.19	0.28	Exp>Fer>Rep*Den
C5	1	0.3	0.03	0.86	Exp>Fer>Rep*Den
C6	1	0.7	0.6	0.8	Exp>Fer>Rep*Den
C7	1	14.32	1.32	0.25	Exp>Fer>Rep*Den
C8	1	0.06	0.01	0.94	Exp>Fer>Rep*Den
C9	1	2.99	0.28	0.6	Exp>Fer>Rep*Den
C10	1	7.45	0.69	0.41	Exp>Fer>Rep*Den
Total	10	49.9	0.9	0.90	Exp>Fer>Rep*Den

CulxFer or AxC	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Interaction Error
C1	1	8.31	1.9	0.16	Model Residual
C2	1	0.05	0.01	0.91	Model Residual
C3	1	0.14	0.03	0.85	Model Residual
C4	1	3.08	0.71	0.4	Model Residual
C5	1	0.02	0.004	0.94	Model Residual
C6	1	6.94	1.59	0.2	Model Residual
C7	1	4.51	1.03	0.31	Model Residual
C8	1	4.35	1	0.32	Model Residual
C9	1	15.23	3.48	0.06	Model Residual
C10	1	8.01	1.83	0.17	Model Residual
Total	10	50.64	1.16	0.32	Model Residual

Mutually orthogonal contrasts for the interaction fertilization x cultivars or AxC.

Mutually orthogonal contrasts for the interaction density x cultivars or BxC.

CulxDen or BxC	Degrees of freedom	Sum of squares	F Value	<i>p</i> value	Interaction Error
C1	1	5.54	1.27	0.26	Model Residual
C2	1	0.14	0.03	0.85	Model Residual
C3	1	1.03	0.24	0.62	Model Residual
C4	1	1.21	0.28	0.59	Model Residual
Total	4	7.92	0.45	0.77	Model Residual

Mutually orthogonal contrasts for the interaction fertilization x density x cultivars.

CulxDenxFer or AxBxC	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Interaction Error
C1	1	0.91	0.21	0.64	Model Residual
C2	1	3.4	0.78	0.37	Model Residual
C3	1	14.76	3.38	0.06	Model Residual
C4	1	0.16	0.04	0.84	Model Residual
C5	1	0.12	0.03	0.87	Model Residual
C6	1	0.61	0.14	0.7	Model Residual
C7	1	2.8	0.64	0.42	Model Residual
C8	1	2.17	0.5	0.48	Model Residual
C9	1	8.06	1.84	0.17	Model Residual
C10	1	7.65	1.75	0.18	Model Residual
C11	1	2.15	0.49	0.48	Model Residual
C12	1	0.33	0.08	0.78	Model Residual

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CulxDenxFer or AxBxC	Degrees of freedom	Sum of squares	F value	<i>p</i> value	Interaction Error
C13	1	0.71	0.16	0.68	Model Residual
C14	1	1.19	0.27	0.6	Model Residual
C15	1	0.57	0.13	0.71	Model Residual
C16	1	1.31	0.3	0.58	Model Residual
C17	1	0.001	0	0.98	Model Residual
C18	1	8.12	1.86	0.17	Model Residual
C19	1	3.65	0.84	0.36	Model Residual
C20	1	0.81	0.18	0.66	Model Residual
Total	20	59.48	0.68	0.84	Model Residual

# Conclusions

The three statistical packages generate similar information for the series of experiments in randomized complete blocks in arrangement of subdivided plots (SE in DBCA in PS) in free or student versions (without cost). The annual license for a PC for SAS is USD \$2 000.00 and for InfoStat or InfoGen it is only USD \$50.00, the last two are friendlier than SAS, requiring less information to obtain statistical analyzes of interest.

The coefficients of mutually orthogonal contrasts (CMO's) must be captured in all three software's, their calculation for interactions is more laborious when the number of levels within each factor increases. The user has the option of constructing only a subset of CMO's or non-orthogonal, that are congruent with the research objectives. The statistical significance of the F values of the CMO's could be considered as a practical guide to design other complementary analyzes, such as multiple comparisons of treatment means or the application of multivariate techniques.

With the information presented in this essay, it will be easier to extend your analysis of SE in DBCA in PS in the case of orthogonal polynomials (PO), response surfaces, or combinations of PO with CMO's.

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