

SAS code to analyze a complete diallelic and heterosis. An environment

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Abstract

The development of statistical analysis system (SAS) programs and their validation with freely available software is essential when there are no financial resources to acquire the license of an appropriate statistical package. In this study a code for SAS is presented and its validation is performed with the program proposed by Zhang and Kang (1997), modified by Saavedra (2019). The code generates an analysis of variance with partition of the effects of treatments on parents (P), direct crosses (CD), reciprocal crosses (CR), P *vs* crosses, and CD *vs* CR. In addition to generating the comparison of treatment means with the Tukey test, the genetic effects for parents or for their crosses are estimated (G_i , S_{ij} , R_{ij} , M_i); as well as, those of heterosis with the average of both parents or with the best of them. Since both codes only coincide in the calculation of the previously indicated genetic effects, their simultaneous application is suggested to carry out a complete analysis of Griffing's method 1 (1956a, b). The code that has been proposed will be especially useful for plant breeders and geneticists and especially for undergraduate and graduate level biological and agricultural science students with little training in the programming language at SAS.

Keywords: Griffing method 1, model 1, randomized complete blocks, Tukey test.

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Introduction

Dialectic crosses were designed before the 1950's, but soon became a powerful tool for plant and animal breeders, who to recognize the merit of various parents evaluated their progenies through the effects and variances of general combinatorial aptitude (ACG) and specific (ACE) (Sprague and Tatum, 1942; Griffing, 1956a, b; González *et al.*, 2007a, b). These define new heterotic patterns or a segregating population from which it is possible to isolate again outstanding plants, predict the response to the selection or the behavior of hybrids or synthetics formed with new lines (Hallauer and Miranda, 1988; Christie and Shattuck, 1992; González *et al.*, 2007a, b).

Analysis of a complete diallel crosses experiment without a personal computer (PC) is laborious and to save time, there are several statistical packages such as SAS (<https://www.sas.com/store/index.ep>), Excel (Microsoft Office), Indostat (<https://www.indostat.org>), AGD-R (<https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10202>), Agrobase II, generation (<http://www.agronomix.com>), PB Tools (<https://pbtools.software.informer.com/2.0/>), TNAUSTAT ([https:// sites. google.com/site/tnaustat](https://sites.google.com/site/tnaustat)) and GSCA (<https://bioseqdata.com/gsca/gsca. htm>), among others; of these, only Agrobase II generation and Indostat must be purchased under license with a cost higher than \$1 000.00 USD, because at least three modules are required to properly operate both softwares. Although SAS is the best statistical package, it is common for breeders and geneticists to use various software to analyze data from experiments designed in the agricultural and biological sciences (Padilla *et al.*, 2019a; Padilla *et al.*, 2019b; Saavedra, 2019).

Also, for many users it is difficult to download free software because there is an incompatibility problem between it and their PCs, there are technical problems during the downloads, the necessary permission is not obtained, the researchers do not respond to the requests or the program does not work in versions old or recent Windows. In this context, it would be desirable to elaborate and validate some codes for SAS, for versions 6.01 or higher (SAS, 1989), that allow complementing the genetic-statistical analysis for experiments of complete dialectic crosses.

Materials and methods

Full dialectic

In methodology 1, described in Saavedra (2019), the analysis of variance (Anova) for a single environment contains repetitions (R), treatments (Trat) and experimental error, its statistical model corresponds to a randomized complete block design. In Anova, the effects of Trat are divided into progenitors (P), direct crosses (CD), reciprocal crosses (CR), P *vs* crosses and CD *vs* CR, as suggested by González *et al.* (2007b), both contrasts estimate average heterosis and maternal and non-maternal effects.

The program calculates the differences between Trat with the Tukey test (SAS, 1989). This code can be easily modified if the user requires other means comparison tests, or various regression and correlation analyzes, these analyzes can be extended to series of experiments in time and space (Saavedra, 2019).

In methodology 2, which corresponds to method 1 of Griffing (1956a, b), the Anova for a single trial has repetitions (R), general combinatorial aptitude (ACG), specific combinatorial aptitude (ACE), maternal effects (EM) and reciprocal effects (ER); in the series of experiments the interactions of these with only two environments could be estimated.

Also, in both cases, the effects of g_i for each parent or of s_{ij} for each cross, the reciprocal and maternal effects would be estimated (Zhang and Kang, 1997). The variance and heritability components, and the prediction of hybrids and synthetics could be estimated with other programs for SAS (Martínez, 1983; González *et al.*, 2007a, b; Montesinos *et al.*, 2007).

Defining variables in code

In the database called “diallel” female, male, YH, YP, YM, X, Y, A, B, C, D and M are defined, in the female and male variables the combinations of each female with each male, YH, YP and YM correspond to the cross, female and male means, respectively. In X, Y the totals for each pair of CD and CR are captured. After the sum over repetitions has been done. In A, B, C, D, each line of the CD or the CR appears twice, as female and as male (Y_i or $Y_{i.}$; $Y_{j.}$ or Y_j). M is the great arithmetic mean, GI, SIJ, RIJ and MI are the same genetic effects that are estimated with the formulas proposed in method 1 of Griffing (1956a, b).

Values used in the code

In this study, 96 data were used, corresponding to four parents, their six direct crosses and their six reciprocal crosses, registered in six repetitions (Saavedra, 2019).

Results and discussion

Since its creation in 1972, SAS programs for the analysis of diallelic cross experiments have been implemented on personal computers PC's by several researchers. The great achievements that have been obtained for PC's are attributed to Schaffer and Usanis (1989); Burow and Coors (1994); Magari and Kang (1994); Zhang and Kang (1997); Martínez (1983, 1991), among others. More recently, Mastache and Martínez (1998a, 1998b, 1999a, 1999b), they refined their algorithms to obtain the best empirical linear and unbiased predictors (MPLI) of the effects of the parents, to help users with little training in programming, when using completely random designs (DCA) and complete random blocks (BCA).

Also, Mastache and Martínez (2003) obtained an integrated algorithm for its simultaneous analysis in balanced experiments for fixed or random effects models. These and other programs could also be used to validate and complement the outputs that were obtained with the code proposed in the present study (Zhang *et al.*, 2005; Montesinos *et al.*, 2007).

Zhang *et al.* (2005) modified the codes of Zhang and Kang (1997); in Diallel-SAS05, they discussed a more efficient program for the genetic-statistical analysis four methods of Griffing's (1956a, b), including those corresponding to designs II and III of Gardner and Eberhart (1966). This program is friendlier and easier to modify than Diallel-SAS, when parents vary from 4 to 12,

when there is no restriction on the number of environments, and when the effects and variances of ACG and ACE for parents and crosses are estimated, as well as their interactions with environments. As with other statistical packages, there are problems in deploying on personal computers with recent versions of Windows (Padilla *et al.*, 2019a, b).

With program 1a the Anova and the comparison of means (Tukey, $p=0.01$) are calculated. Since Trat and its components are considered as fixed effects, the F tests are tested with the mean square of the experimental or residual error of the model. In your code, Data, SET, IF-THEN, Anova, and GLM are used to define subsets of data. The user will be careful to respect the correct order in the database: P, CD and CR, the signs and the coefficients of the contrasts, as for other statistical packages, must be captured within the program. If there is any doubt to design this type of contrasts, it is suggested to consult Padilla *et al.* (2019a).

At the SAS output, if $R=6$ and $\text{Trat}=4$, the Anova corresponds to parents; your hypothesis test is not correct because it was constructed as a subset and its mean square of the residual is a fraction of the 96 data. In this context, a table of F should be consulted at this stage there are no restrictions regarding the number of variables to analyze. The code can be modified to include tests for the least significant difference (DMS or LSD), Dunnett, or mutually orthogonal contrasts, among others. With two or more variables, it is possible to modify the program to perform regression and correlation, estimate simple statistics and apply multivariate methodologies, among others.

The code corresponding to program 1a is presented below:

```
Data corn; Input rep trat PVG;Cards; 1 01 758; 1 02 761; 6 15 768; 6 16 758
DATA PARENTS;SET CORN;IF TRAT>4 THEN DELETE;*parents only;
DATA CD;SET CORN;IF TRAT<5 OR TRAT>10 THEN DELETE;* only direct crosses;
DATA CR; SET CORN; IF TRAT<11 THEN DELETE; *only reciprocal crosses;
PROC ANOVA DATA= CORN; CLASS REP TRAT; MODEL PVG=REP TRAT; MEANS
TRAT/TUKEY LINES ALPHA=0.01;* Analysis with 96 data;
PROC GLM DATA= CORN; CLASS REP TRAT; MODEL PVG=REP TRAT;
CONTRAST "PVSCROSSES"TRAT 12 12 12 12 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4;
CONTRAST "CD VS CR" TRAT 0 0 0 0 1 1 1 1 1 1 -1 -1 -1 -1 -1 -1;
PROC ANOVA DATA= PARENTS; CLASS REP TRAT; MODEL PVG=REP
TRAT;*Analysis of variance for parents;
PROC ANOVA DATA=CD; CLASS REP TRAT; MODEL PVG=REP TRAT;* Analysis of
variance for direct crosses;
PROC ANOVA DATA=CR; CLASS REP TRAT; MODEL PVG=REP TRAT;*Analysis of
variance for reciprocal crosses; RUN;
```

With program 1b, estimates of genetic effects (G_i , S_{ij} , R_{ij} , MI) and heterosis (%) are obtained. The definition of variables before CARDS must be correctly indicated using the data in Tables 1 and 2 (Saavedra, 2019), but the denominator values of the G_i , S_{ij} , R_{ij} and MI formulas should be corrected, if R , P or both of them. At this stage it is essential to resort to the devices that Martínez (1983) used establishing a logical way of relating formulas Griffing (1956a, b) with the programming language in SAS (SAS Institute, 1989).

Table 1. Volumetric weight of the grain (g L⁻¹) of 16 crosses formed with four lines.

Crosses	R1	R2	R3	R4	R5	R6	Total	Mean
1) 1x1	758	734	750	790	758	765	4 555	759.1
2) 2x2	761	762	737	779	763	773	4 575	762.5
3) 3x3	802	812	802	838	793	782	4 829	804.8
4) 4x4	790	768	780	772	783	775	4 668	778
5) 1x2	814	792	770	781	775	755	4 687	781.1
6) 1x3	805	803	806	832	813	824	4 883	813.8
7) 1x4	791	775	777	791	795	780	4 709	784.8
8) 2x3	819	816	793	814	818	786	4 846	807.6
9) 2x4	779	778	758	798	783	755	4 651	775.1
10) 3x4	830	830	850	853	828	806	4 997	832.8
11) 2x1	774	772	786	750	794	769	4 645	774.16
12) 3x1	789	808	816	808	824	806	4 851	808.5
13) 4x1	787	815	815	825	802	796	4 840	806.6
14) 3x2	817	832	808	775	790	797	4 819	803.1
15) 4x2	756	768	756	754	753	768	4 555	759.1
16) 4x3	850	820	840	850	805	758	4 923	820.5
Total	12 722	12 865	12 644	12 810	12 677	12 495	76 033	792.01

Table 2. Values used to estimate genetic effects and heterosis.

Lines	1	2	3	4	Total
1	4 555	4 687	4 883	4 709	18 834
2	4 645	4 575	4 846	4 651	18 717
3	4 851	4 819	4 829	4 997	19 496
4	4 840	4 555	4 923	4 668	18 986
Total	18 891	18 636	19 481	19 025	76 033

Note: added over repetitions and row or column totals are the contribution of each female or male line, respectively.

In some columns, such as for the large arithmetic mean (M), which is a constant for the 12 matings, there are duplicate values, but it is easy to establish which parent or cross they correspond to because the data is shown in descending order (González *et al.*, 2007a, b; Saavedra, 2019).

The code for PROGRAM 1b is presented below:

DATA HETERO; INPUT FEMALE MALE YH YP YM X Y A B C D M;

MP= (YP+YM)/2;*to calculate the mean of the parents (MP);

BP= MAX (YP, YM);*to choose the best parent (BP);

DMP= YH-MP;* to estimate the numerator of the heterosis formula with MP;

HMP= (DMP/MP)*100;*to estimate heterosis with the mean of the parents, in %;

DBP= YH-BP;*calculate the numerator of the heterosis formula with BP;
 HBP= (DBP/BP)*100;*calculates heterosis with the best father, in %;
 GI= (A+B)/48 - M;*estimate the effects of gi;
 SIJ= (X+Y)/12 -(A+B+C+D)/48 + M;*calculate the effects of Sij;
 RIJ= (X-Y)/12;*determines the effects of rij;
 MI= (A-B)/48;* calculate the effects mi;

CARDS;

1 2 781.1 759.1 762.5 4687 4645 18834 18891 18717 18636 792.01
 1 3 813.8 759.1 804.8 4883 4851 18834 18891 19496 19481 792.01
 1 4 784.8 759.1 778.0 4709 4840 18834 18891 18986 19025 792.01
 2 3 807.6 762.5 804.8 4846 4819 18717 18636 19496 19481 792.01
 2 4 775.1 762.5 778.0 4651 4555 18717 18636 18986 19025 792.01
 3 4 832.8 804.8 778.0 4997 4923 19496 19481 18986 19025 792.01
 2 1 774.1 762.5 759.1 4645 4687 18636 18717 18891 18834 792.01
 3 1 808.5 804.8 759.1 4851 4883 19481 19496 18891 18834 792.01
 4 1 806.6 778.0 759.1 4840 4709 19025 18986 18891 18834 792.01
 3 2 803.1 804.8 762.5 4819 4846 19481 19496 18636 18717 792.01
 4 2 759.1 778.0 762.5 4555 4651 19025 18986 18636 18717 792.01
 4 3 820.5 778.0 804.8 4923 4997 19025 18986 19481 19496 792.01

TITLE 'Effects of gi, sij, rij, mi and heterosis for the general dialectic';
 DATA DOS; SET HETERO; PROC PRINT; RUN;

Anova procedure

Dependent variable: PVG
 Sum of square of
 Source DF square mean F-Value Pr > F
 Model 20 51191.16667 2559.55833 9.69 <.0001
 Error 75 19801.82292 264.02431
 Total correct 95 70992.98958
 R-square Coef Var Root MSE PVG Mean
 0.721074 2.051592 16.24882 792.0104
 Square of Source DF Anova SS the mean F-value Pr > F
 rep 5 3365.67708 673.13542 2.55 0.0347
 trat 15 47825.48958 3188.36597 12.08 <.0001

Tukey studentized range test (HSD) for PVG

Note: This test controls the rate of the probability of making an experimentwise Type I error, but usually has a higher Type II error rate than REGWQ.

Alpha 0.01
 Degrees of freedom error 75
 Mean square error 264.0243
 Critical value of the studentized range 5.76634
 Minimal significant difference 38.251
 Means with the same letter are not significantly different.

Tukey Grouping Media N trat

A 832.833 6 10
 B A 820.500 6 16
 B A C 813.833 6 6
 B D A C 808.500 6 12
 B D A C 807.667 6 8
 B D A C 806.667 6 13
 B D A C 804.833 6 3
 B D A C 803.167 6 14
 B D E C 784.833 6 7
 D E C 781.167 6 5
 D E C 778.000 6 4
 D E 775.167 6 9
 D E 774.167 6 11
 E 762.500 6 2
 E 759.167 6 1
 E 759.167 6 15

Contrast Square DF Contrast SS the mean F-Value Pr > F
 P VS CROSSES 1 8075.086806 8075.086806 30.58 <.0001
 CD VS CR 1 272.222222 272.222222 1.03 0.3132

Dependent variable: PVG

Sum of Source Square DF Anova SS the mean F-Value Pr > F
 rep 5 1952.875000 390.575000 2.00 0.1376
 trat 3 7805.458333 2601.819444 13.30 0.0002

Cuadrado de Fuente DF Anova SS la media F-Valor Pr > F
 rep 5 2869.25000 573.85000 3.57 0.0143
 trat 5 15157.25000 3031.45000 18.84 <.0001

Square of Source DF Anova SS the mean F-Value Pr > F
 rep 5 1741.80556 348.36111 0.90 0.4948
 trat 5 16515.47222 3303.09444 8.56 <.000

Effects of GI, SIJ, RIJ, MI and heterosis for methodology 1

Obs FEMALE MALE YH YP YM X Y A B C D M MP
 1 1 2 781.1 759.1 762.5 4687 4645 18834 18891 18717 18636 792.01 760.80
 2 1 3 813.8 759.1 804.8 4883 4851 18834 18891 19496 19481 792.01 781.95
 3 1 4 784.8 759.1 778.0 4709 4840 18834 18891 18986 19025 792.01 768.55
 4 2 3 807.6 762.5 804.8 4846 4819 18717 18636 19496 19481 792.01 783.65
 5 2 4 775.1 762.5 778.0 4651 4555 18717 18636 18986 19025 792.01 770.25
 6 3 4 832.8 804.8 778.0 4997 4923 19496 19481 18986 19025 792.01 791.40
 7 2 1 774.1 762.5 759.1 4645 4687 18636 18717 18891 18834 792.01 760.80
 8 3 1 808.5 804.8 759.1 4851 4883 19481 19496 18891 18834 792.01 781.95
 9 4 1 806.6 778.0 759.1 4840 4709 19025 18986 18891 18834 792.01 768.55
 10 3 2 803.1 804.8 762.5 4819 4846 19481 19496 18636 18717 792.01 783.65
 11 4 2 759.1 778.0 762.5 4555 4651 19025 18986 18636 18717 792.01 770.25
 12 4 3 820.5 778.0 804.8 4923 4997 19025 18986 19481 19496 792.01 791.40

Obs BP DMP HMP DBP HBP GI SIJ RIJ MI

```

1 762.5 20.30 2.66824 18.6 2.43934 -6.0725 5.5517 3.5000 -1.1875
2 804.8 31.85 4.07315 9.0 1.11829 -6.0725 5.2183 2.6667 -1.1875
3 778.0 16.25 2.11437 6.8 0.87404 -6.0725 9.9267 -10.9167 -1.1875
4 804.8 23.95 3.05621 2.8 0.34791 -13.8225 7.2183 2.2500 1.6875
5 778.0 4.85 0.62967 -2.9 -0.37275 -13.8225 -10.9067 8.0000 1.6875
6 804.8 41.40 5.23124 28.0 3.47913 20.0108 14.7600 6.1667 0.3125
7 762.5 13.30 1.74816 11.6 1.52131 -13.8225 5.5517 -3.5000 1.6875
8 804.8 26.55 3.39536 3.7 0.45974 20.0108 5.2183 -2.6667 0.3125
9 778.0 38.05 4.95088 28.6 3.67609 -0.1142 9.9267 10.9167 -0.8125
10 804.8 19.45 2.48198 -1.7 -0.21123 20.0108 7.2183 -2.2500 0.3125
11 778.0 -11.15 -1.44758 -18.9 -2.42931 -0.1142 -10.9067 -8.0000 -0.8125
12 804.8 29.10 3.67703 15.7 1.95080 -0.1142 14.7600 -6.1667 -0.8125

```

The previous results were validated with the program developed by Zhang and Kang (1997). In ANOVA the code allows the partitioning of possible cross effects in ACG, ACE, ER and EM, when SORT, BY, GLM, IF-THEN, DROP, ARRAY, ELSE, GLM, CONTRAST, ESTIMATE and some MACROS were implemented. In Martínez (1991) these and other components are presented to elaborate the reference code.

The program of Zhang and Kang (1997) applies to the four methods of Griffing (1956a, b), for method 1 m variables are analyzed in two environments. In the present study, this was adjusted to a single environment by implementing the restriction I IF ENV > 1 THEN DELETE or IF ENV < 2 THEN DELETE, captured before DROP and after INPUT. Modifying it is more laborious for users with little training in programming and, especially when the analysis extends to series of experiments (Singh, 1973; Mastache and Martínez, 2003; Zhang *et al.*, 2005).

TNAUSTAT Software, in addition to calculating the genetic effects related to parents and their crosses in method 1 of Griffing (1956a, b) also, simultaneously, allows the calculation of hybrid vigor with the mean of both parents, with the best of them and additionally, based on commercial heterosis. This has the additional advantage of estimating the genetic parameters corresponding to the mating design I, proposed by Hayman (1954). However, this software was designed to work properly on a platform with MS Dos, so DOSBox software must be downloaded in advance.

The Zhang and Kang Program (1997), Modified by Saavedra (2019), is presented below:

```

OPTIONS PS=56 LS=78; TITLE 'METHOD 1'; DATA METHOD1;
INPUT I J REP HYBRID YIELD ENV; IF ENV>1 THEN DELETE;DROP N NI NJ P;
P=4;*NUMBER OF PARENTAL LINES? ; ARRAY GCA (N) G1 G2 G3;DO N=1 TO (P-1);
GCA= ((I=N)-(I=P)) + ((J=N)-(J=P)); END;ARRAY SCA(N) S11 S12 S13 S22 S23 S33;
N=0; DO NI=1 TO (P-1); DO NJ=NI TO (P-1); N+1; IF NI=NJ THEN DO;
SCA=(I=NI)*((J=NJ)-(J=P))+I=P*((J=P)-(J=NI));END;ELSE DO;
SCA=(I=NI)*(J=NJ)-(J=P)*((I=NI)+(I=NJ)-(I=P)*2)+(I=NJ)*(J=NI)
-(I=P)*((J=NI)+(J=NJ));END;END;END;
ARRAY REC (N) R12 R13 R14 R23 R24 R34; N=0; DO NI=1 TO (P-1);
DO NJ= (NI+1) TO P; N+1; REC= (I=NI)*(J=NJ)-(j=NI)*(I=NJ); END;END;
ARRAY MAT (N) M1 M2 M3; DO N=1 TO (P-1); MAT= (I=N) + (J=P)-(J=N)-(I=P);

```



```

END;ARRAY NONM (N) N12 N13 N23;N=0;DO NI=1 TO (P-2);DO NJ=(NI+1) TO (P-
1);N+1;NONM=((I=NI)*(J=NJ))-(I=NJ)*(J=NI)-((I=NI)*(J=P))+((I=NJ)*(J=P)
+ ((I=P)*(J=NI)-(J=NJ))); END;END;CARDS;
1 1 1 01 758 1
1 2 1 02 814 1
1 3 1 03 805 1
4 3 6 15 758 1
4 4 6 16 775 1
PROC SORT; BY REP ENV I J; PROC GLM;CLASS REP ENV HYBRID;MODEL
YIELD=ENV REP(ENV) HYBRID HYBRID*ENV;TEST H=HYBRID
E=HYBRID*ENV;LSMEANS HYBRID;
RUN; TITLE 'DIALLEL-SAS 1'; PROC GLM; CLASS REP ENV HYBRID;
MODEL YIELD= ENV REP (ENV) G1 G2 G3 S11 S12 S13 S22 S23 S33 R12 R13 R14 R23
R24 R34 G1*ENV G2*ENV G3*ENV S11*ENV S12*ENV S13*ENV S22*ENV S23*ENV
S33*ENV R12*ENV R13*ENV R14*ENV R23*ENV R24*ENV R34*ENV;
%MACRO GCASCA; CONTRAST 'GCA' G1 1, G2 1, G3 1;
CONTRAST 'SCA' S11 1, S12 1, S13 1, S22 1, S23 1, S33 1;
ESTIMATE 'G1' G1 1; ESTIMATE 'G2' G2 1; ESTIMATE 'G3' G3 1;
Estimate 'G4' G1 -1 G2 -1 G3 -1;
ESTIMATE 'S11' S11 1; ESTIMATE 'S12' S12 1; ESTIMATE 'S13' S13 1;
ESTIMATE 'S22' S22 1; ESTIMATE 'S23' S23 1; ESTIMATE 'S33' S33 1;
Estimate 'S14' S11 -1 S12 -1 S13 -1;
Estimate 'S24' S12 -1 S22 -1 S23 -1;
Estimate 'S34' S13 -1 S23 -1 S33 -1;
Estimate 'S44' S11 1 S12 2 S13 2 S22 1 S23 2 S33 1;
%MEND GCASCA; %GCASCA %MACRO INTERACT;
CONTRAST 'GCA*ENV' G1*ENV 1 -1, G2*ENV 1 -1, G3*ENV 1 -1;
CONTRAST 'SCA*ENV' S11*ENV 1 -1, S12*ENV 1 -1, S13*ENV 1 -1, S22*ENV 1 -1,
S23*ENV 1 -1, S33*ENV 1 -1; %MEND INTERACT; %INTERACT
CONTRAST 'REC' R12 1, R13 1, R14 1, R23 1, R24 1, R34 1;
ESTIMATE 'R12' R12 1; ESTIMATE 'R13' R13 1; ESTIMATE 'R14' R14 1;
ESTIMATE 'R23' R23 1; Estimate 'R24' R24 1; ESTIMATE 'R34' R34 1;
CONTRAST 'REC*ENV' R12*ENV 1 -1,R13*ENV 1 -1,R14*ENV 1 -1,R23*ENV 1 -
1,R24*ENV 1 -1,R34*ENV 1 -1;
CONTRAST 'MAT SS' R12 1 R13 1 R14 1, R12 -1 R23 1 R24 1, R13 -1 R23 -1 R34 1, R14 -1
R24 -1 R34 -1; ESTIMATE 'MAT1' R12 1 R13 1 R14 1/DIVISOR=3;
ESTIMATE 'MAT2' R12 -1 R23 1 R24 1/DIVISOR=3;
ESTIMATE 'MAT3' R13 -1 R23 -1 R34 1/DIVISOR=3;
ESTIMATE 'MAT4' R14 -1 R24 -1 R34 -1/DIVISOR=3; RUN;
TITLE 'DIALLEL-SAS 2'; PROC GLM; CLASS REP ENV HYBRID;
MODEL YIELD= ENV REP (ENV) G1 G2 G3 S11 S12 S13 S22 S23 S33
M1 M2 M3 N12 N13 N23 G1*ENV G2*ENV G3*ENV
S11*ENV S12*ENV S13*ENV S22*ENV S23*ENV S33*ENV
M1*ENV M2*ENV M3*ENV N12*ENV N13*ENV N23*ENV;
%GCASCA %INTERACT
CONTRAST 'MAT SS' M1 1, M2 1, M3 1;
CONTRAST 'NONM SS' N12 1, N13 1, N23 1;

```

```

CONTRAST 'MAT*ENV' M1*ENV 1 -1, M2*ENV 1 -1, M3*ENV 1 -1;
CONTRAST 'NONM*ENV' N12*ENV 1 -1, N13*ENV 1 -1, N23*ENV 1 -1;
ESTIMATE 'M1' M1 1; ESTIMATE 'M2' M2 1; ESTIMATE 'M3' M3 1;
Estimate 'M4' M1 -1 M2 -1 M3 -1;
ESTIMATE 'N12' N12 1; ESTIMATE 'N13' N13 1; ESTIMATE 'N23' N23 1;
Estimate 'N14' N12 -1 N13 -1;
Estimate 'N24' N12 1 N23 -1;
Estimate 'N34' N13 1 N23 1; RUN;

```

To validate the code presented in program 1a, some results are shown that are generated by the program of Zhang and Kang (1997).

GLM procedure

Dependent variable: YIELD

Sum of Square of

Source DF square mean F-Value Pr > F

Model 20 51191.16667 2559.55833 9.69 <.0001

Error 75 19801.82292 264.02431

Total correct 95 70992.98958

R-square Coef Var Root MSE YIELD Mean

0.721074 2.051592 16.24882 792.0104

Source Square DF Type I SS the mean F-Value Pr > F

ENV 0 0.00000

REP (ENV) 5 3365.67708 673.13542 2.55 0.0347

HYBRID 15 47825.48958 3188.36597 12.08 <.0001

ENV*HYBRID 0 0.00000.

Contrast Square DF Contrast SS the mean F-Value Pr > F

GCA 3 30162.39583 10054.13194 38.08 <.0001

SCA 6 14715.59375 2452.59896 9.29 <.0001

REC 6 2947.50000 491.25000 1.86 0.0988

(MAT SS) (3) (240.75000) 80.25000 0.30 0.8224

(NONM SS) (3) (2706.75000) 902.25000 3.42 0.0216

Error Parameter Standard estimate Value t Pr > |t|

G1 -6.0729167 2.03110309 -2.99 0.0038

G2 -13.8229167 2.03110309 -6.81 <.0001

G3 20.0104167 2.03110309 9.85 <.0001

G4 -0.1145833 2.03110309 -0.06 0.9552

S12 5.5520833 3.70826994 1.50 0.1385

S13 5.2187500 3.70826994 1.41 0.1635

S23 7.2187500 3.70826994 1.95 0.0553

S14 9.9270833 3.70826994 2.68 0.0091

S24 -10.9062500 3.70826994 -2.94 0.0043

S34 14.7604167 3.70826994 3.98 0.0002

M1 -1.1875000 2.03110309 -0.58 0.5605

M2 1.6875000 2.03110309 0.83 0.4087

M3 0.3125000 2.03110309 0.15 0.8781

M4 -0.8125000 2.03110309 -0.40 0.6903

R12 3.5000000 4.69063167 0.75 0.4579

R13 2.6666667 4.69063167 0.57 0.5714
 R14 -10.9166667 4.69063167 -2.33 0.0226
 R23 2.2500000 4.69063167 0.48 0.6329
 R24 8.0000000 4.69063167 1.71 0.0922
 R34 6.1666667 4.69063167 1.31 0.1926

Conclusions

The programs '1a' and '1b' are easy to use and modify to carry out an analysis of variance in a single environment, with the subdivision of the effects of the treatments in parents (P), direct crosses (CD), reciprocal crosses (CR), P versus crosses and CD versus CR. It is also useful for comparing treatment means (Tukey, $p=0.01$) and for estimating heterosis with the parents' mean and with the best of these when analyzing a variable.

Zhang and Kang's (1997) program was designed to analyze 'm' variables, but it is more difficult to manipulate when parents and environments are different from 5 and 2, respectively. Due to this restriction, it was necessary to modify the code with $P=4$. The code proposed by Zhang and Kang estimates general and specific combinatorial aptitude, reciprocal and maternal effects, but does not include the Tukey test or the estimation of heterosis.

All three codes for SAS run on versions over 10 years from their commercial release and on the most recent academic proofs. The program of Zhang and Kang (1997) allowed the reliable validation of the code proposed in the present study when the genetic effects were estimated, but the three codes must be used to carry out a more complete diallelic analysis.

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