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# USING THE GRIFFING'S EXPERIMENTAL DESIGN METHOD I, MODEL II. APPLE BREEDING – A CASE STUDY AS A PROPOSED METHODOLOGY OF THE STATISTICAL AND GENETIC ANALYSIS

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The diallel mating design is widely used in plant breeding to estimate combining ability of genitors and useful genetic parameters. In this work, a complete diallel mating design with four apple cultivars used as genitors was set, in order to elaborate a pattern for a quantitative trait, respectively the height of apple hybrids, measured at three months after emergence. Griffing's method I, model II, with random effects, also known as complete diallel mating design, was included in a broader context of a methodology for both statistical and genetic analysis of the experimental data. An algorithm was developed based on the proposed methodology and tested on the experimental data as well as on other two simulated scenarios of complete diallel mating design with respectively three and seven parents fed with random values. The results have illustrated the pattern suitability for the quantitative inherited trait as was the height of the apple seedlings, respectively the speed of growth of the very young plants. In addition, the proposed algorithm can help young researchers to understand and use adequately for different similar traits the complexity of statistical and genetic analysis of the full diallel mating design. The information obtaining using diallel crosses offers the breeders the

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possibility to choose the appropriate breeding and selection strategies for quantitative traits.

*Key words*: algorithm, apple, genetic analysis, phenotypic characteristics, statistical analysis

## INTRODUCTION

In scientific research, the design of experiments is extremely important since any flaws in this process are directly reflected in the collected data and thus incorrect and/or inaccurate and/or misleading conclusion(s). The main aim of the experimental design is to ensure that the experiment will provide valid and reliable experimental data (FISHER, 1947).

Diallel mating designs are commonly used and allow to compute both the general (GCA) and specific (SCA) combining abilities as well as to calculate genetic variance and heritability (SPRAGUE and TATUM, 1942). A series of mathematical models were developed and are applied in the analyses of experimental data resulted from diallel mating designs, each model having its applicability (JINKS and HAYMAN, 1953; HAYMAN, 1954; GRIFFING, 1956 a, b; GARDNER and EBERHART, 1966; SINGH and HINKELMANN, 1995; XIANG and LI, 2001; WU and MATHESON, 2001; MURRAY *et al*., 2003). An essential step in the analysis of experimental data was made in 1956 when Griffing introduced four different methods for data analysis according to upon whether parents, self-pollination and reciprocals are retained or excluded from a particular experimental design (GRIFFING, 1956 a,b). Two distinct models are applied for each method: model I - model with fixed effects (the experimental material is the population on which the inferences are to be drawn) and model II - model with random effects (the parents are a sample of the reference population) (GRIFFING, 1956 a,b). Griffing's experimental method I is applied in both self-pollinations and cross-pollinations no matter if the genetic structure of parents is homozygote or heterozygote (JINKS and HAYMAN, 1953; GARDNER and EBERHART, 1966). This experimental method allows testing and analyzing maternal and paternal effects on the phenotype of a quantitative trait (CRUSIO, 1987). In this design of the experiment, the parents are crossed in all possible combinations resulting in expensive experiments (CRUSIO, 1987; HALLAUER and FILHO, 1988).

The use of a diallel mating design in plant breeding is directly related both to the available resources and to the researcher's ability in the field design, as well as to researcher's knowledge of statistical and genetic analysis (SHARMA, 2006; NDUWUMUREMYI *et al*., 2013). The main issue related to the statistical and genetic analysis of experimental data resulting from applying a diallel design is interconnected with the understanding of the algorithm, its implementation, and interpretation. Efficient implementation of known diallel models could be found in several statistical packages such as DIALL (SCHAFFER and USANIS, 1969), GAREML (HUBER *et al*., 1992), DIOGENE (BARADAT and LABBÉ, 1995), CBE (WOLF, 1997), SAS (ZHANG and KANG, 1997; WU and MATHESON, 2001; ZHANG *et al*., 2005), AGR 21 (AGROBASE, 2001), ASReml (DECHOW *et al*., 2008; MÖHRING *et al*., 2011), SAS PROC MIXED (DE ASSIS *et al*., 2010), GriffingMeth2 (BOLBOACĂ *et al*., 2011), GriffingMeth4 (BOLBOACĂ *et al*., 2010), North Carolina Designs (RODRÍGUEZ *et al*., 2015), and others. However, besides many advantages, these implementations will guide the researcher neither in the use of the algorithm nor its proper interpretation.

Typically, a scientific interpretation of statistical and genetic analysis in diallel designs requires substantial knowledge and expertise on field experimentation as well as advanced

knowledge of applied mathematical models. Starting from the necessity of understanding analytical methods for analysis of experimental data resulting from complete diallel mating designs, our research aimed to develop a methodology of the statistical and genetic analysis based on Griffing's experimental method I, model II (random effects).

*Rationale of the Method*

The requirements for an analysis based on diallel mating include both statistical and genetic analyses. Here is proposed a step-by-step algorithmic. As the convenience, all tests included in the proposed algorithm were carried out at a significance level of 5%, but can be changed to any required level of significance.

Statistical analysis must be applied before the genetic analysis. It is a simple saying if the data are inadequate, if does not meet the requirements (or assumptions) for the analysis then the results are wrong too. This is one of the sensitive steps in the analysis which is the most likely to be omitted by an inexperienced analyst.

The first step of the statistical analysis is the analysis of the distribution of the experimental data. This step is particularly crucial because normality is required for appropriate inferential analysis. Several point estimators could bring information regarding the distribution of experimental data: mean, median, mode, skewness, and kurtosis. A normal distribution is unimodal, has skewness between -0.5 and +0.5 (BULMER, 2012) (-0.2 and +0.2 according to HILDEBRAND (1986)), and kurtosis of 3 (excess kurtosis – Kurt predefined function in Excel equal with zero (CHISSOM, 1970; DECARLO, 1998)). The normality could also be assessed by applying tests as the Z test for skewness and kurtosis (BOLBOACĂ and JÄNTSCHI, 2009), Anderson-Darling (ANDERSON and DARLING, 1952), Kolmogorov-Smirnov (KOLMOGOROV, 1941; SMIRNOV, 1948), Chi-square (PEARSON, 1900), and/or Jarque-Bera (JARQUE and BERA, 1980) test. The agreement between different normality tests is essential (and it may be tricky for inexperienced analysts) and may be tested using the approached provided by Fisher's Chi-square (FISHER, 1948).

The second step of the statistical analysis is inferential statistics. Two tests are to be applied in this step: ANOVA test ($H_0$ (null hypothesis): here 'The offsprings do not differ significantly among themselves' and WELCH (1947) test applied if H0 ANOVA is rejected ($H_0$: The progenies in the sample do not differ significantly among the progenies of all crosses). Both tests are correctly applied if data are normally distributed, and samples are independent.

Depending on the results obtained from inferential statistics, the analysis could be stopped (if the offspring proved no significantly different among themselves) or goes forward to the genetic analysis.

The genetic analysis involves ANOVA test for combining ability and ends with point estimates. On the experimental design considered here, the ANOVA test for combining ability, a test able to partition the variances as main effect (general combining ability, abbreviated as GCA) and interactions effect (specific combining ability, abbreviated as SCA), implemented in our algorithm under genetic analysis is strictly applied to Griffing's experimental design - Method I, Model II (GRIFFING, 1956a).

## MATERIALS AND METHODS

***Method Implementation***

In the full diallel breeding, the parents ($A_i$, $A_j$) make direct ($A_i \times A_j$) and reciprocal

($A_j \times A_i$) crosses as well as self-pollination ($A_i \times A_i$), such that a total of $p^2$ descendants are obtained from $p$ parents. The experiment can be conducted with $n$ repetitions, when the experimental observations (O) may be arranged as presented in Table 1.

*Table 1. The arrangement of experimental observations from Griffing's experimental method I, model II*

| Observations | 1×1 | … | 1×j | … | 1×p | 2×1 | … | 2×j | … | 2×p | i×1 | … | i×j | … | i×p | p×1 | … | p×j | … | p×p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $O_{1,1,1}$ | … | $O_{1,1,j}$ | … | $O_{1,1,p}$ | $O_{1,2,1}$ | … | $O_{1,2,j}$ | … | $O_{1,2,p}$ | $O_{1,i,1}$ | … | $O_{1,i,j}$ | … | $O_{1,i,p}$ | $O_{1,p,1}$ | … | $O_{1,p,j}$ | … | $O_{1,p,p}$ |
| … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| k | $O_{k,1,1}$ | … | $O_{k,1,j}$ | … | $O_{k,1,p}$ | $O_{k,2,1}$ | … | $O_{k,2,j}$ | … | $O_{k,2,p}$ | $O_{k,i,1}$ | … | $O_{k,i,j}$ | … | $O_{k,i,p}$ | $O_{k,p,1}$ | … | $O_{k,p,j}$ | … | $O_{k,p,p}$ |
| … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| n | $O_{n,1,1}$ | … | $O_{n,1,j}$ | … | $O_{n,1,p}$ | $O_{n,2,1}$ | … | $O_{n,2,j}$ | … | $O_{n,2,p}$ | $O_{n,i,1}$ | … | $O_{n,i,j}$ | … | $O_{n,i,p}$ | $O_{n,p,1}$ | … | $O_{n,p,j}$ | … | $O_{n,p,p}$ |
| | | | | | | | | | | | | | | | | | | | | |
| Statistics | Notations: $y_{\cdot,j} = \Sigma_{1\le i\le p} y_{i,j}$, $y_{i,\cdot} = \Sigma_{1\le j\le p} y_{i,j}$, $y_{\cdot,\cdot} = \Sigma_{1\le i\le p}\Sigma_{1\le j\le p} y_{i,j}$; $x_{\cdot,j} = \Sigma_{1\le i\le p} x_{i,j}$, $x_{i,\cdot} = \Sigma_{1\le j\le p} x_{i,j}$, $x_{\cdot,\cdot} = \Sigma_{1\le i\le p}\Sigma_{1\le j\le p} x_{i,j}$ | | | | | | | | | | | | | | | | | | | |
| $y_{i,j} = \Sigma_{1\le k\le n} O_{k,i,j}$ | $y_{1,1}$ | … | $y_{1,j}$ | … | $y_{1,p}$ | $y_{2,1}$ | … | $y_{2,j}$ | … | $y_{2,p}$ | $y_{i,1}$ | … | $y_{i,2}$ | … | $y_{i,p}$ | $y_{p,1}$ | … | $y_{p,j}$ | … | $y_{p,p}$ |
| $n_{i,j} = \Sigma_{1\le k\le n} 1$ | $n_{1,1}$ | … | $n_{1,j}$ | … | $n_{1,p}$ | $n_{2,1}$ | … | $n_{2,j}$ | … | $n_{2,p}$ | $n_{i,1}$ | … | $n_{i,2}$ | … | $n_{i,p}$ | $n_{p,1}$ | … | $n_{p,j}$ | … | $n_{p,p}$ |
| $x_{i,j} = y_{i,j}/n_{i,j}$ | $x_{1,1}$ | … | $x_{1,j}$ | … | $x_{1,p}$ | $x_{2,1}$ | … | $x_{2,j}$ | … | $x_{2,p}$ | $x_{i,1}$ | … | $x_{i,2}$ | … | $x_{i,p}$ | $x_{p,1}$ | … | $x_{p,j}$ | … | $x_{p,p}$ |

Based on the notations from Table 1, in Table 2 the source of variation is decomposed into GCA (general combining ability), SCA (specific combining ability), reciprocal effects (due to the genetic effects of the parents) and error (experimental error).

*Table 2. Genetic analysis of variance for Griffing's experimental method I, model II (random effects)*

| Source of variation | df | SS | MS | Expected mean | F-value | $p_F$ | $H_0$ |
|---|---|---|---|---|---|---|---|
| GCA (effects of general combining ability) | p-1 | $SS_g$ | $MS_g=SS_g/df_g$ | $\hat{\sigma}_e^2 + 2(p-1)\hat{\sigma}_s^2 / p + 2p\hat{\sigma}_g^2$ | $MS_g/M$ | $1\text{-}CDF_F(MS_g/M, df_{GCA}, f)$ | $\hat{\sigma}_g^2 = 0$ |
| SCA (effects of specific combining ability) | p(p-1)/2 | $SS_s$ | $MS_s=SS_s/df_s$ | $\hat{\sigma}_e^2 + 2(p^2-p+1)\hat{\sigma}_s^2 / p^2$ | $MS_s/MS_e$ | $1\text{-}CDF_F(MS_s/MS_e, df_{SCA}, m)$ | $\hat{\sigma}_s^2 = 0$ |
| Reciprocal effects | p(p-1)/2 | $SS_r$ | $MS_r=SS_r/df_r$ | $\hat{\sigma}_e^2 + 2\hat{\sigma}_r^2$ | $MS_r/MS_e$ | $1\text{-}CDF_F(MS_r/MS_e, df_{ReEf}, m)$ | $\hat{\sigma}_r^2 = 0$ |
| Error | $m = \Sigma_i\Sigma_j n_{i,j} - p^2 + 1$ | $SS_e$ | $MS_e=SS_e/df$ | $\hat{\sigma}_e^2$ | | | |

Notations:
df = degrees of freedom; SS & MS = sum & mean of squares; p = number of parents;
p = significance of F-value (FDIST(F,$df_g$/$df_s$,$df_s$/$df_e$) in Excel); $df_t$ = degrees of freedom for totals;
$SS_g = \Sigma(x_{i,\cdot} + x_{\cdot,i})^2/(2p) - 2x_{\cdot,\cdot}^2/p^2$, $SS_s = \Sigma_i\Sigma_j x_{i,j}(x_{i,j} + x_{j,i})/2 - \Sigma(x_{\cdot,i} + x_{i,\cdot})^2/(2p) + x_{\cdot,\cdot}^2/p^2$
$SS_r = \Sigma_i\Sigma_{j>i}(x_{i,j} - x_{j,i})^2/2$, $SS_e = (\Sigma_i\Sigma_j y_{i,j}^2/n_{i,j} - \Sigma_i\Sigma_j y_{i,j}/\Sigma_i\Sigma_j n_{i,j})/[(\Sigma_i\Sigma_j n_{i,j} - \Sigma_i\Sigma_j n_{i,j}^2/\Sigma_i\Sigma_j n_{i,j})\cdot(p^2-1)]$
$u = p^2 - p + 1$, $v = (p^2/u)(MS_s-MS_e)/MS_e/2$, $f = m(p-1)p^3[p+2(p-1)v]^2u^2/[p^3(p-1)+2m(u-1)^2\cdot(p^2+2uv)^2]$
f = degrees of freedom associated with M, $M = MS_e/u + MS_s\cdot(u-1)/u$ and finally $MS_g/M$ is $F_{\{(p-1),f\}}$
expected mean: $\hat{\mu} = X_{\cdot,\cdot}/p^2$ & observed variance $\hat{\sigma}_e^2 = MS_e$

The detailed by components of the approach for genetic analysis (on Griffing's method I, model II, with random effects) is given in Table 3.

*Table 3. Step-by-step genetic analysis for diallel mating design using Griffing's method I, model II (with random effects)*

| | |
|---|---|
| ▪ **ANOVA for combining ability.** *Allow partition of variances as the main effect (general combining ability) and interactions effect (specific combining ability)* | |
| → **IF** statistically significant general effects: calculation of estimated | |
| ▫ effects: | $\hat{g} = (x_{i,\cdot} + x_{\cdot,i} - x_{\cdot,\cdot}/p^2)/(2p)$ |
| ▫ variance: | $\hat{\sigma}^2_g = [MS_g - (MS_e + p(p-1)MS_s)/(p^2 - p - 1)]/(2p)$ |
| ▫ variance for each parent: | $\hat{\sigma}^2_{g_i} = g_i^2 - (p-1)MS_e/(p(p-2))$ |
| ▫ standard error of each effect: | $SE(g_i) = (p-1)MS_e/(2p^2)$; $t(g_i) = g_i/SE(g_i)$; $df = p-1$ |
| → **IF** statistically significant specific effects | |
| ▫ effects: | $\hat{s}_{i,j} = (x_{i,j} + x_{j,i})/2 - (x_{i,\cdot} + x_{\cdot,i} + x_{j,\cdot} + x_{\cdot,j})/(2p) + x_{\cdot,\cdot}/p^2$ |
| ▫ variance: | $\hat{\sigma}^2_s = p^2(MS_s - MS_e)/(2p^2 - 2p + 2)$ |
| ▫ variance for each parent ($i \neq j$): | $\hat{\sigma}^2_{s_i} = [\Sigma_{1\leq j\leq p} s_{i,j}^2 - (p-3)\cdot MS_e]/(p-2)$ |
| ▫ standard error of each effect: | $SE(s_{i,j}) = \sqrt{[(p^2-2p+2)MS_e/(2p^2)]}$; $t(s_{i,j}) = s_{i,j}/SE(s_{i,j})$; $df = p(p-1)/2$ |
| → **IF** statistically significant reciprocal differences | |
| ▫ effects ($i < j$): | $\hat{r}_{ij} = (x_{i,j} - x_{j,i})/2$ |
| ▫ variance: | $\hat{\sigma}^2_r = (MS_r - MS_e)/2$ |
| ▫ standard error of each effect: | $SE(r_{i,j}) = \sqrt{(MS_e/2)}$; $t(r_{i,j}) = r_{i,j}/SE(r_{i,j})$; $df = p(p-1)/2$ |
| → Standard error of difference between two parents & critical differences | |
| ▫ Standard error: | ◦ $SEd(g_i\text{-}g_j) = \sqrt{(MS_e/p)}$ – between two $g_i$'s<br>◦ $SEd(s_{i,j}\text{-}s_{i,k}) = \sqrt{[(p-1)MS_e/p]}$ – crosses with a common parent<br>◦ $SEd(r_{i,j}\text{-}r_{k,l}) = \sqrt{MS_e}$ – reciprocal with different parents |
| ▫ Critical differences: | ◦ $CD = SEd(g_i\text{-}g_j)\cdot t_{5\%}$ - $df = p-1$<br>◦ $CD = SEd(s_{i,j}\text{-}s_{i,k})\cdot t_{5\%}$ - $df = p(p-1)/2$ – between crosses with a common parent<br>◦ $CD = SEd(r_{i,j}\text{-}r_{k,l})\cdot t_{5\%}$ - $df = p(p-1)/2$ – between reciprocal with different parent |
| ▪ **Genetic coefficients of variation.** *A-dimensional measure to compare genetic variability, see (Houle, 1992)* | |
| ▫ Phenotypic ($CV_P$): | $100\sqrt{V_P}/m$, where $V_P$ = phenotypic variance, m = arithmetic mean |
| ▫ Additive ($CV_A$): | $V_A$ (additive variance) $= 2\cdot\sigma_g^2$; $V_D$ (dominance variance) $= \sigma_s^2$<br>$CV_A = 100\sqrt{V_A}/m$<br>$I_A = V_A/m^2 = (CV_A/100)^2$ (survival of the best) |
| ▫ Residual ($CV_R$): | $CV_R = 100\sqrt{V_D}/m$ |
| ▪ **Heritability** *see (Falconer and Mackay, 1996; Holland et al., 2003)* | |
| ▫ Broad-sense heritability ($H^2$): | $H^2 = V_G/V_P$ |
| ▫ Narrow sense heritability ($h^2$): | $h^2 = V_A/(V_A+V_D+V_E)$, where $V_E = MS_e$ |

### *Algorithm Implementation and Evaluation*

Implementation of the algorithm was made with Microsoft Excel due to its availability and flexibility (KAPARTHI and POWER, 2003), a program that is used both for data collection (JULURU and ENG, 2015; STAZIAKI *et al*., 2016) and analysis (FERRAGE, 2016; XIE *et al*., 2016; FÜRTAUER *et al*., 2016). The spreadsheet was created in Microsoft Excel XP for Windows. The testing was conducted under Windows XP on an Intel Pentium Dual CPU @2.20GHz and 2 GB of RAM computer. The created spreadsheet was tested, and it works correctly also on Microsoft Excel 2003, 2007, 2010, 2013, and 2016.

The accuracy and the error analysis of the implemented algorithm were analyzed using

three data sets that resulted from complete diallel mating design (Griffing's experimental method I, models with random effects) with a different number of parents. The first set comprised experimental data related to height (cm) of ten apple hybrids measured at three months after emergence (complete diallel mating design with four parents: Prima (DAYTON *et al*., 1970), Priscilla (WILLIAMS *et al*., 1972), Macfree (ALDWINCKLE, 1974), and Sauron (SESTRAȘ *et al*., 2010). The accuracy of the implemented algorithm was conducted on this set since data were previously analysed at the Fruit Research Station Cluj, Romania (SESTRAȘ, 2010). The second and third data sets were obtained randomly respecting the complete diallel mating design with 3 and 7 parents and were used to identify if the implemented algorithm can display the outputs adequately according to the input data. The accuracy (defined as the "true"/"correct" outputs) of the results of the tests were compared to equivalent tests in Microsoft Excel (such as ANOVA: Single Factor available in [Tool – Data Analysis]) and Statistica (v.8.; Stat. Soft. Inc. USA).

The effectiveness of the implemented algorithm was also tested by asking four researchers to use the spreadsheet, two senior plant researchers who previously work on Griffing's experimental method I, model II and two young plant researchers. All participants received the spreadsheet and were asked to evaluate the program inclusive from the perspective of understanding the concepts of statistical and genetic analysis.

## RESULTS

The flowchart of the implemented algorithm on statistical and genetic analysis for complete diallel mating design is presented in Figure 1.

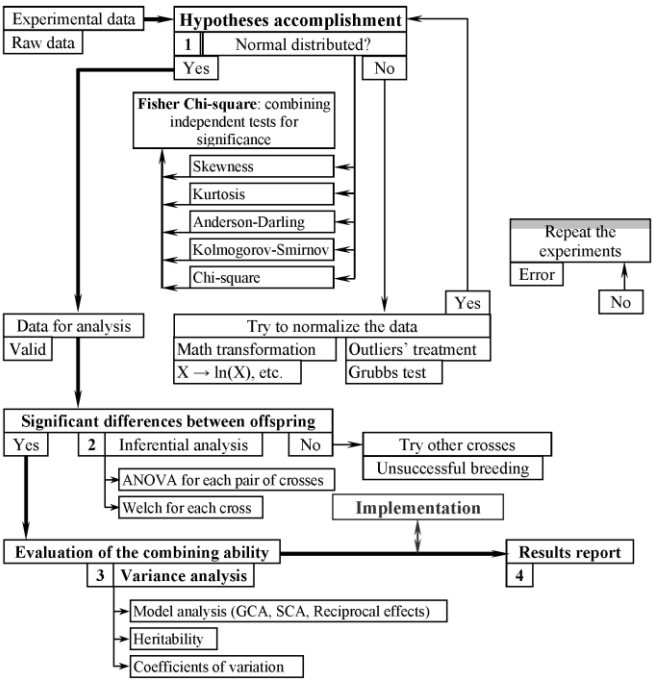


Figure 1. Flowchart of data analysis with Griffing's experimental method I, model II (with random effects)

The algorithm was successfully implemented and the file is available upon request. In our implementation, on complete diallel mating design with a maximum of 7 parents and 80 measurements for each cross is possible. The illustration of the program use is based on the first set used in assessment (complete diallel mating design with four parents, each cross containing 10 measurements). The implemented spreadsheet includes formulae embedded in cells that automatically return the results of both statistical and genetic calculations.

*Results of Statistical Analysis*

The results related to descriptive and inferential statistics are displayed according to the implemented. Sixteen combinations were included in the validation of the implementation with an overall mean of 6.75±1.90 (95%CI [6.45-7.04]) and an overall coefficient of variation of 28% (95%CI [16%-40%]). The normal distribution of experimental data is tested with Jarque-Bera test, and the implementation display as 'yes'/'no' the decision regarding the null hypothesis for this test. Jarque-Bera test is used to check if skewness and kurtosis of experimental data match a normal distribution ($H_0$: Both the skewness and excess kurtosis of experimental data are not significantly different by 0). Despite the result of normality test, the analysis is further performed even if the data is or not normally distributed. The Welch test is also implemented to test the differences between the mean of each cross compared with the overall mean, and if the data proved not to follow the normal distribution, the implemented algorithm would display 'not proper', indicating that the Welch test is not proper to be applied. All results displayed at this stage are summarized in Table 4.

*Table 4. Descriptive parameters: height of hybrids (cm)*

| ♀ × ♂ (n = 10) | cm [95%CI] | Mode | StDev | Min | Max | CV%[95%CI] | JB-ND? | Welch-SD? |
|---|---|---|---|---|---|---|---|---|
| Prima × Prima | 7.43 [6.51-8.30] | 7 | 1.28 | 5 | 9 | 17 [12-32] | yes | no |
| Prima × Sauron | 4.70 [3.67-5.73] | 3 | 1.44 | 3 | 7 | 31 [21-61] | yes | yes |
| Prima × Priscilla | 4.39 [3.63-5.15] | 4 | 1.06 | 3 | 6 | 24 [16-46] | yes | yes |
| Prima × Macfree | 4.55 [3.62-5.48] | 5 | 1.30 | 2 | 7 | 29 [19-56] | yes | yes |
| Sauron × Prima | 7.20 [6.04-8.36] | 7 | 1.62 | 5 | 10 | 22 [15-43] | yes | no |
| Sauron × Sauron | 7.66 [7.18-8.14] | 7 | 0.67 | 7 | 9 | 9 [6-16] | yes | yes |
| Sauron × Priscilla | 7.00 [6.17-7.83] | 7 | 1.15 | 5 | 9 | 16 [11-31] | yes | no |
| Sauron × Macfree | 7.50 [6.53-8.47] | 8 | 1.35 | 5 | 9 | 18 [12-34] | yes | no |
| Priscilla × Prima | 6.50 [4.99-8.01] | 5 | 2.11 | 5 | 12 | 32 [22-65] | yes | no |
| Priscilla × Sauron | 7.20 [5.27-9.13] | 6 | 2.70 | 3 | 12 | 37 [25-77] | yes | no |
| Priscilla × Priscilla | 7.37 [6.46-8.28] | 8 | 1.28 | 5 | 9 | 17 [12-33] | yes | no |
| Priscilla × Macfree | 7.90 [7.17-8.63] | 9 | 1.02 | 6 | 9 | 13 [9-24] | yes | yes |
| Macfree × Prima | 6.80 [5.74-7.86] | 6 | 1.48 | 5 | 9 | 22 [15-41] | yes | no |
| Macfree × Sauron | 5.20 [4.44-5.96] | 4 | 1.06 | 4 | 7 | 20 [14-39] | yes | no |
| Macfree × Priscilla | 8.85 [7.54-10.16] | 9 | 1.83 | 5 | 11.5 | 21 [14-39] | yes | yes |
| Macfree × Macfree | 7.70 [7.02-8.38] | 8 | 0.95 | 6 | 9 | 12 [8-23] | yes | yes |
| Experience (ensemble) | 6.75±1.90 (95%CI [6.45-0.04]) | | | | | 28% (95%CI [16-40%]) | | |

m = arithmetic mean; 95% CI = 95% confidence interval; StDev = Standard deviation; Min = minimum; Max = maximum;
CV% = coefficient of variation / coefficient of relative variability;
JB-ND = Jarque-Bera - Normal distribution?; Welch-SD? = Welch - Statistically different?

If the normal distribution is rejected in one cross, the implemented algorithm will warn the user regarding the appropriateness of ANOVA analysis (we do not have this situation for this first dataset). Regardless this answer, the implemented algorithm will do the ANOVA analysis and the interpretation as well as suitability of variance decomposition is displayed (see Table 5).

*Table 5. ANOVA analysis: computations and results for the first dataset*

| Source of variance | df | SS | MS | F-value | p-value |
|---|---|---|---|---|---|
| Within Groups | 144 | 350.69 | 2.44 | | |
| Between Groups | 15 | 265.32 | 17.69 | 7.26 | 1.01E-11 |
| Error | 159 | 616.00 | 3.87 | | |

df = degrees of freedom, SS = Sum of Squares, MS = Mean of Squares

| | |
|---|---|
| **Is there any statistical significant differences between variances?** | yes |
| **Is the analysis of variance decomposition proper to be conducted?** | yes |

*Results of Genetic Analysis*

The computations on genetic analysis are completed regardless the results obtained in statistical analysis and consequently, the researcher decides to stop the analysis when the results of statistical analysis do not recommend the genetic analysis. The ANOVA test is applied for partitioning the variances in its components (as general, specific, and reciprocal) and a short interpretation is provided as shown in Table 6.

*Table 6. Results on genetic analysis regarding general and specific combining abilities*

| | df | SS | MS | F-value | p-value | Estimated variance | SE |
|---|---|---|---|---|---|---|---|
| General Combining Ability (GCA) | 3 | 6.70 | 2.23 | 0.2272 | 0.8743 | 0.0000 | 0.0227 |
| Specific Combining Ability (SCA) | 6 | 63.79 | 10.63 | 43.9613 | 2.69E-30 | 6.3940 | 0.0486 |
| Reciprocal effects | 6 | 9.83 | 1.64 | 6.7717 | 2.41E-06 | 0.6980 | 0.3477 |
| Errors | 145 | 35.07 | 0.24 | | | | |

df = degrees of freedom, SS = Sum of Squares, MS = Mean of Square, SE = standard error

| | |
|---|---|
| **Effects of general combining ability are statistically significant?** | **no** |
| **Effects of specific combining ability are statistically significant?** | **yes** |
| **Reciprocal effects are statistically significant?** | **yes** |

When the results of general combining ability are statistically significant, the analysis is further conducted and the effects, variances and their significance are computed; otherwise 'not appropriate' is displayed. In the case of the first dataset, the implemented algorithm calculated and displayed the significance of the specific effect of each combination and respectively the specific variance of each parent (see Table 7).

*Table 7. Specific effects ($s_{ij}$): estimated values*

| | $s_{ij}$ (p-value) |
|---|---|
| $s_{Prima \times Sauron}$ | -0.2994 (4.87E-05) |
| $s_{Prima \times Priscilla}$ | -0.8244 (9.45E-10) |
| $s_{Prima \times Macfree}$ | -0.9094 (3.03E-10) |
| $s_{Sauron \times Priscilla}$ | -0.1719 (4.10E-03) |
| $s_{Sauron \times Macfree}$ | -0.0869 (9.91E-02) |
| $s_{Priscilla \times Macfree}$ | 1.0681 (4.62E-11) |

The analysis of errors in the implemented algorithm was conducted with randomly generated data sets to test the qualitative, logic and omission errors. The summary of errors expressed as the number of occurrences is presented in Table 8.

*Table 8. Absolute frequency distribution of errors with the proposed algorithm*

| Category of error (description) | Complete diallel mating design | | |
|---|---|---|---|
| | 4 parents | 3 parents | 7 parents |
| Qualitative (incorrect results or results in the absence of experimental data) | 0 | 0 | 0 |
| Logic (incorrect cell/function/algorithm is choose to obtain the results) | 1* | 0 | 0 |
| Omission (function or algorithm left out of the spreadsheet model) | 0 | 0 | 0 |

* the algorithm of Jarque-Berra was incorrect; its modification was performed before testing with set number 2 and 3

Four researchers, two who previously analyzed data on Griffing's experimental method 1, model II (with random effects) and two without any previous interaction with this diallel mating experimental design evaluated the effectiveness of the spreadsheet. The researchers who previously used this analysis recognized that they were able to identify answers to some unanswered questions regarding statistical and genetic analysis. The young researchers admitted that besides the complexity of the experimental design, the statistical and genetic analysis is even more complex and it is very hard to follow it by reading the algorithms required to be applied. They said that the implemented spreadsheet gave the possibility to visualize how the analysis is conducted, and thus at the end of the analysis, the algorithms became more understandable.

## DISCUSSION

An algorithm useful in the analysis of experimental data resulted in Griffing's experimental method I, model II (with random effects) was successfully developed and implemented. The Griffing-Meth1-Model2 algorithm contains embedded formulae in the cells that automatically return the results of applied statistical and genetic calculations along with decisions at a significance level of 5%. Changing the experimental values entered in the green table led to modification of the displayed results on both statistical and genetic analysis ensuring that the calculated values reflect the experimental data.

The results of the implemented algorithm matched exactly with the results performed by Excel and Statistica. The analysis of the results revealed that the smallest height was observed for Prima × Priscilla hybrids, while the highest value was identified for Macfree × Priscilla and Priscilla × Macfree hybrids. Prima as maternal position provides genitors with a small height compared to Sauron, Priscilla, and Macfree as paternal genitors. The analysis of the mean of the descendants (Table 4) allowed identification of two groups: first group with small height (Prima × Sauron, Prima × Priscilla, Prima × Macfree, Macfree × Sauron) and the second group with higher height (Prima × Prima, Sauron × Prima, Sauron × Sauron, Sauron × Priscilla, Sauron × Macfree, Priscilla × Prima, Priscilla × Sauron, Priscilla × Priscilla, Priscilla × Macfree, Macfree × Prima, Macfree × Priscilla, Macfree × Macfree). The investigation of 95% confidence intervals highlights the overlap of ranges in each group, showing that the mean is not significantly different among descendants in the same group. The relative variability proved to be similar within offsprings with three exceptions: Sauron × Sauron, Macfree × Macfree , and Priscilla × Macfree. Descending classification of offsprings considering the relative variability coefficient was (top 5): Priscilla × Sauron (37%), Priscilla × Prima (32%), Prima × Sauron (31%), Prima × Macfree (29%), Prima × Priscilla (24%). All experimental data proved to follow the normal distribution so the ANOVA test was applied and its results revealed the presence of statistically

significant differences among height of progenies (see Table 5). The genetic analysis proved that the investigated parents had no significant general abilities (Table 6), but statistically significant specific effects were identified for the following crosses: Prima × Sauron, Prima × Priscilla, Prima × Macfree, Sauron × Priscilla, Priscilla × Macfree (Table 7). Macfree as genitor proved to had significantly statistic specific effect in crosses with Prima and Priscilla (in both combinations used as a father). The results obtained by applying the implemented algorithm matched with previously conducted analysis (SESTRAȘ, 2010).

The algorithm can be used for a large number of individuals in each combination (because here, a small number of $F_1$ hybrids was used, just as example), but also for any other quantitative traits such as trees growth and vigour, fruit production and fruits peculiarities (e.g. fruit size, fruit content in sugar), response to diseases attack (also for polygenic inheritance diseases), such as powdery mildew and apple scab etc. (SESTRAȘ *et al*., 2010, 2011; DAN *et al*., 2015a,b).

Do to costs and rigorous experimental design Griffing's experimental method I, model II is not necessary a method widely used, but occasionally researchers from crop science use it (SÁNCHEZ-HERNÁNDEZ *et al*., 2011; HERNÁNDEZ-RAMOS *et al*., 2015). To this target population, our algorithm is addressed too. The main advantages of the implementation are as follows:

- The calculations are executed automatically and quickly. Moreover, the function/formula/algorithm applied to compute each value is available and could be consulted for a proper understanding of the method / mathematical model.
- The user could see how the results were obtained for better understanding the implemented function/algorithm. The visualization of the applied function/algorithm could help plant researchers to lower the threshold caused by the complexity of statistical and genetic analysis of experimental data associated to the use of Griffing's experimental method I, model II (with random effects).
- The spreadsheet provides decisions regarding of the applied tests and informs the user on statistical and/or genetic analysis suitability. The analysis is conducted regardless if it is or not suitable, the spreadsheet just provides a 'yes'/'no' answer regarding the suitability, and therefore the decision of giving the results belongs to the user. In certain situations, it is inappropriate to apply specific tests. For example, the ANOVA test is not properly applied when experimental data did not follow a normal distribution, while the variance decomposition analysis is useless when variances are significantly different.
- The source code is relatively easy to extend/add/modify. No programming knowledge is needed to extend/add/modify the function/formula/algorithm except good Microsoft Excel skills besides information/knowledge that the user desires to implement.

The main limitation of the implemented algorithm is represented by the easiness of introducing errors, especially by users without experience in working with Microsoft Excel. The main types of errors that could be introduced are mechanical (such as simple slips and move from one function to another cell) or accidental (such as deleting formula/functions/algorithm). The results lose accuracy if a formula/function/algorithm is moved to a new location, while output will be empty if formula(s) is accidentally deleted. Furthermore, errors appear in the outputs if the user did not provide the experimental data in the requested form (for example, copy the block of experimental data in the GriffingMeth1Model2 worksheet without respecting the crosses in the columns).

The implementation of known formulas/equations in a workbook is not a big deal from the methodological point of view. Specialized statistical software for analysis of diallel mating designs already exists, but most of them are expensive and did not give the possibility to see in details how the analysis is conducted. The smallest sample size on effectiveness analysis is a limitation of this study, but this was due to the academic and explanations reasons. In fact, the model could be extended to a large hybrids populations. However, positive feedback, especially from the young researchers, is a good result but the analysis needs to be extended to analyze the real effect of using the spreadsheet on reducing the complexity of statistical and genetic analysis of the full diallel mating design.

## CONCLUSIONS

Conducting statistical and genetic analysis using the implemented algorithm offer a dynamic perspective to understand and explore concepts for a proper analysis of experimental results obtained on Griffing's experimental method I, model II (with random effects). The use of the algorithm by the young researchers affirmatively lower the threshold caused by the complexity of statistical and genetic analysis on experimental results associated to the use of full diallel mating design.

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## PRIMENA GRIFING-OVOG EKSPERIMENTALNOG DIZAJNA METOD I, MODEL II. PRIMER U OPLEMENJIVANJU JABUKE: PREDLOG METODOLOGIJE ZA STATISTIČKU I GENETIČKU ANALIZU

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Izvod

Dizajn dialela se široko koristi u oplemenjivanju biljaka da bi se procenila kombinaciona sposobnost roditelja i korisnih genetičkih parametara. U ovom radu, korišćen je kompletan dizajn dialela kod četiri kultivara jabuke, koji su korišćeni kao roditelji, u cilju određivanja kvantitativnih osobina, kao što su visina hibrida jabuke merena tri meseca po pojavljivanju. Grifing-ov metod I, model II sa slučajnim efektima, poznat kao kompletan dialelni dizajn, upotrebljen je u širem kontekstu za statističku i genetičku analizu eksperimentalnih podataka. Razvijen je algoritam na osnovu predložene metodologije i testiran na eksperimentalnim podacima, kao i na dva primera kompletnog dialela sa tri i sedam roditelja. Rezultati su ilustrovali pogodan način nasleđivanja za kvantitativne osobine, kao što su visina klijanaca jabuke i brzina porasta vrlo mladih biljaka. Informacije dobijene upotrebom dialelnih ukrštanja pružaju mogućnost oplemenjivačima da odaberu odgovarajuće oplemenjivačke i selekcione strategije za kvantitativna svojstva.

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