



GRIFFING'S EXPERIMENTAL METHOD II: STEP-BY-STEP DESCRIPTIVE AND INFERENTIAL ANALYSIS OF VARIANCES

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Abstract

This paper presents a step-by-step approach in statistical and genetic analysis applied in diallel mating design using Griffing's experimental Method II, Model II. A two-step approach is developed and implemented in order to assist the researchers in statistical and genetic analysis of diallel crossing using Griffing's experimental Method II, Model II. The approach is exemplified on the corolla diameter of *Calendula officinalis* L. Descriptive and inferential approaches of statistical analysis on experimental data are applied. Statistical analyses describe experimental

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data and compare means among progenies as well as between the crossover mean and the overall mean. The ANOVA results proved the appropriateness of the ANOVA test for combining ability. Statistically significant differences among progenies were identified. Since the ANOVA statistics identified a statistically significant model, the genetic analysis proved to be appropriate. The genetic analysis was able to identify the appropriate parent(s) needed to obtain plants with larger corolla diameter.

Introduction

The term *diallel cross* was introduced in 1919 by Danish geneticist J. Schmidt (Schmidt [35]). This technique was first applied in plant breeding in the 1950s (Christie and Shattuck [9]). The genetic analysis of a diallel mating design operates under five assumptions:

- parents must be diploid and homozygous;
- reciprocal differences should be absent;
- epistasis and non-allelic interactions should be absent;
- multiple allelism should not occur, and
- the genes are independently distributed between the two parents involved in the cross.

The experimental method of diallel crosses has been broadly used in plant breeding (Jinks and Hayman [26]; and Hayman [21, 22]). The main advantage of the diallel mating designs is their ability to carry out a complex approach in order to test and analyze the progenies and to obtain information that could not be found otherwise (Christie and Shattuck [9]). The results of diallel mating designs could be useful in plant breeding if experimental data are analyzed correctly and the results are interpreted appropriately (Hallauer and Filho [20]). Although the methods were introduced many years ago, they are still in use (Benin et al. [4]; Masny et al. [27]; Glala et al. [17]; de Pádua et al. [12]; and Badu-Apraku et al. [2]).

The theoretical concepts of general and specific combining abilities were introduced by Sprague and Tatum [38]. A series of methods were developed and applied in diallel analyses:

- Gardner and Eberhart [15]: The method is applied on inter-variable hybrids

(heterosis) in open-pollinated plants introducing the statistical genetic models for heterosis.

- Hayman [21]: The method used the parental variance and parent-offspring co-variance to identify the relationship among diallel cross progenies (known as the “graphical approach”). The analysis allows the nature (positive/negative) and magnitude of the degree of dominance to be computed using the value of the intercept. Furthermore, parents with dominant or recessive allele could be identified.
- Jinks and Jones [26]: The method uses generation means to assess different types of interactions (additive \times additive, additive \times dominance, dominance \times dominance). It is able to indicate the presence of non-allelic interactions.
- Griffing [18, 19]: His methods embed the theoretical concepts of general and specific combining abilities in statistical approaches for the four diallel mating designs (p^2 full diallel – Method I, $p(p+1)/2$ half diallel – Method II, $p(p-1)$ – Method III, and $p(p-1)/2$ – Method IV). Two models were described for each mating design: the model with fixed-effects (the experimental material is seen as the population about to be inferred; known as Model I) and the model with random effects (the experiment is conducted on random samples and the inference is applied to the population from which the random sample was extracted; known as Model II).

The present paper aims to present a step-by-step approach in statistical and genetic analysis applied on diallel mating design using Griffing's experimental Method II, Model II.

Material and Method

The approach presented in Figure 1 was applied in statistical and genetic analysis of diallel mating design, the second experimental method described by Griffing. Microsoft Excel was used to implement the approach. The normality of the experimental data was tested using the EasyFit 5.1 software. The Tukey test of additivity was applied at a significance level of 5% using SPSS 16.0.

ANOVA for combining ability was implemented in Microsoft Excel using the mathematical model presented in Table 1.

Table 1. Genetic analysis of variance for Griffing's experimental Method II, Model II

Source	<i>df</i>	<i>SS</i>	<i>MS</i>	Expected means squares
General combining ability (GCA) effects	$p - 1$	SS_g	MS_g	$\sigma_e^2 + \sigma_s^2 + (p + 2)\sigma_g^2$
Specific combining ability (SCA) effects	$p(p - 1)/2$	SS_s	MS_s	$\sigma_e^2 + \sigma_s^2$
Error	$\text{Sum}(n_i) - df_g - df_s$	SS_e	MS_e	σ_e^2

$y = p(p + 1)/2$; y = total number of combinations; p = number of parental lines;

$$SS_g = \left[\sum_i (x_i + x_{ii})^2 - 4x_{..}^2/p \right] / (p + 2);$$

$$SS_s = \sum_{i,i \leq j} \sum_{x_{ij}}^2 - \sum_i (x_i + x_{ii})^2 / (p + 2) + 2x_{..}^2 / [(p + 1)(p + 2)];$$

$$SS_e = SPE/n^*, \text{ where } n^* = \left(\sum n_i - \sum n_i^2 / \sum n_i \right) / (y - 1), \text{ SPE from ANOVA (statistics);}$$

σ = expected mean squares; e = for error; s = for SCA effects; g = for GCA effects;

n_i = total number of experimental data

$$MS = SS/df$$

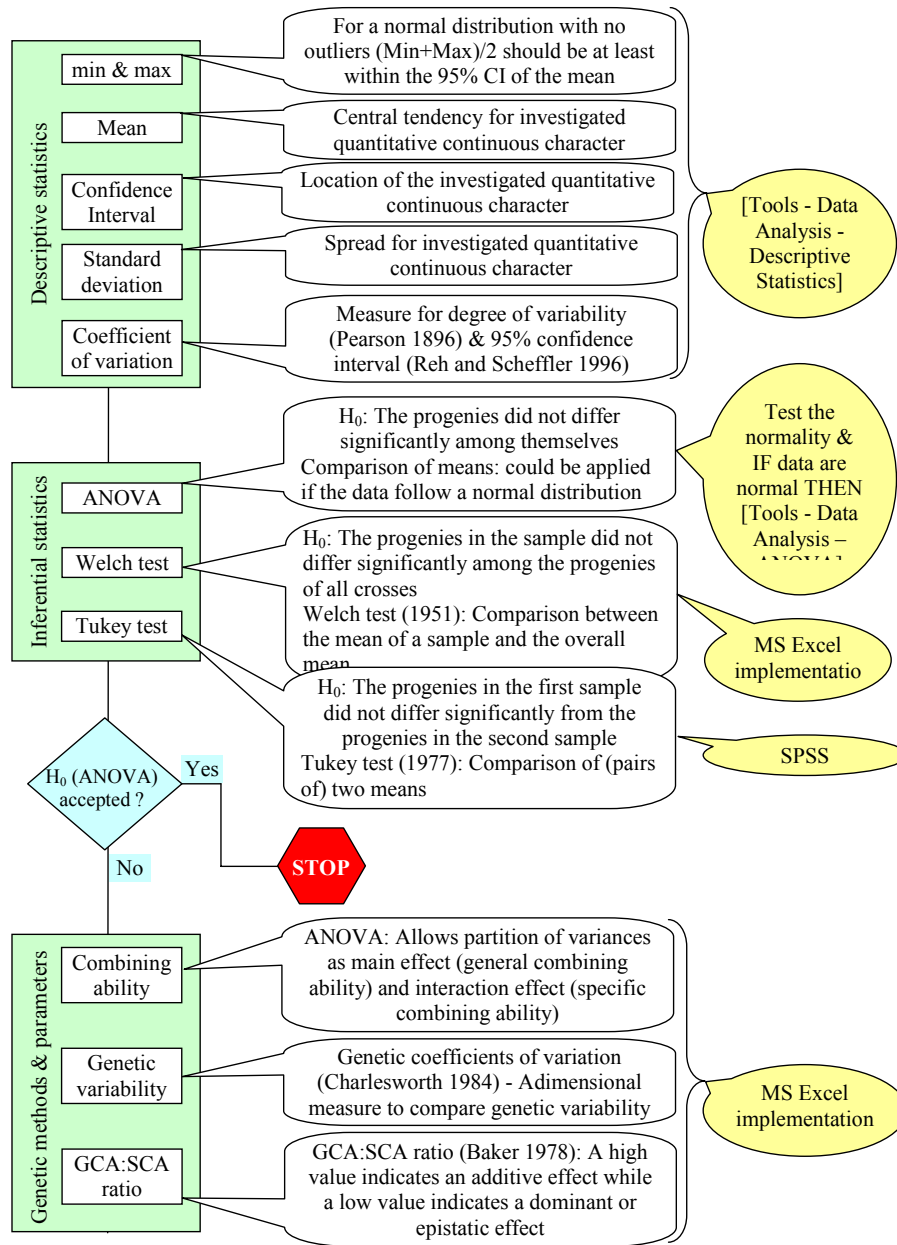


Figure 1. Statistical and genetic approach: Griffing experimental Method II, Model II.

The following estimators were used and implemented in genetic analysis:

- Estimator for mean (Griffing [18, 19]):

$$\hat{u} = \frac{2x_{..}}{p(p-1)}.$$

- Estimators for variance components (Griffing [18, 19]):

- $Est(\sigma_g^2) = (MS_g - MS_s)/(p + 2).$
- $Est(\sigma_s^2) = (MS_s - MS_e).$
- $Est(\sigma_e^2) = MS_e(V_E).$
- V_A (additive genetic variance) = $2 * Est(\sigma_g^2).$
- V_D (dominance genetic variance) = $Est(\sigma_s^2).$
- V_P (total phenotypic variance) = $V_A + V_D.$

- Heritability (Falconer and Mackay [14]; and Holland et al. [24]):

- Broad sense: $H^2 = V_A/V_P.$
- Narrow sense: $h^2 = V_A/(V_A + V_D + V_E).$

- Estimators for combining ability effects (Griffing [18, 19]):

- GCA effects (g_i): $\hat{g} = \frac{1}{p+2} \left(x_{i.} + x_{ij} - \frac{2x_{..}}{p} \right).$
- SCA effects:
 - $s_{ij} : \hat{s}_{ij} = x_{ij} - \frac{1}{p+2} (x_{i.} + x_{ii} + x_{j.} + x_{jj}) + \frac{2x_{..}}{(p+1)(p+2)}.$
 - $s_{ii} : \hat{s}_{ii} = x_{ii} - \frac{1}{p-1} (x_{i.} + x_{ii}) + \frac{2x_{..}}{(p+1)(p+2)}.$

- Estimators for individual GCA and SCA variances (Griffing [18, 19]):

- $Est(\sigma_g^2) = g_i^2 - (p-1)MS_e/[p(p-2)].$
- $Est(\sigma_s^2) = 1/(p-2) \sum s_{ij}^2 - (p-3)MS_e/(p-2).$

- Standard error (SE) of effects: calculated to test the accuracy of g_i and s_{ij} and of significance of differences between any two GCA's or SCA's (Griffing [18, 19]):

$$\circ SE(g_i) = \sqrt{(p-1)MS_e/[p(p+2)]} - t_i = g_i/SE(g_i) - df = p-1.$$

$$\circ SE(s_{ij}) = \sqrt{[p^2 + (p+2)] * MS_e/[(p+1)(p+2)]} - t_{ij} \\ = s_{ij}/SE(s_{ij}) - df = p(p-1)/2.$$

- Genetic coefficient of variation (Charlesworth [8]):
 - Phenotypic coefficient of variation: $CV_P = 100\sqrt{V_P}/m$, where m = arithmetic mean.
 - Additive coefficient of variation: $CV_A = 100\sqrt{V_A}/m$.
- GCA:SCA ratio (Baker [3]): $= 2MS_g/(2MS_g + MS_s)$.

Our model was implemented in Microsoft Excel and was used to analyze the corolla diameter of *Calendula officinalis* L. (Pot Marigold). Four cultivars were used as parental lines (*Prycosnovjenie* – abbreviated as Pry, *Pacific Beauty* – abbreviated as PB, *Bon Bon Mix* – abbreviated as BBM, and *Bon-Bon Orange* – abbreviated as BBO). We used Griffing's experimental Method II, Model II (the seeds of parental lines were randomly extracted from the available population of seeds) (Table 2).

Table 2. Griffing's experimental Method II on *Calendula officinalis* L. cultivars

♂ \ ♀	Pry.	PB	BBM	BBO
Pry.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
PB		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
BBM			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
BBO				<input checked="" type="checkbox"/>

Pry. = Prycosnovjenie; PB = Pacific Beauty;

BBM = Bon Bon Mix; BBO = Bon-Bon Orange

The experiment was carried out between 2007-2009 at Agro Botanical Garden of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

The diameter of corolla (measured in cm) on different progenies of *Calendula officinalis* L. was used to describe our approach as a step-by-step statistical and genetic analysis.

Results

A number of 10 combinations were analyzed. Statistical analysis was carried out first. An overall mean of 1.81 ± 0.29 (95%CI [1.77-1.86]) was obtained. An overall variation coefficient of 6.20 (95%CI [5.21-7.19]) was obtained when all progenies were investigated. Descriptive statistics of corolla diameter are presented in Table 3.

The normal distribution of the progenies resulted from all crosses could not be rejected at a significance level of 5%. Therefore, inferential statistics could be applied on the experimental data. The results of the ANOVA test, which establishes whether progenies are statistically different among themselves, are presented in Table 4.

Table 3. Descriptive statistics

$\text{♀} \times \text{♂} (n)$	m [95%CI]	StDev	Min	Max	CV(%) [95%CI]
Pry. \times Pry. (16)	1.96 [1.84-2.07]	0.23	1.60	2.40	12 [9-19]
Pry. \times PB (21)	1.99 [1.87-2.11]	0.28	1.50	2.50	14 [11-20]
Pry. \times BBM (9)	1.77 [1.53-2.00]	0.34	1.40	2.30	19 [13-39]
Pry. \times BBO (11)	1.67 [1.60-1.74]	0.11	1.50	1.90	7 [5-12]
PB \times PB (18)	1.76 [1.71-1.81]	0.10	1.60	2.00	6 [4-9]
PB \times BBM (12)	2.07 [1.92-2.21]	0.25	1.80	2.50	12 [8-21]
PB \times BBO (2)	1.60 [n.a.]	0.28	1.40	1.80	18 [n.a.]
BBM \times BBM (14)	1.55 [1.47-1.63]	0.15	1.30	1.80	9 [7-15]
BBM \times BBO (28)	1.93 [1.81-2.05]	0.33	1.20	2.60	17 [13-23]
BBO \times BBO (22)	1.57 [1.49-1.66]	0.19	1.20	1.80	12 [9-18]

n = sample size; m = arithmetic mean; 95%CI = 95% confidence interval;

StDev = standard deviation; Min = minimum; Max = maximum;

CV = coefficient of variation; n.a. = not applicable; Pry. = Prycosnovjenie;

PB = Pacific Beauty; BBM = Bon Bon Mix; BBO = Bon-Bon Orange

Table 4. Results of the ANOVA test: statistically significant differences among progenies

Source of variation	SS	df	MS	F*	p
Between groups (SPV)	4.77	9	0.53	9.19	$6.39 \cdot 10^{-11}$
Within groups (SPE = SPT – SPV)	8.25	143	0.06		
Total (SPT)	13.02	152			

* $F_{\text{critical}} = 1.95$; SS = sum of square; df = degree of freedom;

MS = mean of square; $F = F\text{-value}$; $p = p\text{-value}$

The Welch test was applied in order to determine whether the mean of progenies was statistically different from the overall mean. The results are presented in Table 5.

Table 5. Results of the Welch test: statistically significant differences between the mean of progenies and overall mean

	Pry. × Pry.	Pry. × PB	Pry. × BBM	Pry. × BBO	PB × PB	PB × BBM	PB × BBO	BBM × BBM	BBM × BBO	BBO × BBO
<i>t</i>	33.90	33.11	15.46	50.26	72.06	28.65	8.00	39.88	31.41	38.19
<i>p</i>	$1.37 \cdot 10^{-15}$	$6.06 \cdot 10^{-19}$	$3.05 \cdot 10^{-7}$	$2.35 \cdot 10^{-13}$	$1.40 \cdot 10^{-22}$	$1.10 \cdot 10^{-11}$	0.0792	$5.58 \cdot 10^{-15}$	$8.57 \cdot 10^{-23}$	$6.86 \cdot 10^{-21}$

t = *t*-values; *p* = *p*-value associated to *t*-value;

Pry. = Prycosnovjenie; PB = Pacific Beauty; BBM = Bon Bon Mix; BBO = Bon-Bon Orange

Tukey test was applied in order to see whether the means of progenies were statistically different when different parents were crossed. The PB × BBO progenies were not included in this analysis, since only two data were available. The results are presented in Table 6.

Table 6. Tukey test: mean differences (upper right corner of the matrix) and associated significances (lower left corner of the matrix)

	Pry. × Pry.	Pry. × PB	Pry. × BBM	Pry. × BBO	PB × PB	PB × BBM	BBM × BBM	BBM × BBO	BBO × BBO
Pry. × Pry.		-0.0342	0.1896	0.2835	0.1951	-0.1104	0.4063	0.0241	0.3835
Pry. × PB	1.000		0.2238	0.3177	0.2294	-0.0762	0.4405	0.0583	0.4177
Pry. × BBM	0.617	0.324		0.0939	0.0056	-0.3000	0.2167	-0.1655	0.1939
Pry. × BBO	0.072	0.014	0.994		-0.0884	-0.3939	0.1227	-0.2594	0.1000
PB × PB	0.310	0.080	1.000	0.989		-0.3056	0.2111	-0.1710	0.1884
PB × BBM	0.954	0.994	0.114	0.004	0.023		0.5167	0.1345	0.4939
BBM × BBM	$2.77 \cdot 10^{-4}$	$1.36 \cdot 10^{-5}$	0.468	0.938	0.256	$6.76 \cdot 10^{-6}$		-0.3821	-0.0227
BBM × BBO	1.000	0.995	0.682	0.068	0.314	0.789	$1.02 \cdot 10^{-4}$		0.3594
BBO × BBO	$1.02 \cdot 10^{-4}$	$2.26 \cdot 10^{-6}$	0.516	0.969	0.255	$1.96 \cdot 10^{-6}$	1.000	$1.81 \cdot 10^{-5}$	

Pry. = Prycosnovjenie; PB = Pacific Beauty; BBM = Bon Bon Mix; BBO = Bon-Bon Orange

Tukey test identified four classes of homogeneity based on harmonic means in the group:

- **Class 1.** Progenies of Pry. × BBM, Pry. × BBO, PB × PB, BBM × BBM and BBO × BBO ($p = 0.257$).
- **Class 2.** Progenies of Pry. × BBM, Pry. × BBO, PB × PB and BBM × BBO ($p = 0.085$).
- **Class 3.** Progenies of Pry. × Pry., Pry. × PB, Pry. × BBM, PB × PB, BBM × BBO ($p = 0.191$).
- **Class 4.** Progenies of Pry. × Pry., Pry. × PB, PB × BBM and BBM × BBO ($p = 0.839$).

Genetic analysis could be carried out next, since our statistical analysis identified significant differences among progenies. The ANOVA test for combining abilities was applied in order to partition the variances (according to general and specific combining abilities).

The means matrix presented in Figure 2 was used in genetic analysis.

IF		=Y3+Z3+AA3+AB3+Z4+AA4+AB4+AA5+AB5+AB6					
	W	X	Y	Z	AA	AB	AC
1							
2			Pry.	PB	BBM	BBO	Σx_i
3		Pry.	1.9563	1.9905	1.7667	1.6727	7.3861
4		PB	1.9905	1.7611	2.0667	1.6000	7.4183
5		BBM	1.7667	2.0667	1.5500	1.9321	7.3155
6		BBO	1.6727	1.6000	1.9321	1.5727	6.7776
7		Σx_j	7.3861	7.4183	7.3155	6.7776	
8						$x_{..}$	Z3+AA3+AB3+

Figure 2. Means of progenies following replications: example of computed overall mean.

The $x_{..}$ statistics was calculated as follows:

$$\begin{aligned}
 x_{..} &= 1.956 + 1.990 + 1.767 + 1.673 + 1.761 + 2.067 \\
 &+ 1.600 + 1.550 + 1.932 + 1.573 = 17.869.
 \end{aligned}$$

The ANOVA test for combining abilities is presented in Table 7.

Table 7. Step-by-step ANOVA test for combining abilities

Source of variation	df	SS	MS	F	p
General combining ability (GCA)	df_g $= p - 1 = 3$	SS_g $= 0.09$	MS_g $= 0.03$	MS_g/MS_e $= 8.29$	$FDIST(MS_g/MS_e, df_g, df_e)$ $= 3.98 \cdot 10^{-5}$
Specific combining ability (SCA)	df_s $= p(p - 1)/2 = 6$	SS_s $= 0.23$	MS_s $= 0.04$	MS_s/MS_e $= 9.79$	$FDIST(MS_s/MS_e, df_s, df_e)$ $= 4.89 \cdot 10^{-9}$
Error	$df_e = 153 - 3 - 6$ $= 144$	SS_e $= 0.55$	MS_e $= 0.0038$		

$FDIST(\cdot, \cdot) = MS$ Excel function for calculating the probability from Fisher's distribution

Different statistics presented in Table 7 are calculated as follows:

- General combining ability:

$$\begin{aligned} \circ SS_g &= 1/(4 + 2) * ((7.386 + 1.956)^2 + (7.418 + 1.761)^2 \\ &\quad + (7.315 + 1.550)^2 + (6.778 + 1.573)^2 - 17.869^2) = 0.09. \end{aligned}$$

$$\circ MS_g = SS_g / df_g = 0.09/3 = 0.03.$$

- Specific combining ability:

$$\begin{aligned} \circ SS_s &= (1.956^2 + 1.990^2 + 1.767^2 + 1.673^2 + 1.761^2 + 2.067^2 \\ &\quad + 1.600^2 + 1.550^2 + 1.932^2 + 1.573^2) - ((7.386 + 1.956)^2 \\ &\quad + (7.418 + 1.761)^2 + (7.315 + 1.550)^2 + (6.778 + 1.573)^2)/(4 + 2) \\ &\quad + 2 * (17.869^2)/((4 + 1) * (4 + 2)) = 0.23. \end{aligned}$$

$$\circ MS_s = 0.23/6 = 0.04.$$

- Errors:

$$\begin{aligned} \circ SS_e &= SPE (ANOVA statistics)/n'', \text{ where } n'' = 1/(10 - 1) * (153 - \\ &\quad (16^2 + 21^2 + 9^2 + 11^2 + 18^2 + 12^2 + 2^2 + 14^2 + 28^2 + 22^2))/153) = \\ &\quad 14.94 \rightarrow SS_e = 8.25/14.94 = 0.55. \end{aligned}$$

$$\circ MS_e(\sigma_e^2) = 0.05/144 = 0.0038.$$

The genetic estimators were calculated using the formula presented in the material and method section as follows:

- Estimator of mean: $= 2 * 17.869 / (4 * (4 - 1)) = 2.98$.
- Variance components:
 - $Est(\sigma_g^2) = (0.03 - 0.04) / (4 + 2) = -0.0017$.
 - $Est(\sigma_s^2) = 0.04 - 0.0038 = 0.0362$.
 - $Est(\sigma_e^2) = 0.0038$.
 - V_A (additive variance) $= 2 * (-0.0017) = -0.0034$.
 - V_D (dominance variance) $= 0.0362$.
- Heritability:
 - Broad sense: $H^2 = -0.06005$.
 - Narrow sense: $h^2 = -0.03002$.
- Combining ability effects:
 - $g_1 = (7.386 + 1.956 - 2/4 * 17.869) / (2 + 4) = 0.068$.
 - $g_2 = (7.418 + 1.761 - 2/4 * 17.869) / (2 + 4) = 0.041$.
 - $g_3 = (7.315 + 1.550 - 2/4 * 17.869) / (2 + 4) = -0.012$.
 - $g_4 = (6.778 + 1.573 - 2/4 * 17.869) / (2 + 4) = -0.097$.
 - $s_{12} = 1.990 - (7.386 + 1.956 + 7.418 + 1.761) / (4 + 2) + 2 * 17.869 / ((4 + 1) * (4 + 2)) = 0.094$.
 - $s_{13} = 1.767 - (7.386 + 1.956 + 7.315 + 1.550) / (4 + 2) + 2 * 17.869 / ((4 + 1) * (4 + 2)) = -0.076$.
 - $s_{14} = 1.673 - (7.386 + 1.956 + 6.778 + 1.573) / (4 + 2) + 2 * 17.869 / ((4 + 1) * (4 + 2)) = -0.085$.

- $s_{23} = 2.067 - (7.418 + 1.761 + 7.315 + 1.550)/(4 + 2)$
 $+ 2 * 17.869/((4 + 1) * (4 + 2)) = 0.251.$
- $s_{24} = 1.600 - (7.418 + 1.761 + 6.778 + 1.573)/(4 + 2)$
 $+ 2 * 17.869/((4 + 1) * (4 + 2)) = -0.130.$
- $s_{34} = 1.932 - (7.315 + 1.550 + 6.778 + 1.573)/(4 + 2)$
 $+ 2 * 17.869/((4 + 1) * (4 + 2)) = 0.0254.$
- $s_{11} = 1.956 - (7.386 + 1.956)/3 + 2 * 17.869/((4 + 1) * (4 + 2))$
 $= 0.033.$
- $s_{22} = 1.761 - (7.418 + 1.761)/3 + 2 * 17.869/((4 + 1) * (4 + 2))$
 $= -0.107.$
- $s_{33} = 1.550 - (7.315 + 1.550)/3 + 2 * 17.869/((4 + 1) * (4 + 2))$
 $= -0.214.$
- $s_{44} = 1.573 - (6.778 + 1.573)/3 + 2 * 17.869/((4 + 1) * (4 + 2))$
 $= -0.019.$

▪ Individual GCA and SCA variances:

- $Est(\sigma_{g_1}^2) = g_1^2 - (p - 1)MS_e/[p(p - 2)]$
 $= 0.068^2 - (4 - 1) * 0.0038/(4 * (4 - 2)) = 0.0032.$
- $Est(\sigma_{g_2}^2) = g_2^2 - (p - 1)MS_e/[p(p - 2)]$
 $= 0.041^2 - (4 - 1) * 0.0038/(4 * (4 - 2)) = 0.0003.$
- $Est(\sigma_{g_3}^2) = g_3^2 - (p - 1)MS_e/[p(p - 2)]$
 $= (-0.012)^2 - (4 - 1) * 0.0038/(4 * (4 - 2)) = -0.0013.$

- $Est(\sigma_{g4}^2) = g_4^2 - (p-1)MS_e/[p(p-2)]$
 $= (-0.097)^2 - (4-1) * 0.0038/(4 * (4-2)) = 0.0080.$
- $Est(\sigma_{s1}^2) = 1/(4-2) * (s_{12}^2 + s_{13}^2 + s_{14}^2) - 0.0038/(4-2)$
 $= \frac{1}{2} * (0.094^2 + (-0.076)^2 + (-0.085)^2) - 0.0038/2 = 0.009.$
- $Est(\sigma_{s2}^2) = 1/(4-2) * (s_{21}^2 + s_{23}^2 + s_{24}^2) - 0.0038/(4-2)$
 $= \frac{1}{2} * (0.094^2 + 0.251^2 + (-0.130)^2) - 0.0038/2 = 0.042.$
- $Est(\sigma_{s3}^2) = 1/(4-2) * (s_{31}^2 + s_{32}^2 + s_{34}^2) - 0.0038/(4-2)$
 $= \frac{1}{2} * ((-0.076)^2 + 0.251^2 + 0.254^2) - 0.0038/2 = 0.064.$
- $Est(\sigma_{s4}^2) = 1/(4-2) * (s_{41}^2 + s_{42}^2 + s_{43}^2) - 0.0038/(4-2)$
 $= \frac{1}{2} * ((-0.085)^2 + (-0.130)^2 + 0.254^2) - 0.0038/2 = 0.042.$

▪ *SE of effects:*

- $SE(g_i) = \sqrt{(p-1)MS_e/[p(p+2)]}$
 $= ((4-1) * 0.0038/(4 * (4+2)))^{0.5} = 0.022.$
 - $t_{(g1)} = g_1/SE(g_i) = 0.068/0.022 = 3.091, p = 0.0537.$
 - $t_{(g2)} = g_2/SE(g_i) = 0.041/0.022 = 1.864, p = 0.1593.$
 - $t_{(g3)} = g_3/SE(g_i) = (-0.012)/0.022 = -0.545 = 0.6234.$
 - $t_{(g4)} = g_4/SE(g_i) = (-0.097)/0.022 = -4.409, p = 0.0216.$
- $SE(s_{ij}) = \sqrt{[p^2 + (p+2)] * MS_e/[(p+1)(p+2)]}$
 $= ((4^2 + (4+2)) * 0.0038/((4+1) * (4+2)))^{0.5} = 0.053.$
 - $t_{s12} = s_{12}/SE(s_{ij}) = 0.094/0.053 = 1.774,$
 $p = 0.1265$ ($df = p(p-1)/2 = 4 * (4-1)/2 = 6$).

- $t_{s13} = s_{13}/SE(s_{ij}) = -0.076/0.053 = -1.434, p = 0.2016.$
- $t_{s14} = s_{14}/SE(s_{ij}) = -0.085/0.053 = -1.604, p = 0.1599.$
- $t_{s23} = s_{23}/SE(s_{ij}) = 0.251/0.053 = 4.736, p = 0.0032.$
- $t_{s24} = s_{24}/SE(s_{ij}) = -0.130/0.053 = -2.453, p = 0.0496.$
- $t_{s34} = s_{34}/SE(s_{ij}) = 0.254/0.053 = 4.792, p = 0.0030.$
- $GCA:SCA = 2 * 0.03/(0.03 + 0.0038) = 0.6000.$
- Genetic coefficient of variation:
 - Phenotypic coefficient of variation: $CV_P = 100 * \sqrt{0.03/1.81} = 13\%.$
 - Additive coefficient of variation: $CV_A = \text{n.a.},$ since V_A had a negative value.

Discussion

A methodology for statistical and genetic analysis of experimental data resulted by applying the Griffing's experimental Method II, Model II was developed, implemented and described in a step-by-step analysis. The results of variance analyses of diallel mating designs are usually presented only at the level of inferential statistics using ANOVA statistics and post-hoc statistics and are followed by genetic analysis with ANOVA for combining abilities. The descriptive statistics of experimental data are not generally presented as results, even if their interpretation could provide important information. Our methodology is presented as a guideline for researchers who want to improve their knowledge and skills in statistical and genetic analysis. Neither statistical analysis (descriptive and inferential) nor genetic analysis could be omitted from the correct analysis of experimental data in diallel mating designs.

What information could be obtained from the statistical analysis of experimental data? Unfortunately, statistical analysis is seen as an intermediary step towards genetic analysis. This analysis is frequently reduced to the ANOVA test for comparing more than two means and to post-hoc tests. However, descriptive statistics could bring useful information in terms of data description and the need for inferential statistics.

The analysis of means and variance coefficients (also known as relative variability) and their associated confidence intervals revealed the following:

- The highest value of the mean corolla diameter was observed in PB × BBM progenies.
- The lowest value of the mean corolla diameter was noted in BBM × BBM progenies, closely followed by BBO × BBO progenies.
- Statistically significant differences are expected on mean corolla diameters when the Pry. × BBO, PB × PB, BBM × BBM and BBO × BBO progenies are compared to all the other progenies. This finding is supported by the fact that 95% confidence intervals did not overlap.
- The experimental data could be classified in two classes according to the values of the variation coefficients (Botez et al. [7]): the corolla diameter had low variability for the Pry. × BBO, PB × PB, BBM × BBM progenies; the corolla diameter had moderate variability for all the other progenies.

Since the mean corolla diameter obtained was of 1.81cm with a 95% confidence level of [1.77-1.86], most means were expected to be within this range. However, in 9 out of 10 cases, the mean value of the corolla diameter was outside this range. Therefore, inferential statistics for comparing the mean of any cross to the overall mean was required.

The relative variability of progenies could also be compared based on the 95% confidence intervals of the variation coefficients. Since the 95% confidence intervals of variation coefficients overlapped, relative variability did not differ statistically among progenies. Furthermore, the values of the variation coefficients of all progenies belonged to the 95% confidence interval of the overall variation coefficient. Therefore, the variation coefficients of progenies were not statistically different from the overall variation coefficient.

The arithmetic mean and associated 95% confidence intervals are valid statistical parameters if and only if experimental data are normally distributed. Otherwise, the median is the appropriate parameter for describing experimental data and for comparisons. Moreover, normality is a condition of the ANOVA test for comparing means. The variation coefficient should not be used when the arithmetic mean is near zero (Wong and Wu [44]).

The ANOVA test is able to identify statistically significant differences anywhere in the data. The results presented in Table 4 showed the occurrence of statistically significant differences among corolla diameters of progenies ($F = 9.19$, $p = 6.39 \cdot 10^{-11}$). Nevertheless, this result is unable to identify the location of such differences. Since a statistically significant difference was identified among progenies, Tukey and Welch tests could be applied in order to identify differences among progenies as well as differences between progenies and the overall mean. Welch test was applied based on the observation of the 95% confidence intervals associated to the means. The Welch test identified statistically significant differences between corolla diameter means and the overall mean (see Table 5). The results for PB \times BBO progenies could not be considered reliable for this analysis, since only two plants were investigated. Two classes of differences were identified: the progenies had lower mean compared to the overall mean (Pry. \times BBM, Pry. \times BBO, PB \times PB, PB \times BBO, BBM \times BBM, and BBO \times BBO progenies) and the progenies had higher mean compared to the overall mean (Pry. \times Pry., Pry. \times PB, PB \times BBM, and BBM \times BBO progenies). Tukey test identified statistically significant differences among corolla diameter means in the following progenies (Table 6):

- Pry. \times BBO progenies proved statistically lower than Pry. \times PB progenies.
- Pry. \times PB progenies proved statistically higher than Pry. \times BBO and PB \times PB progenies.
- BBM \times BBM progenies proved statistically lower than Pry. \times Pry., Pry. \times PB, PB \times BBM, BBM \times BBO progenies.
- BBO \times BBO progenies proved statistically lower than Pry. \times Pry., Pry. \times PB, PB \times BBM, BBM \times BBO progenies.

The above presented results are in accordance with the 95% confidence intervals associated to the means.

What information could be obtained from the genetic analysis of experimental data? ANOVA for combining abilities is a statistical method able to partition the variance in its components (general and specific combining abilities). The highest corolla diameters were: PB \times BBM, Pry. \times PB, and Pry. \times Pry. Their replications are required if a higher corolla diameter is desired. The lowest corolla diameters were: BBM \times BBM, BBO \times BBO, and PB \times BBO.

The ANOVA test for combining ability identified both a statistically significant additive and dominates effect (Table 8). Nevertheless, the question was whether all or only some replications were statistically significant. To answer this question, the combining ability effects and individual GCA and SCA variances were calculated. The results led to the identification of the appropriate parent based on its performances: a parent with a parent with a high value of $Est(\sigma_{gi}^2)$ and $Est(\sigma_{si}^2)$ is a better parent for creating the specific combination desired. The BBO parent proved to have the highest $Est(\sigma_{gi}^2)$ value while the BBM parent proved to have the highest differential ability to transmit its genes to its progenies. Furthermore, the standard error of effects was computed in order to analyze the accuracy of the effects. Accordingly, the g_4 value (BBO, even if GCA effect was negative) proved to be statistically significant ($p = 0.0216$) while statistically significant abilities to transmit the genes in the hybrid progenies were observed for (BBO \times BBM, PB \times BBO, and PB \times BBM, $p \leq 0.0496$). According to these results, the significance of the general combining ability could only be attributed to BBO while the significance of the specific combining ability was attributed to BBO \times BBM, PB \times BBO, and PB \times BBM replications.

Based on the results of ANOVA for combining abilities, the variance components were identified. Some negative values (e.g., GCA estimated variance, additive variance and heritability) were obtained for some estimators; these negative values should be considered zero (Robinson et al. [33]). Furthermore, when a negative estimator results from using a negative value (e.g., broad and narrow sense heritability), its values should be omitted. Even if the negative values should be considered equal to zero, these values should be reported as they are in order to be properly interpreted when further meta-analysis is needed (Dudley and Moll [13]).

The value of the GCA : SCA ratio suggested the importance of additive effects in the heredity of the corolla diameter. A dominant or epistatic effect was suggested for the corolla diameter, since the GCA : SCA ratio was relatively low (0.6000) (Griffing [18]; and Bhullar et al. [5]).

Statistical and genetic analysis both are required. Statistical analysis describes experimental data and compares means among progenies as well as between the crossover mean and the overall mean. ANOVA results should indicate whether ANOVA for combining ability should be applied. In other terms, statistical analysis

could indicate if genetic analysis should be conducted. If the ANOVA test fails to identify a statistically significant *F*-value, then the genetic analysis will not be conducted as it will be unable to identify valid general and/or specific combining abilities.

Diallel mating designs are frequently used in plant breeding (Tyagi et al. [40]; Yoshioka et al. [45], and Mebrahtu and Devine [28]) due to their capacity to test general and specific combining abilities and other secondary genetic parameters (Viana et al. [42]). They are also used to reduce the costs and the complexity of experiments (Hinkelmann and Kempthorne [23]). Griffing's experimental Method II could be applied in diallel crosses in complete random block design (Aliu et al. [1]; Izge et al. [25]; and Murty et al. [30]), incomplete blocks (Gilbert [16]; and Das et al. [11]) and orthogonal blocks (Mukerjee [29]). Apart from the experimental design, a correctly conducted experiment on which proper statistical and genetic analysis are applied is considered a valid experiment. Thus, statistical analysis in diallel mating design is as important as genetic analysis; both need to be carried out correctly.

The information presented in this paper is not new. A series of programs were developed to assist the researcher in the statistical analysis of experimental data from diallel crosses (Utz [41]; Saxton [34]; Skinner and Stuteville [37]; and Crossa et al. [10]) and there is no need for a new program. Our paper, similarly, with other studies (Bolboacă et al. [6]), aimed to make the researchers truly understand the statistical and genetic analysis of Griffing's experimental Method II, Model II by presenting it step-by-step.

Certain statistically significant differences among progenies were identified. Since the ANOVA statistics identified a statistically significant model, the genetic analysis proved to be appropriate. The genetic analysis was able to identify the appropriate parent(s) needed to obtain plants with larger corolla diameter.

Conflict of Interest

The experiments carried out in this research comply with the Romania current laws.

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