Statistics and Genetic Analysis of Quantitative Traits using Griffing's Experimental Method IV: A practical guideline

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Abstract: Starting from the analysis of the specialty literature, a guideline for statistic and genetic analysis of quantitative traits using Griffing's experimental method IV was developed.

Keywords: Inferential statistics; Genetic parameters; Quantitative traits; Griffing's experimental method IV; Guideline

INTRODUCTION

The aim of the present research was to develop and implement a guideline for statistic and genetic analyses of quantitative traits for Griffing's experimental method IV. The proposed guideline follows the template presented in Figure 1 (Bolboacă and Sestraş, 2010).
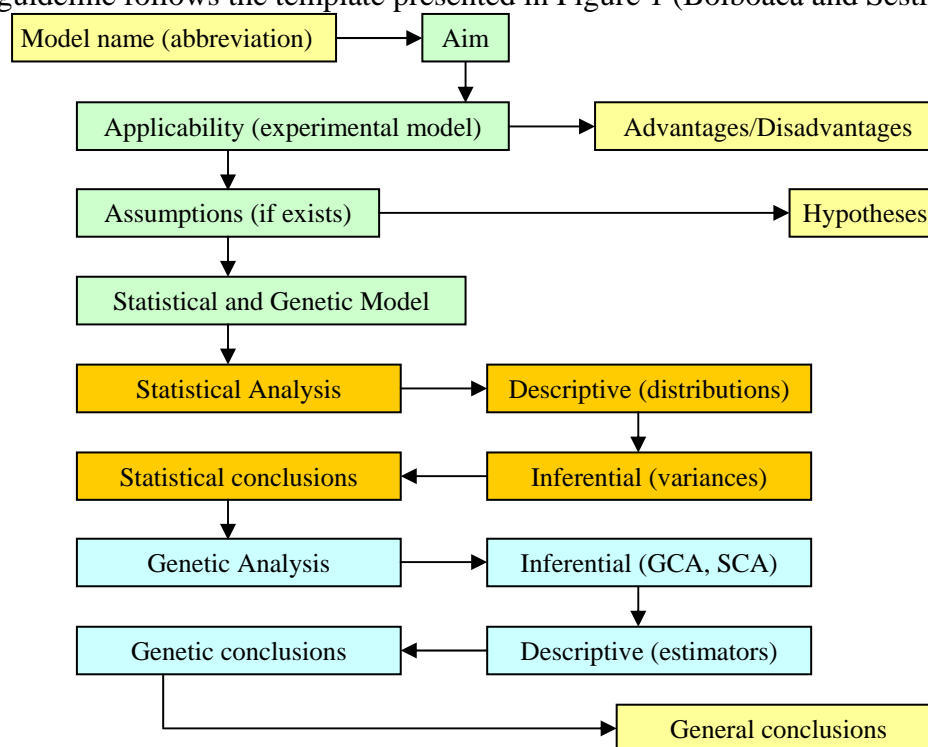

Fig. 1. Template for statistic and genetic analyses of quantitative traits

MATERIALS AND METHODS

Assessment of a quantitative trait. The proposed guideline was applied on growth vigor (as quantitative trait) of apple. It is well known that creation of an apple tree with small growth vigor allows increasing the trees density on surface. Apple production could be increased by increasing the density of trees on surface.

A diallel matting design was carried out at Fruit Production and Research Station Cluj-Napoca (Tab. 1). The growth vigor was assessed on the F_1 hybrids when neither parents nor F_1's reciprocal where included (Griffing's experimental method IV, model I) (Griffing, 1956). In this experimental model the variety and block effects are both random variables.

Tab. 1

Diallel cross: Griffing's experimental method IV, model I

♀/♂	Strk.	GDs.	Fl.	Lib.
Strk.		Strk.×GDs.	Strk.×Lib.	Strk.×Fl.
GDs.			GDs.×Lib.	GDs.×Fl.
Fl.				Lib.×Fl.

combinations = p(p-1)/2 = 6; p = number of parents

The height was measured from ground level to the summit of the tree and was expressed on meters. The measurements were carried out on samples of 50 three-year old F_1 hybrids for each cross. Four varieties of apple trees, two spur (Starkrimson abbreviated as Strk. and Goldenspur abbreviate as Gds.) and two standard (non-spur-type) varieties with genetic resistance to scrab (Liberty abbreviated as Lib. and Florina abbreviated as Fl.), were used in experiments. A total number of six crosses were obtained: Strk.×Gds., Strk.×Lib., Strk.×Fl., Gds.×Lib., Gds.×Fl., and Lib.×Fl. (Table 1).

Guideline for statistics and genetic analysis. The proposed pathway to be followed in analysis of quantitative trait and implemented in the proposed guideline could be summarized as follow:

1. *Descriptive statistics*: mean and associated 95% confidence level, standard deviation, minimum, maximum, sample size. The coefficient of variation (also knows as relative variability and defined as the ration between standard deviation and arithmetic mean) - (Pearson, 1896) - is used as a parameter of variability within cross. The coefficient of variation is not recommended to be computed as parameter when the observed mean is near zero or when the data had positive as well as negative values (Wong and Wu, 2002). The confidence interval for coefficient of variation allows a more reliable interpretation of this descriptive parameter (Bonet, 2006). Coefficient of variation could take values in the range (0, +∞). A value less than 10% indicate that the analyzed trait present a small variability; a value between 10% and 20% indicate a variable trait since a value greater than or equal to 20 indicate that the analyzed trait have a high variability (Botez *et al.*, 1995).

2. *Inferential statistics:*
   a. <u>Statistical analysis</u>: ANOVA test is proper applied after testing of its assumptions when is used to test the equality of means: experimental data are normally distributed; experimental data has equal variances (homoscedancity); and the groups used for comparison are independent. The proposed steps used in ANOVA analysis are as follow:
      ÷ Distribution analysis: applied to experimental data. Three test were applied: Kolmogorov-Smirnov (Kolmogorov, 1941), Anderson Darling (Anderson and

Darling, 1952), and Chi-Squared (Pearson, 1900). The EasyFit software can be used to accomplish this task (http://mathwave.com).

÷ Homoscedacity analysis: applied to experimental data. Bartlett test (Bartlett, 1937) or Levene test (Levene, 1960) can be used to test the variance differences.

÷ Independence of the compared groups. Since different parents were crossed the obtained F_1 hybrids were considered independent.

If all three assumptions proved to be true, the ANOVA test will be applied to test the difference between means.

The ANOVA test could be used to test the following null hypotheses: "The mean of height is not statistically different when different F_1 hybrids are compared" & "The variances are not statistically different when different F_1 hybrids are compared". It is also possible to use some post-hoc test for a further analysis of the experimental data: Tukey HDS test to test pairs of means (Tukey, 1986); Dunnett test (Dunnett, 1955) to compare the values of one cross (as control) with all others (as tests).

A significance level of 5% was used in all statistical analyses.

b. <u>Genetic analysis</u>. The following methods and/or tests are proposed to be used in genetic analysis:

÷ General and specific combining ability (estimation of GCA, SCA, and off reciprocal effects) - (Griffing, 1956; Gardner and Eberhart, 1966). These two concepts were introduced by Sprague and Tatum (1942) in order to distinguish between the average performance of parents in crosses and the deviation of individual crosses from the average of the crosses. Mathematical model used for this analysis is presented in Table 2.

The expected values of variances are given by the following formulas: σ_s^2 = MSs-MSe (V_A, additive variance) & σ_g^2 = (MSg-MSs)/(p-2) (V_{NA}, non-additive variance). The general effect is calculated by applying the formula: u = 2x../[p(p-1)], where p = number of parents. The effect on hybrids of ith parent in terms of GCA is given by formula: g_i = [px$_{i.}$ – 2x..]/p(p-2). The effect of $i^{th} \times j^{th}$ cross in term of SCA (computed for each cross) is given by the formula: s_{ij} = x_{ij} - (x$_{i.}$ + x$_{.j}$)/(p-2) + 2 x$_{..}$/[(p-1)(p-2)]

÷ Heritability coefficients (narrow-sense heritability, h^2).

o Broad-sense heritability (Plomin *et al.*, 1990): H^2 = V_G/V_P, where V_G = genotypic variance, V_P = phenotypic variance. H^2 was considered zero for negative values of V_G.

o Narrow sense heritability (Nyquist, 1991): h^2 = V_A/V_P, V_A = additive genetic variance. h^2 was considered zero for negative values of V_A.

÷ Coefficient of variation (Charlesworth, 1984): adimensional measure for comparison of genetic variability.

o Genotipic (Burton, 1952): CV_G = 100$\sqrt{(V_G)}$/m, where V_G = genotipic variance ; m = arithmetic mean of quantitative trait.

o Phenotypic: CV_P = 100$\sqrt{(V_P}$/m, CV_P = phenotypic coefficient of variation, V_P = phenotypic variance.

o Additive (Charlesworth, 1984; Houle, 1992): CV_A = 100$\sqrt{V_A}$/m.

÷ GCA:SCA ratio (Baker, 1978): GCA:SCA = 2MS$_g$/(2MS$_g$ + MS$_s$). A high value of this ratio indicates a higher importance of additive effect while a small value indicates the presence of a dominant or epistatic effect in inheritance of the quantitative trait (Griffing, 1956a; Bhullar *et al.,* 1979).

Tab. 2

Genetic analysis based on a Griffing's IV experimental method, model I

Sourse of variation	df	SS	MS	EMS	F (p)
GCA	p-1	$=\sum x_{i.}^2/(p-2) -4x_{..}^2/[p(p-2)]$	$= SSg/df_g$	$\sigma^2 + \sigma_s^2 + (p-2)\sigma_g^2$	$=MSg/MSs$ [a]
SCA	p(p-3)/2	$=\sum\sum_{i<j}x_{ij}^2 -\sum x_{i.}^2/(p-2) +2x_{..}^2/[(p-1)(p-2)]$	$= SSs/df_s$	$\sigma^2 + \sigma_s^2$	$=MSs/MSe'$ [b]
Eroare	m	$=SPe/[(n_T -\sum n_i^2/n_T)/(p-1)]$	$= SSe/df_e$	σ^2	

CGC = general combining ability; SCA = specific combining ability; $x_{i.}$ = mean of i th parent; $x_{..}$ = overall mean of all crosses;

x_{ij} = the mean value of the trait for F_1 hybrids resulted from crosses of ith parent with j^{th} parent;

EMS = Expectation of mean square;

p = FDIST(F,df_g/df_s,df_s/df_e); df_T = degrees of freedom from ANOVA table for total;

[a] = testing the effects of GCA ($\sigma_g^2 = 0$) (p(p-3)/n, n degrees of freedom);

[b] = testing the effects of SCA ($\sigma_s^2 = 0$) ((p-1), p(p-3)/2 degrees of freedom);

RESULTS AND DISCUSSION

Statistical analysis. Descriptive statistics associated to apple growth vigor are presented in Table 3.

Tab. 3

Statistical parameters for apple growth vigor

Parameter	Strk.×GDs. (n = 50)	Strk.×Lib. (n = 50)	Strk.×Fl. (n = 50)	GDs.×Lib. (n = 50)	GDs.×Fl. (n = 50)	Lib.×Fl. (n = 50)
m	1.04	1.37	1.23	1.30	1.35	1.45
[95% CI]	[0.90-1.18]	[1.21-1.52]	[1.09-1.37]	[1.14-1.45]	[1.19-1.52]	[1.32-1.58]
StDev	0.49	0.54	0.49	0.53	0.58	0.45
Min	0.20	0.25	0.20	0.20	0.28	0.43
Max	2.10	2.35	2.15	2.30	2.40	2.30
CV	0.47	0.39	0.40	0.41	0.43	0.31
[95% CI]	[0.38-0.62]	[0.32-0.51]	[0.33-0.52]	[0.33-0.53]	[0.35-0.56]	[0.26-0.40]

m = arithmetic mean; CI = confidence interval; StDev = standard deviation;

Min = minimum; Max = maximum value; CV = coefficient of variation/variability;

The results obtained in testing normal distribution of experimental data are presented in Table 4. The following results were obtained when the homogeneity of variances was analyzed:

÷ All hybrids (6 samples): Bartlett statistic = 3.6757 (p = 0.597); Levene statistic = 1.372 (p = 0.2345);

÷ Excluding the hybrids resulted from Strk.×Lib. crosses (not normally distributed experimental data; 5 samples): Bartlett statistic = 3.4483 (p = 0.486).

Tab. 4

Analysis of Experimental Data: Normality Tests

	Kolmogorov-Smirnov		Anderson Darling		Chi-Squared	
	Statistic (p)	Reject H_0?	Stat (CritV$_{5\%}$)	Reject H_0?	Stat (p)	Reject H_0?
Strk.×GDs.	0.1044 (0.6101)	No	0.5585 (2.5018)	No	4.8314 (0.3050)	No
Strk.×Lib.	0.1837 (0.0599)	No	1.9761 (2.5018)	No	12.812 (0.0122)	Yes[a]
Strk.×Fl.	0.1372 (0.2775)	No	1.4472 (2.5018)	Yes[b]	7.7242 (0.1022)	Yes[b]
GDs.×Lib.	0.1312 (0.3267)	No	0.7022 (2.5018)	No	2.3926 (0.6640)	No
GDs.×Fl.	0.0967 (0.7012)	No	0.4777 (2.5018)	No	2.5894 (0.6287)	No
Lib.×Fl.	0.0882 (0.7989)	No	0.2622 (2.5018)	No	1.3796 (0.8477)	No

[a] $\alpha \geq 0.02$; [b] $\alpha \geq 0.20$

H_0 = data follow a normal distribution.

The results obtained by applying ANOVA test are presented in Table 5 (all 6 crosses as well as Strk.×Lib. cross excluded).

ANOVA: All Crosses

Source of Variation	SS	df	MS	F^*	p
All crosses					
Between Groups (Crosses)	5.1046	5	1.0209	3.8374 [a]	0.0022
Within Groups (Errors)	78.2181	294	0.2660		
Total	83.3227	299			
Strk.×Lib. excluded					
Between Groups (Crosses)	4.7387	4	1.1847	4.5348 [b]	0.0015
Within Groups (Errors)	64.0043	245	0.2612		
Total	68.7429	249			

[b] $F_{critic(5\%)} = 2.2447$; [d] $F_{critic(5\%)} = 2.4085$;
SS = sum of squares; df = degrees of freedom; MS = mean of squares;

The results obtained when pairs of means were compared are presented in Table 6. Two homogenous subsets in terms of means were identified:

÷ Tukey HDS test: ▪ Strk.×GDs. - Strk.×Fl.- GDs.×Lib. (p = 0.139); ▪ Strk.×Fl.- GDs.×Lib. - GDs.×Fl. - Strk.×Lib. - Lib.×Fl. (p = 0.246).

÷ Duncan test: ▪ Strk.×GDs. - Strk.×Fl. (p = 0.072); ▪ Strk.×Fl.- GDs.×Lib. - GDs.×Fl. - Strk.×Lib. - Lib.×Fl. (p = 0.050)

Multiple Comparisons (Tukey HSD test): Results

(I) Cross	(J) Cross	Mean Difference (I-J) [95%CI of difference]	Sig.
Strk.×GDs.	Strk.×Lib.	-0.3260 [-0.6219 − -0.0301]	0.021
	Strk.×Fl.	-0.1860 [-0.4819 − 0.1099]	0.465
	GDs.×Lib.	-0.2540 [-0.5499 − 0.0419]	0.139
	GDs.×Fl.	-0.3096 [-0.6055 − -0.0137]	0.034
	Lib.×Fl.	-0.4118 [-0.7077 − -0.1159]	0.001
Strk.×Lib.	Strk.×Fl.	0.1400 [-0.1559 − 0.4359]	0.753
	GDs.×Lib.	0.0720 [-0.2239 − 0.3679]	0.982
	GDs.×Fl.	0.0164 [-0.2795 − 0.3123]	1.000
	Lib.×Fl.	-0.0858 [-0.3817 − 0.2101]	0.961
Strk.×Fl.	GDs.×Lib.	-0.0680 [-0.3639 − 0.2279]	0.986
	GDs.×Fl.	-0.1236 [-0.4195 − 0.1723]	0.838
	Lib.×Fl.	-0.2258 [-0.5217 − 0.0701]	0.246
GDs.×Lib.	GDs.×Fl.	-0.0556 [-0.3515 − 0.2403]	0.995
	Lib.×Fl.	-0.1578 [-0.4537 − 0.1381]	0.645
GDs.×Fl.	Lib.×Fl.	-0.1022 [-0.3981 − 0.1937]	0.921

Genetic analysis. The results obtained in analysis of general and specific combining ability of the apple growth vigor are presented in Table 7. The estimates GCA and SCA effects are presented in Table 8.

General and specific combining abilities: results

Source	SS	df	MS	F (p)
General combining ability (GCA)	0.0872	3	0.0291	5 ($1.14 \cdot 10^{-3}$)
Specific combining ability (SCA)	10.1000	2	5.0500	949 ($5.48 \cdot 10^{-129}$)
Error	1.5644	294	0.0053	

SS = sum of squares; df = degree of freedom; MS = mean squares; F = F-value; p = p-value
$\sigma = 0.0053$; $\sigma_g^2 = (MSg-MSs)/(p-2) = -2.5105$ ($=V_{NA}$); $\sigma_s^2 = (MSs-MSe) = 5.0447$ ($=V_A$)

Tab. 8

Estimates of GCA (g_i) and SCA (s_i) effects

♀/♂	Strk.	GDs.	Lib.	Fl.	g_i [a]
Strk.		1.0420	1.3680	1.2280	-0.1159
GDs.	1.0420		1.2960	1.3516	-0.0901
Lib.	1.3680	1.2960		1.4538	0.1241
Fl.	1.2280	1.3516	1.4538		0.0819
				DL(5%) = 0.0880; DL(1%) = 0.1157	
♀/♂	Strk.	GDs.	Lib.	Fl.	s_i
Strk.		-0.0420	0.0699	-0.0279	0.001054
GDs.	-0.0420		-0.0279	0.0699	0.001054
Lib.	0.0699	-0.0279		-0.0420	0.001054
Fl.	-0.0279	0.0699	-0.0420		0.001054

An intraclass correlation coefficient of 0.059 (95%CI [0.000 - 0.412]) was obtained when average measures were compared while a value of 0.010 (95% CI [0.000 - 0.104]) was obtained when single measures were included in analysis.

Genetic coefficients of variation computed for the apple tree growth vigor proved to have the following values:

÷ $CV_A = 100\sqrt{(V_A)}/m = 100*sqrt(5.0447)/1.2899 = 174.12\%$

÷ $CV_G = 100\sqrt{(V_G)}/m = 100*sqrt(5.0447+(-2.5105))/1.2899 = 123.41\%$

÷ $CV_P = 100\sqrt{(V_P)}/m = 100*sqrt(2.5395)/1.2899 = 123.54\%$

The heritability coefficients had the following values:

÷ Broad-sense heritability: $H^2 = 2.5342/2.5395 = 0.9979 (\sim 100\%)$

÷ Narrow-sense heritability: $h^2 = 5.0447/2.5395 = 1.98$

The GCA:SCA ratio was equal to 0.9162.

The proposed aim of the research was successfully accomplished. The variation of the results in analysis of quantitative traits when the assumptions of some tests are considering to be true and when the assumptions are tested was identify and analyzed. A guideline for reporting results of quantitative traits analysis for Griffing's experimental method IV, model I was proposed and applied.

The present guideline proposed presentation of experimental data using descriptive statistics parameters and split the inferential statistics in statistic analysis and genetic analysis. The description of the data will provide further information needed to apply the proper statistical test.

At it is well known descriptive parameters are used to describe the experimental data. The analysis of descriptive statistic parameters presented in Table 3 revealed the followings:

÷ Three crosses proved to provided the smaller hybrids: Strk.×GDs., Strk.×Fl., and GDs.×Lib.

÷ The GDs.×Fl. cross proved to provide the highest hybrids.

÷ The ascending order of F_1 hybrids in terms of average of height was as followe: Strk.×GDs. − Strk.×Fl. − GDs.×Lib. − GDs.×Fl. − Strk.×Lib. − Lib.×Fl.

÷ The most homogenous F_1 hybrids were obtained by Lib.×Fl. (smallest distance from the average value).

÷ It is expected that Strk.×GDs. hybrids to be statistically smaller compared to Strk.×Lib., GDs.×Fl. and Lib.×Fl. (the 95% confidence intervals did not overlap on each other).

÷ Data resulted from all crosses proved to be relative heterogenous or heterogenous without any exceptions (coefficient of variation higher than 0.30). Relative to their means, the variability of Strk.×GDs. hybrids is higher compared to variability of all other crosses.

The relative variability proved not to be statistically different between crosses since the associated 95% confidence intervals overlap on each other. The apple tree vigor is known to be a polygenic trait (Janick *et al.,* 1977). Moreover, relative variability is known to be dependent by maternal and paternal genitors (Sestraş *et al.,* 2009).

A series of assumptions and/or conditions were applied in order to carry out statistic and genetic inferential analysis. The analysis of test used to verify assumptions and/or conditions are as follow:

÷ Experimental data proved to be normal distributed with one exception, the hybrids resulted from Strk.×Lib. cross. For these hybrids, the Chi-Squared test (Table 4) rejected the null hypothesis of normality with a probability of 1.22%. Since just one test out of tree reject the null hypothesis and since it is well known that the Chi-Squared is a conservative test proper to be used if the sample sizes are higher than 50 (Riffemburgh, 2006), all experimental data were considered normal distributed.

÷ Bartlett and Leven test showed that the variances of experimental data are homogenous. This result is in agreement with the results obtained when 95% confidence intervals for relative variability are compared.

The analysis of the results obtained when statistical inference was applied on experimental data revealed the followings.

÷ The test was proper to be applied for comparing means as well as for comparing variances since the assumptions proved to be true.

÷ A significantly statistic test was obtained when all hybrids were analyzed as well as when the hybrids for which the Chi-Squared test reject the normality (see Table 5). The probability to be in error was of 2.2% and decrease to 1.5% when the data for which the Chi-Squared test rejects the normality were withdrawn.

÷ The variances proved to be significantly different.

The comparison of height means reveled that the Strk.×GDs. F_1 hybrids were significantly smaller compared to Strk.×Lib. (p = 0.021), GDs.×Fl. (p = 0.034), and Lib.×Fl. (p = 0.001) F_1 hybrids. No other significantly differences between height means could be identified on the analyzed sample.

The analysis of genetic inference results revealed the followings:

÷ General and specific combining abilities influence significantly the vigor of apple trees.

÷ The vigor inheritance is more of non-additive nature (dominance and interaction, specific combining ability with smallest probability of being in error) compared to additive nature.

÷ The F_1 hybrids of Liberty and Florina proved to have a higher vigor (positive value of Florina and Liberty effects on F_1 hybrids in terms of general combining abilities) while Starkrimson and Goldenspur provide hybrids with smaller vigor (negative g_i values).

÷ Heredity and fixation of small vigor is observed at Starkrimson.

÷ The broad sense heritability proved to have a high value (almost 100%), unfortunately this result is useful just for the analyzed data (it is not correct to generalize this result) (Hallauer, 2007).

÷ The values of genetic coefficients of variation showed that the additive effect is stronger compared to non-additive effect in inheritance of vigor.

÷ The value of GCA:SCA ratio (0.9162) indicates a high importance of additive effect in inheritance of vigor for apple trees.

In terms of relationship between statistic and genetic analysis for Griffing's experimental method IV, model I the following conclusion could be drawn:

÷ Descriptive statistics parameters bring information about experimental data in terms of description. Correct interpretation of an estimator is proper to be done taking into consideration also its 95% confidence interval. This interval could show if a test is useful to be applied for comparing two estimators.

÷ Inferential statistic and generic inference did not provide the same information. Since the inferential statistic provide information regarding means and variables, genetic inference provide information about additive or non-additive factors for a quantitative trait inheritance.

÷ The absence of significance in statistic inference did not mean the absence of significance in genetic analysis.

÷ The homogeneity test for variances proved to give the same results as analysis of confidence interval of coefficient of variation.

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