Management and evaluation of the vegetative growth of three pumpkin materials (*Cucurbita moschata* Duch. ex. Lam) by non-parametric methods and bootstrap

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Abstract

The morphological diversity of the fruits of *Cucurbita moschata* Duch. ex. Lam can influence the yield and quality of this important crop in Peru, Venezuela and other countries. This research aims to evaluate the vegetative growth of three plant materials of *C. moschata* Duch. ex. Lam with differences in the shape of their fruits (globular, lageniform and oblong) using non-parametric methods for the analysis of longitudinal data (*ANOVA-type* and *Wald-type* statistics). and bootstrap on morphological characteristics. The empirical distribution and type I error of range-based statisticians were determined and compared with parametric procedures. The results showed; robustness of the *ANOVA-type* statistic and its control over the type I error rate for effects where time is involved as a subplot, consistency of the *Wald-type* estimator for sample sizes *nj=60*, and the advantages of the *ANOVA-type* statistic for multiple comparisons of morphological characteristics of *C. moschata* Duch. ex. Lam versus parametric procedures, for sample sizes *nj=12,* data with asymmetric distribution and discrete variable. Differences were observed in stem diameter at the beginning and end of the test between the lageniform and oblong fruits, in the petiole diameter of the globular fruits, in the lamina length of lageniforms, and between the lamina width of the globular and lageniform fruits.

Keywords: Morphology, fruits, *Cucurbitaceae,* longitudinal data, ranges.

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1. Introduction.

Auyama (*Cucurbita moschata* Duch. ex. Lam) is an important crop in Peru, Venezuela and other countries. However, there is morphological diversity of the fruits, which is reflected in a variety of shapes and dimensions (Lira 1995), which could influence aspects of behavior, yield, quality at the time of harvest and limit competitiveness for marketing. For this reason, it is important to investigate morphological aspects and growth over time of existing materials. On the other hand, sometimes, in agricultural trials the experimental unit is observed on more than

one occasion in time. Such data are called longitudinal data. In these cases, Noguchi. et al (2012) point out the typical questions that researchers ask: Do treatments have the same effect? Are the effects of the treatments similar over time? Are the treatment profiles parallel? Alternatively, these practical questions can be translated into statistical language as treatment effects, time effects, and interaction effects between treatment and time, respectively. Diggle, Liang, and Zeger (1994) provide a comprehensive overview of existing methods for longitudinal data analysis: generalized linear models (GLMs) and their extensions, generalized mixed linear models (GLMMs), and generalized estimation equations (Breslow and Clayton 1993; Zeger and Liang 1992; Liang and Zeger 1986). However, all of these (semi)parametric procedures are based on model-specific assumptions, for example, the existence of an expectation or homogeneous variations. In practice, such conditions can rarely be verified, and if the observed measurements do not reflect the conditions imposed, for example, in the case of biased distributions, outliers or small sample sizes, parametric statistical procedures can lead to unreliable or even false conclusions (Noguchi. et al 2012). Alternatively, nonparametric range-based methods can be employed that provide a flexible and robust framework for the analysis of a variety of longitudinal studies. In particular, in contrast to parametric procedures for factorial designs, the range-based methodology is not restricted to data on a continuous scale and allows for the systematic analysis of categorical, dichotomous, and highly biased ordered data (Konietschke, Bathke, Hothorn, and Brunner 2010). In addition, these non-parametric methods are robust in the presence of outliers and exhibit competitive performance for small samples (Brunner, Domhof and Langer 2002). If the basic assumptions are not met or are impossible to verify, the conclusions of the parametric method could be unreliable or even false. In such situations, more widely applicable non-parametric approaches are preferred (Brunner et al. 2002; Robson, 2002; Lehmann, 2009 and Romano, 2009). In the case of three auyama materials with differences in the shape of their fruits, vegetative growth was evaluated using non-parametric methods for the analysis of longitudinal and bootstrap data on morphological characteristics measured at the crop between 21 and 49 days after planting (dds).

2. Materials and methods

2.1 Description of the test

The test was carried out in the Marfilar sector of the municipality of Guanare, Venezuela, geographically located between the coordinates 08° 57' 11''; 8° 57' 33'' N and 69° 43' 14''; 69° 43' 29'' W, with annual precipitation averages of 1700 mm, temperature of 26 °C, evaporation of 1800 mm, relative humidity of 76 % (FAV, 2014). In the area there are two well-defined periods, one dry from December to April and another rainy from May to November. The research was conducted during the rainfed period, as a completely random design with three treatments or materials coming from fruits with different shapes: T1: globular fruits, T2: lageniform, T3: oblong (Figure 1.) and four repetitions. The soil used for planting belongs to the Guanare series, moderately deep phase (fmp), with high fertility and suitable for diversified agricultural use (Quintero et al., 1974). Veiga (2001) reported that, according to studies of the Western Soil Office, it presents frank texture (Fa, F, FL, FA) on granzón at a depth of 76-125 cm; is an Inceptisol and is taxonomically classified as *Typic Haplustept* fine frankish.

The preparation consisted of four rake passes. Sowing was done manually, at the rate of three seeds per point. Each of the materials was planted with a distance of 3 m between furrows and 3 m between plants, for a total of six plants per treatment. At 20 days after planting, the most vigorous plant was flattened and left at each point. Fertilization was carried out with 40g per plant of fertilizer complete formula (15-15-15), in a fractional way, 20g at 20 and 30 days after planting, according to the recommended specifications for the crop. Weed control was performed chemically with the systemic herbicide Glyphosate in doses of $2L \cdot ha^{-1}$ one week before planting and then manually.

Weekly plant growth measurements were recorded at 21, 28, 35, 42 and 49dds, in order to compare the three materials. Leaf number (NH), stem diameter (DT), petiole diameter (DP), sheet length (LH), leaf blade width (HA), and petiole length (LP) were determined.

Figure 1. Types of auyama fruits (*Cucurbita moschata*). 1. Globular; 2. Lageniformes; 3. Oblong

2.2 Non-parametric model and hypothesis.

Below is the idea of the non-parametric marginal model for longitudinal data (Brunner and Puri, 2001 and Brunner et al. 2002). These authors point out that the factor that stratifies the samples into independent groups is called the whole plot factor; whereas the factor, which stratifies repeated measurements, is called the subplot factor. In repeated measurement designs, time is considered as a subplot factor. Based on the models described by Brunner et al. (2002) for this test we consider the model for the F1-LD-F1 design as shown below:

Suppose different groups of homogeneous subjects are observed repeatedly at different time points and each group receives a randomly assigned treatment (treatment *1*, treatment *2*, ..., treatment a). The statistical model underlying this design can be described by independent random vectors$X_{ik} = (X_{ik1}, \dots, X_{ikt})^T$, , with marginal distributions. The total number of observations is $k = 1, \dots, n_i X_{iks} \sim F_{is}, i = 1, \dots a; s = 1, \dots, tN{=}n\,.\,t$, where the data structure for this $n = \sum_{i=1}^{a} n_i$F1-LD-F1 design is shown in Table 1.

The assumptions of no effect of factor A, no effect of time T, and non-effect interaction (AT) between A and T are expressed in terms of the marginal distribution functions:

$$H_0^F(A): \bar{F}_{1.} = \cdots \bar{F}_{a.}$$
$$H_0^F(T): \bar{F}_{.1} = \cdots \bar{F}_{.t}$$
$$H_0^F(AT): F_{is} = \bar{F}_{i.} - \bar{F}_{.s} + \bar{F} \dots, i = 1, \dots, a; s = 1, \dots, t$$

Where denotes the average distribution over time for treatment, denotes the average distribution over treatments over time, and, denotes the overall mean distribution. Note that hypotheses for classical (parametric) linear longitudinal models are expressed in the same way as hopes.$\bar{F}_{i.} = \frac{1}{t}\sum_{i=1}^{t} F_{is}\, i, i = 1 \dots, a\bar{F}_{.s} = \frac{1}{a}\sum_{i=1}^{a} F_{is}\, s, s = 1, \dots, t\bar{F}_{.s} = \frac{1}{at}\sum_{i=}^{q}\sum_{s=1}^{t} F_{is}\, \mu_{is}$

Table 1. F1-LD-F1 design and corresponding marginal distributions.

		Data			Marginal distributions		
		Time Factor (T)			Time		
Factor A	Experimental unit	$s = 1$	...	$s = t$	$s = 1$	...	$s = t$
$i = 1$	$k = 1$	X_{111}	...	X_{11t}	F_{11}	...	F_{1t}
	⋮		⋮			⋮	
	$k = n_1$	X_{1n_11}	...	X_{1n_1t}	F_{11}	...	F_{1t}
⋮	⋮		⋮			⋮	
$i = a$	$k = 1$	X_{a11}	...	X_{a1t}	F_{a1}	...	F_{at}
	⋮		⋮			⋮	
	$k = n_a$	X_{an_a1}	...	X_{an_at}	F_{a1}	...	F_{at}

2.3 Non-parametric effects, estimators and hypothesis testing.

According to Noguchi et al. (2012), the model of this design class does not imply any parameter by which a difference between the distributions can be described. Therefore, the mean distribution function $H(x) = \frac{1}{t}\sum_{s=1}^{t} F_s(x)$ and distribution functions are used to define a treatment effect as a marginal summary measure between and $F_s(x)HF_s$:

$$p_s = \int HdF_s = P(Z < X_{1s}) + 0.5P(Z = X_{1s}), s = 1, \dots, t,$$

Where denotes a randomly chosen observation of the entire dataset independently of. These so-called relative marginal effects can be thought of as the probability that a randomly chosen observation at the point of time $Z{\sim}HX_{1s}p_sX_{sk}s$ will result in a value greater than Z. The interpretation of is quite simple; that is, it tends to result in: A value less than , if , a value greater than , if , a value neither greater nor less than , if $.p_sX_{1j}X_{2s}p_j < p_sX_{2s}p_j > p_sX_{2s}p_j = p_s$

Unknown quantities can be estimated with the general ranges of the data by replacing all observations with their general ranges. In addition, let denote the mean of the ranges in the marginal sample $p_sX_{11}, \dots, X_{ns}R_{11}, \dots, R_{ns}\bar{R}_{.s} = \frac{1}{n}\sum_{k=1}^{n} R_{sk}s$. Then, an asymptotically unbiased and consistent estimator is given byp_s

$$\hat{p}_s = \frac{1}{N}\left(\bar{R}_{.s} - \frac{1}{2}\right), s = 1, \dots, t$$

Where.$N = n.t$

To test the hypothesis, suppose $H_0^F: F_1 = \cdots F_t that\ C$ denotes a contrast matrix, the distribution vector, the relative marginal effects vector, and the vector of the respective estimators. In addition, it denotes the empirical variance-covariance matrix of the ranges, observing that it is a consistent estimator. Then, under the hypothesis, the Wald-type statistic (WTS)$F = (F_1, \dots, F_t)^T p = (p_1, \dots, p_t)^T \hat{p}\hat{V}_n\hat{V}_n H_0^F: CF = 0$

$$Q_n(C) = n\hat{p}^T C^T\left[C\hat{V}_n C^T\right]^+ C\hat{p}$$

It has, asymptotically, a chi-square distribution with degrees of freedom under certain conditions of regularity (Akritas, Arnold and Brunner 1997). Here it denotes the Moore–Penrose inverse of na matrix $\left(\chi_f^2\right)f = rango(C)M = \left[C\hat{V}_n C^T\right]^+ M.$

It is well known that the convergence of the distribution to its limit distribution is relatively slow (Akritas et al. 1997; Akritas and Brunner 1997; Brunner, Munzel and Puri 1999; Brunner et al. 2002). In general, the asymptotic approach deteriorates with an increase of various levels of factors, as well as for smaller sample sizes. Therefore, a small sample modification of this test statistic is also presented that maintains an exact test size even for small samples.$Q_n\chi_f^2(n \geq 7)$

Suppose it denotes the projection matrix obtained from C where it $T = C^T[CC^T]^- CM^-$ denotes the generalized inverse of. M Then, the distribution of the ANOVA-type statistic (ATS)

$$A_n(C) = \frac{n}{tr(T\hat{V})}\hat{p}^T T\hat{p}$$

It can be approximated by the distribution $F_{(\hat{f},\infty)}$, where , (Brunner, Dette, and Munk 1997; Brunner et al. 1999; Brunner and Puri 2001).$\hat{f} = \left[tr(T\hat{V})\right]^2 / tr(T\hat{V}T\hat{V})$

For the main effects of the factors of the main parcel and the interactions involving only the factors of the main parcel, the distribution of ATS can be further approximated by applying a finite denominator for the degrees of freedom, i.e. by distribution, taking advantage of the diagonal variance-covariance matrix resulting from the independence of the subjects, this is what is known as modified $\hat{f}_0 F_{(\hat{f},\hat{f}_0)} ATS$ (Brunner et al. 1999; Brunner et al. 2002).

2.4 Analysis of data with the R nparLD package.

The statistical analyses described above were performed with the help of R's nparLD (Nonparametric Longitudinal Data Analysis) package. The package provides an nparLD() function that automatically identifies the most appropriate design through the formula provided by the user. The nparLD() function creates a class object called nparLD from which users can get short and extended summaries, as well as a graph of the results using print(), summary(), and plot() for the nparLD object, respectively. In particular, the print() function shows basic results on the model formula and the results of the *Wald-type* and *ANOVA-type* statistics; the summary()

function shows the relative treatment effects, in detail in addition to the output provided by print(). Finally, plot() creates graphs of the relative effects of treatment and their corresponding confidence intervals at different time points (Noguchi et al. 2012).

2.5 Empirical distribution of *the Wald-type* and *ANOVA-type statistics*

Based on the sample of data related to the growth of three plant materials of *C. moschata* Duch. ex. Lam, the bootstrap method was used to determine the empirical distribution of the *Wald-type* and *ANOVA-type* statistics. This method was proposed by Efron (1979) and Efron and Tibshirani (1993). The bootstrap allows to approximate the sampling distribution of a statistician and its properties by means of a very simple procedure: Create a large number of samples with replenishment of the observed data. Remember that the sampling distribution of a statistician is key when performing statistical inference tasks (Ledesma, 2008). At the conceptual level, bootstrap can be included within the larger approach to data *resampling*, which comprises a variety of techniques that implement computer simulation processes to estimate empirical probabilities from experiments with the data itself (Rudner and Shafer, 1992). One of the advantages of using this resampling technique lies in the fact that it is not demanding in terms of compliance with theoretical assumptions for its application and is, in this sense, *less restrictive than* conventional techniques. To begin with, it is possible to dispense with assumptions related to distributions, since, instead of assuming a priori a certain theoretical distribution, the original sample is used and a large number of sub-samples are generated that serve as the basis for inductively estimating the shape of the sample distribution of the statisticians (Ledesma, 2008).

2.6 R code for applying the bootstrap method to *C* growth data. *moschata* Duch. ex. Lam

Below is the R code for applying the nparLD package to determine the empirical distribution of the *Wald-type* and *ANOVA-type* statistics using the bootstrap method from data related to vegetative morphological characteristics of *C* plants. *moschata* Duch. ex. Lam.

```
set.seed(1)
indices.train <- sample(x = nrow(datos), size = 0.5*(nrow(datos)), replace = FALSE)
datos.entrenamiento <- datos[indices.train,]
datos.test <-datos[-indices.train,]
library(nparLD)
ex.f1f1np <- nparLD(DT~tiempo*tratamiento,subject="ue",data=datos,description=FALSE)
ex.f1f1np
T=ex.f1f1np<- nparLD(Y~tiempo*tratamiento,subject="ue",data=datos,description=FALSE)$Wald.test[3]
n=nrow(datos)
gl.WTS=ex.f1f1np <- nparLD(Y~tiempo*tratamiento,subject="ue",data=datos,description=FALSE)$ANOVA.test[6]
gl.numATS=ex.f1f1np <- nparLD(Y~tiempo*tratamiento,subject="ue",data=datos,description=FALSE)$ANOVA.test[6]
n1=
n2=
n3=
meanstar1=mean(datos$Y[1:n1])
meanstar2=mean(datos$Y[n1:n1+n2])
meanstar3=mean(datos$Y[n1+n2:n1+n2+n3])
sdstar1=sd(datos$Y[1:n1])
sdstar2=sd(datos$Y[n1:n1+n2])
sdstar3=sd(datos$Y[n1+n2:n1+n3])
R =
Fstar=numeric(R)
for(i in 1:R){
groupA=rnorm(n1,mean=meanstar1,sd=sdstar1)
groupB=rnorm(n2,mean=meanstar2,sd=sdstar2)
groupC=rnorm(n3,mean=meanstar3,sd=sdstar3)
simY=c(groupA,groupB,groupC)
simtratamiento = datos$tratamiento
simtiempo=datos$tiempo
simue=datos$ue
```

```
simdata =
data.frame(simY,simtiempo,simtratamiento,sim
ue)

library(nparLD)

Fstar[i]=nparLD(simY~simtiempo*simtratamie
nto,subject="simue",data=simdata,description=F
ALSE)$Wald.test[3]

}

Pstar = numeric(R)

for(i in 1:R){

groupA=rnorm(n1,mean=meanstar1,sd=sdstar1
)

groupB=rnorm(n2,mean=meanstar2,sd=sdstar2
)

groupC=rnorm(n3,mean=meanstar3,sd=sdstar3
)

simY=c(groupA,groupB,groupC)

simtratamiento = datos$tratamiento

simtiempo=datos$tiempo

simue=datos$ue

simdata=data.frame(simY,simtiempo,simtratami
ento,simue)

library(nparLD)

Pstar[i]=nparLD(simY~simtiempo*simtratamie
nto,subject="simue",data=simdata,description=F
ALSE)$ANOVA.test[7]

}
```

3. Results and discussion

Table 2 shows the results of the verification of assumptions of the model (normality and sphericity of the variance-covariance matrix) on vegetative morphological characteristics of three plant materials of *C. moschata* Duch. ex. Lam. There it is observed that the variance-covariance matrix for all variables does not meet the sphericity assumption. Likewise, it is observed that all the variables, except the number of leaves, show a tendency to normality in the distribution of residues (see Table 2 and Figure 2). The aforementioned results suggest the use of less restrictive tests in relation to compliance with the assumptions of the model, for example, non-parametric methods based on ranges for the analysis of longitudinal data as referred to by Konietschke *et al.* (2002). Figures 3-8 show the box diagrams (left panel) and the relative effect profiles of the material type (right panel) of a test to evaluate vegetative morphological characteristics from three plant materials of *C. moschata* Duch. ex. Lam. Box charts show that the data follows a skewed distribution. Likewise, there is evidence of an increase in the variables in all materials over time, but less pronounced for the NH, especially in the first measurements, In the *Cucurbita* plants the initial growth of the leaves was slow. Vega *et al.* (2012) suggested that this is because plants devote their early stages of development to establishing their photosynthetic machinery, in direct competition with radical development, i.e. they grow simultaneously and in synchrony so that a harmonious root/aerial part development occurs. Valnir *et al*. (2012) found similar results; a slow initial growth for the number of C. *moschata* leaves and explained that this was because the seedlings spent much of their energy in the process of fixing to the ground and then began to emit more leaves and branches. Also these graphs indicated that for DT there was a sustained growth with less variation, while AH, LH, LP and DP, had a rapid increase during the first two measurements at 21 and 28 dds, but then decreased in the final measurements (35; 42 and 50 dds), which could be due to the fact that the stem is an organ of indeterminate growth that has apical and lateral meristems and remains longer in the plant to contribute to the transport of photo assimilated and the formation and support of leaves, flowers and fruits, while the leaves are organs of determined growth that when they reach their maximum dimensions become senescent and the process of abscission occurs generating the fall of the same.

The relative marginal effects graphs of the six variables show that the profiles of the three materials are parallel. As suggested by Noguchi et al. (2012), parallel profiles of these relative effects indicate absence of interaction time*treatment, in this case, the three materials from *C. moschata* Duch. ex. Lam show similar growth over time. This could be due to several factors that, according to several authors, may be: characteristics of the adaptation of the species to wide altitudinal intervals (Lira 1995), as well as certain morphological and physiological factors such as the rapid and indeterminate growth of plants and their developmental plasticity (Tsivelikas et al. 2009). However, Hazra et al.

(2007) reported that morphological variables such as internode length and stem diameter have been useful for the characterization of materials.

When comparing these three materials in relation to vegetative morphological characteristics, the results presented in Table 3 show the non-parametric procedures based on the *Wald-type* and *ANOVA-type* statistics to test the hypothesis of "no interaction" between the material and time formulated in terms of the distribution functions, which helps us to infer about the possible effect of the material on the growth of this species. There it is observed that the hypothesis of non-interaction, that is, parallel material profiles, is accepted ($p > 0.05$), for both *Wald-type* and *ANOVA-type* statistics in relation to the variables number of sheets, petiole diameter, petiole length, sheet length and sheet width. However, in relation to the diameter of the stem, both statisticians report different results in relation to the hypothesis of "no interaction", in this case, the *Wald-type* statistic indicates that the interaction between the material and time is significant ($p < 0.01$), while the *ANOVA-type* statistic indicates that the interaction is not significant ($p > 0.05$). The results of the *Wald-type* and *ANOVA-type* statistics indicate that material type and time have a significant effect ($p < 0.01$) on leaf number, stem diameter, petiole diameter, sheet length and sheet width. However, in relation to the length of the petiole, both statisticians report different results in relation to the hypothesis of "no effect of material" and "no effect of time". In this sense, the *Wald-type* statistic indicates that the type of material does not have a significant effect ($p > 0.05$) on the length of the petiole, while the *ANOVA-type* statistic indicates that time does not have a significant effect ($p > 0.05$) on this variable. These results show the possible effect of the sample size on the *Wald-type* and *ANOVA-type* statistics, especially in the case of the *Wald-type* statistic, which as Pointed out by Akritas et al. (1997); Akritas and Brunner (1997); Brunner, Munzel and Puri (1999); Brunner et al. (2002) the convergence of the distribution of its limit distribution is relatively slow, so its use is recommended for large sample sizes ($n>30$).$Q_n\chi_f^2$

Table 2. Evaluation of assumptions of normality and sphericity of the variance-covariance matrix on vegetative morphological characteristics of plants of three materials of *C. moschata* Duch. ex. Lam.

Variable	Normality (Prueba de Wilk-Shapiro)		Sphericity of the variance-covariance matrix (Mauchly Village)	
	W	P valor	W	P valor
Number of sheets	0.79865	< 0.00001	8.5931e-09	< 0.00001
Stem diameter	0.98079	0.01390	4.1403e-05	< 0.00001
Petiole diameter	0.96028	0.00005	2.3753e-05	< 0.00001
Sheet length	0.96349	0.00012	7.0192e-06	< 0.00001
Sheet width	0.95509	0.00007	0.0002579	0.00213
Petiole length	0.96219	0.00008	0.0023576	0.09235

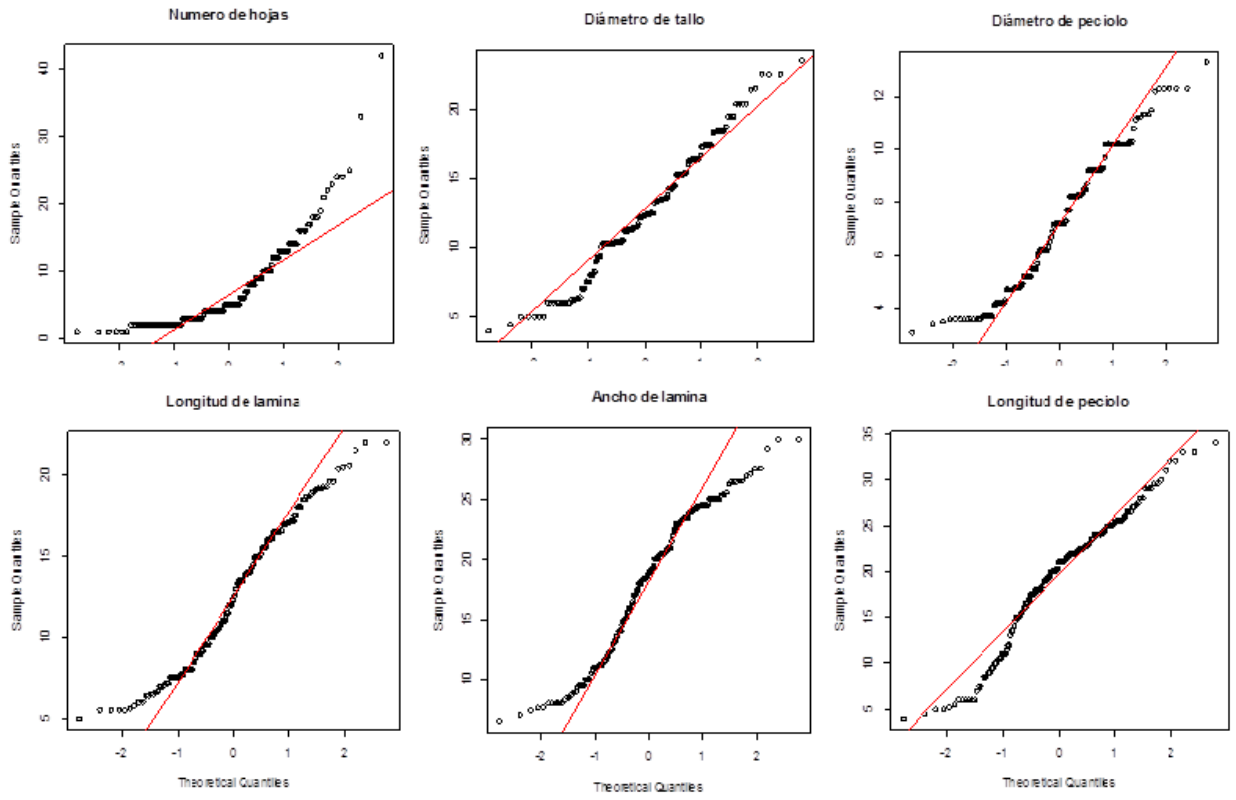

Figure 2. Distribution of residues of vegetative morphological characteristics of plants of three materials of *C. moschata* Duch. ex. Lam.

Table 3. Evaluation of plant growth of three materials of *C. moschata* Duch. ex. Lam based on the statistics *ANOVA-type* and *Wald-type*.

Variable	Effect					
	Material		Time		Material*Time	
	Statistical (*P-value*)		Statistical (*P-value*)		Statistical (*P-value*)	
	Wald-type	*ANOVA-type*	*Wald-type*	*ANOVA-type*	*Wald-type*	*ANOVA-type*
Number of sheets	0,001415	< 0,000001	< 0,000001	0,000281	0,127076	0,147365
Stem diameter	< 0,000001	< 0,000001	< 0,000001	< 0,000001	0,003184	0,114634
Petiole diameter	0,003833	0,002292	< 0,000001	< 0,000001	0,376808	0,892872
Sheet length	0,005544	0,002357	< 0,000001	< 0,000001	0,887229	0,957209
Sheet width	0,007750	0,004131	< 0,000001	< 0,000001	0,272730	0,749856
Petiole length	0,102261	< 0,000001	< 0,000001	0,081318	0,570435	0,723924
n=180; n_j=60; j=1,2,3						

Table 4 shows the results of the *ANOVA-type and Wald-type* statistics to test the "non-interaction" hypothesis between the type of material and time on vegetative morphological characteristics of plants of three materials of *C. moschata* Duch. ex. Lam, obtained by bootstrap. There it is observed that the percentage of times that the hypothesis of "no interaction effect" between the type of

material and time is lower for the procedure based on the *ANOVA-type* statistic is rejected, however, these results are consistent with those shown in Table 3. These results show what Bathke *et al*. pointed out. (2009), who indicate that the procedure based on the *ANOVA-type* statistic is conservative when a sub-parcel factor is involved, in this case, time, as referred to by Noguchi *et al.* (2012) who suggest that such a statistic accurately controls the Type I error rate even for small sample sizes when evaluating treatment interactions*time. On the other hand, Figures 9-14 show the empirical distribution of the bootstrap *Wald-type* and *ANOVA-type* statistics estimated from vegetative plant morphological characteristics of three C. *moschata* Duch. ex. Lam materials to test the hypothesis of "no interaction effect" between material and time. These results show that the *Wald-type* statistic fits a chi-square distribution with degrees of freedom as pointed out by Akritas, Arnold and Brunner (1997), $(\chi_f^2)f = rango(C)$while the *ANOVA-type* statistic fits a distribution $F_{(\hat{f},\infty)}$, where, as referred to by Brunner, Dette, and Munk (1997); Brunner et al. (1999); Brunner and Puri (2001).$\hat{f} = [tr(T\hat{V})]^2/tr(T\hat{V}T\hat{V})$

Table 4. Percentage of times that the procedures based on the *ANOVA-type* and *Wald-type* statistics reject the null hypothesis of "no material effect", "no time effect" and "no interaction" between the type of material and time on vegetative morphological characteristics of plants of three materials of *C. moschata* Duch. ex. Lam, by bootstrap, and variance-covariance matrix is unstructured (UN).

Variable	Effect					
	Material		Time		Material*Time	
	Statistical (*P-value*)		Statistical (*P-value*)		Statistical (*P-value*)	
	Wald-type	*ANOVA-type*	*Wald-type*	*Wald-type*	*ANOVA-type*	*Wald-type*
Number of sheets	0,161	0,145	0,089	0,048	0,162	0,054
Stem diameter	0,377	0,372	0,084	0,051	0,158	0,053
Petiole diameter	0,503	0,515	0,092	0,052	0,155	0,049
Sheet length	0,596	0,621	0,095	0,055	0,155	0,049
Sheet width	0,499	0,476	0,088	0,051	0,155	0,053
Petiole length	0,217	0,194	0,087	0,054	0,155	0,052
n=180; n_j=60; j=1,2,3; R=1000						

Bootstrap replicas (R); Unstructured variance-covariance matrix (UN); Normal distribution of residuals (ε_{ij}).

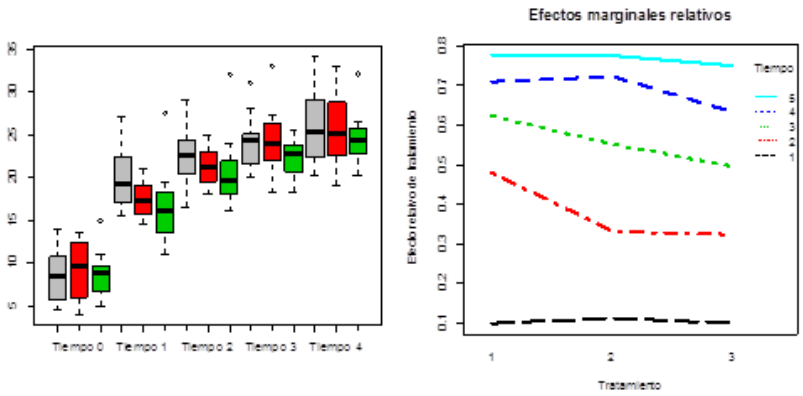

Figure 3. Temporal evolution and relative marginal effects of petiole length of plants of three materials of *C. moschata* Duch. ex. Lam.

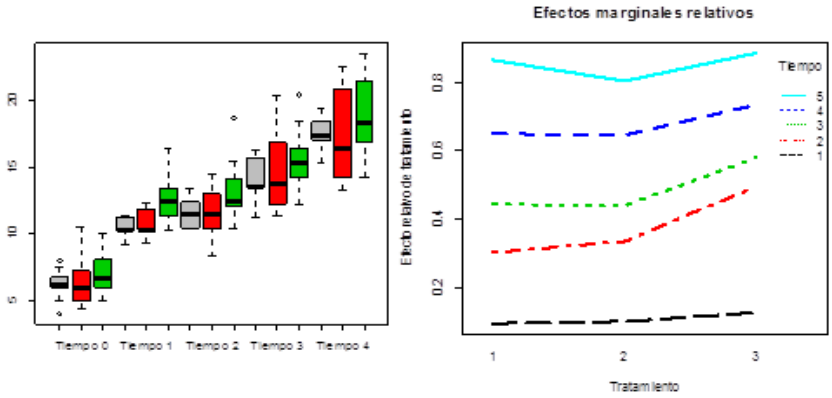

Figure 4. Temporal evolution and relative marginal effects of plant stem diameter of three materials of *C. moschata* Duch. ex. Lam.

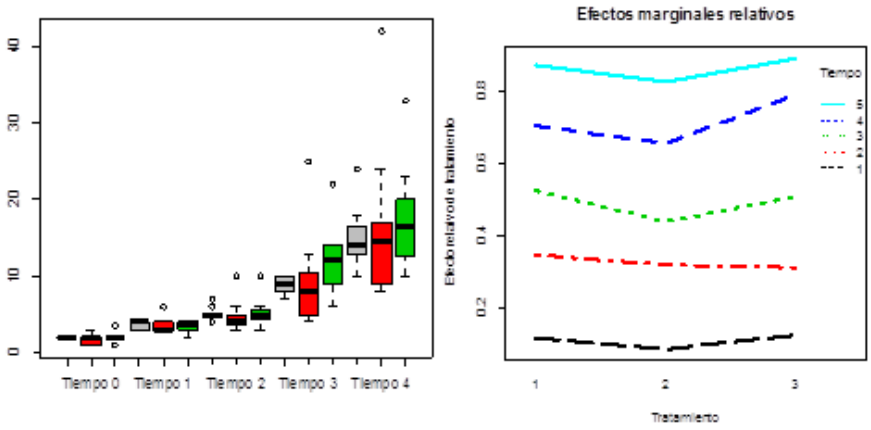

Figure 5. Temporal evolution and relative marginal effects of plant leaf numbers of three materials of *C. moschata* Duch. ex. Lam.

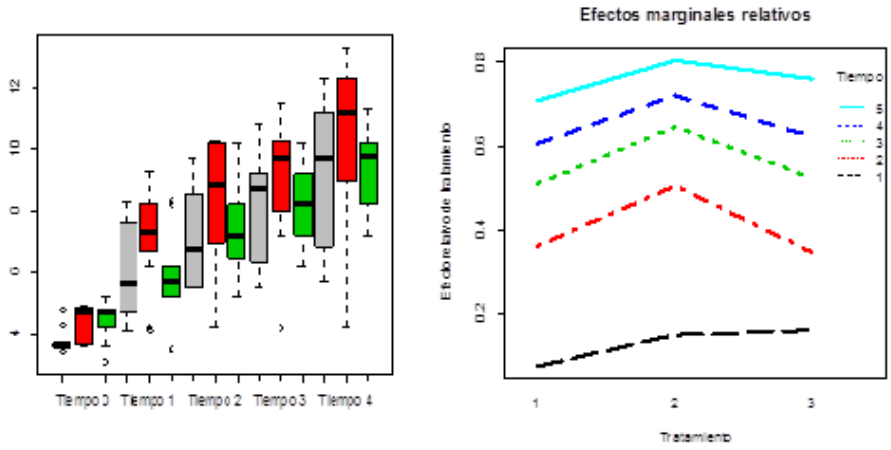

Figure 6. Temporal evolution and relative marginal effects of petiole diameter of plants of three materials of *C. moschata* Duch. ex. Lam.

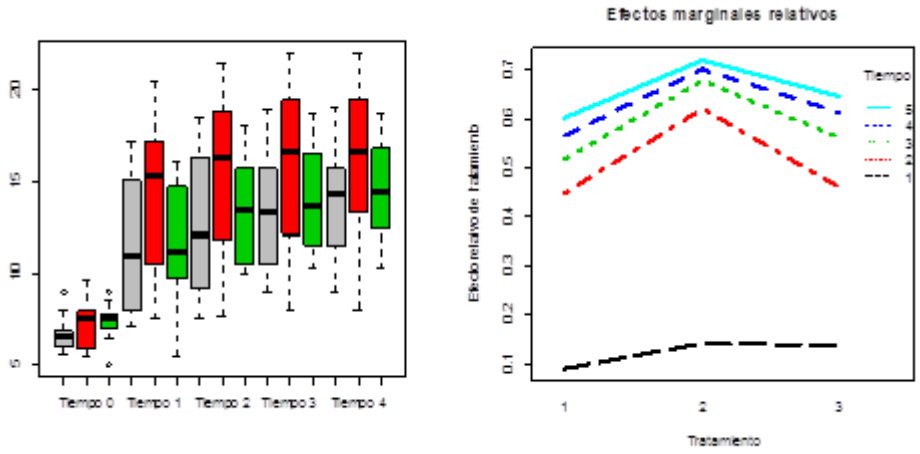

Figure 7. Time evolution and relative marginal effects of plant sheet length of three materials of *C. moschata* Duch. ex. Lam.

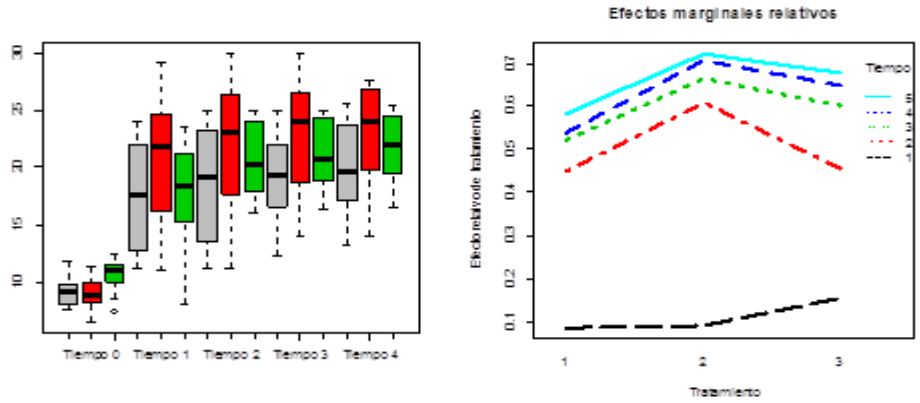

Figure 8. Temporal evolution and relative marginal effects of plant sheet width of three materials of *C. moschata* Duch. ex. Lam.

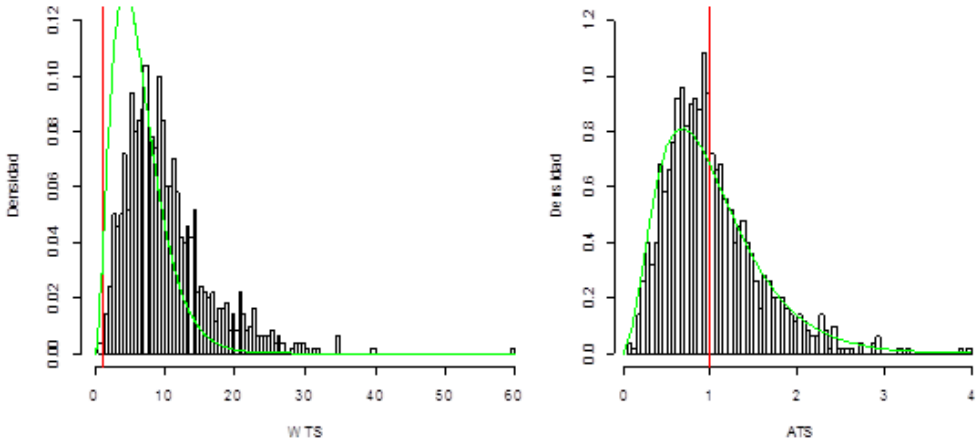

Figure 9. Empirical distribution of the ANOVA-type and Wald-type statistics on the number of plant leaves of three materials of *C. moschata* Duch. ex. Lam by bootstrap.

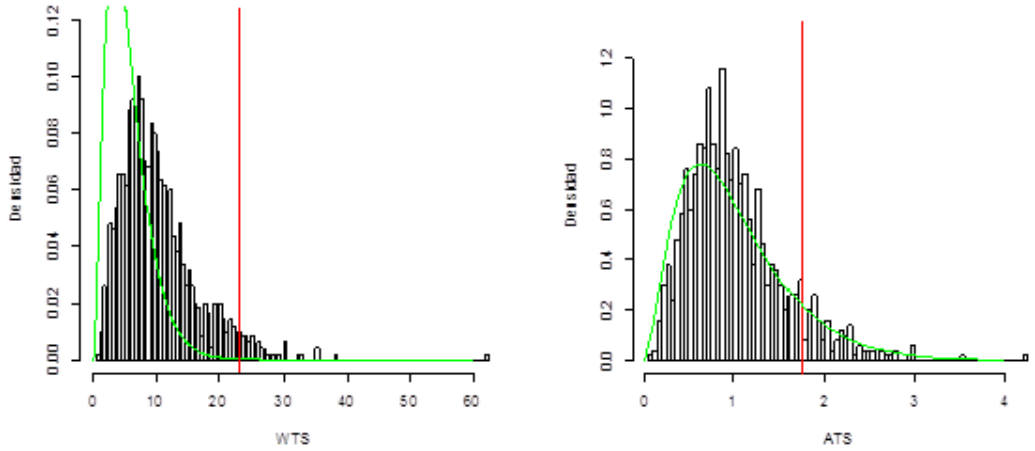

Figure 10. Empirical distribution of the ANOVA-type and Wald-type statistics on the diameter of the plant stem of three C*. moschata* Duch. ex. Lam materials by bootstrap.

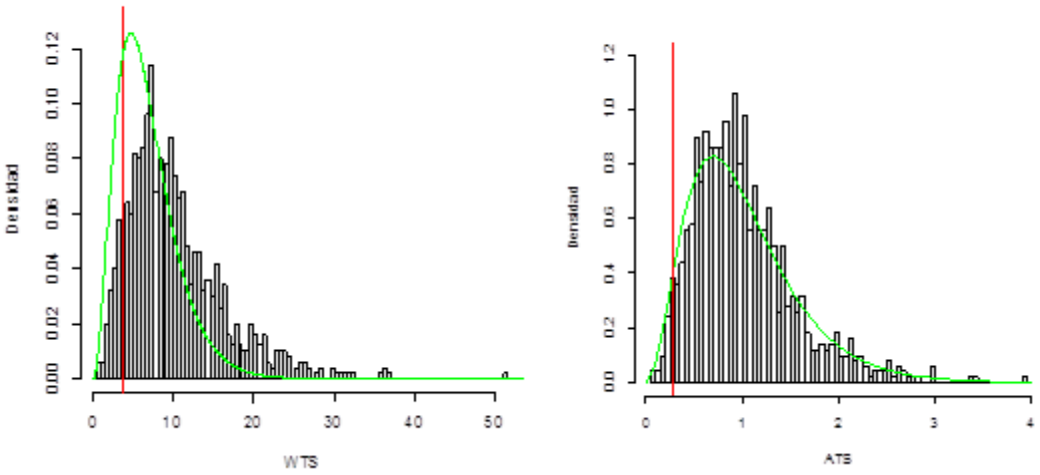

Figure 11. Empirical distribution of the ANOVA-type and Wald-type statistics on the plant sheet length of three C *. moschata* Duch. ex. Lam materials by bootstrap.

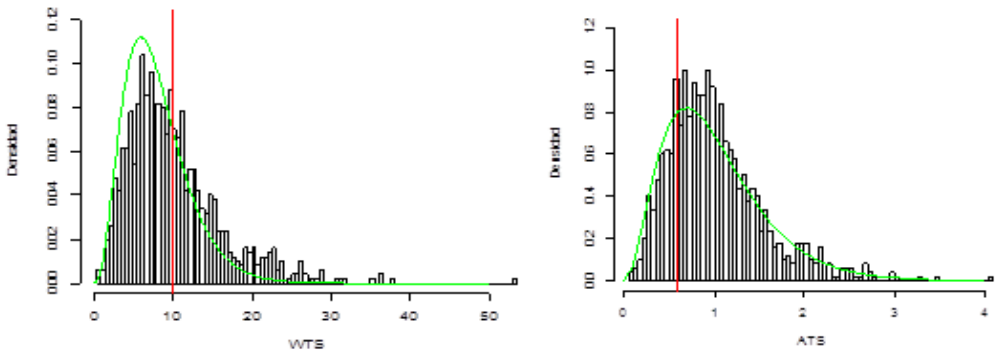

Figure 12. Empirical distribution of the ANOVA-type and Wald-type statistics on the plant sheet width of three C *. moschata* Duch. ex. Lam materials by bootstrap.

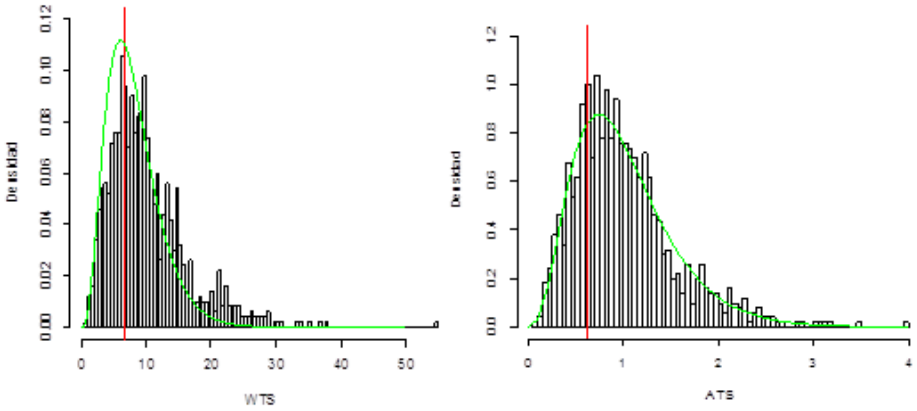

Figure 13. Empirical distribution of the ANOVA-type and Wald-type statistics on the petiole length of plants of three materials of *C. moschata* Duch. ex. Lam by bootstrap.

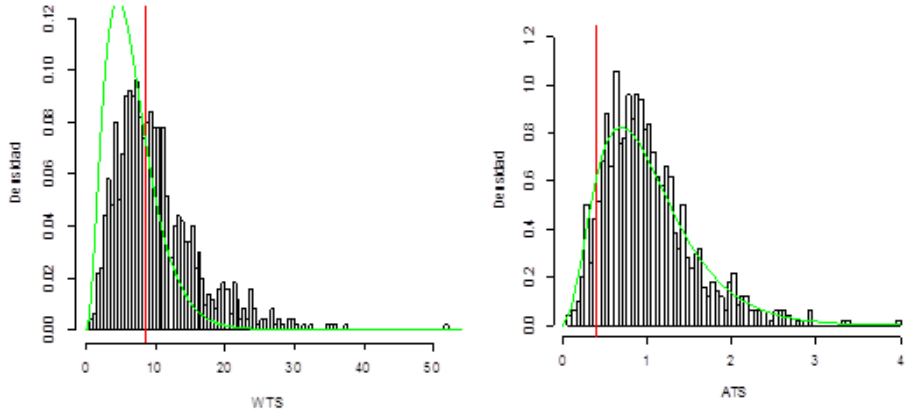

Figure 14. Empirical distribution of the ANOVA-type and Wald-type statistics on the petiole diameter of plants of three materials of *C. moschata* Duch. ex. Lam by bootstrap.

Of the results presented in Table 3 regarding the diameter of the stem, the *Wald-type* statistic showed that the interaction between the material and time was significant ($p < 0.01$); in this case the interaction shown could be determined by the oblong fruit material that consistently showed the highest values during all measurements and also reached the highest in the last measurement in relation to the other two materials. Authors such as Aruah *et al*. (2010) found significant differences for stem diameter, measured at 10 weeks dds, when they evaluated 10 collections of *Cucurbita* in Nigeria, but indicated that growth was very similar for the other variables and Du *et al*. (2011) when analyzing the diversity among 39 lines of *C. moschata* in China, they also reported differences for this variable. In this sense, as suggested by Noguchi et al. (2002) in relation to the use of multiple comparisons with Bonferroni adjustment to infer on which distribution functions differ from each other, Table 5 shows multiple comparisons based on the *ANOVA-type* statistic at each time point with Bonferroni adjustment. There it is observed that, at the beginning (15dds) and at the end (49dds) of the trial differences ($p < 0.05$) were found between the diameter of the stem of the lageniform and oblong fruits, in time 2 and 3 significant differences were reported ($p < 0.05$) between oblongs and the other two types of fruits. At the end of the trial (time 5) significant differences (p

< 0.05) were detected between lageniforms and oblongs. Probably the material of oblong fruits reached faster an adequate and more robust architecture of its plants and therefore had more availability of photosynthesized for the formation, growth and development of fruits; which contributes significantly to the increase in performance, as expressed by Moreno et al. (2016).

The above results (see Table 4) were compared with parametric methods, according to the procedures suggested by Pinheiro *et al*. (2012) regarding the use of the lme() function in the R nlm package (see Table 6). In this regard, both parametric and non-parametric procedures result in similar conclusions regarding the diameter of the stem at each time point, which is acceptable since the data exhibit only a minor degree of asymmetry as indicated by the box graphs (see the left panel of Figure 3). On the other hand, Table 7 and 8 show the comparisons based on the *ANOVA-type* statistic and those based on parametric procedures, respectively, on vegetative morphological characteristics of the three materials regardless of the time with Bonferroni adjustment. The results show significant differences between; the diameter of the petiole of globular fruits in relation to the other two fruits, length of lamina of lageniform fruits with respect to the others, width of the lamina of the globular and lageniform fruits. However, no significant differences were found between the length of the petiole of the three fruits. Similarly, it was observed that both parametric and non-parametric procedures report similar results for the aforementioned variables, except for the number of sheets. This may be a consequence of the scale of the variable (discrete) and the non-normality of the distribution (see Table 2). In this regard, Konietschke *et al*. (2010) refer to the fact that, in contrast to parametric procedures for factorial designs, the range-based methodology is not limited to data on a continuous scale and allows to analyze categorical, dichotomous and highly biased ordered data in a systematic way. These results coincide with those reported by Villegas *et al*. (2015) in relation to the use of statistical *ANOVA-type* to purchase the number of Silver leaves of *Coffea arabica* in an organic fertilization trial.

Table 5. Multiple comparisons based on the *ANOVA-type* statistic on the stem diameter of three plant materials of *C. moschata* Duch. ex. Lam at each time point with Bonferroni adjustment.

Time	Comparison	Hypothesis	P value	P adjusted value
1	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.725522	0.999999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.101089	0.303267
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.035679	0.107037
2	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.6379798	0.999999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.000003	0.000009
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.000376	0.001128
3	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.849788	0.999999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.002183	0.006549
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.004561	0.013683
4	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.999999	0.999999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.030919	0.092757
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.177695	0.533085
5	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.152450	0.457350
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.305919	0.917757
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.024992	0.074976

Table 6. Multiple comparisons based on parametric methods on the diameter of the plant stem of three materials of *C. moschata* Duch. ex. Lam at each time point with Bonferroni adjustment.

Time	Comparison	Hypothesis	P value	P adjusted value
1	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.7387	0.9999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0795	0.2385
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.1435	0.4305
2	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.5662	0.9999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0024	0.0072
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.0069	0.0207
3	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.8949	0.9999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0180	0.0540
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.0138	0.0414
4	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.6862	0.9999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0166	0.0498
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.2959	0.8877
5	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.5917	0.9999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.1200	0.3600
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.1022	0.3066

Table 7. Multiple comparisons based on the *ANOVA-type* statistic on vegetative morphological characteristics of plants of three materials of *C. moschata* Duch. ex. Lam regardless of time with Bonferroni adjustment.

Variable	Comparison	Hypothesis	*P value*	*P adjusted value*
Number of sheets	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.001284	0.003852
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.213848	0.641544
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.000662	0.001986
Petiole diameter	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.000781	0.002343
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.310601	0.931803
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.007388	0.022164
Petiole length	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.268860	0.806580
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.038373	0,115119
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.153097	0.459291
Sheet length	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.000652	0.001956
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.237502	0.712506
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.012159	0.036477
Sheet width	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.001331	0.003993
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.029867	0.089601
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.124905	0.374715

Table 8. Multiple comparisons based on parametric methods on vegetative morphological characteristics of plants of three materials of *C. moschata* Duch. ex. Lam regardless of time with Bonferroni adjustment.

Variable	Comparación	Hipótesis	*P valor*	*P valor* ajustado
Number of sheets	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	1.0000	1.0000
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0036	0.0108
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.1033	0.3099
Petiole diameter	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.001	0.003
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.3204	0.9612
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.0064	0.0192
Petiole length	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.3546	0.9999
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0347	0.1041
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.119	0.357
Sheet length	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.0009	0.0027
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.2429	0.7287
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.0113	0.0339
Sheet width	Globular vs. Lageniformes	$H_0^F: F_1 = F_2$	0.0033	0.0099
	Globular vs. Oblong	$H_0^F: F_1 = F_3$	0.0163	0.0489
	Lageniformes vs. Oblong	$H_0^F: F_2 = F_3$	0.2265	0.6795

4. Conclusions

By bootstrap on morphological characteristics of three plant materials of *C. moschata* Duch. ex. Lam and matrix of variance-unstructured covariance (UN) se evidenced the robustness of the *ANOVA-type* statistic and its ability to precisely control the error rate type I for effects where time is involved as a subplot, in this case, "no effect of time" and "no interaction" between time and material type, as well as the consistency of the *Wald-type* estimator for sample sizes $_{nj}$*=60*, and the asymptotic distribution of the *Wald-type* and *ANOVA-type* statistics, and $\chi_f^2 F_{(\hat{f},\infty)}$, respectively, were verified. The advantages of the *ANOVA-type* statistic as an alternative for multiple comparisons of morphological characteristics of three materials of *C. moschata* Duch. ex. Lam versus parametric procedures were evidenced, for sample sizes *nj=12,* when the distribution of the data is not normal and the variable is not continuous, in this case, the number of leaves, and when the interaction between time and the type of material is significant, in this case the diameter of the stem. In relation to the diameter of the stem, differences were observed at the beginning and end of the trial between the lageniform and oblong fruits. Differences were found between; the diameter of the petiole of globular fruits and the others, length of lamina of lageniformes with respect to the rest of the fruits, and between the width of the lamina of the globular and lageniformes. Finally, the results of this research demonstrate the need for more research to determine the real importance of vegetative morphological variables for the identification or separation of genotypes and for the detection of desirable characteristics to be incorporated to improve promising materials of different species within the genus *Cucurbita* , as well as the robustness of non-parametric procedures for the analysis of longitudinal data, especially discrete variables with asymmetric distribution.

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