Original Article

STATISTICS APPLIED TO PLANT MICROPROPAGATION: A CRITICAL REVIEW OF INADEQUATE USE

ESTATÍSTICAS APLICADAS A MICROPROPAGAÇÃO DE PLANTAS: UMA REVISÃO CRÍTICA SOBRE USOS INADEQUADOS

Vanderley José PEREIRA[1]; Simone Abreu ASMAR[2]; Nádia Giaretta BIASE[3]; José Magno Queiroz Luz[4]; Berildo de Melo[4]

1. Doctoral student in Plant Production, Instituto de Ciências Agrárias - ICIAG, Universidade Federal de Uberlândia - UFU, Uberlândia, MG, Brazil. vamceres.vanderley@gmail.com; 2. PhD in Plant Science, ICIAG - UFU, Uberlândia, MG, Brazil; 3. Professor, FAMAT - UFU, Uberlândia, MG, Brazil; 4. Professor, ICIAG - UFU, Uberlândia, MG, Brazil;

ABSTRACT: Statistical analyses are an essential part of scientific research. Several choices, including the setup of the experiment, influence the selection of statistical procedures. Thus, successful planning implies accurate analysis. We used a 95% confidence interval and a 7% error margin to sample and characterize the statistical techniques used in studies on micropropagation and to discuss the effects of misusing these techniques. We quantified the following: sample size, number of replications, design, scheme (factorial or not factorial) and number of treatments, whether data transformation was used, transformation type and criteria for selection; variable type (quantitative or qualitative); statistical test and regression types. Although statistics were consistently used in these micropropagation experiments, there were several limitations such as small plot sizes, low replication numbers, employing data transformation while neglecting to inform the criteria used or even using the wrong criteria. Although statistical approaches were applied homogeneously, neglecting to use blocking can lead to errors. Blocking is recommended to increase sample size. For example, the times of an experiment or the number of people needed to set up an experiment can be used as blocks. Micropropagation studies typically employ factorial experiments to identify plant regulator types and application rates. Thus, these experiments have numerous treatments. The Tukey test is used for qualitative data while regression models (linear and quadratic) are more frequently used for quantitative data.

KEYWORDS: Experimental designs. Plant biotechnology. *In vitro* experimental planning.

INTRODUCTION

Biotechnology is a synthesis of subjects that emerged with the fusion of different but complementary technological paradigms, including those found in the drug and seed industries (ARBIX, 2007). Biotechnology yields advances in several fields but especially in chemical engineering, which is fundamental to the study of bioprocesses in the pharmaceutical, food, and oil industries. In recent years, biotechnology has also relied on computer engineering (bioinformatics) to develop software with a strong mathematical base that can be used to address the challenges of genetic sequencing and describe relationships between genes and proteins (PAUGH; LAFRANCE, 1997). Moreover, mathematics (especially statistics) is involved in every scientific process and improves the reliability of research results.

Micropropagation is significant because it can be used to exchange genetic material, rescue germplasm, preserve threatened material and multiply propagules that do not germinate under any other condition. Furthermore, the technique reduces germination time, is not affected by pests and yields uniform seedlings (MELO, 2000).

Statistical analysis is fundamental to research because it can be used to estimate experimental error and verify the significance of tested factors (CARDELLINO; SIEWERDT, 1992). However, the inferences made in these experiments must be compatible with the quality of the data obtained (CAEIRÃO, 2006).

Several statistical tests allow researchers to infer experimental results. However, to apply these tests correctly, the types of factors, response variables, treatments, and experimental design need to be known (BERTOLDO, 2008). Usually, the statistical procedure chosen is not ideal for the experiment type, especially when means comparison tests are applied to quantitative factors such as application rates, concentrations, densities, etc. (CAEIRÃO, 2006).Inappropriate selection of analysis type may hinder the interpretation of the experimental data by limiting inferences or even producing misleading results (CARDELLINO; SIEWERDT, 1992).

To define protocols, micropropagation studies test numerous treatments and application rates. These experiments are costly and time-consuming, and yet the results are contradictory with non-accurate data analyses. Data obtained from *in vitro* cultures usually present problems that make them difficult to analyze. Typically, the

Received: 12/06/17
Accepted: 15/06/18

variables in this type of experiment are not measured on a continuous scale (MIZE et al., 1999). For instance, measurements of seedlings under glass are inaccurate and require the establishment of data categories. In other cases, the sheer number of variables and levels within each variable mean that not all combinations are tested, resulting in unbalanced data. However, in most cases, standard analyses (ANOVA) are applied to these problematic data types, which lead to misinterpretations (IBAÑEZ, 2003). Sample size (*n*) is another limitation in micropropagation, but few references specifically address this subject. Apparently, experiments are sized according to material availability or researcher experience (MORAIS et al., 2014).

Micropropagation studies that use statistics correctly are stand outs in the scientific literature. Ibanez (2003) used a case study to demonstrate the feasibility of using generalized linear models instead of statistical analysis in cases where ANOVA was not appropriate. Compton (1994) discussed different parameters for the correct use of statistics. Kuklin et al. (1993) reported the use of incomplete block designs. Compton and Mize (1999) explained factors related to experiment planning. In summary, these studies are references for the correct use of statistics in micropropagation. Nevertheless, information on the most frequent techniques has not been reported.

Given the importance of micropropagation to modern agriculture, studies on this topic should be accurate; implying that means comparison tests and other statistical procedures must be used correctly. To this end, we characterized the statistics used in micropropagation studies and discuss the consequences of misusing these tools.

MATERIAL AND METHODS

Given the sheer number of yearly publications, our sampling strategy admitted a 95% confidence interval to estimate results for the entire research population. An additional7% margin of error was stipulated for the number of papers collected. Thus, the expression used to calculate the sample size was:

$$n = \frac{Z^2 \hat{p}(1-\hat{p})}{e^2} \qquad 1]$$

Where n is the calculated sample; Z is a standardized normal variable associated with the confidence level; $\hat{P}$ is the probability estimate of an event and e is the sampling error. Here, it is assumed that the upper limit of $\hat{p}(1-\hat{p})$ is 0.25, which occurs when $\hat{p}$ is equal to 0.5. Furthermore, Z equals 1.96 at a significance level of 5%, meaning that there is a 95% probability that the confidence interval will contain the real population mean and 5% probability that the confidence interval would be outside the confidence interval.

Equation [1] showed that the sample size should include 196 papers (248 experiments) on plant tissue culture techniques that would be randomly sampled from broadly disseminated Brazilian and international journals. The number of papers sampled per journal depended on the results of a search in "Google Scholar" on "plant tissue culture/culture de tecidos vegetais". Thus, papers of interest were sequentially sampled regardless of the year and place of publication. Therefore, this study sampled papers from 2009 to 2015 published in 4 Brazilian journals [Revista Brasileira de Plantas Medicinais, Theoretical and Experimental Plant Physiology (formerly Revista Brasileira de Fisiologia Vegetal), Pesquisa Agropecuária Brasileira, Plant Cell Culture & Micropropagation] and5 international journals (Plant Cell, Tissue & Organ Culture, Bioresource Technology, Plant Physiology and Biochemistry, Biotechnology Advances, Acta Horticulturae)

The following data were collected from these publications: 1) sample size; 2) number of replications; 3) design; 4) scheme (factorial or not factorial) and number of treatments; 5) use of transformation or not, transformation criteria and transformation type; 6) type of variable (quantitative or qualitative), test, and regression types.

Since some papers present more than a single test, transformation type, sample size, etc., the final value of each variable (*n*) exceeded the total number of papers and experiments sampled. For experiments designed in a factorial scheme, the number of treatments was found by multiplying by the number of factors involved. Relative frequencies were calculated for each variable using expression [2]:

$$f_p = \frac{f_i}{\sum_{i=1}^{n} f_i} \times 100 \qquad 2]$$

Where f_p is the relative frequency (percentage) f_i is the simple absolute frequency (i.e., the number of observations within a given range) and *n* is the total number of observations. Pie charts showing relative frequencies were created for comparisons.

RESULTS AND DISCUSSION

One of the objectives of statistics is to draw conclusions about an entire population based on a smaller sample (BANZATTO; KRONKA, 2006). Given the manual labor demands of micropropagation, small samples are typically used to make inferences about plant development. Most studies evaluate up to 10 explants per plot (Figure 1). According to Morais et al. (2014), the definition of sample size does not follow any statistical criteria; instead, it is based on technical feasibility and practical availability. The authors also discussed discrepancies in the literature regarding sample size, which varied from 1 to 12 seedlings for the same species.

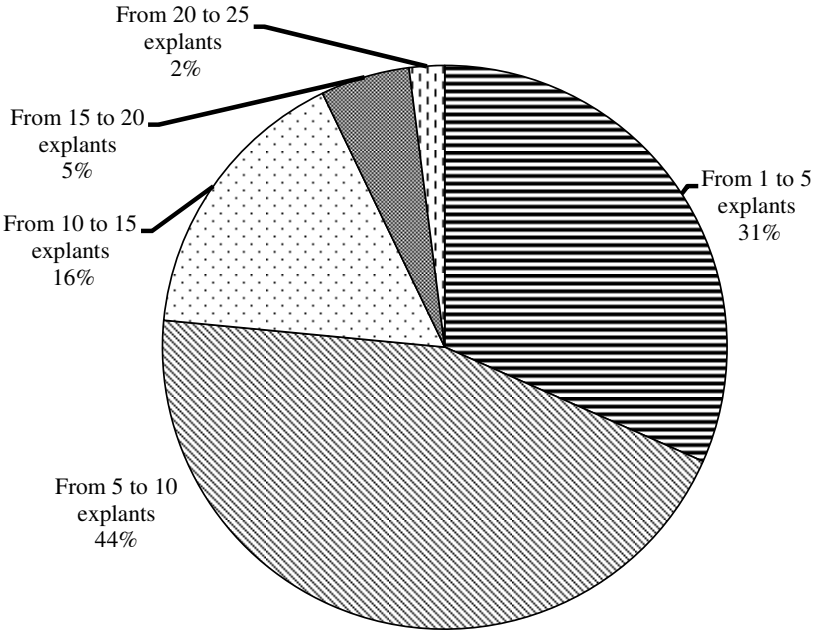

Figure 1. Plot sizes in micropropagation experiments

Studies by Morais et al. (2014) and Peixoto (2009) are essential since they recommend minimum plot sizes needed for statistical inference. Minimum plot size is achieved when variability is minimal and greater plot sizes do not confer significant reductions in variability. Optimal plot size in vine micropropagation is 12 explants in individual glass bottles (MORAIS et al., 2014). Peixoto (2009) used various methods to find that the optimal plot size for the *in vitro* conservation of *Passiflora giberti* N. E. Brown was 10 explants. Using these values as a reference, 31% of the plot sizes used for the variables in our sample was lower than that postulated by Peixoto (2009).

It is essential that researchers use only one explant per bottle when determining optimal sample size. This requirement helps guarantee that observations are independent by avoiding pseudoreplications that can lead to under/overestimations. These inaccuracies occur because of bottle contamination or oxidation that may hinder the development or lead to the death of the explants. These explants are discarded, producing many zero values that affect variances and directly influence the mean square of the error and the F test. In most cases, data transformations do not correct the variability generated by these values (COUTO, 2009).

Filamentous fungi, bacteria, and yeasts are the most frequent contaminants of *in vitro* plant cultivation. Most of these contaminants are not pathogenic to plants in the field; however, they become pathogenic *in vitro* experiments during micropropagation (LEIFERT et al., 1994). The basic difference between these contaminants is that fungi and yeasts are easily perceived in the culture medium a few days after cultivation, facilitating elimination of the contaminated material (LEIFERT; WOODWARD, 1998). On the other hand, bacteria are not always evident before the beginning of the cultivation and, therefore, can spread among the materials during the multiplication stages (MONTARROYOS, 2000).

Large plot sizes (greater than 20), which result in more precise observations, are uncommon (4% of the sample). Plot sizes are usually smaller due to the costs of labor, time, reagents and growth regulators, and space limitations in laboratories. For these reasons, replications were less than six in 77% of the sampled experiments (Figure 2).

However, three to six replications may be statistically enough for plots of representative size, especially when dealing with continuous variables and low intrinsic variability.

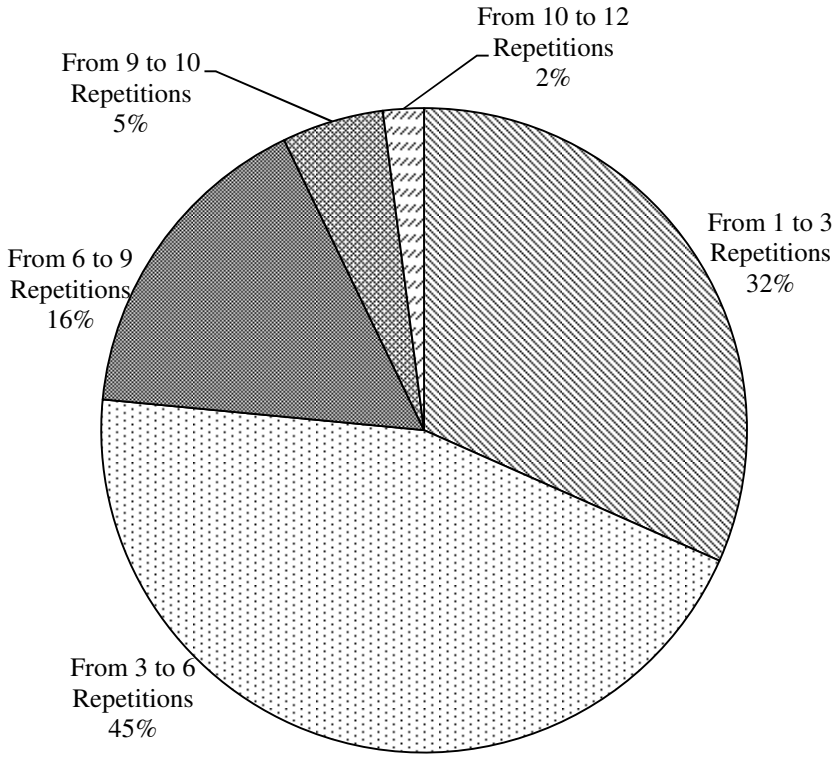

Figure 2. Number of replications in the micropropagation experiments

The number of replications is counterbalanced by sample size since the sample (*n*) is the product of these variables. Given the upper limits of plot size (10) and replication number (6), we inferred that more than 70% of the studies used sample sizes of 60 units. However, if few replications are used, significance might be detected when it does not actually exist (SILESHI, 2012). Conversely, if sample sizes are excessive, even trivial differences may become statistically significant (TRYON; LEWIS, 2009).

Most traits tested in micropropagation studies are expressed as percentages (e.g. explant survival, mortality, contamination, oxidation, callus formation, and seedling formation). Thus, small sample sizes (<100) can exaggerate errors in analysis. All variables recorded by Morais et al. (2014) and Peixoto (2009) (seedling size, number of shoots, and mass) were continuous, while replication numbers and sample sizes were not reported. Studies have not yet been developed for traits expressed as percentages; however, it can be inferred that sample sizes would have to be larger in such experiments. Ribeiro-Oliveira et al. (2016) developed a method to determine sample sizes for proportional variables. The method was based on the germination of *Bowdichia virgilioides* Kunth seeds of different qualities, and showed that 78 to 239 seeds were needed for accurate analyses (*n*). Since micropropagation also uses seeds as explants, these values may also be applicable to both research fields. Furthermore, a variable expressed as a percentage requires a sample size close to 100. A sample size of four units can yield only 0, 25, 50 and 100% while a sample size of ten units can yield 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%, and so on. These observations show that the weight given to a single observation decreases as sample size increases, which consequently reduces error. Therefore, the indetermination of the sample size for this type of variable is still a bottleneck. For this reason, large sample sizes are recommended to guarantee reliable analyses.

Micropropagation is highly dependent on controlled conditions, mainly temperature and luminosity, since an optimal environment for plant development is also ideal for microbial development. Regarding controls, 58% of the experiments were performed with unrestricted randomization (Figure 3). This behavior is worthy of criticismas the random manipulation of materials by more than one person is common in micropropagation (but not reported in scientific papers), and produces variability (errors). Unlike the randomized complete design, the randomized block design uses three basic principles of experimentation: replication, randomization, and

local control (blocks). Thus, the block is a set of homogeneous plots that is equal to or a multiple of the number of treatments. (PIMENTEL-GOMES, 2000).

Time can also be a blocking factor. Here, one part of the replications is installed in one moment (day), and the other part is installed in another moment, each part corresponding to a block. Another example of blocking is when more than one experimenter participates in the inoculation of explants in a laminar flow chamber; in this case, each person corresponds to one block. This type of blocking may allow the installation of more precise experiments, with a larger number of replications and plots.

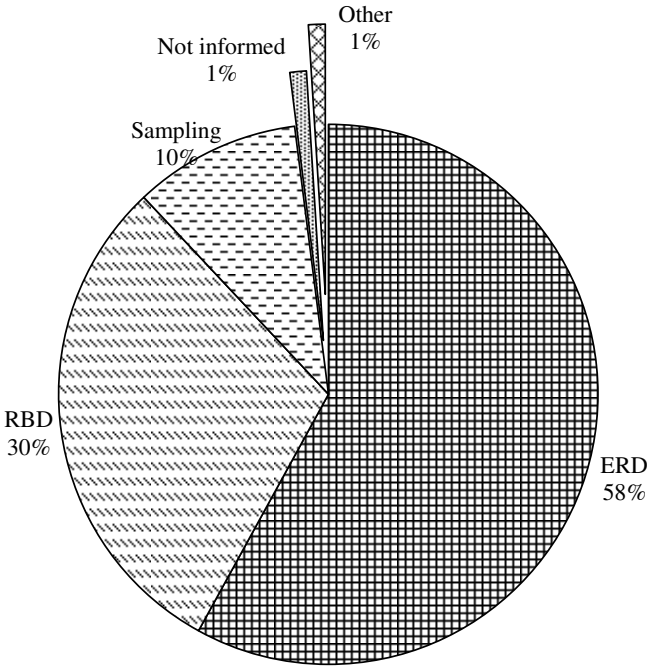

Figure 3. Statistical designs used in micropropagation experiments. RCD and RBD: Randomized complete design and randomized block design, respectively.

Non-blocking, when required, affects mean squares (MSE), leading to incorrect values and consequently incorrect inferences (SILVA, 1999). Consequently low precision mislead researchers and increase the probability of a type II error. This error indicates that differences between treatments are not significant when significant differences actually exist. The most troubling consequence of these errors is that producers may fail to adopt optimal technologies (JUDICE, 2002). In micropropagation studies, blocks were used in 30% of the published papers (Figure 3).

Some of the species used in micropropagation present incompatibility problems such as seasonality of fruit and seed production and may undergo anthropic effects, mainly at the reproductive stage, which limit explant supply. The technical difficulties of working with large samples limit the use of experimental designs, such as the completely randomized block design, which requires at least 20 plots and ten degrees of freedom (PIMENTEL-GOMES 2000; BANZATTO; KRONKA 2006). Sampling is recommended when the number of explants does not meet the requirements of these experimental design techniques. Possibly for these reasons, 10% of the experiments were analyzed as samples. Finally, 1% of the studies did not indicate which design was used, and 1% used other design types, such as the Latin square design and the incomplete block design.

Lowreplication numbers and plot sizes may be characteristic of micropropagation studies that employ numerous treatments and test several types and application rates of growth regulators,mainly in factorial schemes. Factorial schemes were used in 68% of the experiments, and the combination of factors was high, reaching over 16 treatments in 4% of the experiments (Figure 4). The most common number of treatments (six to eight) was found in 40% of the studies, followed by ten to twelve treatments in 30%of the studies. Thirty-two percent of the studies did not use a factorial scheme and employed two to ten treatments. The highest proportion of studies (44%) used three to four treatments, while 26% of the studies used one or two treatments. One study on the development of a protocol tested only one treatment.

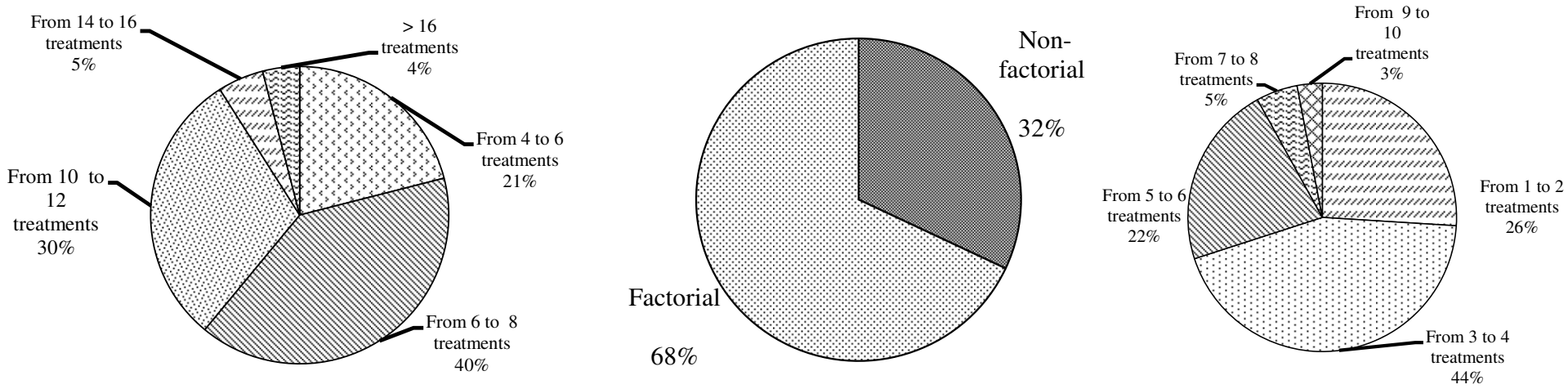

Figure 4. Number of treatments and schemes in micropropagation experiments.

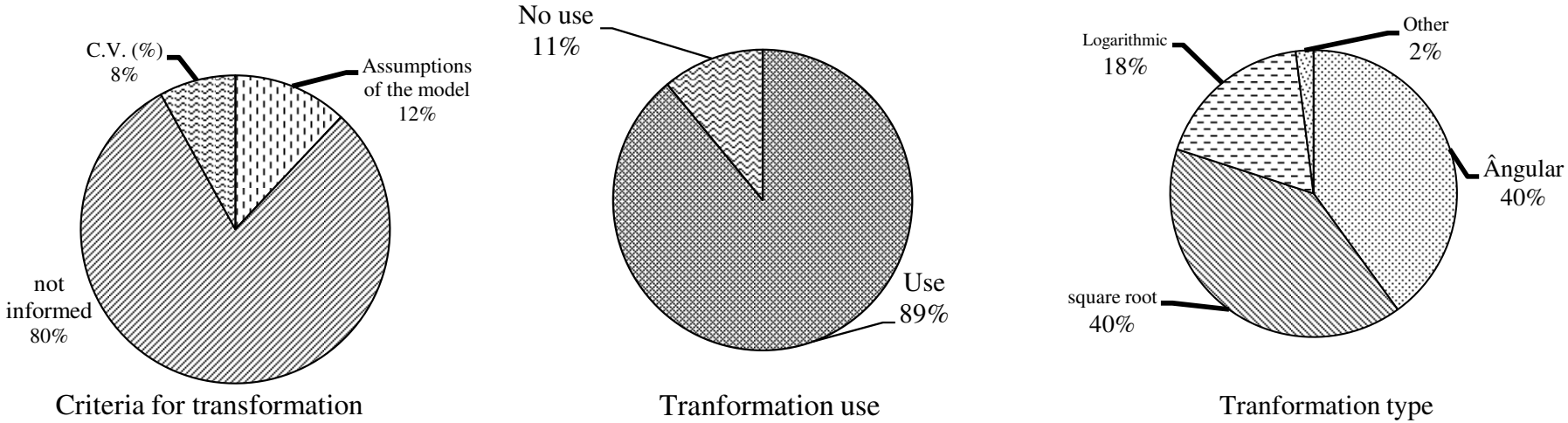

Figure 5. Considerations on data transformation in micropropagation experiments.

Pre-testing before narrowing the focus of a study is an alternative method of reducing the number of treatments. These pre-tests can be performed in a sampling scheme that respects statistical principles and allows comparisons between treatments. Another alternative for factorial experiments is the installation of different experiments using joint analysis of variance to identify interaction among factors. This procedure is common in plant breeding where the same experiment is carried out at different locations. In plant tissue culture this type of analysis is used in works with *in vitro* germplasm conservation, in which experiments are installed in different environments, such as growth room and BOD, with different luminosity intensity and temperature (PIRES, 2017). Another example is the installation of experiments under colored Light Emitting Diode sources, which has been recently used in micropropagation (SANTANA, 2017[1]), being strongly associated with technological innovations to provide light with specific wavelength, intensity, and distribution, stimulating the growth and development of *in vitro* cultures. In addition, these light sources reduce energy consumption, waste production, and environmental pollution. Joint analysis assumes that variance in the experiments is homogenous. Thus, if the ratio between the largest and the smallest mean square of the residue is 7:1, the joint analysis of variance and the statistical tests can easily be performed (PIMENTEL-GOMES, 2000).

Data transformation was widely used to enable parametric analysis of data (89% of the traits tested) (Figure 5). Despite its frequent use, 89% of these data transformations were applied without observing statistical assumptions. Of this total, the criteria for data transformation were not informed for 80% of the traits, while the coefficient of variation was used incorrectly as a transformation criterion for 8% of the traits. In short, only 12% of the studies used data transformation properly based on model assumptions (normality, additivity and homogeneity). CV is a measure of the quality of an experiment (Pimentel-Gomes, 2000; Oliveira et al., 2009). Thus, data transformation is used to reduce high variability. In addition, high coefficients of variation may allow analysis without data transformation, whereas low coefficients may require data transformation since they do not meet the assumptions of the statistical model (PEREIRA; SANTANA, 2013). Angular and square root transformations were the most common (both 40%), based on the type of variable tested. References on agricultural statistics and experimentation recommend angular transformations for data expressed as percentages and root transformations for continuous data. Logarithmic transformations are recommended for greatly dispersed continuous data (PIMENTEL-GOMES2000; BANZATTO; KRONKA, 2006).

Of the experiments whose factors were tested qualitatively (44% of the variables); the Tukey's test was the most common (75%), followed by the Student's t-test (14%) (Figure 6). A study on the comparison procedures used in the Journal Horticultura Brasileira revealed that the Tukey test was most frequently used (57.1%), followed by the Duncan's test (32.6%) (BEZZERA NETO, 2002). These results concur with those observed in papers published from 1980 to 1994in the Brazilian Agricultural Research Journal (PAB) (SANTOS et al, 1998).

More frequent use of the Tukey test is consistent with the design type used in micropropagation experiments, since this test can be applied to either randomized block or randomized complete designs. The Tukey test is used to compare two means that may overlap (i.e., the same treatment may belong to two groups of treatments) (BANZATTO; KRONKA, 2006). Among tests commonly used to compare means, the Tukey test is the strictest (SANTANA; RANAL, 2000). Another reason that the Tukey test is more common is that the Duncan test is applied under the same conditions as those of the Tukey test and the Scott-Knott test requires a minimum number of treatments.

According to Carvalho (2005), infrequent use of the t-test is explained by the objections of many researchers to using small samples. However, the study showed that even with small samples, inferences can be made without violating principles and assumptions. The same study also out lined the procedures available for making comparisons using the "t" test when working with binomial data converted to percentages. This methodology is known as an approximation of a binomial distribution via a normal distribution, and thus respects statistical principles and assumptions.

[1]SANTANA, D.G. Comunicação Pessoal, 2017.

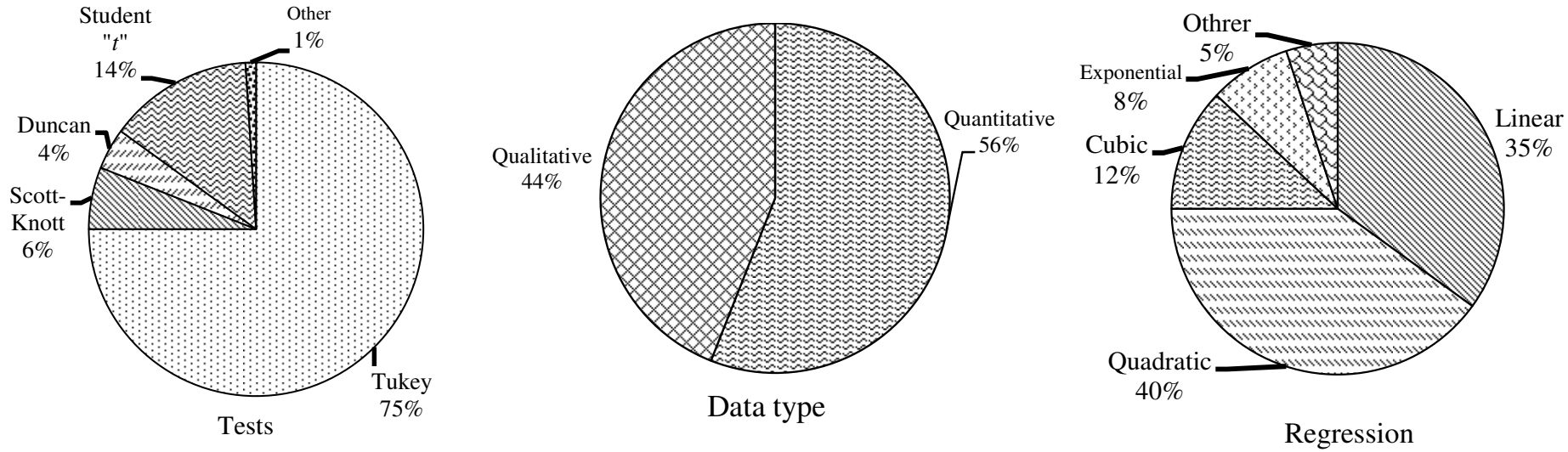

Figure 6. Types of data and tests used in micropropagation experiments.

The most frequent models used for or quantitative variables (56% of variables), were quadratic and linear (40 and 35%, respectively). These models are preferred for their easy interpretation and biological sense. Linear models easily approximate cause and effect relationships (REGAZZI, 2004), (*i.e.*, predicting dependent variables using predictive variables). However, in biological sciences and especially in growth modeling (such as trait analysis in micropropagation), non-linear functions (usually quadratic) must be fit to better explain growth (REGAZZI, 2004). Thus, quadratic regression is widely used to define micropropagation protocols since using at least four points enables the estimation of several other points. Moreover, it also allows the estimation of the point where further increases in application rates start damaging seedlings.

CONCLUSION

Even though statistics have been consistently used in micropropagation experiments, limitations such as insufficient plot sizes and replication numbers remain. Furthermore, many studies neglect to report the criteria used for data transformation or sometimes employ incorrect criteria. Failure to use blocking can also lead to errors. Blocking is recommended to increase sample size by using multiple times or experimenters in the experiment setup. Factorial experiments are characteristically used in micropropagation to determine optimal plant regulator types and application rates. Thus, these experiments have numerous treatments. The Tukey test is used for qualitative data, while regression models (linear and quadratic models) are more frequently used for quantitative data.

ACKNOWLEDGMENTS

This study was financed in part by the Coorde nação deAperfeiçoamento de Pessoal de Nível Supe rior - Brasil (CAPES) - Finance Code 001 and also by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

RESUMO: As análises estatísticas são uma parte essencial da pesquisa científica. Vários procedimentos desde a elaboração do experimento têm impacto nos procedimentos estatísticos finais adotados, assim um correto planejamento implica em uma análise precisa. Por meio de uma amostragem utilizando intervalo de confiança a 95% e margem de erro de 7% objetivou caracterizar as estatísticas utilizadas pelos pesquisadores da área de cultura de tecidos vegetais, e com base nos resultados discutir os principais impactos do mau uso. Foram quantificadas as informações referentes ao tamanho da amostra; número de repetições; delineamento; esquema adotado (fatorial ou não) e número de tratamentos; uso ou não de transformação, critério para adoção e tipo de transformação; tipo da variável (quantitativo ou qualitativo), teste e tipos de regressão. Mesmo com uso consistente da estatística nos experimentos de micropropagação alguns gargalos permanecem, como o tamanho das parcelas e o número reduzido de repetições. Soma-se a isto, a transformação de dados na qual não são informados os critérios para a adoção, ou usam-se critérios equivocados. Mesmo considerando as condições homogêneas, o não uso da blocagem nos experimentos pode configurar em um erro. Recomenda-se a blocagem para aumentar o tamanho da amostra, tendo como bloco o tempo e, ou, o fator humano envolvido na instalação do experimento. Como característica da área tem-se o uso de experimentos fatoriais, visando definir doses e reguladores vegetais, assim os experimentos apresentam grande número de tratamentos. Para comparação dos dados qualitativos utiliza-se o teste de Tukey e no quantitativo as regressões preferidas são as lineares e quadráticas.

Palavras-chave: Delineamentos experimentais. Biotecnologia vegetal. Planejamento experimental *in vitro*.

REFERENCES

ARBIX, G. Biotecnologia sem fronteiras. **Novos Estudos**-CEBRAP, São Paulo, n. 78, p. 5-10, 2007. http://dx.doi.org/10.1590/S0101-33002007000200001

BANZATTO, D. A.; KRONKA, S. N. **Experimentação agrícola**. 4. ed. Jaboticabal: Funep, 2006. 244 p.

BERTOLDO, J. G. COIMBRA, J. L. M.; GUIDOIN, J. L. M.; GUIDOLIN, A. F.; MANTOVANI, A.; VALE N. M. Problemas relacionados com o uso de testes de comparação de médias em artigos científicos. **Biotemas**, Florianópolis, v. 21, n. 2, p. 145-153, 2008. https://doi.org/10.5007/2175-7925.2008v21n2p145

CAEIRÃO, E. Aplicação dos testes de comparação de médias em ensaios de cevada. **Pesquisa Agropecuária Gaúcha**, Porto alegre, v. 12, n. l-2, p. 51-55, 2006.

CARDELLINO, R.; SIEWERDT, F. Utilização Correta e Incorreta dos Testes de Comparação de Médias. **Revista da Sociedade Brasileira Zootecnia**. Viçosa, v. 21, n. 6, p. 985 - 995, 1992.

CARVALHO, M. P.; SANTANA, D. G.; RANAL, M. A. Emergência de plântulas de *Anacardiumhumile* A. St.-Hil.(Anacardiaceae) avaliada por meio de amostras pequenas. **Revista Brasileira de Botânica**, São Paulo, v. 28, n. 3, p. 627-633, 2005. http://dx.doi.org/10.1590/S0100-84042005000300018

COMPTON, M. E. Statistical methods suitable for the analysis of plant tissue culture data. **Plant Cell, Tissue and Organ Culture**, New York, v. 37, n. 3, p. 217-242, 1994.https://doi.org/10.1007/BF00042336

COMPTON, M. E.; MIZE, C. W. Statistical considerations for in vitro research: I—Birth of an idea to collecting data. **In Vitro Cellular & Developmental Biology-Plant**, Berlin, v. 35, n. 2, p. 115-121, 1999.https://doi.org/10.1007/s11627-999-0020-2

COUTO, M. R. M. Transformação de dados com excesso de zero em experimentos com culturas oleícolas. **Ciência Rural**, Santa Maria, v. 39, n. 6, 2009. http://dx.doi.org/10.1590/S0103-84782009005000110

IBAÑEZ, M. A.; MARTIN, C.; PÉREZ, C. Alternative statistical analyses for micropropagation: a practical case of proliferation and rooting phases in Viburnum opulus. **In Vitro Cellular & Developmental Biology-Plant**, Berlin, v. 39, n. 5, p. 429-436, 2003. https://doi.org/10.1079/IVP2003435

JUDICE, M. G.; MUNIZ, J. A.; AQUINO, L. H.; BEARZOTTI, E. Avaliação da precisão experimental em ensaios com bovinos de corte. **Ciência e Agrotecnologia**, Lavras, v. 26, n. 5, p. 1035-1040, 2002.

KUKLIN, A. I.; TRIGIANO, R. N.; SANDERS, W. L.; CONGER, B. V. Incomplete block design in plant tissue culture research. **Journal of tissue culture methods**, Dordrecht, v. 15, n. 4, p. 204-209, 1993.https://doi.org/10.1007/BF02388321

LEIFERT, C.; MORRIS, C. E.; WAITES, W. M. Ecology of microbial saprophytes and pathogens in tissue culture and field grown plants: reason for contamination problems in vitro. **Critical Reviews in plant Sciences**, Philadelphia, v. 13, p. 139-83, 1994. https://doi.org/10.1080/07352689409701912

LEIFERT, C.; WOODWARD, S. Laboratory contamination management: the requirement for microbiological quality assurance. **Plant Cell, Tissue and Organ Culture**. New York, v. 52, n. 01/02, p. 83- 88, 1998. https://doi.org/10.1023/A: 1005905604043

MELO, B. **Cultivo de embrião in vitro da gabirobeira (*Syagrus Oleraceae* (Mart.) Becc.).** Tese (Doutorado em Agronomia) - Universidade Federal de Lavras, MG. 2000. 117f.
MIZE, C. W.; CHUN, Y. W. Analysing treatment means in plant tissue culture research. **Plant Cell, Tissue and Organ Culture**, New York, v. 13, n. 3, p. 201-217, 1988.https://doi.org/10.1007/BF00043669

MONTARROYOS, A. V. V. **Contaminação in vitro Laboratório de Cultura de Tecidos Vegetais**. Empresa Pernambucana de Pesquisa Agropecuária (IPA). p. 5-10. 2000

MORAIS, A. R.; ARAÚJO, A. G.; PASQUAL, M.; PEIXOTO, A. P. B. Estimação do tamanho de parcela para experimento com cultura de tecidos em videira. **Semina:** Ciências Agrárias, Recife, v. 35, n. 1, p. 113-124, 2014. https://doi.org/10.5433/1679-0359.2014v35n1p113

OLIVEIRA, R. L., MUNIZ, J. A.; ANDRADE, M. J. B.; REIS, R. L. Precisão experimental em ensaios com a cultura do feijão. **Ciência e Agrotecnologia**, Lavras, v. 33, p. 113-119. 2009.

PAUGH, J.; LAFRANCE, J. C. **The U.S. Biotechnology Industry**.U.S. Department of Commerce — Office of Technology Policy, 1997.

PEIXOTO, A. P. B. **Estimação do tamanho de parcelas para experimentos de conservação in vitro de maracujazeiro**. Dissertação de mestrado. Lavras, UFLA. 2009. 98f.

PEREIRA V. J.; SANTANA D. G. Coefficient of variation of normal seedlings obtained from the validation of methods for the seed germination testing of 20 species belonging to the family Fabaceae. **Journal of Seed Science**. Londrina, v. 35, p. 161-170. 2013. http://dx.doi.org/10.1590/S2317-15372013000200004

PIMENTEL-GOMES, F. **Curso de estatística experimental**. Editora da Universidade de São Paulo, Piracicaba. 2000.

PIRES, D. C. M. **Conservação *in vitro* por crescimento lento de mangabeira do Cerrado (*Hancornia speciosa* Gomes)** 2017. 66 p. Dissertação (Mestrado em Agronomia) Universidade Federal de Uberlândia, Uberlândia, 2017.

REGAZZI, A. J.; SILVA, C. H. O. Teste para verificar a igualdade de parâmetros e a identidade de modelos de regressão não-linear. I. dados no delineamento inteiramente casualizado. **Revista de Matemática e Estatística**, Lavras, v. 22, n. 3, p. 33-45, 2004. http://dx.doi.org/10.1590/S0034-737X2010000300005.

RIBEIRO-OLIVEIRA, J. P.; RANAL, M. A.; SANTANA, D. G.; PEREIRA, L. A.Sufficient sample size to study seed germination. **Australian Journal of Botany**, Melbourne, p. 1-15, 2016. https://doi.org/10.1071/BT15254

SANTANA, D. G.; RANAL, M. A. Análise estatística na germinação. **Revista Brasileira de Fisiologia Vegetal**, Brasília, v. 12, n. 4, p. 205-237, 2000.

SANTOS, J. W.; MOREIRA, J. D. A. N.; BELTÃO, M. N. E. Avaliação do emprego dos testes de comparação de médias na Revista Pesquisa Agropecuária Brasileira (PAB) de 1980 a 1994. **Pesquisa Agropecuária Brasileira**, Brasília, v. 33, n. 3, p. 225-230, 1998.

SILESHI, G. W. A critique of current trends in the statistical analysis of seed germination and viability data. **Seed Science Research**, Cambridge, v. 22, n. 03, p. 145-159, 2012. https://doi.org/10.1017/S0960258512000025

SILVA, J. G. C. A consideração da estrutura das unidades em inferências derivadas do experimento. **Pesquisa Agropecuária Brasileira**, Brasília, v. 34, n. 3, p. 911-925. 1999. http://dx.doi.org/10.1590/S0100-204X1999000600001.

TRYON, W. W.; LEWIS, C. Evaluating independent proportions for statistical difference, equivalence, indeterminacy, and trivial difference using inferential confidence intervals. **Journal of Educational and Behavioral Statistics**. Londres, v. 34, p. 171–189, 2009. https://doi.org/10.3102/1076998609332753