Revista Internacional de BOTANICA EXPERIMENTAL ΦYTON International Journal of EXPERIMENTAL BOTANY

Fundada en 1951 por Founded 1951 by
Miguel Raggio & Nora Moro-Raggio
Editor: Dr. Miguel Raggio
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina

49° ANIVERSARIO 68 (2000) 117-122 49th ANNIVERSARY

Multivariate analysis applied to *in vitro* culture

(with one figure & 2 tables)

Marconi Patricia L; Silvia Radice

Abstract. Discriminant analysis is a multivariate method used to maximize differences among groups under study (variables, cultivars, treatments) and observations or variables (weight of organs or tissues; number of buds, leaves or embryos; foliar area; etc. (6). In order to improve the performance of tissue culture experiments a discussion about entry, screening, reduction and analysis of data is included.

Univariate analysis is used in most tissue and organ culture assays involving more than one observation or variable for each individual or unit under study, an experimental design with multiple factors may be used, but the restriction of this method is that number of treatments to be analyzed cannot be higher than three (3).

Assays involving three treatments are frequently found in micropropagation or root microcutting experiments. As an additional inconvenience, under unvariate analysis the responses of many variables can not be related. Each one must be analyzed separately. As a result of such analysis, infinite data contrasted by different comparison tests are obtained (3). It is very difficult to analyze and draw conclusions from the multiple results obtained.

Multivariate Analysis (MA) synthesizes and represents all results obtained from the same assay in two or more dimensions. This method can be used to better understand the relationships among variables of the same experiment (1). Discriminant Analysis (DA) is a multivariate method used to maximize differences among groups under

This work was supported by CONICET. Centro de Ecofisiología Vegetal (CEVEG – CONICET). Serrano 669, C.P. 1414, Bs. As. Argentina, pmarconi@mail.retina.ar. Telfax: 54-011-4-956-7110

Received 11.XII.2000; accepted 29.XII.2000

study (variables, cultivars, treatments) and observations or variables (weight of organs and tissues; number of buds, leaves and/or embryos; foliar area, etc.) (6). DA is used to classify individuals into two or more groups or populations on the basis of a set of measurements; by using DA, variables that contribute to the aforementioned classification can be found (1). In this way, multiple experimental situations and their surroundings can be described. We also have used it for prediction and description (6). Variables and their combinations can be infinite in micropropagation trials. Application of DA allows interpretation of all data in sets from a graph with two or more dimensions (Figure 1). For instance, this statistical analysis can be applied to classical culture media optimization trials.

Explants do not show stress symptoms *in vitro* when nutrient level and ionic balance are adequate. Nevertheless, it has been reported that numerous problems may appear during the axillary bud sprouting stage (2). Results obtained during the multiplication stage can be manipulated. This is possible, first by selecting the culture medium salts and second, by using different organic compounds and hormones (7). Results obtained by the application of alternative treatments are frequently assessed through different variables. The aim of this work is to prove that DA can be applied as the synthesis of results obtained from the assays performed. Furthermore, we will prove that this method can be used to reduce the number of variables on which the ANOVA can be applied. For a better understanding of the application of the method, three different salt treatments we analyzed in the multiplication stage of two *Abelia grandiflora* (André) – Rehde varieties.

MATERIAL & METHODS

Plant material and culture conditions. Experiments were carried out *in vitro* with explants of *Abelia grandiflora* (André) Rehder cv. grandiflora and cv. variegate. Microcuttings with two nodes were taken during the multiplication stage of both *Abelia* sp. plants. They were cultured in three salt media combinations: Chalupa (1981) (**CH**), Lloyd & Mc Cown (1981) (**WPM**) and Murashige & Skoog (1962) (**MS**) (5). All salt combinations were added together with the following organic components and growth regulators (mg/L): thiamine, 0.4; myo-inositol, 100; glycine, 2; benzyladenine (BA), 1; sucrose 3% and agar, 0.8%.

Media (50 ml) were dispensed into jam-flasks (375 cc capacity). Five explants per flask were cultivated during 30 days. Ten repetitions per experiment were made, and each experiment was replicated three times.

Before autoclaving at 137 KPa during 15 min, pH was adjusted to 5.8. All cultures were maintained at 24 ± 1°C, under a 16 h photoperiod of cool white fluorescent light (57 mE.m.$^{-2}$s.$^{-1}$) of TLT 110 W/54 RS Philips day light tubes.

Data compilation & analysis. Cultured plant material was collected after 45 days. Variables measured were fresh and dry stem weight (**FSW** and **DSW**); fresh and dry leaves weight (**FLW** and **DLW**); number of secondary stems (branches)(**NSS**); number of leaves (**NL**); increment plant material (**IPM**); stem length (**SL**); number of buds (**NB**) and foliar area (**FA**, Licor Li-3000).

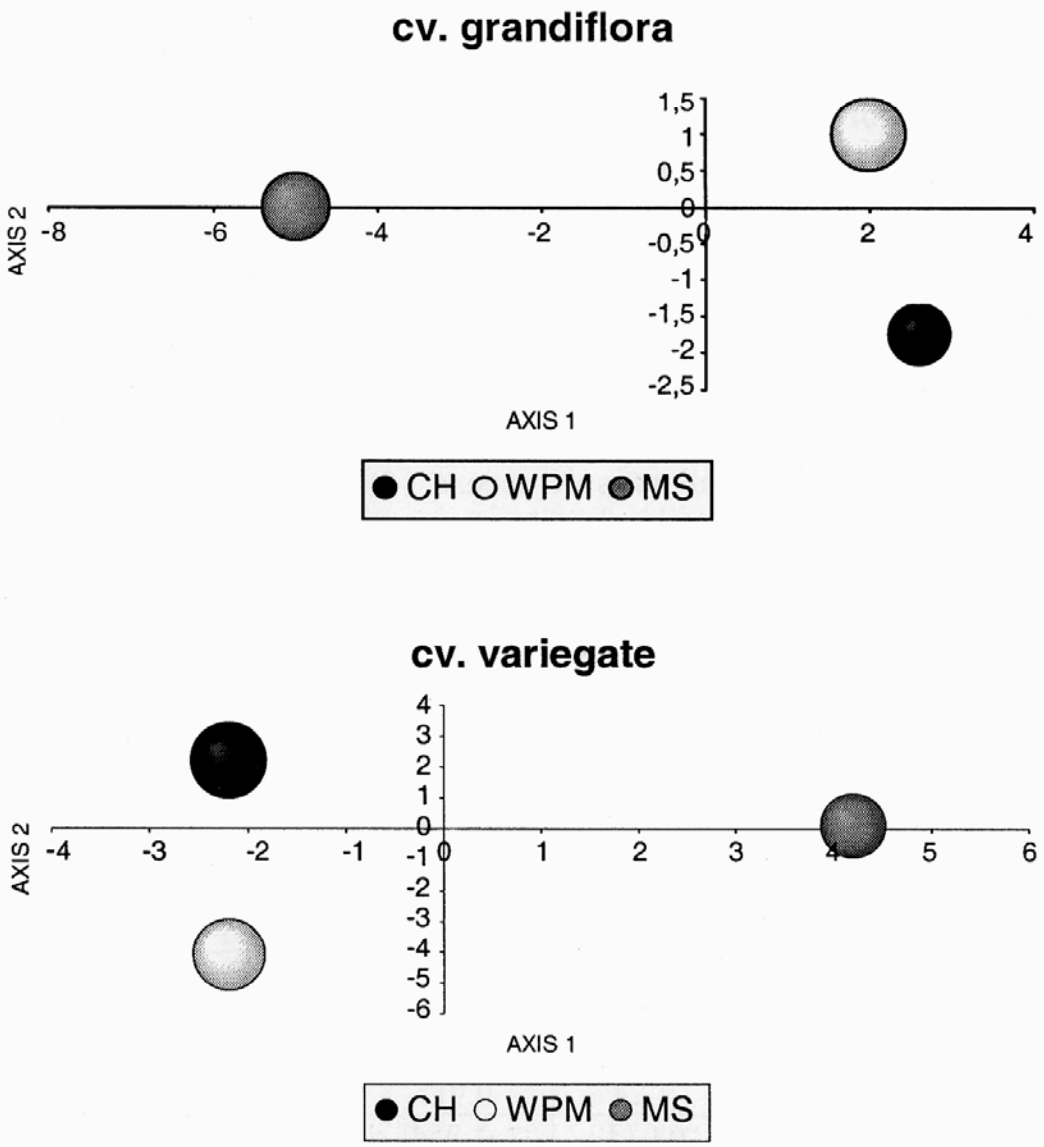

Figure 1.- Effect of salt treatments (**CH**, **WPM**, **MS**) in the multiplication stage for *Abelia* sp. Set 1, **CH** salts treatment; set 2, **WPM**; and set 3, **MS** for cv. grandiflora. Set 4 represent **CH** salt treatment; set 5, **WPM** and set 6, **MS** for cv. variegate.

Data were analyzed by DA and ANOVA. Tukey's test was used for multiple comparisons. Homogeneity of variance was supported by Barlett's test. For numerical analysis, the STATGRAPHICS program was utilized (Statistical Graph Corp, 1992) (8).

RESULTS & DISCUSSION

Statistical analysis. The results of the assay on *Abelia* sp. stem segments multiplication were estimated through nine directly measured variables. The first step in analyzing the results was to assess data was to assess data through a DA. The first step in analyzing the results was to assess data through a DA. Each treatment was separated in a well-defined istlet (Fig. 1). The DA offers a weighted average of each variable and shows a discrimination coefficient (Table 1). Coefficients with extreme values for each treatment offer a better contribution to the separation of treatments. In the given example, **WPM** and **CH** salts treatment can be distinguished from **MS** by the **SL** ($Cest._{eje2}$.1034) and **IPM** ($Cest._{eje2}$.0.197, Fig. 1 A, same table). The results of which show that plants treated with MS salts had longer internodes and, in consequence, longer stems but less internodes due to low IPM. Moreover, chlorosis in the margins of leaves and damages caused by necrosis were observed in plants of this treatment. Such symptoms have also been observed in other species when **MS** salts have been utilized, possibly due to salt stress (4). On the contrary, plants

Table 1.- Standarized discriminant function coefficient for *Abelia grandiflora* vr. grandiflora and variegate.

	Abelia grandiflora vr			
	grandiflora		variegate	
VARIABLE	axis 1	axis 2	axis 1	axis 2
LS	2.716	-0.675	-0.94	1.478
FSW	0.355	1.034	0.176	-0.897
FLW	0.212	-0.739	1.885	0.341
DSW	0.415	-0.153	-.0763	-0.016
DLW	1.749	-0.189	-0.49	0.346
IPM	-1.24	0.917	0.129	-1.903
LN	0.673	-1.354	-0.619	0.402
BN	1.847	0.442	0.102	0.929
LA	-0.24	-0.007	-0.129	0.212

Table 2.- Salt treatments effect (**CH**, **WPM**, **MS**) in the multiplication stage of the *Abelia grandiflora* vr. grandiflora and variegate.

Cultivar	Treat..	**SL** (cm)	**IPM**	**FSW**
grandiflora	**CH**	4.333 ± 0.105 a	5.00 ± 0.683 b	1.45 ± 0.07 b
	WPM	3.916 ± 0.271 a	8.83 ± 1.108 a	1.77 ± 0.30 a
	MS	2.916 ± 0.153 b	3.00 ± 0.447 b	0.78 ± 0.18 c
variegate	**CH**	5.666 ± 0.494 a	7.166 ± 0.477 a	1.18 ± 0.08 b
	WPM	5.500 ± 0.619 a	7.666 ± 0.614 a	2.87 ± 0.24 a
	MS	3.416 ± 0.506 b	4.000 ± 0.966 b	0.91 ± 0.16 c

cultured with **WPM** and **CH** salts had more internodes and, consequently, higher **IPM**. The difference between the last treatments was that plants cultured with **WPM** salts had a greater **FSW** than those cultured with CH salts.

The ANOVA revealed important differences among the three more discriminant variables (Figure 2). In the cv. variegate, only a few variables coincided with the results of cv. grandiflora. Genetic difference(s) between the two cultivars may account for this (4). The three islets that represent each salt treatment are shown in fig. 1 B. As in cv. grandiflora, the difference between the **MS** salt treatment, and the others, was the **SL** (Cest.$_{axis1}$.-0.94, Table 1). The difference between the **CH** salt treatment and the **WPM** salt treatment was the **IPM** (Cest.$_{axis2}$.-1.903) and the **FSW** (Cest.$_{axis2}$.-0.897). Also, plants cultured with **WPM** salts were higher, with more internodes, a higher level of **IPM** and a greater **FSW** than those treated with **CH** salts. As with *Abelia* sp., the ANOVA confirmed the results obtained through the DA (Table 2).

CONCLUSIONS

DA is a method allows in all treatments to relate all variables under study. Under this procedure, original data entered in a spreadsheet or software do not require previous calculations. The first result observed is a bidimensional figure (as Fig. 1). From such figure and the discrimination coefficient, many fast and numerous conclusions can be drawn. The idea of working with all variables at the same time is to allow the analysis as a whole of the results corresponding to each individual. Partializzation of the ANOVA can be confusing and show contradictory results. For instance, if we analyze the results, variables SL and IPM, CH and WPM will be the same for *Abelia* cv. variegate. The ANOVA conclusion would be that use of any culture medium will multiply the material, without

detriment to the multiplication rate. Nevertheless DA, through the combination of variables under study, clearly shows differences among treatments.

From the results analyzed by DA, the following conclusions may be drawn:

WPM media enhanced growth in both *Abelia* sp. cultivars; the existing genotypical differences between them were shown in the variables measured directly.

Treatment with **MS** salts delayed growth and multiplication of plants belonging to both cultivars. The reason could be the induction of salt stress, possibly brought about by the high level of N (as ammonium and nitrate), or the higher ionic power of this salt medium with respect to the **WPM** and **CH** salt media (4).

DA is certainly a powerful tool for analysing multiple measurement variables in tissue culture experiments. When analyzed by DA, the number of variables is reduced, thus simplifying the ANOVA analysis.

REFERENCES

1. Afifi A, Clark V, In: Computer-Aided multivariate analysis, 4 Learning Publications, Belmont, Calif, USA, (1984) 246
2. Albrecht C, *Comb Proc Intl Plant Prop* (1985) 196
3. Compton ME, *Plant Cell Tissue and Org Culture* 37 (1994) 217
4. George EF, *In*: Plant propagation by tissue culture. Part 1, The technology, 1993, Exegetics Ltd, Edington, UK, (1993) 573
5. George EF, Puttock DJM, George HJ, In: Plant Culture Media, Formulations and Uses, Exegetics Ltd., Edington, UK, 567 pp.
6. James FC, McCulloch CE, *Annu Rev Ecol Syst*, 21 (1990) 129
7. Preece JE, *Int Asoc Plant Tiss Cult*, 1 (1995) 26
8. Statistical Graphics Corp, STSC Inc. Rockville, Md., Software (1992)