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METHODOLOGY

Multivariate analysis as a tool for measuring the stability of morphometric traits in *Lycopersicon* plants from *in vitro* culture

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Abstract

The phenotypic stability of morphometric traits in *Lycopersicon* spp. (stem perimeter at the base, middle and top, and number of flowers per cluster) was measured by multivariate analysis through a progeny test in order to estimate the genetic stability of these traits. Principal components were calculated for two groups of *Lycopersicon* spp., non-regenerated plants and the progeny of regenerated plants. Analysis of variance was performed to support principal component analysis. Both groups presented similar eigenvalues and eigenvectors, while no significant differences were found between any of the traits studied. These results indicated that the phenotypic structure was the same among the progeny of regenerated and non-regenerated plants, so that no variation would occur in *in vitro* culture. Multivariate analysis proved to be an appropriate methodology for the measurement of the stability of morphometric traits after one regeneration cycle.

INTRODUCTION

One of the aims of plant regeneration by tissue culture is to produce individuals genetically identical to the explant's donors and to themselves (i.e., a clone). The efficiency of this biotechnological process depends not only on the number of plants obtained *in vitro* but also on the genetic stability after the regeneration cycles. Somaclonal variants may be produced by *in vitro* culture (Evans and Sharp, 1983; D'Amato, 1985; Lee and Phillips, 1988), and these heritable changes might produce an increase in genetic variability available for breeding programs, but if cloning genotypes was desired this variation should be minimized (Skirvin *et al*., 1994). Phenotypic markers showing a known inheritance pattern may be considered to evaluate the genetic stability of the regenerated plants (Gavazzi *et al.*, 1987), and De Klerk (1990) emphasized the importance of the use of metric traits as phenotypic markers to evaluate genetic stability.

Multivariate analysis gives a measure of the total variation in different samples of individuals (Chatfield and Collins, 1986), and it is possible to apply this methodology to the evaluation of modifications of regenerated plants in respect to the explant's donors.

The goal of this experiment was to use principal component analysis to measure the stability of metric traits in tomato (*Lycopersicon* spp.) plants obtained from *in vitro* culture after one regeneration cycle with the aim of developing a new approach for detecting the effects of *in vitro* culture on these traits.

MATERIAL AND METHODS

Regenerated plants of different genotypes of *L. esculentum*, *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium*, *L. peruvianum* and *L. hirsutum* (Pratta *et al*., 1997) were used (Table I), the *in vivo* adaptation of the regenerated plants being accomplished according to the protocol described in Pratta *et al.* (1995). Acclimatized plants were kept in a greenhouse for flowering and fructification.

The stability of metric traits in the *in vitro* regenerated plants was assessed through a progeny test, for which two groups of plants were assayed: group 1, the experimental group, was non-regenerated plants (NP) consisting of the explant's donor plants (NP_1) and their progeny (NP_2) (*L. esculentum* (N = 24), *L. esculentum* var. *cerasiforme* (N = 19), *L. pimpinellifolium* (N = 13), *L. peruvianum* (N = 9) and *L. hirsutum* (N = 7) (N = 72)). Group 2 consisted of the progeny of the regenerated plants (PRP) (*L. esculentum* (N = 12), *L. esculentum* var. *cerasiforme* (N = 43), *L. pimpinellifolium* (N = 14), *L. peruvianum* (N = 1) and *L. hirsutum* (N = 9) (N = 79)).

The metric traits evaluated in both groups were stem perimeter (in cm) at the base (PB), the middle (PM) and the top (PA) of the plant and the number of flowers per cluster (FC). Seeds of NP_1 were sown in the first crop cycle, while seeds of NP_2 and PRP were sown in the next cycle. Annual effect was assessed by comparing the mean values for each variable in NP_1 plants and NP_2 plants with the Student *t*-test (Snedecor, 1964). Analysis of the effect of *in vitro* culture on plant stability was accomplished by princi-

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pal components (PC) analysis (Chatfield and Collins, 1986), which was applied firstly to the groups to compare respective PC values, then to the total data in order to test if any modification was produced in the values and lastly to the genotypes within each group. In the latter case genotypic variances for the PC (i.e., the respective eigenvalues) were compared by the homogeneity of variance test (Snedecor, 1964).

An ANOVA test was applied to compare the mean values for each variable in the NP and PRP groups in order to verify the principal component analysis.

RESULTS

No significant difference was found between NP_1 and NP_2 for any variable, indicating that there were no significant differences between years which influenced the expression of the traits. It was therefore possible to pool both subgroups in the NP sample.

No significant differences were detected by ANOVA between NP plants and PRP plants for PB, PM, PA and FC (Table II).

The first two PC (PC1 and PC2) accounted for a high proportion of the total variability (92%) in NP plants (Table III). The subsequent PC had a small contribution to the whole and will not, therefore, be considered further. Figure 1A displays the NP plants following multivariate analysis on the basis of these two first PC.

Results corresponding to the PRP plants are shown in Table IV. The respective eigenvectors and eigenvalues were the same as those of the NP plants. PC1 and PC2 values for PRP plants are plotted in Figure 1B. In this case, associations of individuals were similar to those in NP plants.

No modification was observed when PC values were calculated for the total data (Table V). A similar arrangement of plants was again obtained (Figure 2). Additionally, it was not possible to observe any separation of the individuals according to their origin (i.e., regenerated plants vs. non-regenerated plants).

The first two eigenvalues of the PC by genotype are presented in Table VI. No significant difference among groups was detected by the homogeneity of variance test.

Table I - Genotypes of *Lycopersicon* investigated.

Species	Genotype
Lycopersicon esculentum	cv. Nor (E1)
	cv. Platense Italiano (E2)
	cv. Caimanta (E3)
L. esculentum var. *cerasiforme*	LA 1385 (C1)
	LA 1673 (C2)
	Z 1994 (C3)
L. pimpinellifolium	LA 722 (Pi1)
	Z 1995 (Pi2)
L. peruvianum	LA 2151 (Pe1)
L. hirsutum	LA 2128 (H1)

DISCUSSION

No modification was observed for the mean values and the variances of any trait in the progeny of the regenerated plants in respect to the non-regenerated plants. This fact was demonstrated by the ANOVA as well as by the multivariate analysis. However, the latter method would have additional advantages, since it would permit the in-

Table II - Mean values ± standard deviation of the morphometric traits in the non-regenerated plants (NP) and in the progeny of the regenerated plants (PRP) by genotype of different *Lycopersicon* species.

Genotype		PB	PM	PA	FC
E1	NP	3.23 ± 0.23	2.73 ± 0.27	2.25 ± 0.30	5.67 ± 1.86
	PRP	3.05 ± 0.21	2.55 ± 0.21	2.20 ± 0.10	4.50 ± 2.12
E2	NP	2.92 ± 0.49	2.69 ± 0.29	2.12 ± 0.22	5.15 ± 1.07
	PRP	2.98 ± 0.39	2.70 ± 0.61	2.05 ± 0.59	6.50 ± 1.29
E3	NP	3.12 ± 0.42	2.78 ± 0.35	2.25 ± 0.27	4.33 ± 0.52
	PRP	2.80 ± 0.07	2.24 ± 0.13	2.02 ± 0.13	3.60 ± 0.55
F-value		0.63 n.s.	0.46 n.s.	0.12 n.s.	0.11 n.s.
C1	NP	2.70 ± 0.34	2.37 ± 0.30	1.98 ± 0.30	10.00 ± 2.53
	PRP	2.55 ± 0.35	2.18 ± 0.27	1.80 ± 0.29	10.12 ± 2.85
C2	NP	3.05 ± 0.56	2.50 ± 0.44	2.02 ± 0.36	8.33 ± 1.75
	PRP	2.57 ± 0.27	2.02 ± 0.30	1.80 ± 0.29	7.38 ± 1.12
C3	NP	2.91 ± 0.58	2.23 ± 0.59	2.07 ± 0.59	7.57 ± 2.07
	PRP	2.36 ± 0.40	1.98 ± 0.29	1.68 ± 0.49	5.80 ± 0.45
F-value		0.10 n.s.	0.37 n.s.	0.25 n.s.	0.58 n.s.
Pi1	NP	2.43 ± 0.37	1.69 ± 0.25	1.31 ± 0.39	8.57 ± 1.51
	PRP	2.48 ± 0.45	1.95 ± 0.24	1.80 ± 0.43	7.75 ± 0.96
Pi2	NP	2.12 ± 0.24	1.72 ± 0.21	1.35 ± 0.27	13.50 ± 2.42
	PRP	2.32 ± 0.32	1.81 ± 0.27	1.54 ± 0.20	13.20 ± 2.35
F-value		0.65 n.s.	0.16 n.s.	0.46 n.s.	0.80 n.s.
Pe1	NP	1.53 ± 0.19	1.06 ± 0.34	0.89 ± 0.34	9.11 ± 2.42
	PRP	1.50*	0.50*	0.50*	7.00*
F-value		0.74 n.s.	0.38 n.s.	0.28 n.s.	1.00 n.s.
H1	NP	2.27 ± 0.53	1.64 ± 0.30	1.41 ± 0.26	13.57 ± 2.23
	PRP	2.01 ± 0.21	1.56 ± 0.23	1.18 ± 0.27	13.89 ± 1.76
F-value		0.24 n.s.	0.89 n.s.	0.20 n.s.	0.69 n.s.

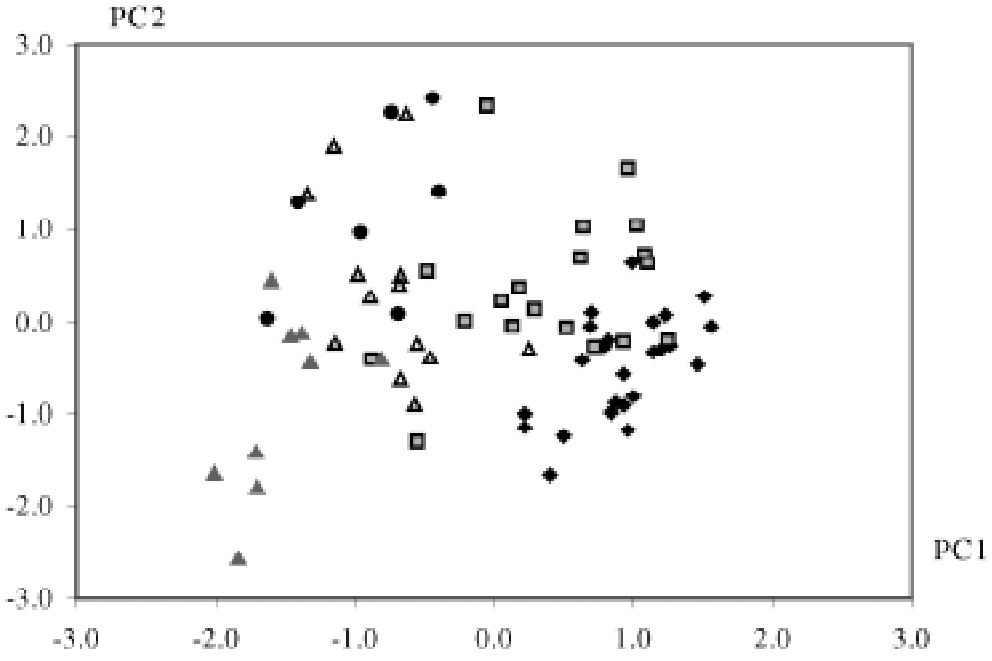
List of genotypes by species: *L. esculentum*: E1: cv. Nor, E2: cv. Platense Italiano, E3: cv. Caimanta; *L. esculentum* var. *cerasiforme*: C1: LA 1385, C2: LA 1673, C3: Z 1994; *L. pimpinellifolium*: Pi1: LA 722, Pi2: Z 1995; *L. peruvianum*: Pe1: LA 2151; *L. hirsutum*: H1: LA 2128. PB: Stem perimeter at the base (cm); PM: stem perimeter in the middle (cm); PA: stem perimeter at the top (cm); FC: mean number of flowers per cluster. *Data corresponding to only one plant. n.s.Nonsignificant.

Table III - Principal components (PC), eigenvalues (E) and proportions of explained variances (EV) and accumulated variances (AV) for the non-regenerated plants (NP) of different *Lycopersicon* species.

	PC1	PC2	PC3	PC4
PB	0.53 (0.91)	0.25 (0.21)	-0.74 (-0.33)	-0.33 (-0.11)
PM	0.54 (0.93)	0.18 (0.15)	0.67 (0.30)	-0.48 (-0.17)
PA	0.55 (0.94)	0.13 (0.15)	0.09 (0.04)	0.81 (0.28)
FC	-0.35 (-0.60)	0.93 (0.79)	0.05 (0.02)	0.03 (0.01)
E	2.95	0.73	0.20	0.12
EV	0.74	0.18	0.05	0.03
AV	0.74	0.92	0.97	1.00

Correlation coefficient between the PC and each variable in parentheses. For abbreviations see legend to Table II.

A. Non-regenerated plants



B. Progeny of the regenerated plants

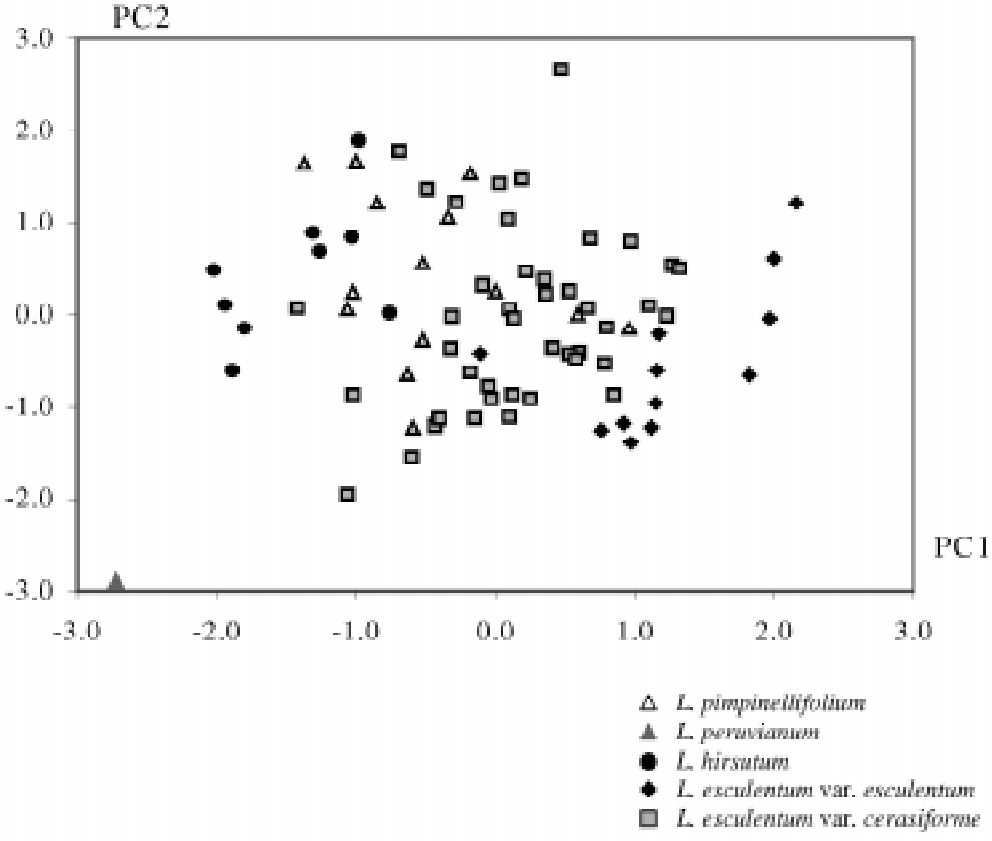


Figure 1 - Individual plants by species on the basis of principal component 1 (PC1) and principal component 2 (PC2).

Table IV - Principal components (PC), eigenvalues (E) and proportions of explained variances (EV) and accumulated variances (AV) for the progeny of the regenerated plants (PRP) of different *Lycopersicon* species.

	PC1	PC2	PC3	PC4
PB	0.53 (0.88)	0.19 (0.16)	-0.71 (-0.41)	0.43 (0.19)
PM	0.54 (0.90)	0.12 (0.10)	0.70 (0.40)	0.45 (0.20)
PA	0.53 (0.88)	0.34 (0.28)	0.03 (0.02)	-0.77 (-0.34)
FC	-0.38 (-0.63)	0.91 (0.76)	0.04 (0.02)	0.14 (0.06)
E	2.78	0.70	0.33	0.19
EV	0.69	0.18	0.08	0.05
AV	0.69	0.87	0.95	1.00

Correlation coefficient between the PC and each variable in parentheses. For abbreviations see legend to Table II.

Table V - Principal components (PC), eigenvalues (E) and proportions of explained variances (EV) and accumulated variances (AV) for all data (non-regenerated and progeny of regenerated plants) of different *Lycopersicon* species.

	PC1	PC2	PC3	PC4
PB	0.53 (0.90)	0.23 (0.20)	-0.72 (-0.35)	0.39 (0.15)
PM	0.54 (0.92)	0.15 (0.13)	0.69 (0.34)	0.45 (0.17)
PA	0.55 (0.94)	0.24 (0.20)	0.05 (0.02)	-0.80 (-0.31)
FC	-0.36 (-0.61)	0.93 (0.79)	0.05 (0.02)	0.04 (0.02)
E	2.89	0.72	0.24	0.15
EV	0.72	0.18	0.06	0.04
AV	0.72	0.90	0.96	1.00

Correlation coefficient between the PC and each variable in parentheses. For abbreviations see legend to Table II.

terpretation of the phenotypic structure (and any eventual changes) of the groups. In this case, PC1 may be considered as a general variability trait, since it represented about 74% of the total phenotypic variation observed in both groups and was highly correlated to all the variables. It allowed differentiation of the species according to the ratio vegetative development/reproductive development, and was positively associated with PB, PM and PA, and negatively associated with FC. The species with high PB, PM and PA values and low FR values showed the greatest PC1 values. For instance, *L. esculentum* and *L. esculentum* var. *cerasiforme* were both situated in the extreme right of the PC1 axis of both Figures 1 and 2. On the other hand, PC2 was narrowly associated to FC, which could be interpreted as a potential productivity factor. Even though PC2 explained a smaller percentage of the total variability (18%), it permitted differentiation within the larger arrangements defined by PC1. Indeed, *L. esculentum* var. *cerasiforme* (which had a greater number of flowers per cluster) was situated above *L. esculentum* in both Figures 1 and 2. Also, *L. pimpinellifolium* and *L. hirsutum* were situated in the upper left quadrant of both Figures 1 and 2 while *L. peruvianum* was below them.

Multivariate analysis appeared to be an appropriate methodology for measuring the stability of metric traits after one regeneration cycle. Similar arrangement of NP plants and PRP plants based on the principal components was observed. These associations were preserved when analyzing all the data, suggesting that neither the means nor the variances of the regenerated plants were modified by effect of *in vitro* culture. It follows that both groups of plants

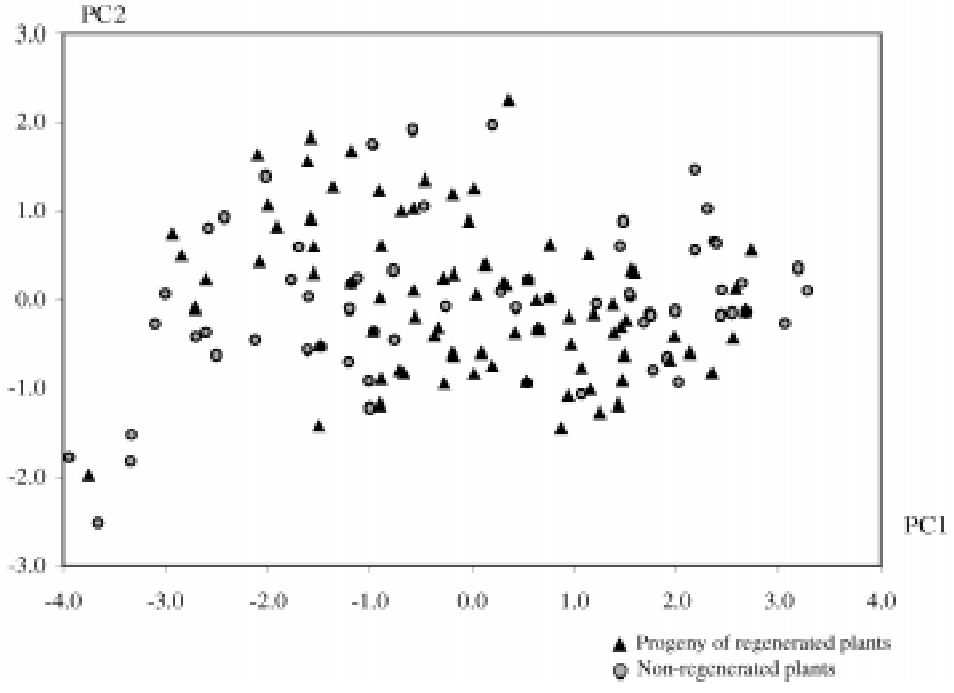


Figure 2 - Plot of the individuals on the basis of principal component 1 (PC1) and principal component 2 (PC2).

Table VI - Eigenvalues (λ) of principal component 1 and principal component 2 by genotype within group of different *Lycopersicon* species and F-values for the homogeneity of variance test.

Genotype	λ_1			λ_2		
	NP	PRP	F-value	NP	PRP	F-value
E1	1.91	2.47	1.29 n.s.	1.26	0.59	2.14 n.s.
E2	1.91	2.26	1.18 n.s.	1.24	1.71	1.38 n.s.
E3	2.55	2.00	1.28 n.s.	0.94	1.05	1.12 n.s.
C1	2.26	2.17	1.04 n.s.	1.32	0.93	1.42 n.s.
C2	2.40	2.11	1.14 n.s.	1.10	0.96	1.15 n.s.
C3	2.66	2.91	1.09 n.s.	1.00	0.74	1.35 n.s.
Pi1	2.04	2.77	1.36 n.s.	1.12	0.86	1.30 n.s.
Pi2	1.90	1.52	1.25 n.s.	1.72	1.31	1.31 n.s.
Pe1	3.31	-	-	0.54	-	-
H1	3.15	2.53	1.25 n.s.	0.79	0.94	1.19 n.s.

NP: Non-regenerated plants; PRP: progeny of the regenerated plants. For list of genotypes by species, see legend to Table II. n.s. Nonsignificant.

showed the same magnitude and source of variation in the phenotypic structure. According to De Klerk (1990), no variation would have been produced in the genetic systems influencing those traits.

CONCLUSIONS

No significant effect of *in vitro* culture on the morphometric traits was detected in *Lycopersicon* plants obtained from *in vitro* culture by principal component analysis.

Multivariate analysis is an appropriate methodology for the measurement of the stability of morphometric traits after one regeneration cycle.

RESUMO

Nesta experiência a estabilidade fenotípica dos caracteres métricos perímetro do culmo na base, no meio e no alto e número de flores por racemo foi medida mediante a prova de progênie com análise multivariada. Desta forma, uma nova metodologia para avaliação da estabilidade genética depois de um ciclo de regeneração *in vitro* foi proposta. Os componentes principais foram calculados para dois grupos de plantas de *Lycopersicon* spp.: o não regenerado e a progênie das plantas regeneradas. A ANOVA foi empregada como controle da análise de componentes principais. As diferenças entre grupos não foram estatisticamente significantes para nenhum caráter. Ambos os grupos apresentaram autovalores e autovetores similares. Os resultados obtidos indicaram que a estrutura fenotípica das plantas não foi modificada. Assim, nenhuma variação foi causada pela cultura *in vitro*. A análise multivariada mostrou ser uma metodologia apropriada para a medida da estabilidade dos caracteres métricos depois de um ciclo de regeneração.

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