

# Tissue culture effects on quantitative traits in *Stylosanthes guianensis* (Leguminosae)

Luciano Consoli, Maria Lúcia Carneiro Vieira, Cláudio Lopes de Souza Jr.  
and Antônio Augusto Franco Garcia

## ABSTRACT

Sixty-three tissue culture-derived progenies ( $R_1$ ) and 53 progenies from the original population ( $O_1$ ) of *Stylosanthes guianensis* were assessed for six traits of interest for pasture. An increase of 50.7%, 102.2% and 61.9% in the genetic variability was observed, respectively, for the traits basal area (BA), basal diameter (BD) and plant vigor (PV) in the regenerated population. A decrease in the genetic variability of 45.5% for fresh matter yield (FMY) and 32.9% for dry matter yield (DMY) was noted in the regenerated populations, while no difference was found for main stem height (MSH). Except for MSH, the means of all traits in the regenerated population were lower than those in the original population. Thus, the expected means of  $R(\mu_{sr})$  population were lower than those of control ( $\mu_{so}$ ) for the traits FMY ( $\mu_{so} = 306.37$  and  $\mu_{sr} = 248.76$  g/plant) and DMY ( $\mu_{so} = 72.12$  and  $\mu_{sr} = 61.61$  g/plant).

## INTRODUCTION

The genus *Stylosanthes* (Leguminosae) is native to the tropical and subtropical regions of the American continent. Some species from Africa and Asia also occur. The center of genetic diversity is located in Brazil, where extensive morphological variation exists. The genus *Stylosanthes* is considered one of the most important sources of natural tropical pastures, including *S. guianensis* (Aubl.) Sw., a perennial and self-pollinating species, which is adapted to acid and low fertility soils. *S. guianensis* has been widely used in Australia, where a large number of accessions potentially useful for forage were introduced (Williams *et al.*, 1984; Edye and Cameron, 1984).

Tissue culture techniques and genetic manipulation methodologies have been indicated to com-

plement some *Stylosanthes* breeding programs. Several studies reported the regeneration of *Stylosanthes* plants from leaf, hypocotyl and cotyledon explants (Meijer and Szabados, 1990), and also from protoplasts (Meijer and Steinbiss, 1983; Szabados and Roca, 1986; Vieira *et al.*, 1990). Regeneration occurs by organogenesis with shoot development on the surface of the calli. The genus *Stylosanthes* may be considered, among leguminous plants, as a model for *in vitro* regeneration. Under the hormone treatment, the morphological responses, in terms of callus development and shoot and root formation, depend on the part of the plant from which the explanted material was obtained. Dornelas *et al.* (1992) also found morphological differences between *Stylosanthes scabra* Vog. callus cultures obtained from cotyledonary explants. As for many legumes, the response depends on which type and concentration of auxin was used to supplement the culture medium. *Stylosanthes* species represent clear examples of how manipulation of chemical and physical environments,

and genetic factors, can influence the reorganization of cells in culture.

Tissue culture techniques have great potential for generating somaclonal variation (Larkin and Scowcroft, 1981; Evans *et al.*, 1984; Sree Ramulu *et al.*, 1986; van den Bulk *et al.*, 1990). Several authors suggest that the mechanisms involved in the process of variability generation are related to chromosomal abnormalities, activation of transposable elements, DNA methylation and point mutation (Lee and Phillips, 1988; Peschke and Phillips, 1992). However, the genetic basis of somaclonal variation has not yet been completely determined.

Most of the regenerants reported in the literature have been assessed for qualitative traits (Barwale and Widholm, 1987; Freytag *et al.*, 1989; Linacero and Vázquez, 1993), cytological alterations (Karp and Maddock, 1984; Lee and Phillips, 1987a,b) and biochemical modifications (Shenoy and Vasil, 1992; Amberger *et al.*, 1992). Studies related to the assessment of quantitative traits have been limited to the comparison of averages between the regenerated population and the control (Ryan *et al.*, 1987; Lee *et al.*, 1988; Adkins *et al.*, 1990; Mohmand and Nabors, 1990; Dahleen *et al.*, 1991; Stephens *et al.*, 1991, Hawbaker *et al.*, 1993), or estimates of phenotypic variance (Godwin *et al.*, 1987; Jackson and Dale, 1989; Dale and McPartlan, 1992). However, very few studies on somaclonal variation assessment based on genetic parameters have been reported (Liu and Chen, 1978; Baenziger *et al.*, 1989). The objective of this study was to evaluate the genetic variability of quantitative traits in progenies derived from original and regenerated populations of *S. guianensis*, in order to verify whether tissue culture is capable of generating a higher genetic variance than that of the original population.

## MATERIAL AND METHODS

*S. guianensis* (Aubl.) Sw. seeds cv IRI 1022 provided by the IRI Institute of Research, Brazil, were scarified in sulfuric acid for 2 min and then sterilized in 70% (v/v) ethanol for 40 sec and in 2% (v/v) NaOCl solution for 8 min. After washing three times in sterilized water, seeds were germinated in half-strength MS medium (Murashige and Skoog, 1962), containing 0.9% (w/v) agar and 1.5% (w/v) sucrose, under 16 h light regime ( $30 \mu\text{Em}^{-2}\cdot\text{s}^{-1}$ ), at  $27 \pm 2^\circ\text{C}$ . Hypocotyledonary and leaf tissues were excised at 10 and 30 days, respectively, from *in vitro* germinated seedlings, and placed on MS medium supplemented with  $2.0 \text{ mg}\cdot\text{l}^{-1}$  BAP (6-benzyl amino purine) for hypocotyls and

$0.4 \text{ mg}\cdot\text{l}^{-1}$  BAP +  $0.1 \text{ mg}\cdot\text{l}^{-1}$  NAA (1-naphthalene acetic acid) for leaves. Shoot regeneration occurred after 30 and 60 days, with a short callus phase in cultures derived from hypocotyledonary and leaf explants, respectively. Shoots were isolated and transferred to the same basal medium containing  $0.2 \text{ mg}\cdot\text{l}^{-1}$  IBA (indole-3-butyric acid) to promote root formation.

The  $R_0$  population (first regenerants) was made up of 133 regenerated plants, that were kept in greenhouse conditions along with a population of 100 plants obtained from seeds ( $O_0$  generation). Progenies were produced from seeds of individual plants collected after two flowering cycles, in both populations. Seeds of each progeny were sown in plastic pots containing a mixture of soil, sand and manure. The progenies  $R_1$  and  $O_1$  were evaluated in two randomized complete block experiments, with three replications at Piracicaba, SP, in 1994. Plots were 1.6-m rows, spaced 1.0 m apart with 0.4 m within rows, and with four plants per plot. In one experiment, 33  $R_1$  progenies and 26  $O_1$  progenies were assessed, while in a second experiment 30  $R_1$  progenies and 27  $O_1$  progenies were evaluated. Progenies were randomly assigned in the field experiments.

The following traits were recorded: main stem height (MSH, in cm), basal diameter (BD, in m), basal area ( $\text{BA} = (\text{BD}^2/4)\pi$ , in  $\text{m}^2$ ), plant vigor (PV, evaluated on a scale of 1 = poor vigor to 10 = good plant vigor), fresh matter yield (FMY, in g/plant) and dry matter yield (DMY, in g/plant).

Individual variance and covariance analysis were performed, and pooled into a single analysis for all traits by using Proc ANOVA from SAS program (SAS Institute Inc., 1988). The following genetic and phenotypic parameters were estimated by manipulating the mean squares as follows: genetic variance of progenies [ $\sigma_g^2 = \text{MSg} - \text{MSe}/r$ ], phenotypic variance at the mean progeny level [ $\sigma_{\text{Ph}}^2 = \text{MSg}/r$ ], heritability coefficients [ $h^2\% = (\sigma_g^2 / \sigma_{\text{Ph}}^2)100$ ], expected response to selection  $\text{RS} = i (\sigma_g^2 / \sigma_{\text{Ph}})$  and  $\text{RS}\% = (\text{RS}/\bar{X})100$ . In these expressions, MSg and MSe are the mean squares of the progenies and the experimental error of the joint analysis,  $r$  is the number of replications, and  $i = 1.755$  is the standardized selection differential for a 10% intensity of selection. The expected selected populations means were both obtained by the expressions  $\mu_{\text{so}} = \mu_o + \text{RS}_o$  for the original and  $\mu_{\text{sr}} = \mu_r + \text{RS}_r$  for the regenerated population, where  $\mu_o$  and  $\mu_r$  stand for the general means of the original and regenerated populations, respectively. Estimates of genetic [ $r_{g(x,y)} = \text{cov}_{g(x,y)} / \sigma_{g(x)} \cdot \sigma_{g(y)}$ ] and phenotypic [ $r_{\text{Ph}(x,y)} = \text{cov}_{\text{Ph}(x,y)} / \sigma_{g(x)} \cdot \sigma_{g(y)}$ ] correlations for both populations were obtained from the covariance analysis

between traits, resulting in  $COV_g = (MP_g - MP_e)/r$  and  $COV_{Ph} = (MP_g)/r$ , where  $MP_g$  and  $MP_e$  refer to the mean products of progenies and experimental error, respectively (Falconer, 1989).

## RESULTS AND DISCUSSION

Acclimation conditions, the 50% plant survival rate in greenhouse conditions and variable reproduction rates, which included seed bareness of some  $R_0$  plants, resulted in a reduction in the number of regenerated progenies from 133 to 63. Similarly, the original progenies were reduced to 53.

The analysis of variance (not shown) detected genetic variability for all the traits. The contrasts among  $O_1$  and  $R_1$  progenies were highly significant ( $P < 0.01$ )

for all the traits, except for MSH (Table I). The averages for BA, BD, PV, FMY and DMY in the original ( $\mu_o$ ) and regenerated populations ( $\mu_r$ ) differed significantly ( $P < 0.01$ ). The regenerated population showed a lower range of variation than the original one for all traits, although the range of variation for MSH and PV were similar. The experimental coefficients of variation were within the acceptable values for these traits, in this species (Table I).

There were increases in the genetic variabilities in the regenerated populations for BA, BD and PV as compared to those recorded, for the same characters, in the original populations (Table II). Therefore,  $h^2\%$  and  $RS\%$  also increased in the regenerated population. However, the means of these traits in the regenerated population were lower than those of the original population. For the yield traits FMY and DMY,

**Table I** - Means, ranges and coefficients of variation (CV%) for the traits: main stem height (MSH), basal area (BA), basal diameter (BD), plant vigor (PV), fresh matter yield (FMY) and dry matter yield (DMY), for the regenerated and original populations.

Traits	Populations				CV%
	Original		Regenerated		
	Means	Ranges	Means <sup>a</sup>	Ranges	
MSH (cm)	43.14 ± 3.42	29.17 - 61.08	42.37 ± 3.42 ns	26.72 - 54.50	13.88
BA (m <sup>2</sup> )	0.31 ± 0.05	0.15 - 0.45	0.26 ± 0.05**	0.08 - 0.44	29.87
BD (m)	0.61 ± 0.05	0.43 - 0.74	0.54 ± 0.05**	0.30 - 0.73	15.48
PV (1-10)	6.19 ± 0.52	5.17 - 7.92	5.87 ± 0.52**	4.08 - 7.41	14.87
FMY (g/plant)	228.57 ± 49.09	106.76 - 112.61	199.29 ± 49.09**	67.62 - 403.60	39.98
DMY (g/plant)	54.26 ± 12.07	24.32 - 100.45	48.35 ± 12.07**	15.24 - 86.49	40.96

a: ns - Non-significant; \*\* $P < 0.01$ , referring to the significance of the mean squares of the joint analysis of variance (F test) contrasting regenerated versus original progenies.

**Table II** - Estimates of genetic ( $\sigma_g^2$ ) and phenotypic ( $\sigma_{Ph}^2$ ) variances, heritability ( $h^2\%$ ) and responses to selection ( $RS\%$ ) for all traits in the original and regenerated populations.

Traits	Populations							
	Original				Regenerated			
	$\sigma_g^2$	$\sigma_{Ph}^2$	$h^2\%$	$RS\%$ <sup>a</sup>	$\sigma_g^2$	$\sigma_{Ph}^2$	$h^2\%$	$RS\%$ <sup>a</sup>
MSH	28.56	40.29	70.89	18.30	27.22	38.94	69.89	18.07
BA <sup>b</sup>	3.04	5.38	56.55	23.72	4.58	6.92	66.24	37.47
BD <sup>b</sup>	3.07	5.69	53.97	11.77	6.20	8.82	70.33	21.34
PV	0.20	0.46	42.53	8.22	0.32	0.59	54.45	12.45
FMY	3370.63	5780.82	58.31	34.04	1836.88	4247.07	43.25	24.82
DMY	185.13	330.88	55.95	32.92	124.15	269.90	46.00	27.43

<sup>a</sup>Selection intensity = 10%; <sup>b</sup> $\sigma_g^2$  and  $\sigma_{Ph}^2$  values are multiplied by  $10^3$  for both populations.

reduction in the genetic variability, heritability coefficients, and responses to selection values in the regenerated plants were also lower than those of the original population. The reductions in genetic variability were 45.5% and 32.9% for FMY and DMY traits, respectively. However, for the MSH trait these parameters were quite similar for the O<sub>1</sub> and R<sub>1</sub> populations (Table II).

The estimates of the genetic ( $r_{go}$  and  $r_{gr}$ ) and phenotypic ( $r_{Pho}$  and  $r_{Phr}$ ) correlation between traits in both population types were positive and high. The genetic correlations apparently did not differ between the populations, except for MSH x BA and MSH x BD, which showed lower genetic correlation values for O<sub>1</sub> population compared to the R<sub>1</sub> population (Table III). We found that high values of  $r_g$  and  $r_{ph}$  for this species were reported by Pontes *et al.* (1983), involving all combinations among the traits BD, BA, FMY and DMY. However, lower genetic ( $r_g = 0.172$ ) and phenotypic ( $r_{ph} = 0.322$ ) correlation values were detected between the traits MSH x DMY (Barros, 1978).

The expected means of the original and regenerated selected populations were similar ( $\mu_{so}$  and  $\mu_{sr}$ ) for the MSH, BA, BD and PV traits, whereas for the yield traits, FMY and DMY, tissue culture seems to have had an adverse effect, reducing the expected means of the regenerated selected population, for both fresh and dry matter yield traits (Table IV).

**Table III** - Estimates of the genetic ( $r_g$ ) and phenotypic ( $r_{ph}$ ) correlations involving all traits for the original (upper) and regenerated population (lower diagonal).

	Genetic correlations					
	MSH	BA	BD	PV	FMY	DMY
MSH	-	0.35	0.38	0.90	0.48	0.52
BA	0.66	-	1.00	0.80	0.93	0.95
BD	0.72	0.99	-	0.81	0.94	0.94
PV	0.85	0.70	0.78	-	0.99	1.01
FMY	0.66	0.86	0.86	0.85	-	0.99
DMY	0.70	0.93	0.93	0.84	0.99	-

  

	Phenotypic correlations					
	MSH	BA	BD	PV	FMY	DMY
MSH	-	0.41	0.43	0.73	0.50	0.52
BA	0.62	-	0.99	0.72	0.85	0.86
BD	0.66	0.98	-	0.74	0.84	0.85
PV	0.73	0.68	0.74	-	0.81	0.83
FMY	0.59	0.78	0.77	0.74	-	0.98
DMY	0.62	0.83	0.82	0.76	0.98	-

**Table IV** - Expected means of the selected original and regenerated populations for the traits MSH, BA, BD, PV, FMY and DMY.

Traits	Improved populations <sup>a</sup>	
	Original	Regenerated
MSH (cm)	51.04	50.02
BA (m <sup>2</sup> )	0.38	0.36
BD (m)	0.68	0.66
PV (scale)	6.70	6.60
FMY (g/plant)	306.37	248.76
DMY (g/plant)	72.12	61.61

<sup>a</sup>Selection intensity = 10%.

These results indicated that the methodology used for *S. guianensis* plant regeneration increased the genetic variability in 50% of the traits assessed (BA, BD and PV), reduced the variability for the traits of FMY and DMY, and did not significantly alter the MSH trait. The data indicate a significant reduction in the mean of the regenerated population for all the traits, except MSH. Godwin *et al.* (1987, 1990) reported significant reduction in the means of the DMY trait in 33.7% and 9.5% of the R<sub>2</sub> progenies of *S. guianensis* and *S. scabra* and none R<sub>2</sub> progeny means were superior to the control; however, 12.5% of R<sub>2</sub> progeny means of *S. hamata* had greater and 2.5% had lower yields than the parental controls.

The majority of studies on plant yield have shown an adverse effect of the somaclonal variation, with a reduction in the mean of regenerated wheat (Baenziger *et al.*, 1989; Mohmand and Nabors, 1990), potato (Dale and McPartlan, 1992), maize (Lee *et al.*, 1988) and oat (Dahleen *et al.*, 1991) populations. However, Stephens *et al.* (1991) did not find differences in yield among regenerated and control lines of soybean, while Hawbaker *et al.* (1993) observed a bi-directional variation for all traits studied, with increase and decrease in soybean yield for different cultivars. Beneficial alterations were observed for maturity in maize (Lee *et al.*, 1988), sorghum (Bhaskaran *et al.*, 1987) and flooding tolerance in rice (Adkins *et al.*, 1990). Dale and McParthan (1992) reported a significant reduction in the means of plant height, tuber weight, tuber number, large tuber weight and large tuber number per plant in regenerated potato plants.

Variation among regenerated and original plants was also found in *Lolium* (Jackson and Dale, 1989), *Stylosanthes* (Godwin *et al.*, 1987) and sugar cane (Liu and Chen, 1978). In the latter, genetic variability for several traits related to sugar cane production was estimated and only one clone showed a mean significantly higher in relation to the control. Baenziger

*et al.* (1989) reported genetic variance for di-haploid lines (DHLs) derived from wheat cultivars. The DHLs had a lower grain yield, but showed higher genetic variance than the control.

*S. guianensis* plant regeneration caused a significant reduction in the mean of all the traits except MSH, an increase in the genetic variability for BA, BD and PV, and a decrease for FMY and DMY. Comparing the expected means of the populations after selection, there is a similarity among the means for MSH, BA, BD and PV. However, the means of the regenerated population were 18.81% and 14.57% lower, for the traits FMY and DMY, respectively, indicating that tissue culture was not able to generate an increase in genetic variability great enough to be used in breeding programs.

The experiments were conducted in just one year and location due to the low amount of seeds produced per plant. Therefore, the estimates of genetic parameters could be biased by genotype X location and genotype X year interactions, though these interactions are expected to influence O<sub>1</sub> and R<sub>1</sub> populations similarly.

Modifications in the culture conditions (callus phase, hormone concentrations) can increase the level of somaclonal variation. There are also indications in the literature of a genetic component influencing the type and magnitude of the induced variation, resulting in a higher degree of variability for certain wheat cultivars (Ryan *et al.*, 1987; Mohmand and Nabors, 1990). Thus, alterations in the methodology in the process of *Stylosanthes* plant regeneration may generate high levels of genetic variability, which may justify its use in breeding programs. New studies need to be carried out to identify cultivars more responsive to genetic alterations and to detect the main *in vitro* factors responsible for the increase of genetic variance in regenerated populations. These studies will allow us to make a better decision in future somaclone generation and use.

## ACKNOWLEDGMENTS

To Mr. Carlos Alberto de Oliveira, for helping in the installation of the field experiments and in data collection. This research was supported by the Brazilian Institutions, FAPESP (grant 88/3816-8), CNPq (grant 52.2399/94.0) and FINEP (grant 6.4.93.172.00).

Publication supported by FAPESP.

## RESUMO

Sessenta e três progênies derivadas da cultura de tecidos (R<sub>1</sub>) e 53 derivadas da população original (O<sub>1</sub>) de

*Stylosanthes guianensis* (Leguminosae) foram avaliadas para seis caracteres de interesse forrageiro. Verificou-se um aumento na variabilidade genética da população original de 50,7, 102,2 e 61,6% para os caracteres área basal (BA), diâmetro basal (BD) e vigor de planta (PV), respectivamente. Já para os caracteres produção de matéria verde (FMY) e produção de matéria seca (DMY), ocorreu um decréscimo na variabilidade genética da população regenerada de 45,5 e 32,9%, respectivamente. Para o caráter comprimento da haste principal (MSH), não verificou-se diferença. Para todos os caracteres avaliados, com exceção de MSH, a média da população regenerada foi inferior à média da população original. Para os caracteres de produção, as médias esperadas da população regenerada melhorada foram inferiores (FMY = 248,76 e DMY = 61,61 g/planta) em relação às médias esperadas da população controle (FMY = 306,37 e DMY = 72,12 g/planta).

## REFERENCES

- Adkins, S.W., Shiraishi, T., McComb, J.A., Ratanopol, S., Kupkanchanakul, T., Armstrong, L.J. and Schultz, A.L. (1990). Somaclonal variation in rice - Submergence tolerance and other agronomic characters. *Physiol. Plant.* 80: 647-654.
- Amberger, L.A., Shoemaker, R.C. and Palmer, R.G. (1992). Inheritance of two independent isozyme variants in soybean plants derived from tissue culture. *Theor. Appl. Genet.* 84: 600-607.
- Baenziger, P.S., Wesenberg, D.M., Smail, V.M., Alexander, W.L. and Schaeffer, G.W. (1989). Agronomic performance of wheat doubled-haploid lines derived from cultivars by anther culture. *Plant Breed.* 103: 101-109.
- Barros, L.M. (1978). Avaliação da variabilidade de caracteres agrônômicos em populações de *Stylosanthes guianensis* (Aubl.) Sw. Master's thesis, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba.
- Barwale, U.B. and Widholm, J.M. (1987). Somaclonal variation in plants regenerated from cultures of soybean. *Plant Cell Rep.* 6: 365-368.
- Bhaskaran, S., Smith, R.H., Paliwol, S. and Schertz, K.F. (1987). Somaclonal variation from *Shorgum bicolor* (L.) Moench cell culture. *Plant Cell Tissue Organ Cult.* 9: 189-196.
- Dahleen, L.S., Stuthman, D.D. and Rines, H.W. (1991). Agronomic trait variation in oat lines derived from tissue culture. *Crop Sci.* 31: 90-94.
- Dale, P.J. and McPartlan, H.C. (1992). Field performance of transgenic potato plants compared with controls regenerated from tuber discs and shoot cuttings. *Theor. Appl. Genet.* 84: 585-591.
- Dornelas, M.C., Vieira, M.L.C. and Apezato da Glória, B. (1992). Histological analysis of organogenesis and somatic embryogenesis induced in immature tissues of *Stylosanthes scabra* Vog. *Ann. Bot.* 70: 477-482.

- Edey, L.A. and Cameron, D.C.** (1984). Prospects of *Stylosanthes* improvement and utilization. In: *The Biology and Agronomy of Stylosanthes* (Stace, H.M. and Edey, L.A., eds.). Academic Press, Sydney, pp. 571-589.
- Evans, D.A., Sharp, W.R. and Medina-Filho, H.P.** (1984). Somaclonal and gametoclonal variation. *Amer. J. Bot.* 71: 759-774.
- Falconer, D.S.** (1989). *Introduction to Quantitative Genetics*. Longman Scientific & Technical, Harlow.
- Freytag, A.H., Rao-Arelli, A.P., Anand, S.C., Wrather, J.A. and Owens, L.D.** (1989). Somaclonal variation in soybean plants regenerated from tissue culture. *Plant Cell Rep.* 8: 199-202.
- Godwin, I.D., Gordon, G.H. and Cameron, D.F.** (1987). Callus culture-derived somaclonal variation in the tropical pasture legume *Stylosanthes guianensis* (Aubl.) Sw. *Plant Breed.* 98: 220-227.
- Godwin, I.D., Cameron, D.F. and Gordon, G.H.** (1990). Variation among somaclonal progenies from three species of *Stylosanthes*. *Aust. J. Agricult. Res.* 41: 645-656.
- Hawbaker, M.S., Fehr, W.R., Mansur, L.M. and Shoemaker, R.C.** (1993). Genetic variation for quantitative traits in soybean lines derived from tissue culture. *Theor. Appl. Genet.* 87: 49-53.
- Jackson, J.A. and Dale, P.J.** (1989). Somaclonal variation in *Lolium multiflorum* L. and *temulentum* L. *Plant Cell Rep.* 8: 161-164.
- Karp, A. and Maddock, S.E.** (1984). Chromosome variation in wheat plants regenerated from cultured immature embryos. *Theor. Appl. Genet.* 67: 249-255.
- Larkin, P.J. and Scowcroft, W.R.** (1981). Somaclonal variation - a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.* 60: 197-214.
- Lee, M. and Phillips, R.L.** (1987a). Genetic variants in progeny of regenerated maize plants. *Genome* 29: 834-838.
- Lee, M. and Phillips, R.L.** (1987b). Genomic rearrangements in maize induced by tissue culture. *Genome* 29: 122-128.
- Lee, M. and Phillips, R.L.** (1988). The chromosomal basis of somaclonal variation. *Ann. Rev. Plant Physiol. Plant. Mol. Biol.* 39: 413-437.
- Lee, M., Gadelmann, J.L. and Phillips, R.L.** (1988). Agronomic evaluation of inbred lines derived from tissue cultures of maize. *Theor. Appl. Genet.* 75: 841-849.
- Linacero, R. and Vázquez, A.M.** (1993). Somaclonal variation in rye. *Mutation Res.* 302: 201-205.
- Liu, M.C. and Chen, W.H.** (1978). Tissue and cell culture as aids to sugarcane breeding. II. Performance and yield potential of callus derived lines. *Euphytica* 27: 273-282.
- Meijer, E.G.M. and Steinbiss, H.H.** (1983). Plantlet regeneration from suspension and protoplast cultures of the tropical pasture legume *Stylosanthes guianensis* (Aubl.) Sw. *Ann. Bot.* 52: 305-310.
- Meijer, E.G.M. and Szabados, L.** (1990). Cell and tissue culture of *Stylosanthes* spp. In: *Biotechnology in Agriculture and Forestry 10. Legumes and Oilseed Crops I* (Bajai, Y.P.S., ed.). Springer Verlag, London, pp. 312-322.
- Mohmand, A.S. and Nabors, M.W.** (1990). Somaclonal variant plants of wheat derived from mature embryo explants of three genotypes. *Plant Cell Rep.* 8: 558-560.
- Murashige, T. and Skoog, F.** (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.
- Peschke, V.M. and Phillips, R.L.** (1992). Genetic implications of somaclonal variation in plants. In: *Adv. Genet.* 30: 41-75.
- Pontes, O.F.S., Martins, P.S. and Vello, N.A.** (1983). Melhoramento genético de populações de *Stylosanthes guianensis*. *Pesq. Agrop. Bras.* 18: 413-420.
- Ryan, S.A., Larkin, P.J. and Elleson, F.W.** (1987). Somaclonal variation in some agronomic and quality characters in wheat. *Theor. Appl. Genet.* 74: 77-82.
- SAS Institute Inc. SAS/STAT** (1988). *User's Guide*. Version 6.03, Cary, NC.
- Shenoy, V.B. and Vasil, I.K.** (1992). Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napier grass (*Pennisetum purpureum* K. Schum). *Theor. Appl. Genet.* 83: 947-955.
- Sree Ramulu, K., Dijkhuis, P., Poest, S., Bokelmann, G.S. and Groot, A.B.** (1986). Variation in phenotype and chromosome number of plants regenerated from protoplasts of dihaploid end tetraploid potato. *Plant Breed.* 97: 118-128.
- Stephens, P.A., Nickell, C.D. and Widholm, J.M.** (1991). Agronomic evaluation of tissue-culture-derived soybean plants. *Theor. Appl. Genet.* 82: 633-635.
- Szabados, L. and Roca, W.M.** (1986). Regeneration of isolated mesophyll and cell suspension protoplasts to plants in *Stylosanthes guianensis*. A tropical forage legume. *Plant Cell Rep.* 3: 174-177.
- Vieira, M.L.C., Jones, B., Cocking, E.C. and Davey, M.R.** (1990). Plant regeneration from protoplasts isolated from seedling cotyledons of *Stylosanthes guianensis*, *S. macrocephala* and *S. scabra*. *Plant Cell Rep.* 9: 289-292.
- van den Bulk, R.W., Laffler, F.J.M., Lindhout, W.H. and Koornneef, M.** (1990). Somaclonal variation in tomato: Effect of explant source and comparison with chemical mutagenesis. *Theor. Appl. Genet.* 80: 817-825.
- Williams, R.J., Reid, R., Schultze-Kraft, R., Costa, N.M.S. and Tomas, B.D.** (1984). Natural distribution of *Stylosanthes*. In: *The Biology and Agronomy of Stylosanthes* (Stace, H.M. and Edey, L.A., eds.). Academic Press, Sydney, pp. 73-101.

(Received September 1, 1995)