



Chromosomal and morphological studies of diploid and polyploid cytotypes of *Stevia rebaudiana* (Bertoni) Bertoni (Eupatorieae, Asteraceae)

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Abstract

In this study, we examined the chromosome number and some morphological features of strains of *Stevia rebaudiana*. The chromosomes were analyzed during mitosis and diakinesis, and the tetrad normality and pollen viability were also assessed. In addition, stomata and pollen were measured and some plant features were studied morphometrically. All of the strains had $2n = 22$, except for two, which had $2n = 33$ and $2n = 44$. Pairing at diakinesis was $n = 11_{II}$ for all of the diploid strains, whereas the triploid and tetraploid strains had $n = 11_{III}$ and $n = 11_{IV}$, respectively. Triploid and tetraploid plants had a lower tetrad normality rate than the diploids. All of the strains had inviable pollen. Thus, the higher the ploidy number, the greater the size of the pollen and the stomata, and the lower their number per unit area. The triploid strain produced the shortest plants and the lowest number of inflorescences, whereas the tetraploid strain had the largest leaves. Analysis of variance revealed highly significant differences among the strains, with a positive correlation between the level of ploidy and all of the morphological features examined.

Key words: Asteraceae, pollen, polyploidy, *Stevia rebaudiana*, stevioside, stomata.

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Introduction

The genus *Stevia* Cav. (Eupatorieae, Asteraceae), which consists of approximately 150-200 species of herbaceous, shrub and sub-shrub plants (Gentry, 1996), is one of the most distinctive genera within the tribe Eupatorieae, mainly because of the morphological uniformity of its flowers and capitula, which consist of five tubular flowers and five involucral bracts (King and Robinson, 1987). Although geographically widespread, this genus occurs exclusively in tropical and subtropical regions of the United States and Central and South America (Robinson and King, 1977). Randi (1980) reviewed the potential uses of *Stevia rebaudiana*, which produces stevioside, a non-caloric sweetener that does not ferment in the human body.

Thirteen distinct strains of *S. rebaudiana* are currently being domesticated and assessed agronomically by the Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA/UNICAMP). Although this

species has not yet been extensively studied agronomically, considerable genetic variability has been observed in plant size, flowering period, and stevioside content, which may vary from 2% to 10% (Magalhães, 2000).

Several of the known strains of *S. rebaudiana* resulted from an experiment done at the University of New Mexico designed to develop polyploids that were richer in stevioside (Valois, 1992). In Brazil, plants from this experiment were initially cultivated at the CENARGEN/EMBRAPA germplasm bank before part of the collection was transferred to CPQBA/UNICAMP. The induction of polyploidy to improve agronomic yields is a process commonly used in plants of economic interest (Allard, 1960) and has been applied to other species such as *Nicandra physaloides* (Gupta and Roy, 1986), coffee (Cruz *et al.*, 1993), *Clitoria ternatea* (Ghandi and Patil, 1997) and orange (Romero-Aranda *et al.*, 1997).

A better adaptability of individuals and increased organ and cell sizes are usually associated with polyploidy (Guerra, 1988). In *Andropogon gerardii*, a species with hexaploid ($2n = 6x = 60$) and enneaploid ($2n = 9x = 90$) cytotypes, differences in plant size have been observed, and although seed production per unit area is the same in both

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cytotypes, it is more efficient in hexaploids (Keeler and Davis, 1999). In some species of coffee with different numbers of chromosomes, higher levels of ploidy generally produce larger stomatal cells with a greater number of chloroplasts (Boaventura *et al.*, 1981).

In Gramineae such as *Paspalum*, diploid and tetraploid races within a species are basically distinguished by their reproductive system, which is sexual in diploids and apomictic in polyploids (Burson, 1975). Crossbreeding between individuals with different numbers of chromosomes usually leads to sterile progeny. According to Allard (1960), in various agricultural plants with diploid and tetraploid individuals, the progeny are partially or completely sterile triploids that do not bear seeds.

The main objective of this study was to determine the chromosome numbers of the strains of *S. rebaudiana* cultivated at CPQBA/UNICAMP in order to obtain information that could be useful in breeding programs. The tetrad normality and pollen viability were used to estimate the regularity of meiosis. Some morphological features of each strain were also analyzed morphometrically in order to assess the relationship with the level of ploidy.

Material and Methods

The plant material (floral buds, achenes and roots) for the cytogenetic studies was collected from strains of *S. rebaudiana* cultivated *in vivo* or *in vitro* at CPQBA/UNICAMP. These strains were obtained mainly from CENARGEN/EMBRAPA, in Brasília (Table 1), and originally derived from experiments by Valois (1992), who exposed seeds of *S. rebaudiana* to nine treatments by soaking them for 18 h in a solution of colchicine (an antimutagenic agent), the concentration of which ranged from 0.5% (treatment T1) to 0.001% (treatment T9). A control treatment with no colchicine (T0) was also done. After the treatment, all of the seeds were kept under conditions appropriate for germination.

In this study, the original designations of Valois (1992) for the plants resulting from each treatment have been maintained: the letter T followed by a number refers to the concentration of colchicine used and the letter V followed by a number identifies each vase (Table 1). Voucher specimens of each strain cultivated in the field were deposited in the herbarium at UEC (Universidade Estadual de Campinas) (Table 1).

Mitotic and meiotic studies

For the mitotic studies, root tips were obtained from plants cultivated *in vitro* or from dark achenes germinated in gerbox with gibberellic acid (50 ppm), at room temperature. Light achenes are sterile (Felippe *et al.*, 1971; Monteiro, 1980). The root tips were pretreated with paradichlorobenzene (PDB) for 4 h at 16 °C-18 °C and fixed in ethanol-acetic acid (3:1, v/v). Cytological preparations were obtained using the Giemsa technique (Guerra, 1983). For meiotic analysis, floral buds were fixed in ethanol-acetic acid (3:1, v/v) for approximately 24 h and the anthers then squashed onto slides in acetic carmine (Medina and Conagin, 1964).

The cytological preparations were examined by light microscopy. All cells in which it was possible to determine the chromosome number, the meiotic behavior and morphological features, were included in the analysis. Chromosome numbers were based on counts from an average of 10 cells per strain. The karyotypes were determined from a single cell of the T0 (control) treatment. Chromosome measurements in other strains were also obtained using a camera lucida.

Tetrad analysis and pollen viability

Tetrad analysis and pollen viability were assessed as described by Medina and Conagin (1964). The technique of Alexander (1980) was also used to estimate pollen viability in order to allow comparison between the results obtained with both techniques (Dafni, 1992).

Table 1 - Chromosome number (2n), pairing at diakinesis, tetrad normality and pollen viability (%) in strains of *Stevia rebaudiana* (Bertoni) Bertoni.

Access	Origin	2n	Pairing at diakinesis	Tetrad normality (%)	Pollen variability (%)
Brasília F1	CENARGEN/EMBRAPA	22	11 _{II}	98	0
Maringá F1	Maringá-PR	22	11 _{II}	99	0
Canada F1	Canada	22	11 _{II}	96	0
T0 (control)	Maringá-PR	22	11 _{II}	95	0
T5V1	CENARGEN/EMBRAPA	22	11 _{II}	96	0
T6V1	CENARGEN/EMBRAPA	22	11 _{II}	99	0
T6V3	CENARGEN/EMBRAPA	44	11 _{IV}	80	0
T7V1	CENARGEN/EMBRAPA	33	11 _{III}	64.5	0
T8V1	CENARGEN/EMBRAPA	22	11 _{II}	93	0
T8V2	CENARGEN/EMBRAPA	22	11 _{II}	97.5	0
T9V1	CENARGEN/EMBRAPA	22	11 _{II}	98	0

The total number of normal and abnormal tetrads per slide (approximately 1,000 tetrads from five floral buds) was used to estimate tetrad normality. Pollen viability was assessed for each strain by counting the number of viable and non-viable grains in 200 pollen grains per bud collected from five floral buds of different inflorescences. Each slide was prepared with the anthers of a single bud.

Morphometric analysis

The morphometric analysis focused on stomata size, pollen diameter, and on some vegetative (leaf size and plant height) and reproductive (inflorescences per plant) features.

The number and size of stomata were determined using the third leaf of the primary branch, from the apex down (Figure 1B), of plants from each strain cultivated in the field. The stomata were observed from the impressions made on a thin film of transparent cellulose wetted with acetone and applied to the adaxial surface of the leaf. Approximately 10 leaf impressions from 10 individuals of each strain were collected. To determine stomata size, 100 stomata from each leaf impression were drawn using a camera lucida, and their length and width then measured (Figure 1A). The stomata density was calculated by counting the stomata in five microscopic fields per slide using a 40X objective and a 10X ocular (area of 0.23 mm² per field).

Pollen diameter was determined by drawing 100 pollen grains from each of five floral buds using a camera lucida (total of 500 pollen grains per strain). The height of all of the plants was measured (Figure 1B). Leaf size (length and width) was assessed using the first two leaves of the lowest secondary branch of each plant in all of the strains. The number of inflorescences was established using the second secondary branch, at the third tertiary branch of each plant (Figure 1B).

Variations in the measurements among plants of each strain were compared by calculating the coefficient of variation (CV%). Variations between strains were assessed using ANOVA to determine whether there was a relationship between the level of ploidy and the parameters measured (pollen and stomata size, leaf size, plant height and number of inflorescences per branch). A value of $p < 0.05$ indicated significance.

Results

Almost all of the *S. rebaudiana* strains studied had $2n = 22$, except for two, which had $2n = 33$ and $2n = 44$ (Table 1, Figure 2). The karyotype of the T0 plants consisted of 11 pairs of chromosomes (six metacentrics, four submetacentrics and one acrocentric pair). The chromosomal measurements for the T6V3, T5V1 and T8V2 strains were similar (1.1-2.3 μm). A satellite was observed on the short arm of the third pair of chromosomes.

Diakinesis was the only phase analyzed in meiosis. Pairing was $n = 11_{II}$ for all strains of *S. rebaudiana*, except for two, which had $n = 11_{III}$ and $n = 11_{IV}$, that corresponded to $2n = 33$ and $2n = 44$, respectively (Table 1, Figure 2). The tetrad normality rate varied from 64.5% to 99% (Table 1). Abnormal tetrads generally had three but never four nuclei. The pollen of all strains was inviable (Table 1, Figure 2).

In the 12 strains examined, the stomata were 30-52 μm long and 18-28 μm wide, with a density of 12 to 22 stomata per unit area. The pollen diameter varied from 21.2 to 34 μm (Table 2, Figure 2).

Morphometric analysis showed that plant height varied from 27.8 to 96.8 cm, the number of inflorescences ranged from 5 to 22 (per unit), and leaf length and width ranged from 1.8 to 4.9 cm and from 0.6 to 2.24 cm, respectively (Table 2).

The coefficients of variation for the features measured were: 1.64-2.77% and 1.69-5.30% for stomata length and width, respectively, 8.5-38.6% for pollen diameter, 8-41.1% for plant height, 23-51.9% for inflorescence number, and 11.1-30.7% and 13.8-32.4% for leaf length and width, respectively. Analysis of variance showed that all of the features quantified differed significantly among strains ($p \leq 0.001$) (Table 2).

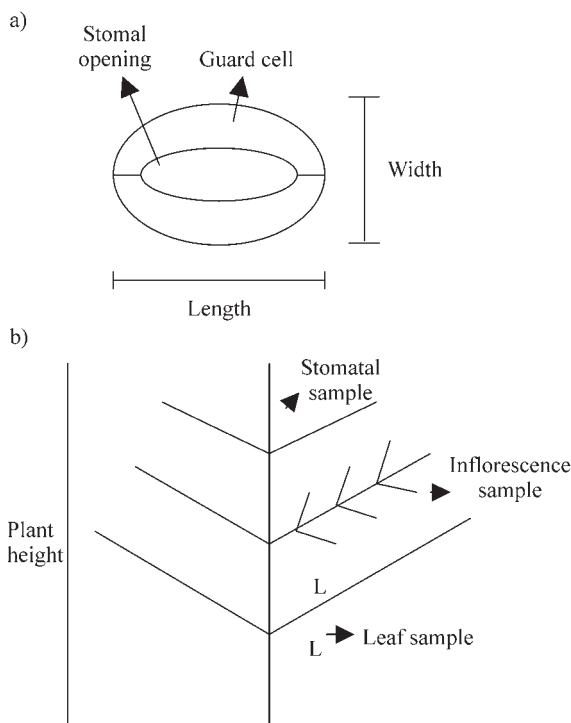


Figure 1 - a) Diagram of a stoma indicating the positions used to measure the length and width. b) Diagram of *Stevia rebaudiana* (Bertoni) Bertoni, indicating the location of the points of stomatal, leaf and inflorescence sampling and the procedure used to determine plant height (L = leaf).

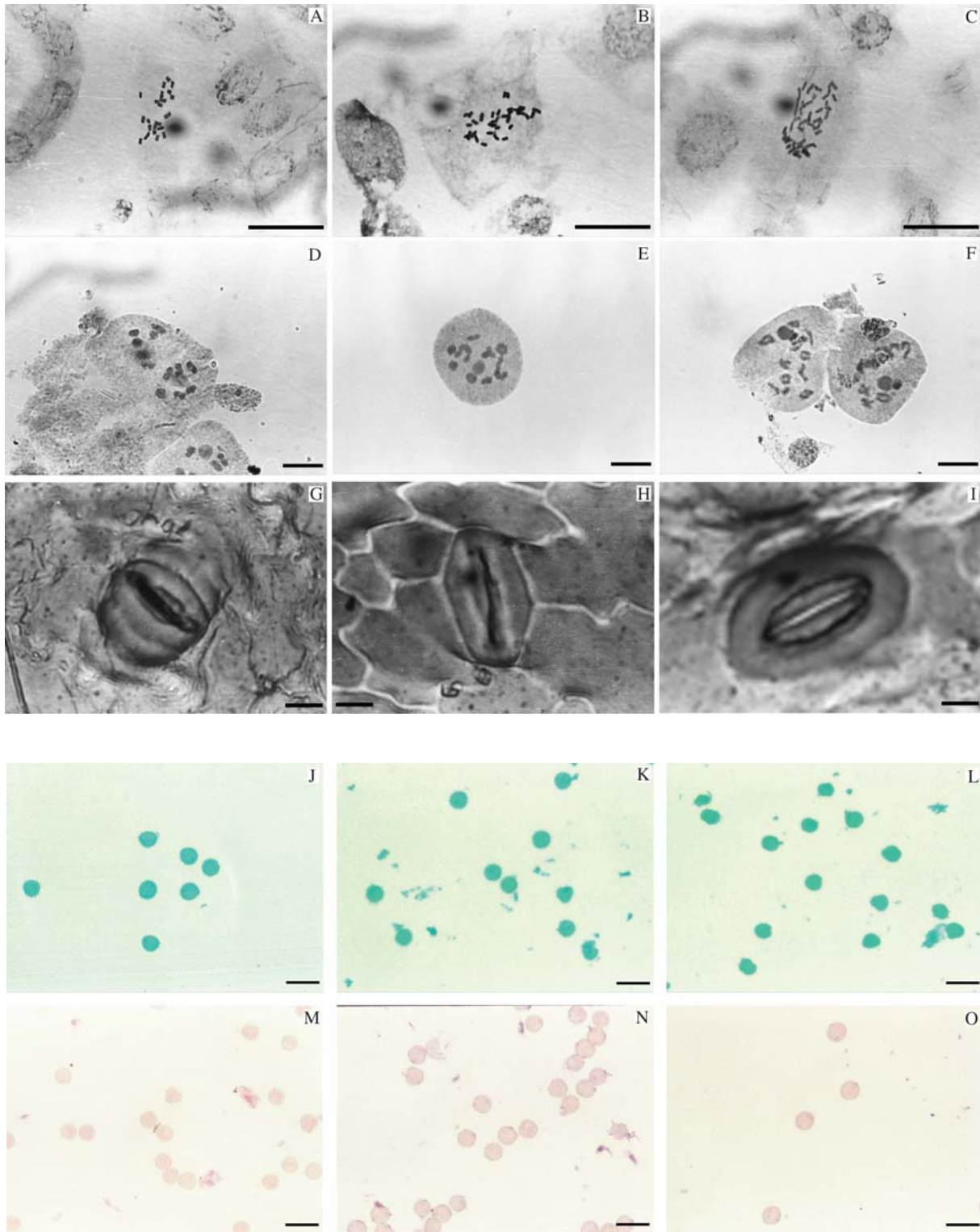


Figure 2 - Mitosis (A-C), meiosis (D-F), stoma (G-I) and pollen stained according to Alexander (J-L) or with acetic carmine (M-O) for strains of *Stevia rebaudiana* (Bertoni) Bertoni. A, D, G, J, M = Maringá F1 ($2n = 22$, $n = 11_{II}$), B, E, H, K, N = T7V1 ($2n = 33$, $n = 11_{III}$) and C, F, I, L, O = T6V3 ($2n = 44$, $n = 11_{IV}$). N = nucleolus. Bars = 10 μ m in A-F and 20 μ m in G-O.

Discussion

The genus *Stevia* shows great variation in chromosome number. Although most reports indicate that $n = 11$ ($2n = 22$), values of $2n = 24, 33, 34, 44, 48, 66, 70$ have also been observed (Darlington and Wylie, 1955; Bolkhovskikh *et al.*, 1969; Moore, 1973, 1974, 1977; Golblatt, 1981, 1984, 1985, 1988; Goldblatt and Johnson, 1990, 1991, 1996, 1998).

Galiano (1987) considered *Stevia* as a multibasic genus, with $x = 11$, $x = 12$ and $x = 17$, while Frederico *et al.* (1996) considered its basic chromosome number as $x = 11$. According to This variation reflects numerical (aneuploidy and polyploidy) and structural changes, mainly pericentric inversions (Frederico *et al.*, 1996). Aneuploidy also occurs in *Picris babylonica*, a plant that belongs to the same family as *Stevia* (Malallah *et al.*, 2001).

For *S. rebaudiana*, the chromosome number of $2n = 22$ ($n = 11$) previously reported by Frederico *et al.* (1996) and Monteiro (1980, 1982) was confirmed here for various strains (Table 1). However, two strains had $2n = 33$ and $2n = 44$, and represented triploid and tetraploid cytotypes, respectively (Table 1). For most strains, pairing at diakinesis was $n = 11_{II}$, which agrees with the literature, while pairing at diakinesis for the triploid and tetraploid strains was $n = 11_{III}$ and $n = 11_{IV}$, respectively (Table 1). This formation of multivalents suggested that these strains may have an autopolyploid origin.

Details of the chromosomal morphology of six Brazilian species of *Stevia*, including *S. rebaudiana*, were recently published by Frederico *et al.* (1996). The karyotypes were very similar in chromosome number ($2n = 22$) and size (1.0-2.4 μm). Most of the chromosomes were metacentric, with a variable number of submedian ones. Only *S. ophryophylla* and *S. rebaudiana* had a pair with a subterminal centromere (Frederico *et al.*, 1996). These details of the

chromosome size and centromere position were confirmed here for the T0 (control) strain. Chromosome lengths were similar in other strains with $2n = 22$ (T5V1 and T8V2) or $2n = 44$ (T6V3). The presence of a nucleolus organizing region on the short arm of the third major chromosome pair (Frederico *et al.*, 1996) was also confirmed for *S. rebaudiana*.

Different cytotypes or chromosomal races within the same species have been reported for various plants, such as *Duguetia furfuraceae* ($2n = 16, 24$ and 32) (Annonaceae) from the Brazilian cerrado (Morawetz, 1984). Some Gramineae have also been reported to have cytotypes with diploid and tetraploid individuals; tetraploids are often associated with asexual reproduction through agamospermy.

Unlike the above cytotypes which occur spontaneously in nature, the *Stevia* cytotypes analyzed here were obtained artificially by inducing polyploidy (Valois, 1992). Polyploidization was only confirmed in two treatments, T6 and T7, which corresponded to the lowest colchicine concentrations. Polyploidy is often induced in plants of economical interest in order to produce variability that can improve the yields (Allard, 1960). This approach has been applied to coffee (Cruz *et al.*, 1993), orange (Romero and Aranda *et al.*, 1997), *Nicandra physaloides* (Gupta and Roy, 1986) and *Clitoria ternatea* (Gandhi and Patil, 1997).

Attempts to induce polyploidy are not always successful and often lead to a small number of plants with chromosomal duplications. Seeds of *Jatropha curcas* (Euphorbiaceae) exposed to various concentrations of colchicine (Forni-Martins and Cruz, 1985) gave results similar to those obtained by Valois (1992) for *S. rebaudiana*. The plantlets of *J. curcas* showed various morphological changes and a very low survival rate (10 plants out of the 300 seeds used in the experiment). Only three of the surviving plants showed polyploidization.

Table 2 - Means (\bar{x}) and coefficients of variation (CV%) for plant height, leaf length and width (in cm), inflorescence number (in units), stomal length and width, and pollen grain diameter (in μm) in strains of *Stevia rebaudiana* (Bertoni) Bertoni grown at CPQBA/UNICAMP.

Strains	Leaf length		Leaf width		Inflorescence number		Plant height		Stomal length		Stomal width		Pollen grain diameter	
	\bar{x} (cm)	CV%	\bar{x} (cm)	CV%	\bar{x} (units)	CV%	\bar{x} (cm)	CV%	\bar{x} (μm)	CV%	\bar{x} (μm)	CV%	\bar{x} (μm)	CV%
Brasília F1	2.05	20	0.53	16.5	16.88	23.7	83.2	6.9	36	2.77	20	3.67	22.2	12.8
Maringá F1	2.60	11.7	0.98	28.9	16.58	33.5	88.2	23.4	36	2.75	20	3.0	23.8	12.1
Canada F1	2.44	15.4	0.55	29.9	22.2	44.4	70.4	8	34	2.16	20	2.89	23.2	11.7
T0 (control)	1.83	23.1	0.60	13.8	17.57	35.6	86.6	17.5	34	2.18	20	2.69	22.6	17.5
T5V1	3.42	19.8	1.25	42.6	8.50	41.5	78.0	5.4	30	2.59	18	4.64	27.6	20.1
T6V1	-	-	-	-	-	-	-	-	34	1.91	20	3.39	24	8.5
T6V3	4.94	30.7	2.24	32.4	30.86	44.1	52.75	32.9	52	1.64	28	3.05	29	38.6
T7V1	2.41	30.5	1.10	29.5	5.30	51.9	27.80	38	40	2.08	24	3.97	34	12
T8V1	1.95	29.1	0.47	21.9	11.25	44.1	65.75	41.1	36	2.45	20	4.23	21.4	19.4
T8V2	-	-	-	-	-	-	-	-	36	2.34	20	4.15	21.2	13.3
T9V1	2.95	21.6	0.96	26.8	17.20	34.4	96.37	23.3	36	2.27	20	5.3	22.2	11.9

Multivalents at diakinesis would be expected in autoployploids, since the latter are derived from a single genome and result in three or four homologous sets of chromosomes in triploid and tetraploids, respectively (Stace, 1980).

Normal meiosis can be inferred from the pollen viability. Although pairing at diakinesis was normal, with the formation of bivalents in all diploids, in none of the *S. rebaudiana* strains studied was the pollen viable. High rates of tetrad normality (>93%) were also observed in the diploid strains. The lowest tetrad normality rates were those of the tetraploid (80%) and triploid (64.5%) strains. Because of irregularities which produce unbalanced gametes during meiosis, polyploids with an odd number of chromosome sets have a high level of sterility (Lawrence, 1980).

The low tetrad normality rates and the lack of viable pollen in the triploid and tetraploid cytotypes may be related to the pairing of multivalents (tetravalents and trivalents) at diakinesis. Other meiotic anomalies, such as irregular chromosomal disjunction in anaphase, could also explain these results. However, as with the diploid cytotypes, the lack of viable pollen cannot necessarily be linked with regular pairing at diakinesis (bivalents) and high rates of tetrad normality.

Using the acetic carmine technique, Monteiro (1980) observed that in some diploid individuals of *S. rebaudiana* the pollen viability was 65%, which differs from the results of the present study. This discrepancy may reflect how pollen viability tests are interpreted. Although pollen reacts in the acetic carmine technique, the staining is quite weak and may not be enough to characterize viable grains. For this reason, we also used the technique of Alexander (1980), which allowed a comparison between the results of the two tests and increased the reliability of our conclusions. This second technique, which produces a much greater difference in color between viable and inviable grains (red cytoplasmic staining for viable grains and green cell wall staining for inviable grains), also showed that there were no viable grains.

Our results on pollen viability are even less consistent when compared to the high tetrad normality rates (>93%) of the diploid strains. Since the tetrad phase antecedes that of pollen, the difficulty in interpreting pollen viability remains. Indeed, staining techniques do not always allow pollen viability to be assessed as accurately as the true germination capacity under natural conditions or in appropriate environments (Dafni, 1992).

According to Forni-Martins *et al.* (1992), the analysis of sporogenesis allows the detection of irregularities that can lead to the formation of inviable gametes. In *Peixotoa* sp. (Malpighiaceae), the presence of trivalents is directly related to high pollen grain inviability and, consequently, to the probable formation of inviable seeds (Forni-Martins *et al.*, 1992). Lopes *et al.* (2002) observed the formation of multivalents with low pollen viability (40.6%) as well as

considerable variation in pollen size in a species of *Senecio* (Asteraceae).

Stevia rebaudiana has light and dark achenes with the former being completely sterile. The fertility rate of dark achenes is 50% (Monteiro, 1980). Agamospermy, *i.e.* the asexual formation of seeds (Lumaret, 1988), could explain the reproductive capacity of this species, even if one takes into account the total lack of viable pollen observed here. According to Mogie (1992), apomictic species are common in the Asteraceae, Rosaceae and Poaceae, which possess a “pre-adaptation” set that allows the development of apomictic embryos.

Grashoff (1974 *apud* Monteiro, 1980) and Monteiro (1980) reported agamospermy in *S. rebaudiana*. According to Monteiro (1980), the presence of apomixis in *S. rebaudiana* shown by embryological studies may be related to specific physiological and/or ecological factors. According to Grashoff (1974 *apud* Monteiro, 1980), sexual and apomictic plants of *S. rebaudiana* produce normal and malformed pollen, respectively.

Cytotypes (or chromosomal races) with diploid and tetraploid individuals have been reported for some Gramineae and are often associated with asexual seed reproduction, *i.e.* agamospermy (Burson, 1975). According to Stebbins (1971), other Asteraceae, such as *Crepis*, *Taraxacum* and *Hieracium*, also show apomixis. All of the agamospermic species in *Taraxacum* and *Hieracium* are polyploid, with a few of them having odd ploidy numbers and sometimes highly irregular meiosis (Stace, 1980). In the genus *Parthenium*, the apomictic species are also associated with polyploidy. *Eupatorium* and *Ageratina* (King *et al.*, 1977) contain sexual and apomictic species, the latter being characterized by irregular meiosis and inviable pollen.

According to Stebbins (1971), polyploid populations show considerable ecological, morphological and genetic differences compared to their corresponding diploids. Although a higher ploidy number has been associated with larger pollen (Muller, 1979), this correlation is only consistent for autoployploids (Stebbins, 1971). In some coffee (Boaventura *et al.*, 1981) and cassava (Carvalho *et al.*, 1999) plants with different chromosome numbers, there is a tendency for stomata cell size to increase with the level of ploidy.

Many domestic autoployploid plants are larger than their corresponding diploids (Lawrence, 1980). Keeler and Davis (1999) noted differences in the size of *Andropogon gerardii*, a species with hexaploid ($2n = 6x = 60$) and enneaploid ($2n = 9x = 90$) cytotypes, in which the biggest individuals had the highest chromosome number. Because of the different plant sizes, the production of seeds per unit area was equivalent for both cytotypes, but was more efficient in hexaploids. The leaves in enneaploid plants were larger than in the hexaploid cytotype.

The coefficients of variation (CV%) for the features quantified here gave an idea of the variation involved (Gomes, 1978). The sample size may have influenced the CV% since a sample of 1,000 pollen grains per strain, considered to be large, was used to analyze the pollen diameter and stomata size. Most of the strains had an intermediate CV% (between 10% and 20%) for pollen diameter, while all had a low CV% (<10%) for stomata size. In contrast, the smallest sample size used for all of the other features (leaf size, plant height and inflorescence number) reflected the fact that there were few plants in the field (approximately 10 plants per strain), and meant that in most strains these parameters had high (20-30%) or very high (>30%) coefficients of variation. A further complicating factor was that *S. rebaudiana* plants lose their leaves completely in the winter and only resprout in the spring. The inherent high genetic variability in vegetative features (such as plant size), flowering period and content of active principles, such as stevioside (Magalhães, 2000) and rebaudioside A (Stefanini and Rodrigues, 1999), also contributed to the high CVs.

ANOVA showed that among strains with different levels of ploidy, there was significant ($p \leq 0.001$) variation in all of the features quantified here. These significant morphological differences among the diploid, triploid and tetraploid strains meant that some features, especially pollen and stomata sizes, could be used to diagnose the level of ploidy much quicker and easier than chromosomal analysis.

There was a positive correlation between the size of the pollen or stomata and the level of ploidy, whereas this relationship was negative for plant size since diploids were larger than their corresponding autopolyploids. Compared to diploids, the tetraploid strain had larger leaves but there was no significant difference between the triploid and diploid strains. Similarly, the triploid and tetraploid strains had lower and higher numbers of inflorescences, respectively, compared to diploid plants.

The results obtained here open new possibilities for genetic breeding programs of *S. rebaudiana* since polyploid individuals may have a higher content of stevioside than diploid plants and the selection of plants for commercial production could possibly increase the level of these compounds. Despite the male sterility present in various strains of *S. rebaudiana*, agamospermy (Monteiro, 1980) could ensure a sufficient seed yield to allow the genetic breeding of this species.

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