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Morphological patterns in *Petunia hybrida* plants regenerated from tissue cultures and differing by their ploidy

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Summary. Quantitative variation in seven morphological characteristics (leaf length and width, leaf length/width ratio, flower, petal and stomata length, and number of chloroplasts in guard cells) were studied in *Petunia hybrida* plants regenerated from anther tissue culture and belonging to four different classes of ploidy (2n, 2n–3n, 3n–2n, 4n–8n). Results showed that leaf size is not a good characteristic for discriminating between plants of different ploidy – flower and stomata characteristics being more adequate for this purpose. After applying stepwise discriminant analysis the association "chloroplast number – leaf length/width ratio – petal length" was verified to be more appropriate for the discrimination of ploidy classes.

Key words: *Petunia* – Tissue culture – Ploidy

Introduction

Petunia plants can be easily regenerated from stem and leaf explants (Rao et al. 1973), protoplasts (Power et al. 1976) and anther cultures (Mitchell et al. 1980). Recently, Santos et al. (1983) used anther calluses to regenerate plants belonging to different classes of ploidy, and related some peculiar morphological features of these plants to their ploidy level.

In plants regenerated from calluses, morphological variations frequently result from modifications in the genotype of cells in culture. It seems that the variability of morphogenetic responses is due mainly to quantitative alterations in the set of chromosomes and not to gene mutations (Buiatti 1977). Thus the association between ploidy level and morphological characteristic may be of interest in the isolation of plants as demonstrated by Eigsti and Dustin (1955) who indicated 16 characteristics for the isolation of polyploids. Many of these characteristics are related to increase of cell size or whole structures, as a result of polyploidization. In plants regenerated from tissue cultures such a relationship has been demonstrated by Nishi and Mitsuoka (1969) with *Oryza;* Nitsch (1969), Tsikov et al. (1974) and Hell (1979) with *Nicotiana;* Sree Ramulu et al. (1976) with *Lycopersicum;* Rajhathy (1976) with *Linum;* Fassuolis (1977) with *Solanum*, etc.

In *Petunia,* a parallel between ploidy and morphological patterns was noticed by Steere (1931) after comparing diploids with tetraploids. He suggested that triploids could have intermediary characteristics. In this work we have used four groups of *Petunia* plants with peculiar chromosome number in attempts to find what morphological characteristics could be better related to ploidy level.

Materials and methods

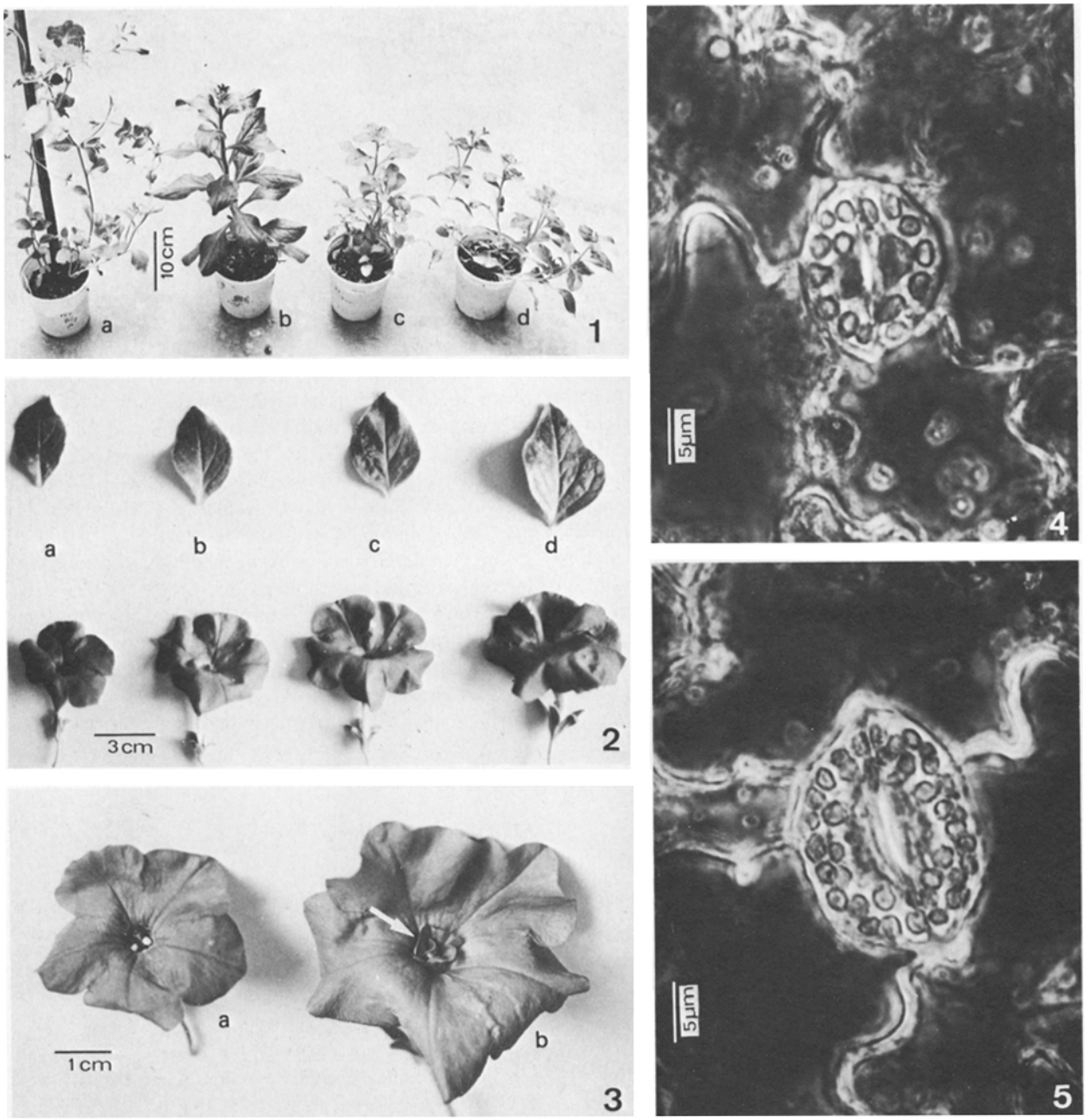
Plants of *Petunia hybrida* with different ploidy levels were regenerated from anther callus tissues as described by Santos et al. (1983). Four sets of five plants each were used for this work: A – diploid plants (2n = 14); B – plants in which chromosome counts in root tips ranged from 2n to 3n, with major incidence of 2n cells; C – plants with 2n and 3n cells, predominating the last ones; D – plants predominantly tetraploid (4n = 28), with several counts nearing 56 (8n). After regeneration from tissue cultures, the plants were potted and kept in a greenhouse until flowering. Seven characteristics were analysed in these plants: 1 – leaf length; 2 – leaf width; 3 – leaf length/width ratio ("foliar ratio"); 4 – flower length (recorded for the largest petal); 5 – length of the petal (only the free part); 6 – length of guard cells; 7 – number of chloroplasts in guard cells. The leaves were taken at the same stem node in all plants. The chloroplasts were stained with 1% $AgNO_3$ (Molisch 1918 cited in Butterfass 1959) in epidermal

preparations. Twenty counts or measurements per plant were made for each parameter.

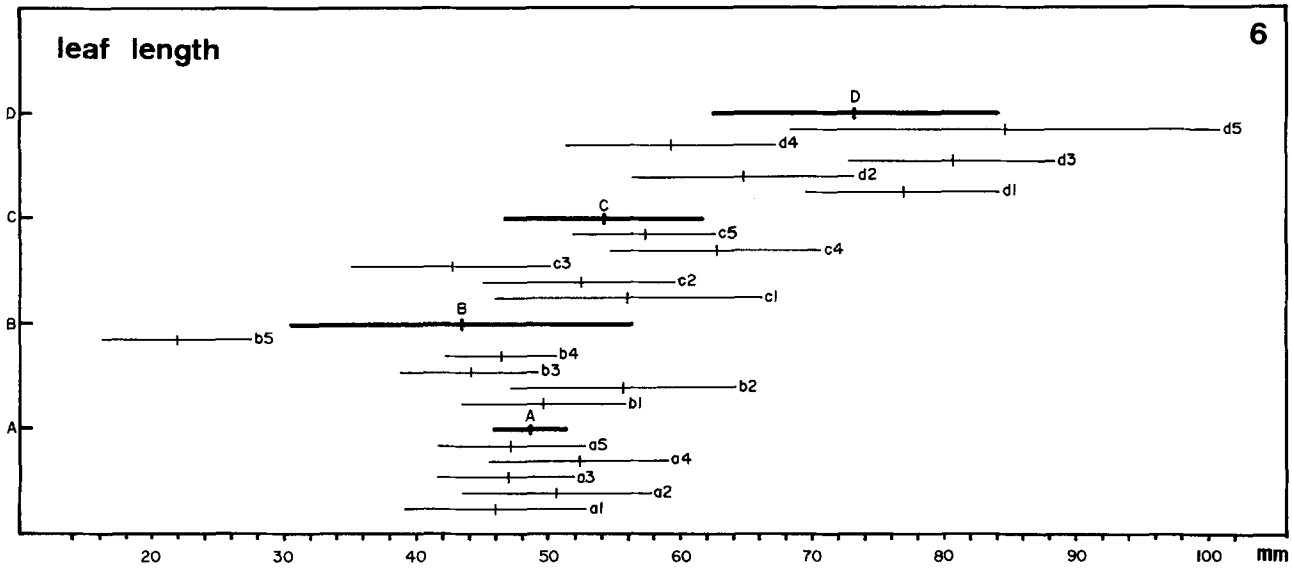
A two-factor hierarchal model was employed in the analysis of variance followed by a stepwise discriminant analysis to characterize the set of variates suitable for better discrimination of ploidy groups (Morrison 1967; Winter 1971; Afifi and Azen 1972).

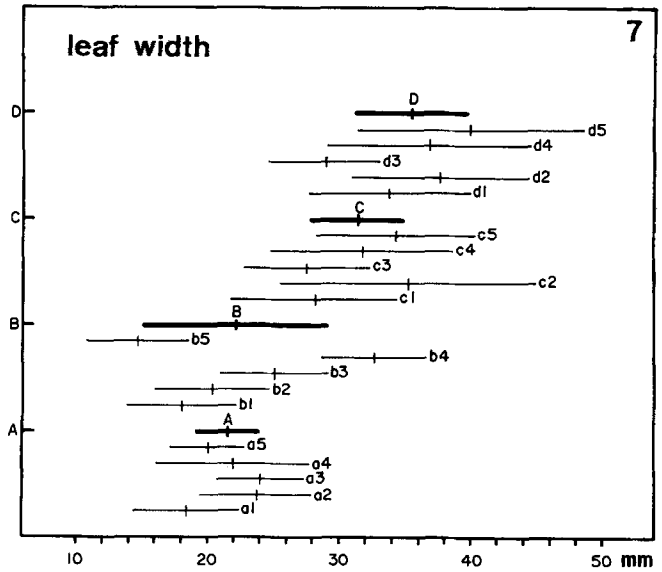
Results

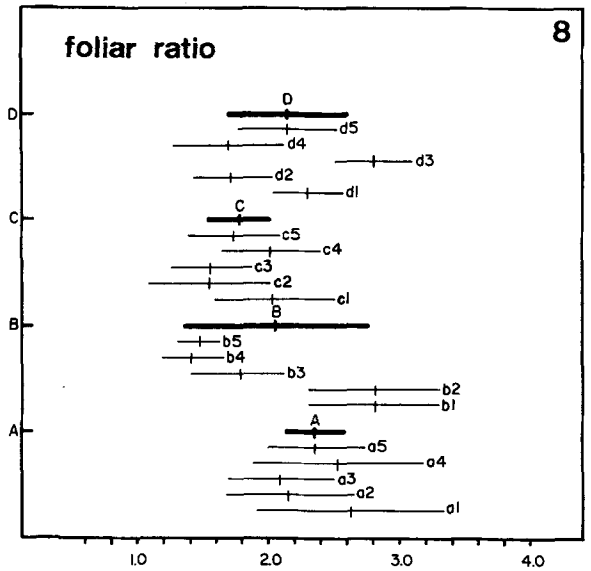
Most of plants belonging to each group of peculiar ploidy level showed some well defined morphological characteristics such as habit (Fig. 1), leaf and flower size (Fig. 2), flower morphology (Fig. 3), and size of



Figs. 1–5. Morphological characteristics in *Petunia* plants of different ploidy. **1** Plants produced by tissue culture: *a* 2n, group A plant; *b* 4n – 8n, group D plant; *c* 2n – 4n plant (not included in this study); *d* 3n – 2n, group C plant. **2** Aspect of leaves and flowers from plants of different groups: *a* (2n); *b* (2n – 3n); *c* (3n – 2n); *d* (4n – 8n). **3** Flowers from plant of: *a* group A (2n) and *b* group D (4n – 8n). *Arrow* indicates staminodes. **4** Stomata from a group A plant (2n), with about 15 chloroplasts. **5** Stomata from a group D plant (4n – 8n), with almost 40 chloroplasts







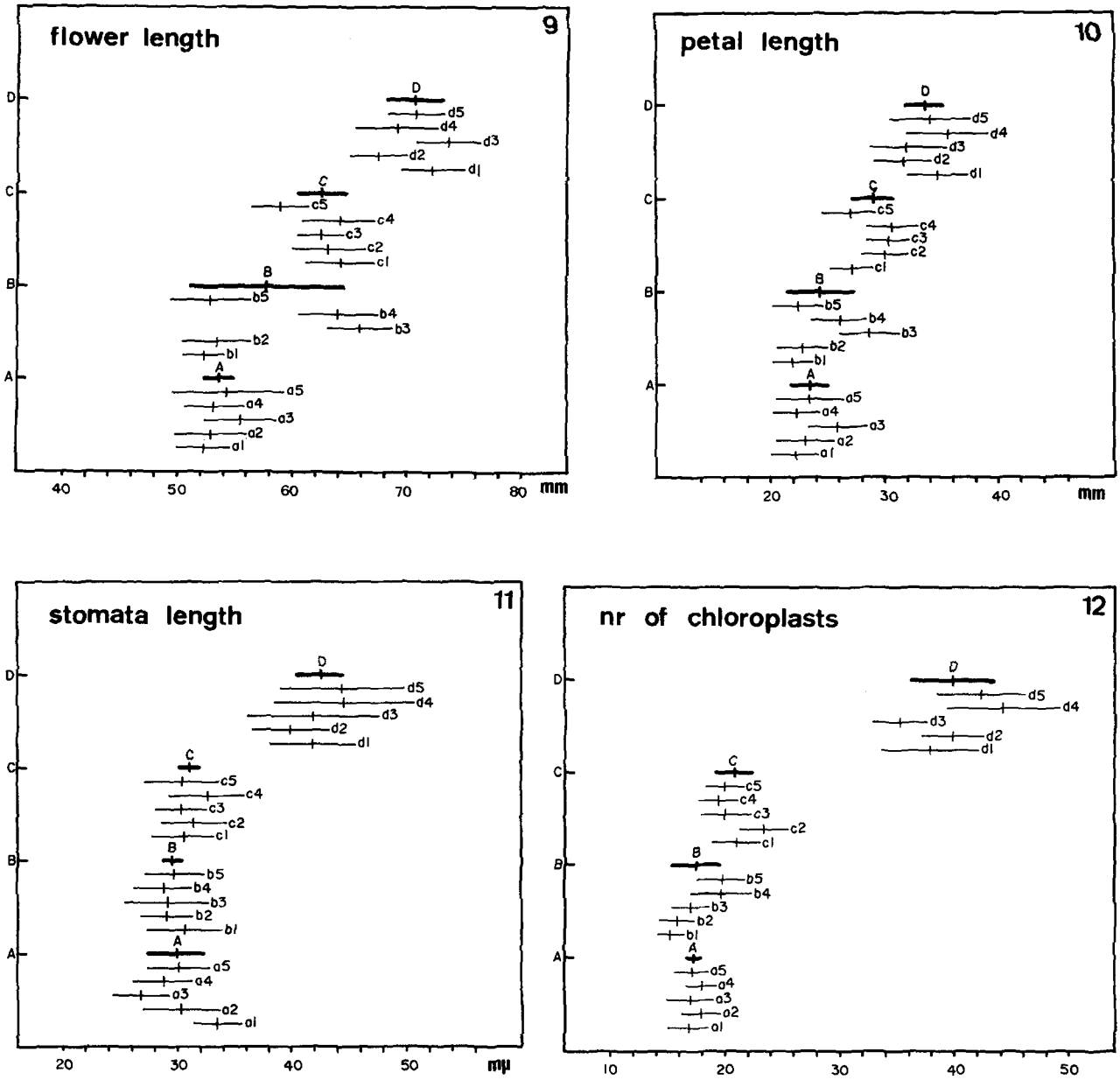
Figs. 6–8. Average and SD of leaf characteristics in plants of groups with different ploidy: *A* (2n), *B* (2n – 3n, with 2n predominance), *C* (3n – 2n, most of cells nearing 3n number), *D* (4n – 8n). Thick bars represent the data of five plants; thin bars represent the data for each plant

stomata and number of chloroplasts in guard cells (Figs. 4–5). Some parameters related to these characteristics were analysed quantitatively; the results are shown in Figs. 6–12.

Plants of group A (2n) appeared to be more homogeneous in the expression of their characteristics as is indicated by the lower values of standard deviation. Group B (2n–3n), on the contrary, was the more heterogeneous, frequently overlapping groups A and C. Plants of group C as compared with B were more uniform in their characteristics and can be isolated from A by five different parameters among seven, and from D, by four parameters. Group D plants differ from A in all characteristics except by foliar ratio, and can also be separated from C by leaf length, flower and stomata characteristics.

The results demonstrated that leaf size is not a good characteristic for isolation of groups of ploidy; it seems that flower characteristics are more adequate for this purpose. Stomata characteristics clearly isolate D plants from the others.

According to the analysis of variance (Table 1) only the foliar ratio did not reveal significant differences when different groups were compared. When the stepwise discriminant analysis was employed the best association of characteristics for discrimination of one



Figs. 9 – 12. Average and SD of flower and stomata charateristics, in groups *A*, *B*, *C* and *D*, as described for Figs. 6–8

Table 1. Values of F from the analysis of variance for each of the seven characteristics

	Leaf length	Leaf width	Foliar ratio	Flower length	Petal length	Stomata length	Chloroplast no.
F for groups ($F_{5\%}=3.24$)	9.83	11.00	1.24[a]	19.11	25.93	67.59	140.99
F for plants ($F_{5\%}=1.63$)	28.78	13.60	23.31	33.39	14.14	4.60	15.47

[a] Not significant at 5%

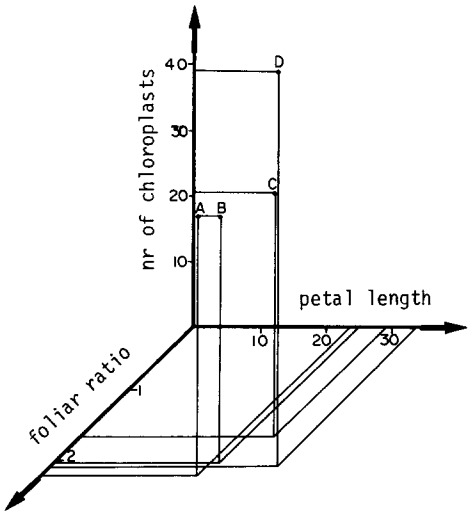


Fig. 13. Discrimination of the four groups of ploidy (*A, B, C, D*) in relation with the threee variates selected by stepwise discriminant analysis

Table 2. Values of F in the stepwise discriminant analysis

Step	Variate included	F
1	nr of chloroplasts	140.99
2	foliar ratio	7.77
3	petal length	4.22

group was "chloroplast number – foliar ratio – petal length" (Table 2). This set of characteristics, however, does not discriminate A perfectly from B (Fig. 13).

Discussion

Our results have shown that plants of group A (2n) were more uniform as compared with those which had exhibited higher variability and increase in chromosome number. Similar results were found by Hell (1979) in tobacco, where polyploids were more heterogeneous than diploids. Group B, which overlaps A even after discriminant analysis presented a high range of variation, probably due to data from plant b5. This specimen had ca. 50% cells with 14 chromosomes, and seems to be dwarf when compared with plants of group A. Steere (1931) described a similar case in two *Petunias* in a lot of triploids. Engvild (1973) also found 2n–3n plants from *Petunia* anther cultures which were smaller than triploids but with some characteristics similar to diploids. It must be emphasized that group B is relatively heterogeneous; probably the predominance of 2n cells make these plants similar to diploids in most of their characteristics, but a high degree of mixoploidy increases the variability in this group when compared with the others. The data recorded for plants of group D (except those of foliar ratio) suggests that the values were actually a duplication of those recorded in the group A, and directly related to the duplication of ploidy level.

Similar results were found by Medina et al. (1972) with *Solanum melongena,* when diploid plants could be discriminated only from tetraploids or 4n–8n mosaic plants, and not from 2n–4n plants. Sree Ramulu et al. (1976) showed that in *Lycopersicum,* cytochimeras 4n–2n–2n were similar to diploids whereas those 2n–4n–4n were similar to tetraploids. Hell (1979) reported that in tobacco the number of chloroplasts in the stomata could be used for the characterization of euploid forms, but not to discriminate aneuploids.

In our study the statistical analysis showed that the number of chloroplasts in guard cells is the best characteristic for separating the groups. Nevertheless, the petal length is the best characteristic to isolate group C. The stepwise discriminant analysis demonstrated that group C could be isolated from the others only by association of at least two characteristics: number of chloroplasts and foliar ratio. Thus, the separation of plants of a certain ploidy level by morphological characteristics must take in account not only the plant species, but also the number of parameters to be analysed or/and the specific characteristics that could isolate the class of ploidy.

Characteristics such as number of chloroplasts and stomatal cell length have been used for identification of ploidy classes (e.g. Butterfass 1959; Schank and Knowles 1961; Hermsen and Boer 1971; Medina et al. 1972; Tann and Dunn 1973; Breukelen et al. 1975; Hell 1979). On the other hand, flower characteristics are infrequently used although they have been described as quantitatively related to polyploidization (Eigsti and Dustin 1955; Medina et al. 1958; Narayanaswamy and Chandy 1971; Medina et al. 1972).

It seems clear from our study that a preliminary screening of ploidy in *Petunia* in a large population of plants is feasible by using the association of some morphological parameters, especially when great numbers of plants must be rapidly identified. Nevertheless, a confirmation of the actual ploidy level can only be made by chromosome counting.

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