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Morphology of artificial hybrids of *Vriesea splendens* × *Tillandsia cyanea* and *V. splendens* × *Guzmania lingulata* (Bromeliaceae)

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Intergeneric hybrids *Vriesea splendens* × *Tillandsia cyanea* and *V. splendens* × *Guzmania lingulata* within the subfamily Tillandsioideae of Bromeliaceae were studied. Multivariate analysis of flower morphology and leaf and bract color place the intergeneric hybrids in relation to their parents, although the bract color resembled more that of the paternal parent. Other characteristics such as petal color and offshoot formation were not intermediate. Important flower characteristics for bromeliad taxonomy such as stigma type, petal appendages and pollen are discussed in relation to their parents. Pollen of the intergeneric hybrids was completely sterile.

Key words: Bromeliaceae, intergeneric hybrids, petal appendages, pollen fertility, ovules, stigma

Introduction

Three subfamilies are currently recognized within the Bromeliaceae: Bromelioideae, Pitcairnioideae, and Tillandsioideae (Smith & Downs 1974, 1977, 1979). The genera *Vriesea*, *Tillandsia* and *Guzmania* belong to the 'core group' of Tillandsioideae, which proved to be genetically very homogenous (< 1.8% sequence divergence), but is morphologically and ecologically highly diverse. One possible explanation is a relative recent radiation into new ecological niches, e.g. as epiphytes (Benzing 2000).

In general, the difficulty in obtaining interspecific hybrids increases with the phylogenetic distance between the parental taxa involved. In some cases, however, barriers are not very strong among species that have evolved in spatial isolation (Sharma 1995). The plants in this study are ornamental cultivars and have been cultured in Europe for a long time. For example *Guzmania lingulata* was introduced in 1776 and *Vriesea splendens* in 1840 (Benzing 1980). Unimproved specimens of these cultivars can still be found in their natural habitats in Central and South America.

A number of artificial and natural intergeneric hybrids are known in the Bromeliaceae (Smith & Downs 1974), which confirms that intergeneric barriers are not always present or that they can be overcome by minor manipulations. For example different intergeneric hybrids of *Vriesea* × *Tillandsia* and *Vriesea* × *Guzmania* were produced by removing a part of the style ('cut-style' pollination) (Vervaeke 2002, Vervaeke *et al*. 2002).

The aim of this study was to evaluate two intergeneric bromeliad hybrids and their parents. Therefore flower characteristics and bract and leaf color were studied. In addition important features in bromeliad taxonomy such as stigma type, petal appendages, and pollen and ovule appearance were examined.

Material and methods

Intergeneric hybridizations in the subfamily Tillandsioideae were attempted using cut-style pollination: *Vriesea splendens* (VS) × *Tillandsia cyanea* (TC) and *V. splendens* (VS) × *Guzmania lingulata* (GL). Parent plants and hybrids were cultivated in greenhouses and maintained at an average day and night temperature of 21 °C and 19.5 °C respectively, and at 65% relative humidity. Normal nursery practice for watering, ventilation, fertilization and pest control were followed. Flowering was induced by ethylene treatment (De Proft *et al*. 1986).

Plant and flower characteristics were determined on respectively 30 and 20 different seedlings of *Vriesea splendens* × *Tillandsia cyanea* and *V. splendens* × *Guzmania lingulata*. Bract and leaf color of the intergeneric hybrids and their progenitors was determined with the Minolta chroma meter (CR-200). *L*, *a* and *b* values were obtained; *L* indicates lightness, and the color space of *a* and *b* runs from –60 to +60 with an achromatic centre at zero. The direction –*a* to +*a* is red–achromatic–green and +*b* to –*b* is yellow–achromatic–green.

To study stigmas, ovaries, pollen and petal appendages, flowers were fixed in ethanol (70%). After dehydration, flower parts were critical-point dried. Different flower parts were examined using a JEOL JSM-5800 LV scanning electron microscope at 15 kV (Nationale Plantentuin, Meise, Belgium). Significant differences between morphometric flower characteristics were determined by Duncan's multiple range tests. Canonical discriminant analysis was performed to examine multivariate morphometric differences among *Vriesea splendens*, *Tillandsia cyanea*, *Guzmania lingulata* and the intergeneric hybrids *V. splendens* × *T. cyanea* and *V. splendens* × *G. lingulata*. All statistical tests were conducted by SAS (version 8).

Results

Cut-style pollination of *Vriesea splendens* (Fig. 1A) × *Tillandsia cyanea* (Fig. 1B) and *V. splendens* × *Guzmania lingulata* (Fig. 1C) resulted in the intergeneric hybrids: *V. splendens* × *T. cyanea* (Fig. 1F) and *V. splendens* × *G. lingulata* (Fig. 1D–E). Morphometric data on flower characteristics are presented in Tables 1 and 2. The data for *V. splendens* × *G. lingulata* were never intermediate between the parent plants (Table 1). In the canonical the three groups of plants were separated (Fig. 2), but the hybrid was more located towards *V. splendens*. Bract, stamen, style and pistil length were intermediate between the parentals for *V. splendens* × *T. cyanea* (Table 2). In the canonical space the hybrid was between the progenitors, but closer to *T. cyanea* (Fig. 3).

For both bract and leaf color the scatter plots of *Vriesea splendens*, *Guzmania lingulata* and the intergeneric hybrid were not well separated (Figs. 4 and 5). *Vriesea splendens* × *G. lingulata* had an intermediate position for leaf color, but not for bract color. Scatter plots of *V. splendens*, *Tillandsia cyanea* and their hybrid for bract and leaf color were more separated in the canonical space with an intermediate position for the hybrid (Figs. 6 and 7).

Bract color of *Vriesea splendens* × *Tillandsia cyanea* resembled more the latter. Objective color measures showed that there was no blue color in the hybrid as observed in *T. cyanea* (data not shown). *Vriesea splendens* × *Guzmania lingulata* (Fig. 1D) had red bracts as *G. lingulata* (Fig. 1C). Petal color in the intergeneric hybrids was yellow, as in *V. splendens*. *Vriesea splend-*

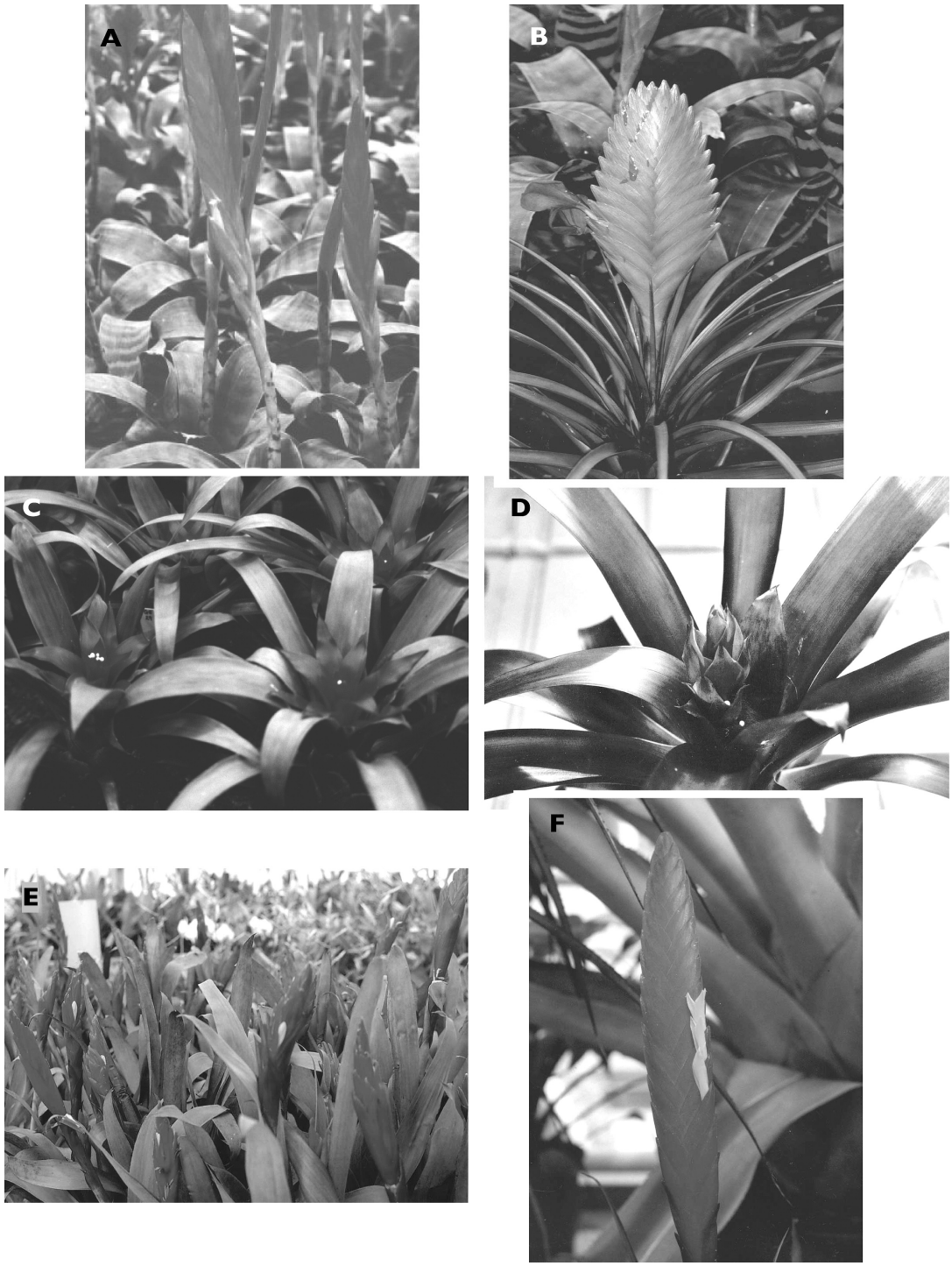

Fig. 1. — **A**: *Vriesea splendens*. — **B**: *Tillandsia cyanea*. — **C**: *Guzmania lingulata*. — **D**: *Vriesea splendens* × *Guzmania lingulata* (developing inflorescence). — **E**: *Vriesea splendens* × *Guzmania lingulata*. — **F**: *Vriesea splendens* × *Tillandsia cyanea* (inflorescence).

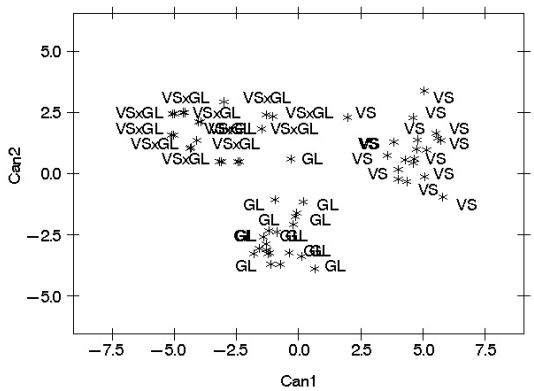

Fig. 2. Scatter plot of scores from canonical discriminant analysis (can1 and can2) applied to 9 morphometric characteristics for VS, GL and VS × GL.

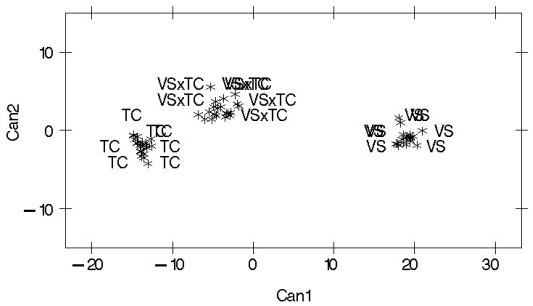

Fig. 3. Scatter plot of scores from canonical discriminant analysis (can1 and can2) applied to 9 morphometric characteristics for VS, TC and VS × TC.

ens and *T. cyanea* have both a distichous, flat inflorescence. The inflorescence of *V. splendens* × *T. cyanea* had the same features but was more long-drawn, as in *V. splendens* (Fig. 1A). *Guzmania lingulata* possessed a polystichous spike (Fig. 1C); the intergeneric hybrid with *V. splendens* had the same inflorescence type, but was very irregular (Fig. 1D–E). Leaf shape of *V.*

Table 1. Flower characteristics of *Vriesea splendens* (VS), *Guzmania lingulata* (GL) and *V. splendens* × *G. lingulata* (VS × GL) at anthesis (n = 20). Same letters in parentheses indicate that there is no significant difference within a column based on Duncan's multiple range test.

Flower characteristics	VS	GL	VS × GL
Length of bract (cm)	6.3 ± 0.3 (A)	–	5.9 ± 0.8 (A)
Length of flower (cm)	6.6 ± 0.2 (A)	5.1 ± 0.3 (B)	5.1 ± 0.4 (B)
Length of sepal (cm)	2.4 ± 0.2 (B)	2.2 ± 0.4 (B)	2.9 ± 0.3 (A)
Length of petal (cm)	6.6 ± 0.2 (A)	5.1 ± 0.3 (B)	5.1 ± 0.4 (B)
Length of stamen (cm)	6.3 ± 0.6 (A)	4.5 ± 0.3 (B)	3.6 ± 0.6 (C)
Length of anther (cm)	0.5 ± 0.0 (C)	0.6 ± 0.0 (B)	0.6 ± 0.1 (A)
Length of stigma (cm)	0.2 ± 0.0 (A)	0.1 ± 0.0 (B)	0.2 ± 0.1 (A)
Length of style (cm)	5.7 ± 0.2 (A)	4.1 ± 0.2 (B)	3.4 ± 0.5 (C)
Length of pistil (cm)	6.9 ± 0.3 (A)	5.1 ± 0.2 (B)	4.7 ± 0.5 (C)
Ovary width (cm)	0.5 ± 0.2 (A)	0.3 ± 0.0 (B)	0.3 ± 0.1 (B)
Pollen germination (%)	48 ± 21 (A)	46 ± 22 (A)	0 ± 0 (B)

Table 2. Flower characteristics of *Vriesea splendens* (VS), *Tillandsia cyanea* (TC) and *V. splendens* × *T. cyanea* (VS × TC) at anthesis (n = 20). Same letters in parentheses indicate that there is no significant difference within a column based on Duncan's multiple range test.

Flower characteristics	VS	TC	VS × TC
Length of bract (cm)	6.3 ± 0.3 (A)	5.1 ± 0.4 (C)	5.9 ± 0.5 (B)
Length of flower (cm)	6.6 ± 0.2 (B)	7.9 ± 0.5 (A)	7.8 ± 0.7 (A)
Length of sepal (cm)	2.4 ± 0.2 (C)	4.2 ± 0.3 (A)	4.1 ± 0.1 (B)
Length of petal (cm)	6.6 ± 0.2 (B)	7.9 ± 0.5 (A)	7.8 ± 0.7 (A)
Length of stamen (cm)	6.3 ± 0.6 (A)	2.7 ± 0.2 (C)	3.2 ± 0.8 (B)
Length of anther (cm)	0.5 ± 0.0 (B)	0.7 ± 0.1 (A)	0.7 ± 0.1 (A)
Length of stigma (cm)	0.2 ± 0.0 (B)	0.2 ± 0.0 (B)	0.3 ± 0.0 (A)
Length of style (cm)	5.7 ± 0.2 (A)	0.6 ± 0.1 (C)	2.1 ± 0.3 (B)
Length of pistil (cm)	6.9 ± 0.3 (A)	1.7 ± 0.1 (C)	3.5 ± 0.4 (B)
Ovary width (cm)	0.5 ± 0.2 (A)	0.3 ± 0.0 (B)	0.4 ± 0.0 (B)
Pollen germination (%)	48 ± 21 (A)	56 ± 18 (A)	0 ± 0 (B)

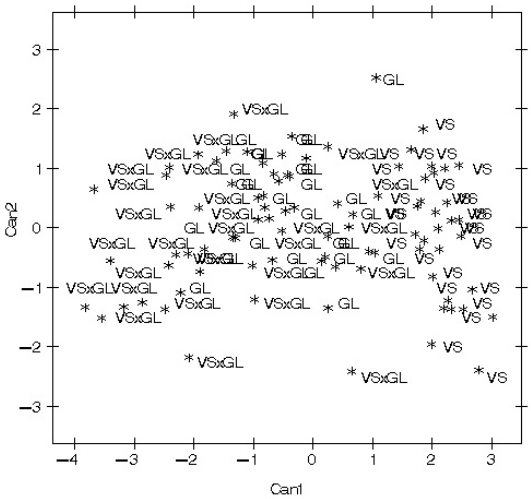

Fig. 4. Scatter plot of scores from canonical discriminant analysis (can1 and can2) applied to bract color for VS, GL and VS × GL.

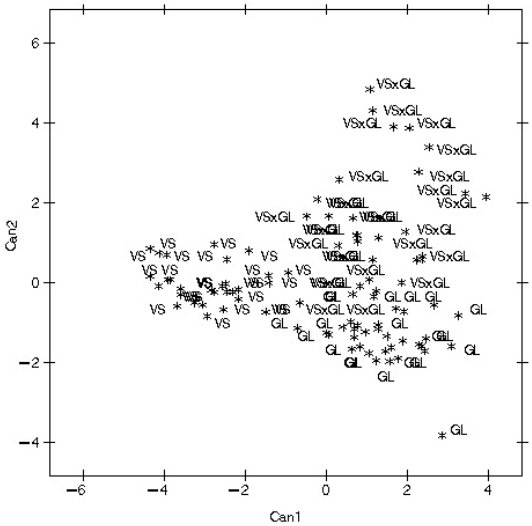

Fig. 5. Scatter plot of scores from canonical discriminant analysis (can1 and can2) applied to leaf color for VS, GL and VS × GL.

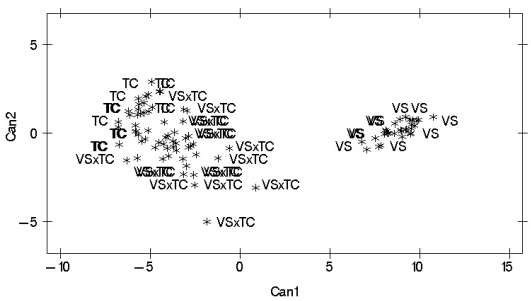

Fig. 6. Scatter plot of scores from canonical discriminant analysis (can1 and can2) applied to bract color for VS, TC and VS × TC.

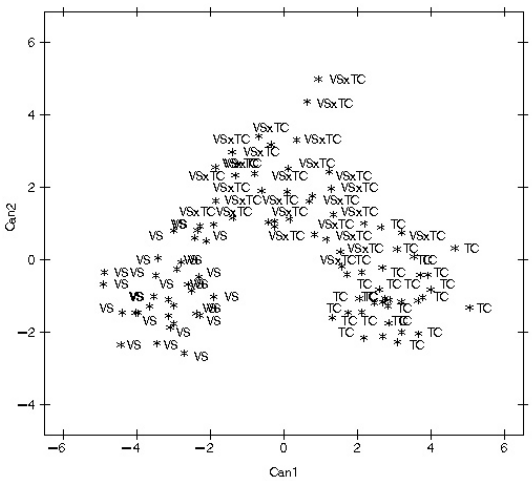

Fig. 7. Scatter plot of scores from canonical discriminant analysis (can1 and can2) applied to leaf color for VS, TC and VS × TC.

splendens × *T. cyanea* was intermediate between its parents. *Vriesea splendens* × *T. cyanea* plantlets showed a lot of 'tubing', meaning that the developing leaves were folded together and consequently the youngest leaves were inhibited in their growth. Tubing arrested also growth of the inflorescence. The phenomenon often occurs in *T. cyanea* but not in *V. splendens*. The horizontal cyanic stripes on leaves of *V. splendens* were never found in the intergeneric hybrids. Offshoot formation of *V. splendens* × *T. cyanea* resembled that of the former; little offshoots were formed in the tank-shaped rosette (phytotelma). In *V. splendens* × *G. lingulata* offshoots were formed on the base from the main shoot as in the latter.

SEM images of stigma, petal appendages, pollen and ovules are presented in Figs. 8 and 9. The stigma of *Vriesea splendens* × *Tillandsia cyanea* (Fig. 8A) resembled more the coralliform stigma of the latter, but was slightly spiraled as the conduplicate-spiral stigma of *V. splendens* (Vervaeke *et al*. 2003). The stigma of *V. splendens* × *T. cyanea* was completely covered with papillae (Fig. 8B). Ovules were attached on a placenta in three ovary locules with a central placenta. The

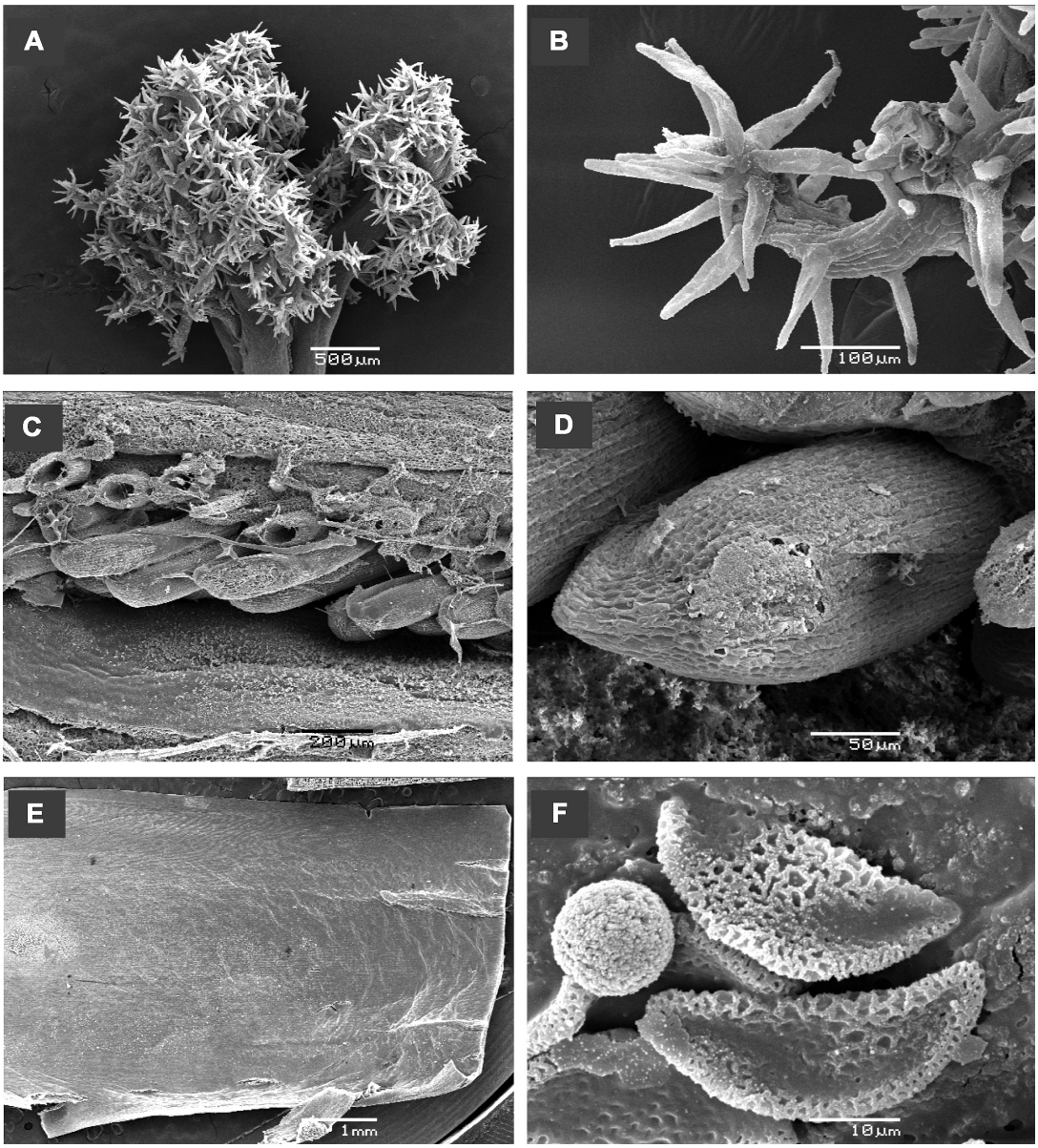

Fig. 8. *Vriesea splendens* × *Tillandsia cyanea*. — **A**: Stigma. — **B**: Papillae. — **C**: Ovules in ovary. — **D**: Ovule. — **E**: Inner side of petal. — **F**: Collapsed pollen.

ovules seemed fully developed (Fig. 8C–D). No developed petal appendages were found, common in *Vriesea* (Fig. 8E). There were two grooves present where normally petal appendages occur. Pollen grains of *V. splendens* × *T. cyanea* were undeveloped and collapsed (Fig. 8F).

The stigma of *Vriesea splendens* × *Guzmania lingulata* (Fig. 9A) resembled more the conduplicate spiraled stigma of *V. splendens* and was not simple-erect as that of *G. lingulata* (Vervaeke *et al*. 2003). Papillae were formed on the edges of the stigma lobes (Fig. 9B). The ovary was separated in three locules (Fig. 9C) with apparently fully developed ovules (Fig. 9D). No petal appendages were present in the hybrid (Fig. 9E), only small grooves could be distinguished. In the anther (Fig. 9F) no pollen or pollen-like structures were found. Pollen was completely absent.

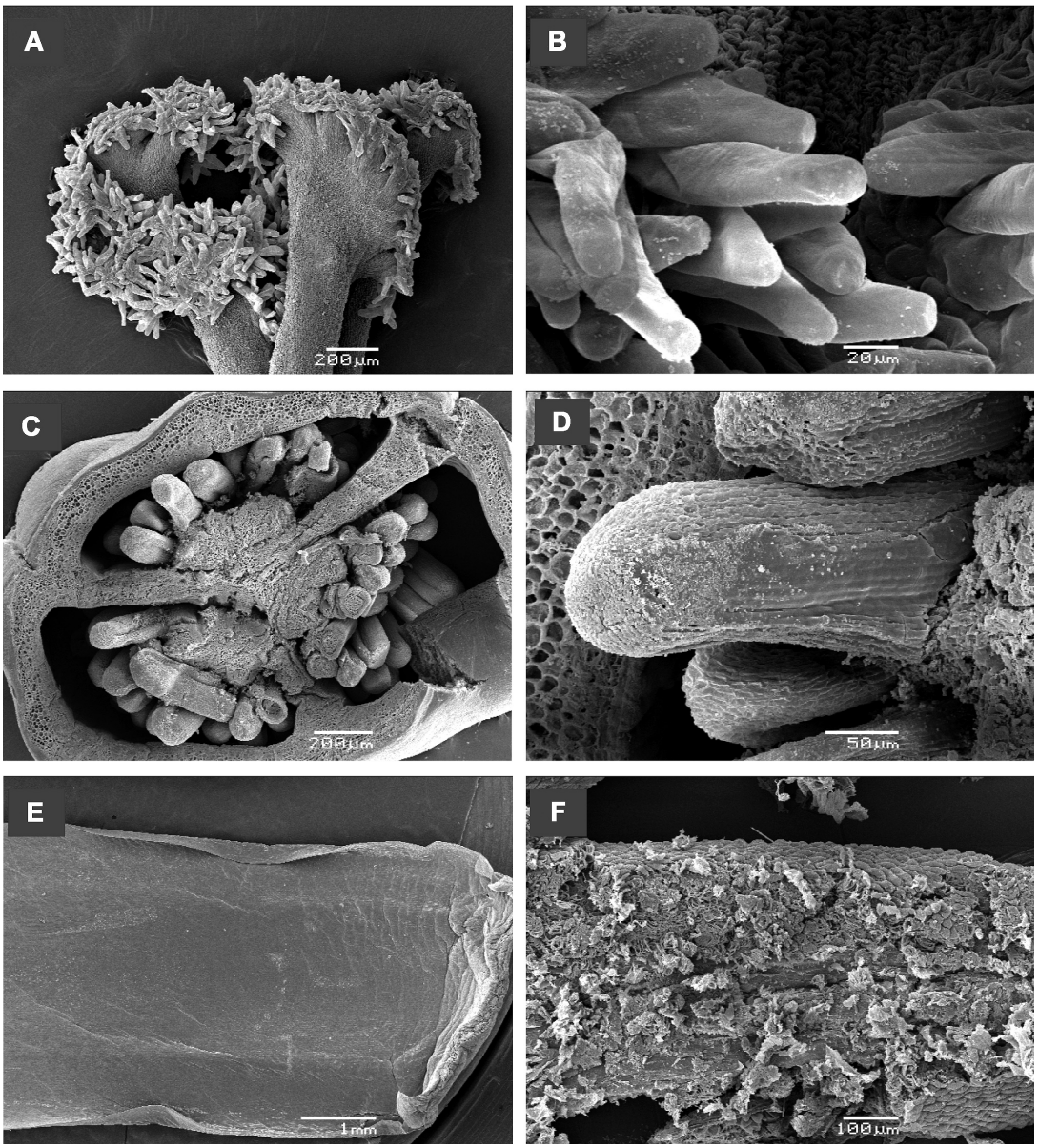

Fig. 9. *Vriesea splendens* × *Guzmania lingulata*. — **A**: Stigma. — **B**: Papillae. — **C**: Ovules in ovary. — **D**: Ovule. — **E**: Inner side of petal. — **F**: Anther without pollen.

Discussion

The intergeneric hybrids were obtained by means of cut-style pollination. This indicates that the reproductive barrier between the cultivars that was situated in the stigma and/or style (Vervaeke *et al*. 2001) could be overcome by technical manipulation. Once pollen tubes of *Tillandsia cyanea* or *Guzmania lingulata* reached the ovary of *Vriesea splendens*, fertilization and seed formation occurred. No post-fertilization barriers or hybrid breakdown were observed.

Plant and flower characteristics of *Vriesea splendens* × *Guzmania lingulata* and *V. splendens* × *Tillandsia cyanea* were not always intermediate between the parent plants. The offspring

population had uniform characteristics, because the parents were genetically very homozygous. Hybrids are generally thought to be morphologically intermediate between their parents, which is a misconception. The expression of paternal or maternal versus intermediate characters in hybrids will depend on the nature of the genetic control of a particular character, as well as interactions with the environment (Rieseberg 1995). A possible explanation for the high proportion of parental characters expressed in hybrids is that many morphological traits that differentiate closely related species display dominant inheritance patterns (Rieseberg & Carney 1998).

Petal appendages are small, paired or single outgrowths from the base of each petal. Approximately 35% of the bromeliad species are known to have petal appendages (Brown & Terry 1992). There has been an overemphasis in the use of the presence of petal appendages in the circumscription of generic limits in Bromeliaceae, and especially in Tillandsioideae. *Tillandsia* and *Vriesea*, for example, differ only by the absence (*Tillandsia*) and presence (*Vriesea*) of petal appendages (Brown & Terry 1992, Benzing 2000). In *Vriesea splendens* petal appendages are almost absent (Vervaeke *et al*. 2003). In the intergeneric hybrids only the grooves for the onset of petal appendages were found. The ease of creating intergeneric hybrids between *Vriesea* × *Tillandsia* and × *Guzmania* indicates a close phylogenetic distance.

Pollen in the intergeneric hybrids was poorly developed or not formed at all, and it was completely sterile; no germination on *in vitro* media occurred. Hybrids render sterile because homologous chromosomes are not conjugated, which prevents their correct distribution in two successive meiotic divisions. The resulting microspores contain aneuploid sets of chromosomes and tend to be non-viable (Shamina *et al*. 1999). Ovules seemed normal, but pollination on stigma of *Vriesea splendens* × *Tillandsia cyanea* with both pollen of mother and father did not result in seed formation. F_1-sterility in interspecific and intergeneric crosses is very common. Despite survival or even vigorous growth of F_1-hybrids, sterility may result in a major barrier to gene flow between species. Among species the degree of F_1-sterility is normally a fair measure of the closeness of the evolutionary relationship between the parents (Benzing 1980).

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