

## Ultrastructural morphologic description of the wild rice species *Oryza latifolia* (Poaceae) in Costa Rica

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Received 25-IX-2001. Corrected 23-VIII-2002. Accepted 30-X-2002.

**Abstract:** The wild rice species *Oryza latifolia* is endemic to Tropical America, allotetraploid and has a CCDD genome type. It belongs to the *officinalis* group of the genus *Oryza*. This species is widely distributed throughout the lowlands of Costa Rica and it is found on different life zones, having great morphologic diversity. The purpose of this research is to perform a morphologic description of *O. latifolia* samples of three Costa Rican localities (Carara, Liberia and Cañas) and to see if the phenotypic diversity of the species is reflected at the ultrastructure level. Structures such as the leaf blade, ligule, auricles and spikelet were analyzed. Leaf blade morphology of the specimens from the three localities is characterized by the presence of diamond-shaped stomata with papillae, zipper-like rows of silica cells; a variety of evenly distributed epicuticular wax papillae and bulky prickle trichomes. The central vein of the leaf blade from the Cañas populations is glabrous, while those from Carara and Liberia have abundant papillae. There are also differences among the borders of the leaf blade between these locations. Cañas and Liberia present alternating large and small prickle trichomes *ca.* 81 and 150  $\mu\text{m}$ , while Carara exhibits even sized prickle trichomes of *ca.* 93  $\mu\text{m}$ . Auricles from Cañas are rectangular and present long trichomes along the surface *ca.* 1.5 mm, while those of Liberia and Carara wrap the culm and exhibit trichomes only in the borders. The ligule from the plants of Carara has an acute distal tip, while that of Cañas and Liberia is blunt. The Liberia spikelet has large lignified spines while Cañas and Carara show flexible trichomes.

**Key words:** Rice, *Oryza latifolia*, ultrastructure, morphology, anatomy, diversity.

Four wild species of the genus *Oryza* native Desv. of Central and South America and the Caribbean have been described: *O. latifolia*, *O. alta*, *O. grandiglumis* and *O. glumaepatula*. The first three are tetraploid (CCDD) and the latter is diploid (AA) (Vaughan 1994). In Costa Rica, all species are present except *O. alta* (Zamora *et al.* 1998, Zamora 2001).

Some of these wild relatives are resistant to diseases and pests that affect cultivated rice (Vaughan 1989, 1994), therefore, they represent useful sources of genes for the improve-

ment of cultivated rice (*O. sativa*). The incorporation of these genes through interspecific crosses could improve yield, confer resistance to diseases and pests and would permit rice cultivation in areas currently inadequate for its production.

*O. latifolia* is distributed from Mexico to Brazil and the Caribbean Islands (Tateoka 1962a, 1962b). It is found in clear patches in the forests, in secondary forests, savannas, grasslands, cultivated fields, muddy soils, along the shores of rivers, lagoons and creeks (Pohl 1978, 1980). It is commonly known by

the name of "arroz pato" and it flowers throughout the year (Zamora *et al.* 1998). In Costa Rica it is found in the lowlands, occupying diverse life zones, from the tropical dry forest (1200 mm annual precipitation) to the humid tropical forest (4000 mm annual precipitation) and in altitudes ranging between 0 m and 650 m above sea level (Zamora *et al.* 1998). The largest populations is found at Palo Verde National Park, Tempisque Conservation Area, Guanacaste, along the Tempisque River, in areas subjected to periodic floods. It is also very abundant in the Atlantic slopes, in the Tortuguero National Park, at the Tortuguero Conservation Area, Limón (Zamora *et al.* 1998).

This species presents morphologic and genetic variability, detected in the plant size (X:  $178 \pm 64.2$  cms SD), spikelet size (ranging from 4.8 to 7.2 mm) awn length (X:  $18 \pm 18$  mm SD), node color and size and number of branches in the panicle, among others (Zamora *et al.* 1999 a and b, Zamora 2001). The ligule is a stiff membrane (X:  $3.29 \pm 1$ mm SD), blunt, rigid and pubescent. The spikelet is composed of two sterile lemmas (glumes) and two fertile lemmas. The lemma, palea and floret include an oblong ovary with two feathery stigmas and six anthers composed of a filament and four lobes (Pohl 1978, 1980). Variability in isozyme patterns among Costa Rican populations have also been documented (Quesada *et al.* 1999, Quesada 2001).

The purpose of this work is to describe the ultrastructure of the leaf blade, ligule, auricles and caryopses from three populations of *O. latifolia* from three Costa Rican locations: Carara, Liberia and Cañas, to determine if intraspecific ultrastructural variability can be found.

## MATERIALS AND METHODS

**Material sampling:** *O. latifolia* plants were collected at the Palo Verde National Park, Cañas, Guanacaste (accession CIBCM-122); at the Carara Biological Reserve, Orotina,

Alajuela (accession CIBCM-022) and at Liberia, Guanacaste (accession CIBCM-004). Seeds of the collected samples were grown in the greenhouse at the Centro de Investigación en Biología Celular y Molecular, San Pedro de Montes de Oca, San José, Costa Rica.

**Electron microscopy:** Samples of the leaf, auricles, ligule and inflorescences were fixed in a 2.5% glutaraldehyde and 2% paraformaldehyde solution in a 0.1 M sodium phosphate buffer, pH 7.4 (Karnovsky 1965) and were rinsed with the buffer solution. The samples were post-fixed with 1% osmium tetroxide in the sodium phosphate buffer, were rinsed with distilled water and dehydrated in a ethanol gradient. Four washes were performed with terbutylic alcohol and were later dried by sublimation (Sublimate Eiko ID-2, Japan). The samples were placed on aluminum bases and were covered with 20nm platinum (Ionic Cover Eiko IB-5, Japan). The samples were then observed in two Scanning Electron Microscopes (Hitachi S-570 y S-2360N), with an acceleration voltage of 15 KV.

Anthers were homogenized with a 10% sodium hydroxide solution, rinsed with distilled water and then centrifuged at 2000 rpm (Hitachi 05 PR-22, Japan). They were later cleaned by ultrasound (Astrason 11H, Japan), fixed with 1% osmium tetroxide in 0.1M sodium phosphate buffer and processed as previously described.

The caryopses were placed in an incubator at 37°C for 48 hours. They were then mounted, covered, and observed on Hitachi S-570 and S-2360N electron microscopes, with an acceleration voltage of 15 KV.

## RESULTS

The ultrastructure of the adaxial surface of the *Oryza latifolia* leaf blade has very well-defined parallel vascular bundles, with a central and parallel to this are the lateral bundles. In the intercostal areas, long cells covered with abundant papillae and zipper-like silica cells were observed (Fig. 1b and 1c). These alternate with

bulky prickle trichomes that measure between *ca.* 40  $\mu\text{m}$  and *ca.* 63  $\mu\text{m}$  (Figs. 1a and 1c). In addition, small trichomes of *ca.* 15  $\mu\text{m}$  in length could be observed (Fig. 1c and 1d). The epicuticular wax pattern is rodlet-shaped (Fig. 1f), and stomata are distinguished as rosettes at low magnification (Fig. 1e) and measure about 17  $\mu\text{m}$ , and the subsidiary cells present various papillae (Fig. 1f).

The borders of the leaf blade show a row of prickle trichomes (Fig. 2a and 2b). Those of Cañas and Liberia samples measure 65 to 81 and 120 to 150  $\mu\text{m}$  in length and are arranged alternatively (Fig. 2a), while in Carara, they are an even in size and measure about 93  $\mu\text{m}$  (Fig. 2b). From the border inward, the epidermal pattern is as follows: several pairs of rows of wax papillae organized in groups, a row of stomata surrounded by papillae followed by another row of papillae and a row of silica cells. This pattern is repeated towards the interior of the leaf blade (Fig. 2a).

In the abaxial surface of the leaf blade, the following epidermal pattern can be observed: rows of stomata, trichomes, papillae and silica cells. This pattern is redundant along the blade surface and it is very similar to that of the adaxial surface. It is composed of papillae that measure about 6 by 9  $\mu\text{m}$ , and in some cases, rows or groups of three to eight tombstone-shaped papillae can be observed. These papillae measure about 12 by 9  $\mu\text{m}$  (Fig. 2c). The central surface of the central vein is composed of cells lacking epicuticular wax and papillae (Fig. 2e). The borders of the central vein have papillae, a row of silica cells and another set of papillae that increase height as they are located further away from the vein (Fig. 2e). Carara and Liberia have a central vein decorated with few small papillae that are distributed irregularly (Fig. 2f).

The auricles of Cañas are rectangular and their surface contain small, pointed trichomes and long attenuated trichomes. The latter are more abundant at the edges of the auricles (Fig. 3a). The auricles of Carara and Liberia wrap the culm, with long trichomes along the edges and small prickle trichomes on the surface (Fig. 3b).

The ligule of Cañas and Liberia is a stiff blunted membrane with prominent lateral vascular tissue, with abundant attenuated trichomes on its distal end (Fig. 3c), while at its surface, small and flexible trichomes are observed (Fig. 3d). The ligule from the Carara samples is, on the other hand, cone-shaped, pubescent and its distal tip is sharp with abundant attenuated trichomes (Fig. 3c). Additionally, it has short prickle trichomes that measure *ca.* 47  $\mu\text{m}$  in length on its surface (Fig. 3e).

The sterile lemmas of the spikelet are arrow-shaped with serrated edges (Fig. 3f). The fertile lemmas are covered with rigid spines in Carara and Liberia samples (Fig. 3f, 4c), while those from Cañas exhibit flexible trichomes (Fig. 4d). The awn is covered by spiny trichomes and lignified papillae (Fig. 4c). The anthers have four lobes covered with long reticulate cells and abundant wax (Fig. 4a), and on tips have scaly surface. The feathery stigmata of the ovary are covered with short, irregular cells (Fig. 4a and 4b).

## DISCUSSION

After ultrastructural analysis of the three *Oryza latifolia* populations, intra-specific variability in the leaf blade was observed in relation to the ornamentation of the central vein, the trichomes of the borders of the leaf blade, and in the shapes of the auricles and ligule. This diversity was previously observed macroscopically by Zamora and co-workers (1998, 1999 a, b and c) and Zamora (2001). In addition, molecular tests using isoenzymes also show those modifications (Quesada *et al.* 1999, 2001). Considering that the variability occurs in all evolutionary and speciation process, Samuel (1988) claim that it is possible that a gradual divergence on a population system may occur, as well as in a primary homogeneous species in two or more different population systems. This divergence is generally related to the adaptation to different geographical areas, climates, or well-defined ecological

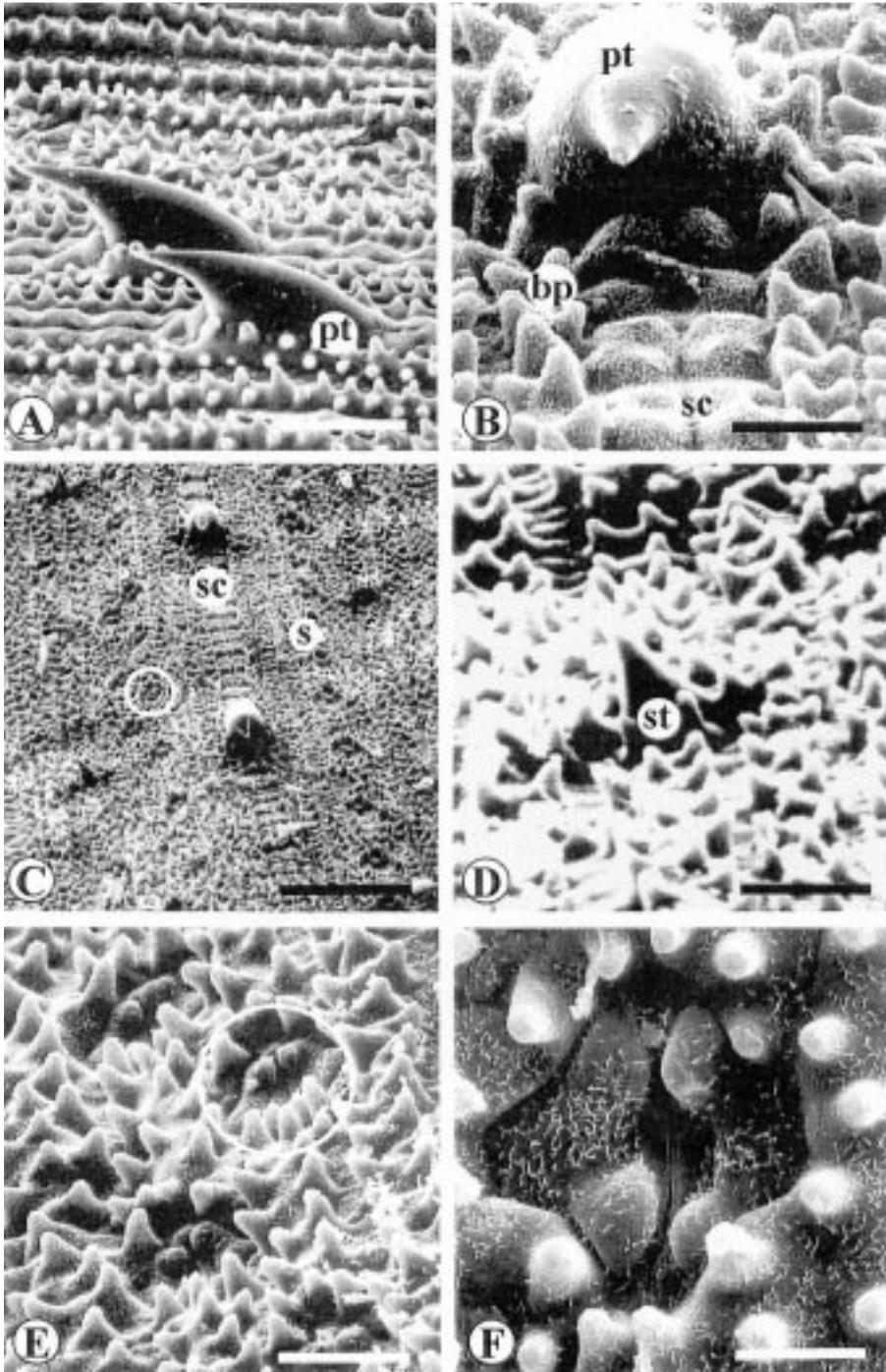


Fig. 1. Adaxial surface of the leaf blade of *Oryza latifolia*. a. Lateral view showing two bulky prickles (pt). (Bar = 43  $\mu$ m). b. Frontal view of the prickly trichome (pt), silica cells (sc), and bilobed papillae (bp). (Bar = 15  $\mu$ m). c. Row of rosette-shaped stomata (s), flanking the row of silica cells. (Bar = 86  $\mu$ m). d. Small spiny trichome (st). (Bar = 17  $\mu$ m). e. Stomata (s) surrounded by papillae (Bar = 17.6  $\mu$ m). f. Close-up of the guard cells of a stoma (Bar = 10  $\mu$ m).

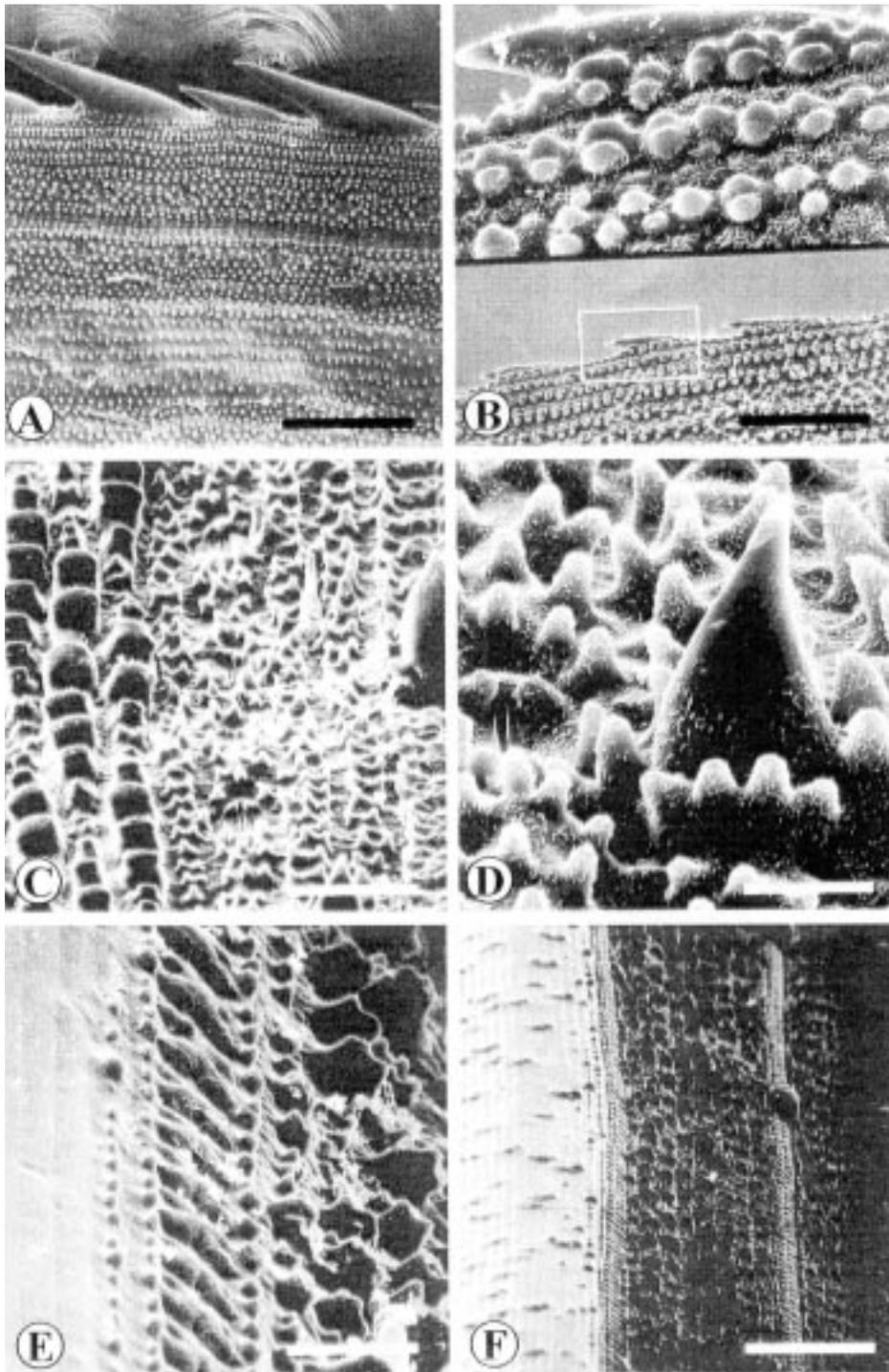


Fig. 2. Abaxial surface of the leaf blade of *Oryza latifolia* a. Border of the leaf blade from Cañas (Bar = 136  $\mu$ m). b. Border of the leaf blade from Carara (Bar = 86  $\mu$ m). c. Tombstone-shaped papillae from Cañas (Bar = 136  $\mu$ m). d. Small pointed trichome (Barra = 12  $\mu$ m). e. Central vein of Cañas (Barra = 27  $\mu$ m). f. Central vein of Carara (Barra = 136  $\mu$ m).

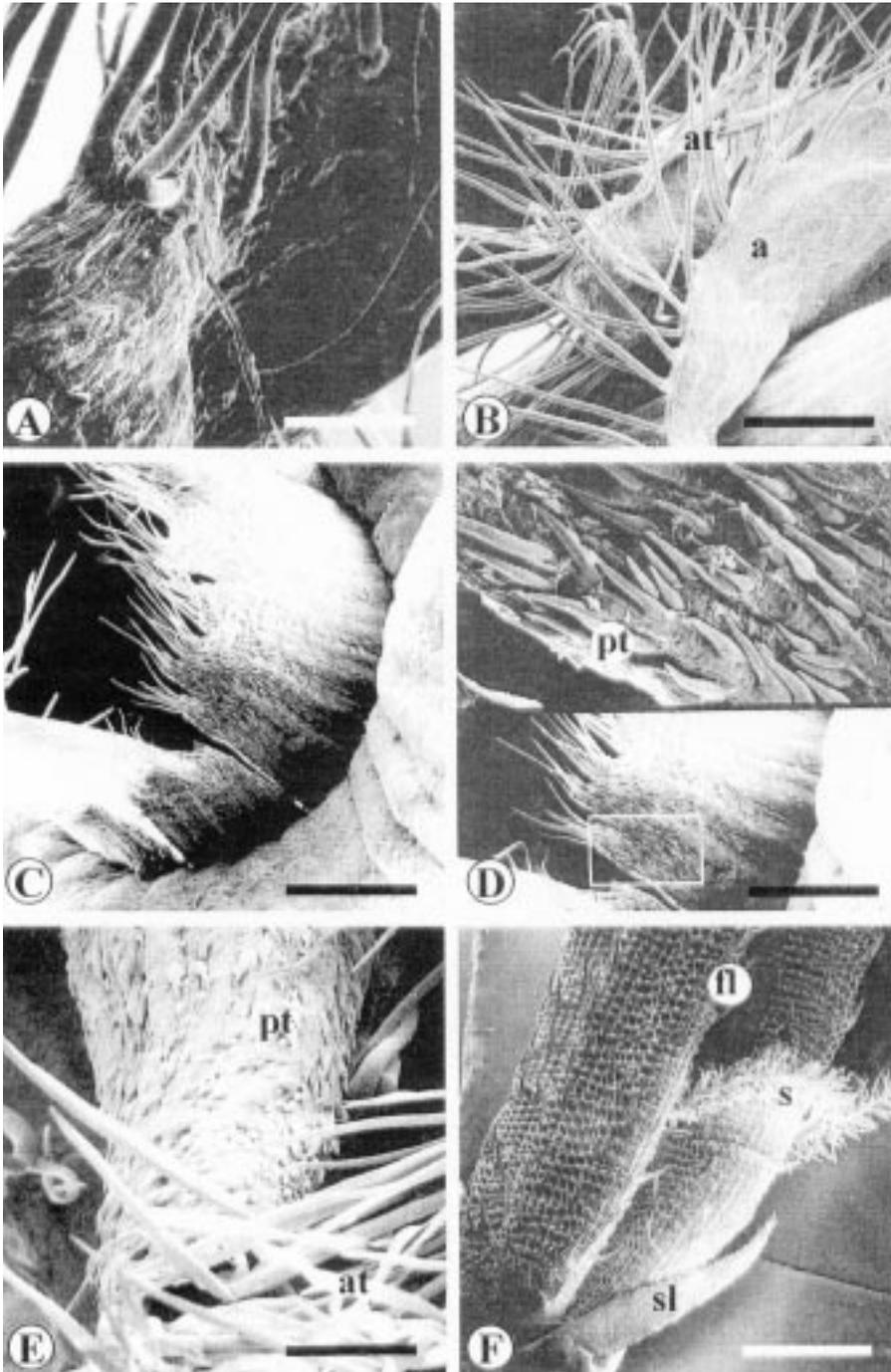


Fig. 3. Auricles, ligule and spikelet of *Oryza latifolia*. a. Rectangular auricle from Cañas (Bar = 231  $\mu$ m). b. Auricle from Carara, showing attenuated trichomes (at) (Bar = 0.86 mm). c. Blunt ligule from Liberia (Bar = 0.86 mm). d. Detail of prickle trichomes (pt) of the ligule surface from Liberia (Bar = 0.86 mm). e. Ligule from Carara, showing attenuated trichomes (at) on the border and prickle trichomes (pt) on the surface (Bar = 231  $\mu$ m). f. Spikelet from Cañas, showing sterile (sl) and fertile lemmas (fl), and feathery stigmata (s) (Bar = 1.00 mm).

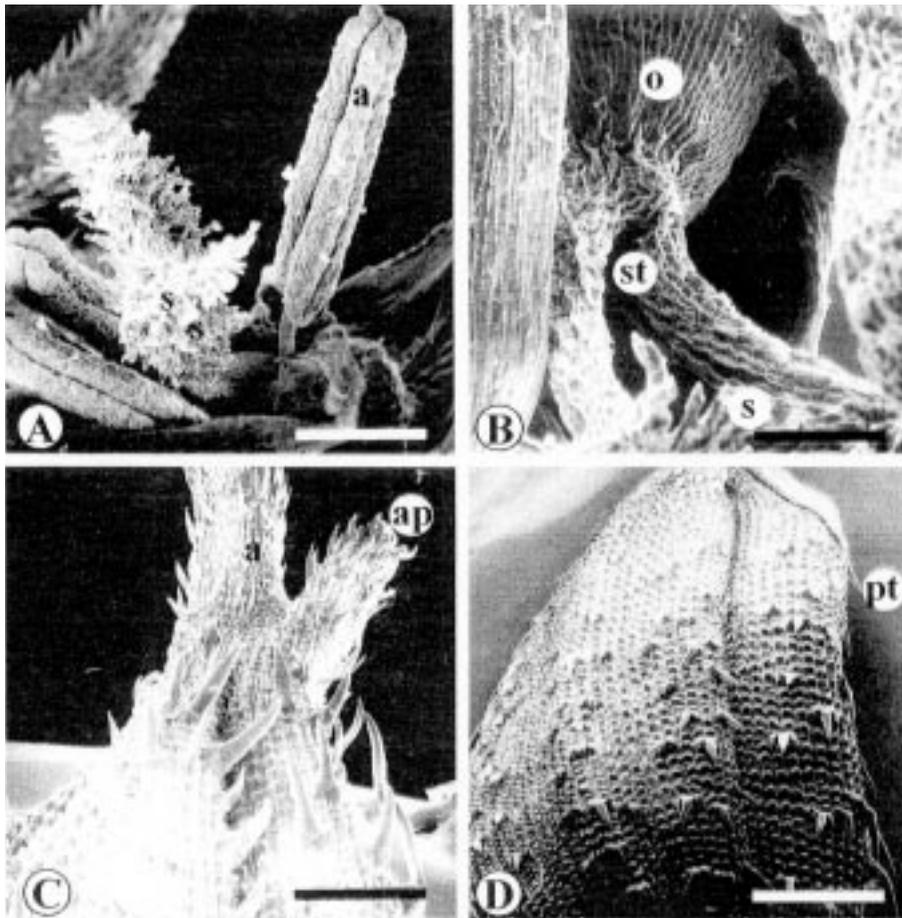


Fig. 4. Spikelet from *Oryza latifolia*. a. Anthers (an) and feathery stigmata (s) (Bar=0.43 mm). b. Ovary (o), stylum (st), feathery stigmata (s). (Bar=120  $\mu$ m). c. Distal end of the spikelet, apiculum (ap), awn base (a) and the rigid spines that wrap the fertile lemmas (Bar=0.43 mm). d. Caryopsis from Cañas, showing flexible prickles (pt) (Bar=1.00 mm).

habitats, resulting in a genetic differentiation that is expressed both in the morphology and in the physiology of the species. As a result, it is possible to find divergent variations in the population depending on the environmental gradient at which the species is kept (Samuel 1988, Zamora 2001). The present analysis was done in the populations of *O. latifolia* from the South-Pacific zone and North zone of Costa Rica, so it is advisable to continue the analysis of the populations from other areas in order to determine the present variability in the species.

In relation to the other structures found in the leaf blade of *O. latifolia*, it was found that

they are similar to the descriptions of Metcalfe (1960) and Hoagland and Paul (1978) for the botanical species *Oryza* and the morphology of the ligule, auricles and spikelet are in agreement with the descriptions done by Stand (1973), Pohl (1978), Hoagland and Paul (1978), Montiel and Kozuka (1994), Terrel, Peterson and Wergin (2001).

#### ACKNOWLEDGEMENTS

The authors want to acknowledge Enrique Freer for value collaboration, to Tania Quesada

and Ricardo Jiménez for the translation of the manuscript to English, to Alejandro Zamora for valuable discussions, to Jéssica Coto for the art work.

## RESUMEN

La especie silvestre *Oryza latifolia* es endémica de América, tetraploide y de genoma CCDD. Pertenece a las especies del género *Oryza* del grupo *officinalis*. Presenta una amplia distribución en las tierras bajas de Costa Rica y se le encuentra en varias zonas de vida, mostrando una gran diversidad morfológica. El propósito de este trabajo fue realizar una descripción ultraestructural de plantas de *O. latifolia* provenientes de las localidades Carara, Liberia y Cañas con el fin de determinar si hay variación intrapoblacional. Se analizó la lámina foliar, lígula, aurículas y espiguilla, con énfasis en las estructuras de valor taxonómico. La morfología de la lámina foliar de los especímenes de las tres localidades se caracteriza por la presencia de estomas romboidales con papilas, células de sílice de forma crenada, variedad de papilas de cera, distribuidas en forma regular y tricomas espinosos abultados de ca 53 µm. La vena central de la lámina foliar de *O. latifolia*, localidad Cañas, es lisa, mientras que en Carara y Liberia presenta papilas. Los bordes de la lámina foliar difieren entre localidades; Cañas y Liberia presentan tricomas ganchudos grandes de ca. 150 µm y pequeños de ca 81 µm que alternan, mientras que Carara tiene tricomas ganchudos pequeños de igual tamaño (ca. 93 µm). Las aurículas de Cañas son rectangulares y presentan tricomas atenuados largos de ca. 1.5mm en toda la superficie, mientras las de Liberia y Carara son envolventes y presentan dichos tricomas solamente en los bordes. La lígula de Carara tiene el extremo distal agudo, mientras que Cañas y Liberia lo tienen truncado. La espiguilla de Liberia presenta tricomas espinosos lignificados grandes, mientras que Cañas y Carara tienen tricomas flexibles.

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