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INFLORESCENCE, SPIKELET, AND FLORAL DEVELOPMENT IN *PANICUM MAXIMUM* AND *UROCHLOA PLANTAGINEA* (POACEAE)[1]

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Inflorescence development in *Panicum maximum* and *Urochloa plantaginea* was comparatively studied with scanning electron and light microscopy to test the transfer of *P. maximum* to *Urochloa* and to look for developmental features applicable to future cladistic studies of the phosphoenol pyruvate carboxykinase (PCK) subtype of C_4 photosynthesis clade (*P. maximum* and some species of *Brachiaria*, *Chaetium*, *Eriochloa*, *Melinis*, and *Urochloa*). Eleven developmental features not discernable in the mature inflorescence were found: direction of branch differentiation; origins of primary branches; apical vs. intercalary development of the main axis; direction of spikelet differentiation; direction of glume, lemma and palea differentiation; position of the lower glume (in some cases); size of the floret meristem; pattern of distal floret development; pattern of gynoecium abortion; differential pollen development between proximal and distal floret; and glume elongation. Inflorescence homologies between *P. maximum* and *U. plantaginea* are also clarified. *Panicum maximum* and *U. plantaginea* differ not only in their mature inflorescence structure but also in eight fundamental developmental features that exclude *P. maximum* from *Urochloa*. The following developmental events are related to sex expression: size of floret meristem, gynoecium abortion, pollen development delay in the proximal floret, glume elongation and basipetal floret maturation at anthesis.

Key words: development; homology, inflorescence; Paniceae; *Panicum maximum*; Poaceae; sex expression; *Urochloa plantaginea*.

The grass subfamily Panicoideae includes approximately 208 genera grouped in several tribes; among these, Paniceae, with more than 110 genera, and Andropogoneae, with 85 genera, are the largest and most important ones (Clayton and Renvoize, 1986; Watson and Dallwitz, 1992). Because the tribe Paniceae is highly diverse in morphological, physiological, anatomical, and karyological characters (Zuloaga et al., 2000; Duvall et al., 2001; Giussani et al., 2001), different evolutionary schemes have been proposed for this tribe and its genera (Aliscioni et al., 2003). According to recent findings and the increase of samples studied, the phylogeny of Paniceae is undergoing several changes, even though the taxonomical delimitation of some of its genera is still unclear (Zuloaga et al., 2000; Duvall et al., 2001; Giussani et al., 2001; Aliscioni et al., 2003).

Recent studies on the phylogeny of Paniceae (Zuloaga et al., 2000; Duvall et al., 2001; Giussani et al., 2001) showed that *Brachiaria eruciformis* (Smith) Griseb., *Chaetium bromoides* (J. Presl.) Benth. ex Hemsl., *Eriochloa punctata* (L.) Desv. Ex Hamilton f. *intermedia* Parodi, *Melinis repens* (Willdenow) Zizka, *Urochloa acuminata* (Renvoize) Morrone & Zuloaga, *U. plantaginea* (Link) Webster, *U. mutica* (Forsskal) Nguyen, and *Panicum maximum* Jacq. form a monophyletic group with high bootstrap support. This was called ''the PCK clade'' because all the taxa use the phosphoenol pyruvate carboxykinase (PCK) subtype of the C_4 photosynthetic pathway (Aliscioni et al., 2003). In spite of strong support for the monophyly of the PCK clade, relationships among these taxa are still unclear. An example of this problem is the controversial taxonomic affiliation of *P. maximum*, which has been referred to as *Urochloa* (Webster, 1987; Giussani et al., 2001; Aliscioni et al., 2003) as well as to the subgenus *Megathyrsus* Pilger of *Panicum*, recently upgraded to a new independent genus (Simon and Jacobs, 2003). Except for the anatomy related to the photosynthetic pathway, no other morphological features distinguish the PCK clade. Among the different morphological features of the taxa involved in the PCK clade, the structure of the inflorescence is remarkably diverse. However, the morphology of mature inflorescences of Poaceae is not enough to understand their morphological diversity and relationships (LeRoux and Kellogg, 1999; Kellogg, 2000a, b, 2003, 2004; Doust and Kellogg, 2002). A comparative analysis of inflorescence development in *Setaria*, *Pennisetum*, and *Cenchrus*, also closely related members of the tribe Paniceae, showed that only a few changes in the pattern of development account for the considerable range of variation seen at maturity (Doust and Kellogg, 2002).

Considering the potential value of the inflorescence in determining systematic relationships within Paniceae, a comparative study of inflorescence development in two members of the PCK clade, *P. maximum* and *U. plantaginea*, is carried out with two aims: (1) to test if inflorescence development supports inclusion of *P. maximum* in *Urochloa* or its segregation in a new, independent genus *Megathyrsus* and (2) to search for features in the development of inflorescences that could be used in future cladistic studies of the PCK clade.

Panicum maximum was selected for study because of its uncertain taxonomic affiliation. Among the species of *Urochloa*, *U. plantaginea* is one of the closest species to *P. maximum* in the analyses of Giussani et al. (2001) and Aliscioni et al. (2003), but its mature inflorescence differs greatly from that of *P. maximum*. *Urochloa plantaginea* is an annual herb with bilateral inflorescences and spikelets on short pedicels (Morrone and Zuloaga, 1992). *Panicum maximum* is a perennial

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herb with radiate, lax inflorescences and spikelets on long pedicels (Zuloaga, 1979; Zuloaga and Morrone, 1995). Both species have bifloral spikelets in which the distal floret is hermaphroditic and the proximal one is male in *P. maximum* and neutral (only a lemma and a palea are observed) in *U. plantaginea* (Zuloaga, 1979; Morrone and Zuloaga, 1992).

MATERIALS AND METHODS

Fresh inflorescences of *Panicum maximum* and *Urochloa plantaginea* were collected from natural populations in Santa Fe, Argentina between September 2001 and March 2002. Twenty-five plants were studied per accession. About 150 samples of inflorescences (in total) were fixed in FAA (formalin : acetic acid : 70% ethanol, 10 : 5 : 85, v/v) to be studied with a stereomicroscope. About 25 samples were selected from the original stock for scanning electron microscopy (SEM) and light microscopy studies.

For SEM observations, fixed inflorescences were dissected and classified with a stereomicroscope Zeiss DV4 (Jena, Germany), according to the different stages of development. After that, the samples were dehydrated in an alcohol series plus two final changes of 100% acetone. Dehydrated material was dried by critical point with CO_2 as transitional fluid and coated with gold-palladium using a BAL-TEC SCD 050 (Balzers, Switzerland). All samples of inflorescences, spikelets, and florets were observed and photographed using a JEOL JSM-T 100 (Kent, UK) scanning electron microscope from the Electron Microscopy Service of La Plata Museum, Buenos Aires, Argentina. Measurements of the floral meristems were standardized following the instructions provided by the Electron Microscopy Service of La Plata Museum.

For studies with light microscopy, fixed samples were dehydrated with isobutyl alcohol, and infiltrated with and embedded in Histoplast (Ruzin, 1999). Longitudinal and transverse sections 10 μm thick were stained with safranin, fast green, and Mayer's haematoxylin (Johansen, 1940), and mounted with Eukitt (Hatfield, PA, USA) on a glass slide.

RESULTS

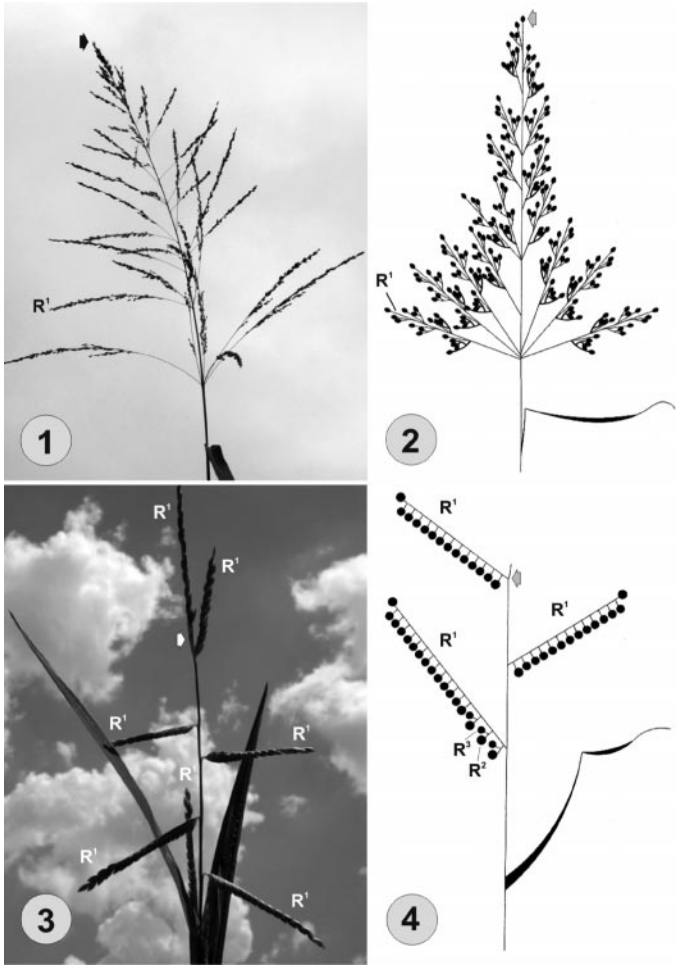
Morphology of the mature inflorescence—The structure of the mature inflorescence of both species has been previously described by Zuloaga (1989), Zuloaga and Morrone (1995), Morrone and Zuloaga (1992), Reinheimer and Vegetti (R. Reinheimer, unpublished data, National University of Litoral) and will be briefly mentioned here.

Panicum maximum has a lax and radiate inflorescence (Fig. 1) where the main axis ends in a terminal spikelet (Fig. 2). The inflorescence includes 18–56 primary branches, each one ending in a terminal spikelet (Figs. 1, 2). The highest branch degree observed is the fifth-order (Fig. 2). Primary branches are alternate; characteristically, they are pseudoverticillate in the proximal region of the inflorescence and sometimes subopposite in the middle region of the inflorescence (Fig. 2).

The inflorescence of *U. plantaginea* is bilateral (Fig. 3) and the main axis lacks a terminal spikelet (Fig. 4). The inflorescence includes 2–14 primary branches, which alternate along the main axis (Figs. 3, 4). The primary branches have terminal spikelets and bear secondary or, less frequently, tertiary branches (Fig. 4). Spikelets are subtended by short pedicels and are distributed in two rows along the abaxial side of the primary branches.

Branch system development—A comparison of inflorescence branch system development between *P. maximum* and *U. plantaginea* is presented in Table 1. During vegetative growth, the apical meristem of the shoot of *P. maximum* and *U. plantaginea* elongates intravaginally and produces leaf primordia in two ranks (distichous) (Fig. 5). The transition from the vegetative state to the flowering one is evident when the apical meristem elongates beyond the last formed leaf primordium to form the main axis of the inflorescence. In addition, the apical meristem of *P. maximum* (90.105 μm diam.) is larger than the apical meristems of *U. plantaginea* (68.586 μm diam.) (Figs. 6, 11).

Development of the *P. maximum* inflorescence starts with the production of first-order branch primordia along the main axis (Fig. 6). Phyllotaxis of the main axis shifts from distichous production of leaves to polystichous production of primary branches when primary branch primordia initiate in additional rows along the main axis. The first formed primary branch is produced from the axillary bud of a small bract

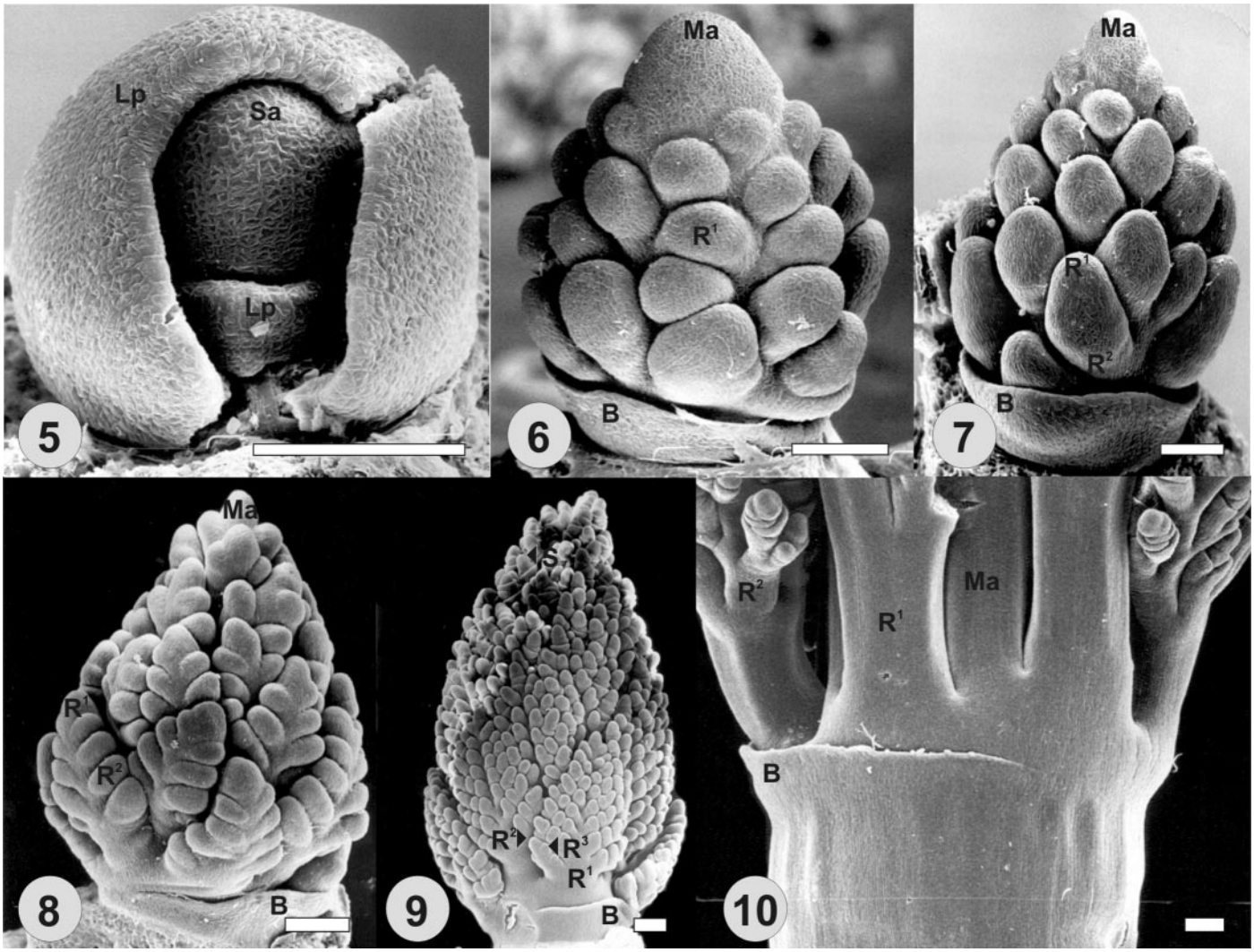


Figs. 1–4. Morphology and structure of the mature inflorescence. **1.** *Panicum maximum*. **2.** Diagram of the inflorescence of *P. maximum* showing main axis ending in a terminal spikelets (arrow), and first and higher order branches. **3.** *Urochloa plantaginea*. **4.** Diagram of the inflorescence of *U. plantaginea* showing main axis without a terminal spikelet, and primary branches, the proximal one bearing some third-order branches.

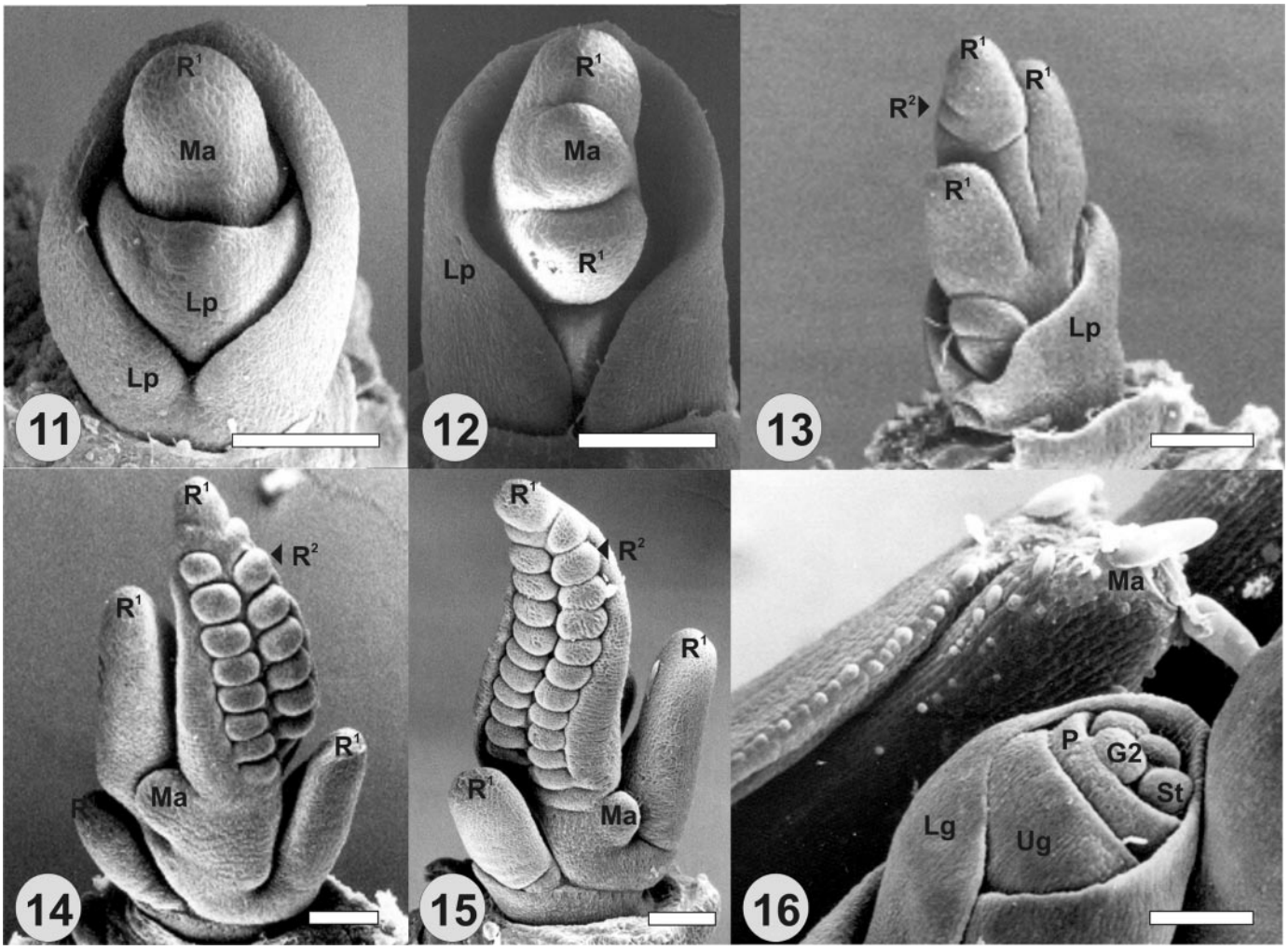
Figure abbreviations: A, anther; B, bract; Fm1, floral meristem of the proximal floret; Fm2, floral meristem of the distal floret; G1, gynoecium primordium of the proximal floret; G1a, aborting gynoecium of the proximal floret; G2, gynoecium primordium of the distal floret; Gr, gynoecial ridge; Ms, microspores; L1, lemma of the proximal floret; L2, lemma of the distal floret; Lg, lower glume; Lo, lodicule; Lp, leaf primordium; Ma, main axis of the inflorescence; Ov, ovule; P, palea; PMCs, pollen mother cells; R^1, primary branch primordium; R^2, secondary branch primordium; R^3, tertiary branch primordium; Ra, rachilla; S, spikelet primordium; Sa, shoot apex; St, stamen primordium; Sta, aborting stamen; Stf, staminate flower; Ug, upper glume; 1CP, 1-celled pollen.

TABLE 1. Main developmental features of the branch system of the inflorescences of *Panicum maximum* and *Urochloa plantaginea*.

Structure/Feature	*P. maximum*	*U. plantaginea*
Main axis		
Diameter of the apical meristem	90.105 μm	68.586 μm
Origin	Elongation of several contiguous internodes formed by the apical meristem	Intercalary growth of the first formed internode
Primary branches		
Origin		
First formed primary branch	Axillary (regular) bud	Axillary (regular) bud
Second and following primary branches	Axillary (regular) buds	Adventitious buds
Quantity	Many (more than 14)	2–14
Phyllotaxis	Polystichous	Distichous
Initiation and maturation	Acropetal	Basipetal
Secondary branches		
Initiation and maturation		
Along the inflorescence	Acropetal	Basipetal
Along the primary branches	Acropetal	Amphipetal
Phyllotaxis	Distichous	Unilateral



Figs. 5–10. Scanning electron micrographs of inflorescence development in *Panicum maximum*. **5.** Main apical meristem and two leaf primordia. **6.** Elongation of the main axis and differentiation of primary branch primordia. **7.** Elongation of primary branch primordia and differentiation of secondary branch primordia. **8.** Bract at the base of the first formed (most proximal) primary branch and differentiation of secondary branch primordia. **9.** Differentiation of tertiary branch primordia and spikelet initiation. **10.** Subverticil of primary branches after elongation of the internodes of the main axis. Bar = 100 μm.



Figs. 11–16. Scanning electron micrographs of inflorescence development in *Urochloa plantaginea*. **11.** Transition to flowering of the apical meristem. **12.** Differentiation of primary branch primordia and displacement of the apical meristem of the main axis. **13.** Elongation of primary branches and differentiation of a secondary branch primordium (the apical meristem of the main axis cannot be seen because it is covered by primary branches and is on the opposite side of the figure). **14.** Arrested apical meristem of the main axis and differentiation of secondary branch primordia in amphipetal succession. **15.** Distal primary branch more advanced in the development than the remaining primary branches. **16.** Apex of the main axis without terminal spikelet. Bar = 100 μm.

primordium (Fig. 6), which stop developing when the primary branch is still elongating (Figs. 7–10). While the main axis elongates, the apical meristem produces additional primary branches in acropetal succession all around the rachis (Fig. 7). In *U. plantaginea*, the apical meristem begins to elongate during transition, but its stops growing almost immediately (Figs. 11–16). A first, single, axillary bud develops as the first primary branch opposite to the last formed vegetative leaf (Fig. 11). The second and subsequent primary branches do not arise from the apical meristem, but from buds arising downward along the elongating first internode of the inflorescence (the one restricted between the last vegetative leaf and the first primary branch). During elongation of the second primary branch, the apical meristem is displaced to a lateral position above the second formed primary branch, probably due to the elongation of the first formed primary branch, which exceeds the length of the main axis early and adopts the main axis position (Figs. 12–15). Except the first primary branch, nothing else is produced by the apical meristem of the main axis. Based on the samples examined of *U. plantaginea*, 2–14 primary branches with distichous dispositions (like the vegetative leaves of the shoot), are initiated and developed in basipetal succession below one another (Figs. 11–13). The first formed (most distal) primary branch is the only one produced by an axillary bud of the apical meristem. In contrast to *P. maximum*, in which the main axis elongates at the expense of the apical meristem, in *U. plantaginea* the main axis increases in length by the elongation of the internode below the first formed primary branch and the last vegetative leaf.

In *P. maximum*, while the first-order branches are increasing in length and the apical meristem is still elongating and producing primary branches, second-order branches are initiated distichously at the base of first-order branches in the proximal region of the inflorescence (Figs. 7, 8). Initiation of the second-order branches is acropetal in the whole inflorescence and along first-order branches. Meanwhile, the main axis is still elongating, new second-order branches are being produced on distal primary branches, and third-order branches are originating distichously at the base of the first formed (proximal) secondary branches (Fig. 9). The inflorescence of *P. maximum*

TABLE 2. Main developmental features of spikelets and florets of *Panicum maximum* and *Urochloa plantaginea*.

Structure/Feature	*P. maximum*	*U. plantaginea*
Spikelet		
Terminal spikelet on main axis	Present	Absent
Order of branch primordia bearing spikelets	1–5	1–3
Floret		
Diameter of proximal/distal floret's meristem	27.259 μm/58.113 μm	19,762 μm/68,972 μm
Meristems covered or not by glumes at the time of gynoecial ridge formation (proximal/distal floret)	Covered/exposed	Covered/covered
Sex (floret primordia → floret at anthesis)		
Proximal floret	Hermaphroditic → Male	Hermaphroditic → Neutral
Distal floret	Hermaphroditic → Hermaphroditic	Hermaphroditic → Hermaphroditic

becomes more complex when new higher-order branches are produced. Among the samples observed, the maximum branch degree in *P. maximum* is up to the fifth-order. Third-, fourth- and fifth-order branches also are initiated in acropetal succession on both the whole inflorescence and on their subtending branches. In contrast to *P. maximum*, secondary branch primordia of *U. plantaginea* are amphipetaly initiated in two ranks on the abaxial side of every primary branch (Figs. 13–15). All secondary branches in *U. plantaginea* differentiate in basipetal direction on the whole inflorescence, but in amphipetal succession on the primary subtending branch. The primary branches flatten as they elongate, and new secondary branches are initiated (Figs. 14, 15). The secondary branches arrest their development at the moment that glumes differentiate at their top. Less frequently, tertiary branches may be initiated on the proximal region of the basal primary branch. Hence, paired spikelets can be found at the proximal region of the last formed basal, primary branch in the inflorescence of *U. plantaginea*.

In both species, the inflorescences emerge from the sheath by elongation of internodes. As a result of the differential elongation of the internodes of the main axis, the inflorescence of *P. maximum* characteristically shows a subverticillate arrangement of primary branches at the proximal region of the inflorescence and, sometimes, opposite the middle of the inflorescence (Fig. 10).

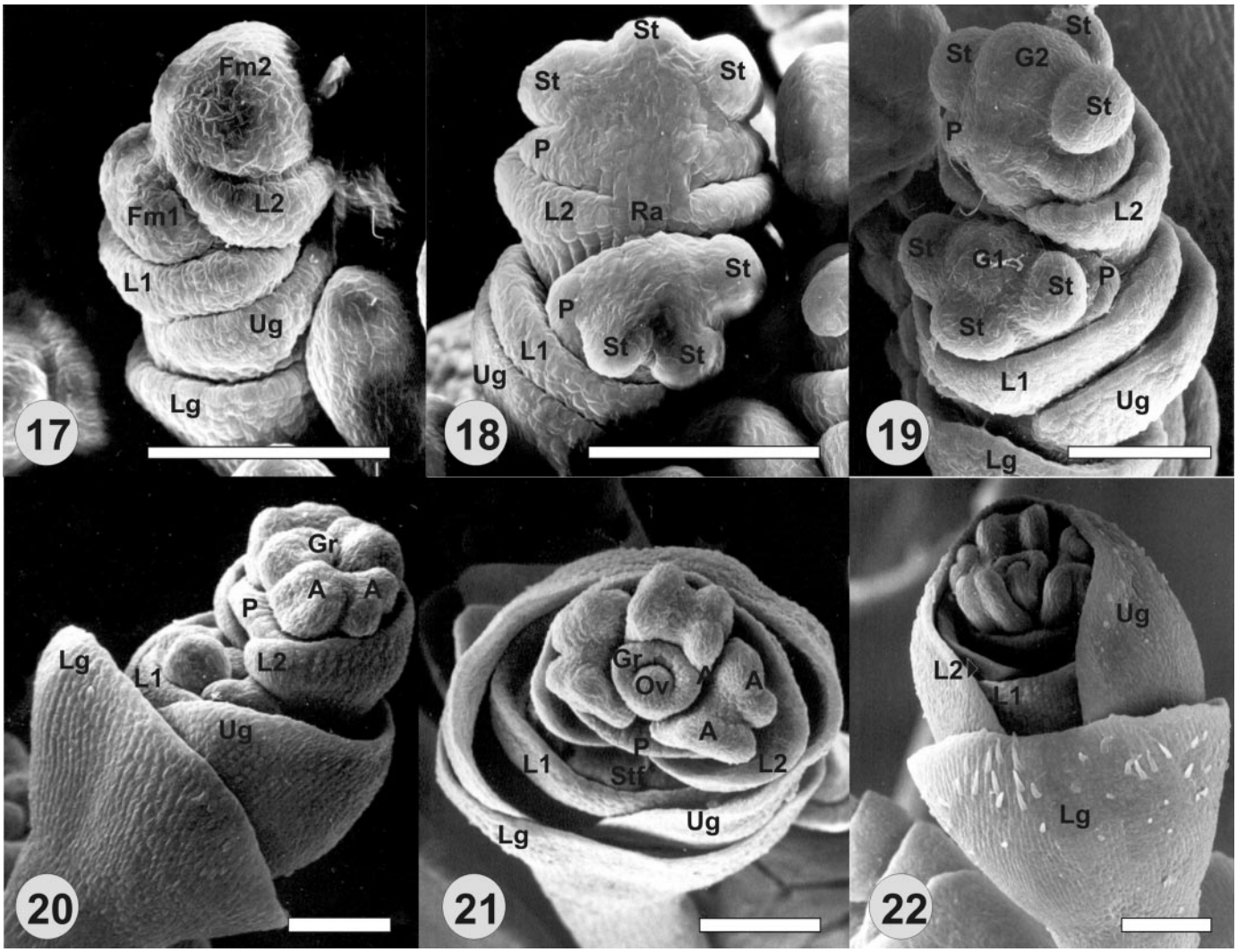
Spikelet development—A comparison of spikelet development between *P. maximum* and *U. plantaginea* is presented in Table 2. During the development of spikelets, *P. maximum* and *U. plantaginea* differed in the order of branch primordia on which spikelets are differentiated and the size of meristems from which the floral organs were initiated. Spikelet differentiation on the whole inflorescence and on branches is basipetal. In both species, glumes and lemmas are initiated acropetally on the spikelet axis.

In *P. maximum*, spikelets are initiated at every order of branching and also at the top of the main axis (Fig. 9), while in *U. plantaginea* spikelets differentiate at the tip of the primary branches and on secondary branches (Fig. 23) or less frequently on third-order branches, but the main axis never ends in a terminal spikelet (Fig. 16).

Spikelet formation starts with a change in the shape of distal branches related to the inception of two alternate concave glume initials. The first formed primordium develops into a lower glume and the second one into an upper glume (Figs. 17, 23). In *U. plantaginea*, the lower glume of the terminal spikelet of a primary branch is abaxial, in contrast to the adaxial lower glume of spikelets on secondary branches (Fig. 25). While the axis of the spikelet elongates, the lemma primordium of the lower floret arises (Figs. 17, 23). Almost simultaneously, the lemma primordium of the upper floret differentiates (Figs. 17, 23). The lemmas initiate alternately (Figs. 17, 23) and subsequently, meristems of the proximal and distal floret are visible. In *P. maximum*, the proximal floret meristem (about 27.25 μm diam.) is 50% smaller than the distal one (about 58.11 μm diam., Fig. 17), while in *U. plantaginea*, the proximal floret meristem (about 19.76 μm diam.) is less than one-third of the upper one (about 68.97 μm diam.), and is covered by the lower lemma early in the development (Fig. 23).

Floret development—A comparison of floret development between *P. maximum* and *U. plantaginea* is presented in Table 2. After the lemmas have differentiated, primordia of floral organs arise. Floral development in *P. maximum* and *U. plantaginea* differs in many characteristics, including the size of the floret meristem, patterns of floret development, sex expression, and elongation of glumes.

In *P. maximum*, differentiation and maturation of the floral organs within the spikelet is basipetal (Figs. 18, 19). Three stamen primordia develop first in the distal floret. Two of them are initiated on the lateral flanks of the meristem and one, abaxially (Fig. 18). Just after the inception of stamen primordia, one palea differentiates on the floret axis alternately with the lemma and surrounding the floret meristem (Figs. 18, 19). Meanwhile, three stamen primordia and a palea are initiated in the proximal floret following the same pattern as the distal one (Figs. 18, 19). Later, the stamen primordia expand to form thecae (Fig. 20). Before the two lodicules differentiate in a whorl outside the stamen primordia, the gynoecial primordium develops from the remaining floret meristem (Figs. 20, 21). The gynoecium of the distal floret develops a gynoecial ridge on the same side as the upper lemma, surrounding the ovule primordium (Figs. 20, 21). At the same time, the proximal floret is enveloped by the glumes, while the distal floret remains exposed and the anthers of both florets elongate above the gynoecium (Figs. 20, 22). The gynoecium of the lower floret arrests its development before the gynoecial ridge becomes evident (Figs. 30, 31). After that, filaments of the stamens gradually elongate and, in the distal floret, the branches of the style and stigmas develop. Thus, in *P. maximum* both florets arise as hermaphroditic primordium, and while the distal floret remains hermaphroditic up to anthesis, the proximal



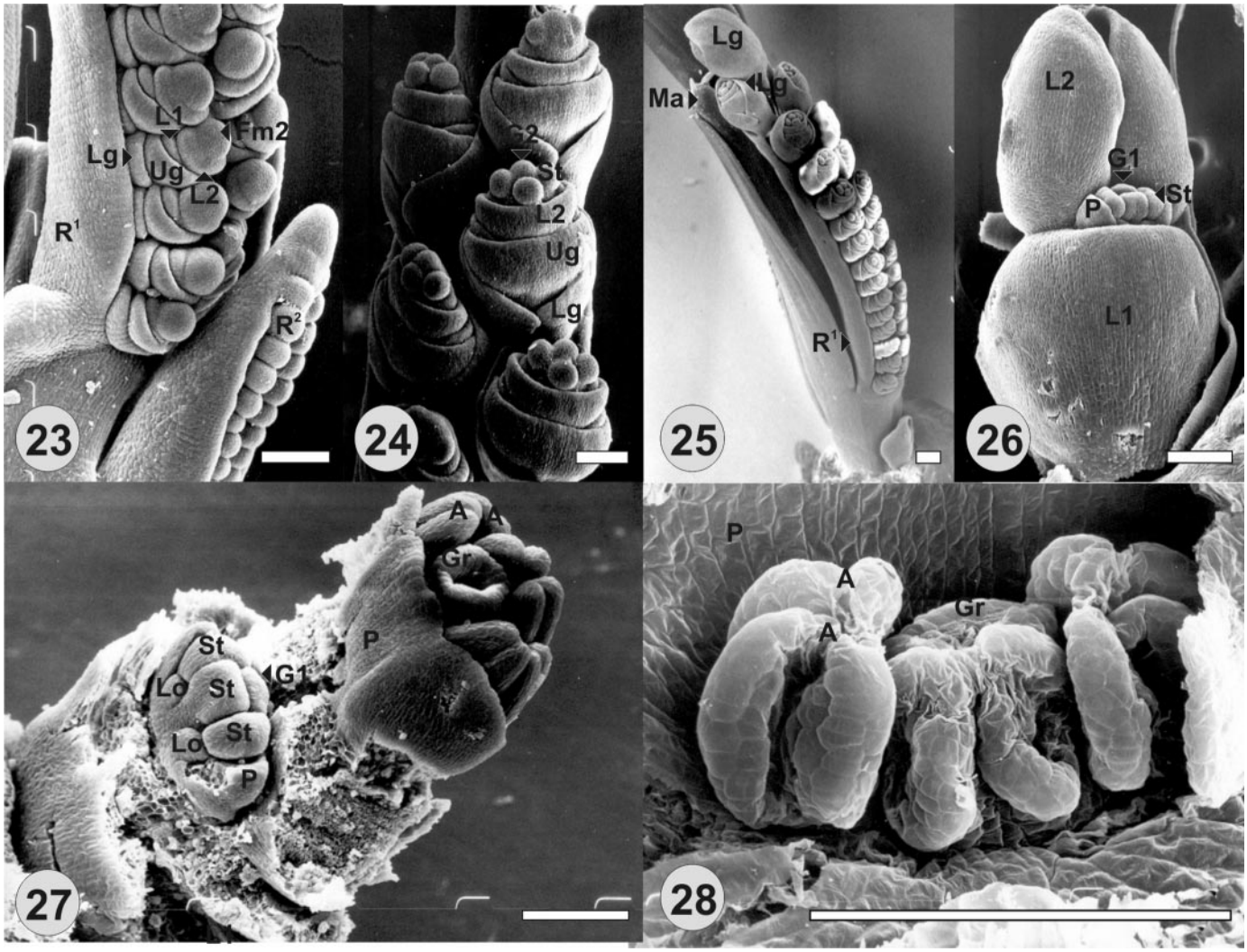
Figs. 17–22. Scanning electron micrographs of spikelet and floral development in *Panicum maximum*. **17.** Floral meristem and differentiation of glumes and lemmas. **18.** Palea and stamen initiation. **19.** Initiation of the gynoecium and beginning of the elongation of glumes. **20.** Glume elongation, anther and gynoecium differentiation. **21.** Apical view of the distal floret and glumes totally covering the proximal floret. **22.** Distal floret partially covered by glumes. Bar = 100 μm.

one develops as a male floret by abortion of the gynoecium primordium.

The stamens, palea, lodicules, and gynoecium in the distal floret of *U. plantaginea* initiate as in *P. maximum* (Figs. 24, 25). After the initiation of the palea in the upper floret, the lower glume elongates, surpassing the stamens of the distal floret (Figs. 16, 25). Whereas the lower glume elongates, the upper glume increases in length slowly until it totally envelopes the distal floret (Figs. 16–25). In contrast to *P. maximum*, the floret organ primordia of *U. plantaginea* continue their development completely protected by the glumes. In *U. plantaginea*, the floral organs of the distal floret follow the same developmental pathway as the distal floret in *P. maximum*, producing a hermaphroditic floret (Fig. 27). The proximal floret meristem does not remain inactive but progresses into a hermaphroditic floret primordium (Fig. 26), which ceases its development and begins to abort when the thecae of the three stamens are differentiated and the gynoecial ridge is just arising (Figs. 26–28). Meanwhile, the lower lemma, the upper lemma and paleas increase in length, enveloping all the floral organs. Hence, in *U. plantaginea* the proximal and the distal florets of every spikelet develop as hermaphroditic, but later, both stamens and gynoecium of the proximal florets cease development to form a proximal sterile floret.

By anthesis, the floral organs of every spikelet are completely enveloped by glumes in both species.

Histological development of floral organs—Histological development of florets differs within and between each species. In *P. maximum*, microsporangial development starts at the same time in both florets and continues simultaneously up to the pollen mother cell stage (PMC, Figs. 30, 37a). Afterward, the proximal floret delays pollen development at the PMC stage, while the distal floret continues pollen development up to the 1-celled stage (Figs. 32–34, 37b). After that, pollen development in the distal floret is temporary delayed at 1-celled stage, while pollen development restarts in the proximal floret reaching the 1-celled stage (Figs. 35, 36, 37c). Fi-



Figs. 23–28. Scanning electron micrographs of spikelet and floral development in *Urochloa plantaginea*. **23.** Floral meristem and differentiation of glumes and lemmas. **24.** Palea and stamen initiation. **25.** Glume elongation and differences in the orientation of the lower glume between the terminal spikelet of the primary branches and the spikelets on secondary branches. **26.** Proximal hermaphroditic primordium. **27.** Differences in development between the proximal and distal floret of the spikelet. **28.** Differentiation of anthers and gynoecium in the proximal floret. Bar = 100 μm.

nally, pollen in the distal floret restarts its development, reaching the 3-celled stage even before the pollen of the proximal floret. At anthesis, the distal floret opens before the proximal one (Fig. 37d, e).

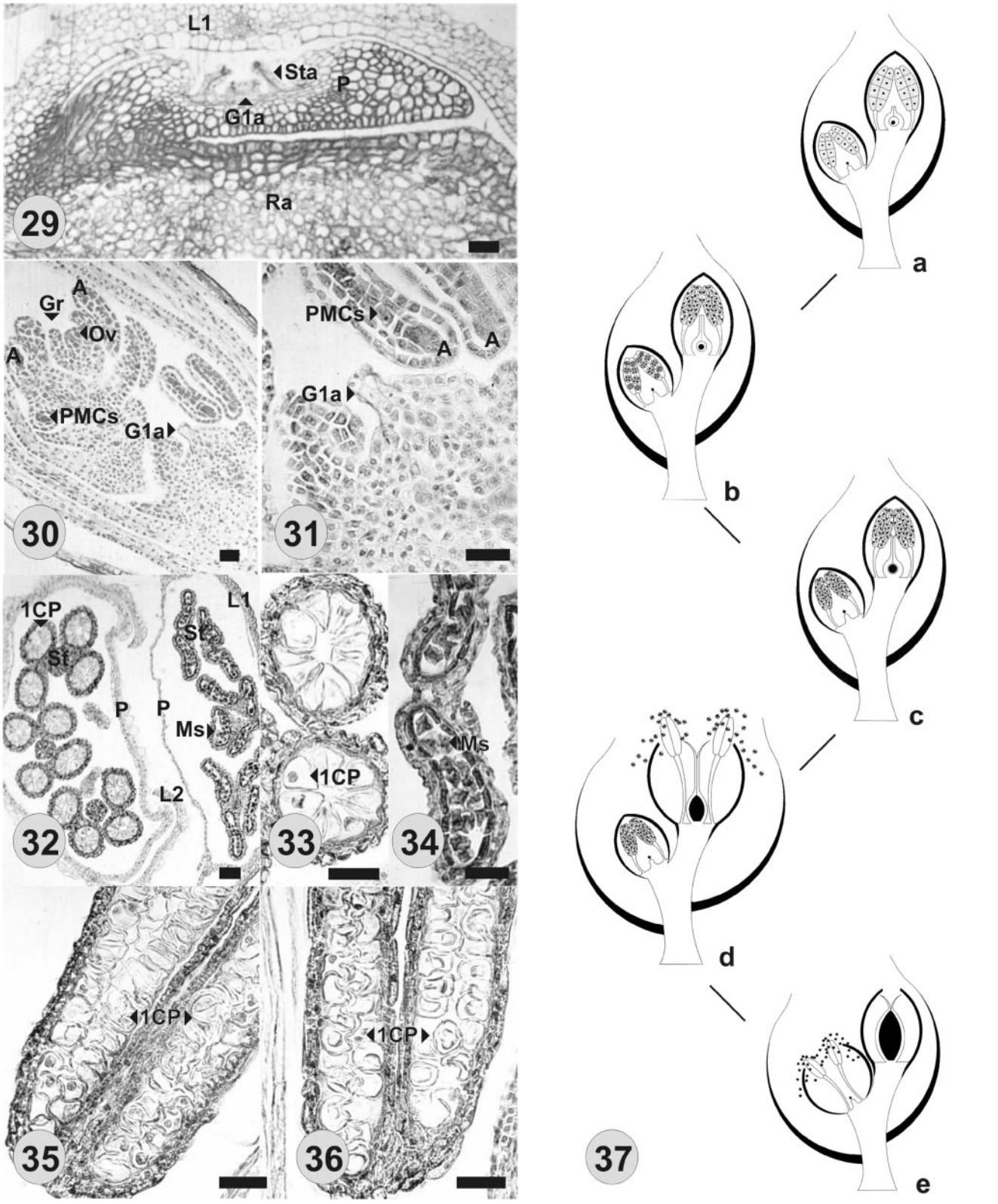
When pollen in the proximal and distal floret of *P. maximum* is at the PMC stage, the gynoecium of the proximal floret ceases development (Fig. 30). A reconstruction of the arrested gynoecium based on longitudinal seriate sections (15 florets) shows cellular death, involving both epidermal and subepidermal cells, in a subapical, transversal plate one cell thick. Cells of this plate gradually lose their nuclei and cytoplasm (Fig. 31). Only the cell walls remain intact (Fig. 31). In contrast, during distal floret development, all cells of the gynoecium maintain the integrity of their cytoplasm and nucleus (Fig. 30).

A reconstruction based on transverse serial sections of the proximal sterile anthecium of *U. plantaginea* (15 florets) shows cells of the stamen and the gynoecium totally collapsed, without inner structure (Fig. 29).

DISCUSSION

Changes in the shoot apex related to flowering transition—The transition to flowering in shoots of *Panicum maximum* and *Urochloa plantaginea* involves the same meristem elongation observed in other members of the Poaceae family (Stür, 1986; Fraser and Kokko, 1993; Orr and Sundberg, 1994; Sundberg et al., 1995; Sundberg and Orr, 1996; Doust and Kellogg, 2002). The change of phyllotaxis in *P. maximum* correlates with the increase in diameter of the apical meristem and the number of orthostichies. (cf. Sundberg and Orr, 1996).

Branch system development—Development of the branch system of *P. maximum* and *U. plantaginea* differs with respect to the growth of the main axis, number and disposition of primary branch primordia, direction of branch differentiation in the whole inflorescence and on branches from which they are originated, and the degree of ramification and disposition of secondary branch on the primary branch. These develop-



Figs. 29–37. Light micrographs of longitudinal and transversal sections of spikelets during floral development stained with safranin, fast green, and Mayer's haematoxylin. **29.** Transversal section of the proximal floret in *Urochloa plantaginea*. **30.** Longitudinal section of the spikelet of *Panicum maximum* showing the aborted gynoecium of the proximal floret and anthers of both proximal and distal floret at pollen mother cell stage. **31.** Aborted gynoecium of the proximal floret in *P. maximum*. **32.** Transversal section of the spikelet of *P. maximum* in which the proximal floret (left) is delayed at the pollen mother cell stage, while the distal floret (right) shows tetrads or microspores. **33.** Microsporangia of the distal floret at 1-celled pollen stage. **34.** The same section as Fig. 33 showing microsporangia of the proximal floret at tetrad-microspore stage. **35, 36.** Longitudinal sections of anthers of *P. maximum* when both florets are at the 1-celled pollen stage. **35.** Distal floret. **36.** Proximal floret. **37.** Diagrams showing pollen maturation in proximal and distal floret in *P. maximum*. (a) Anthers of both

mental differences produce the two different mature morphologies described by Zuloaga (1989), Zuloaga and Morrone (1995), Morrone and Zuloaga (1992), and Reinheimer and Vegetti (unpublished data, National University of Litoral) (Figs. 1–4). However, there are some additional differences in the development of the branching system of *P. maximum* and *U. plantaginea* that cannot be seen in mature inflorescences: (1) the origin of primary branches and development of the main axis, and (2) the direction of the differentiation of branch primordia.

Primary branches in the inflorescence of *P. maximum* develop from ''regular'' buds derived from the apical meristem. The main axis of the inflorescence is the result of contiguous, elongated internodes also produced by the apical meristem, which finally differentiates a terminal spikelet. In *U. plantaginea* the apical meristem of the main axis only produces the first primary branch. After the arising of the second primary branch, the apical meristem of the main axis is laterally displaced, remaining inactive and not producing any further structure. The main axis of *U. plantaginea*'s inflorescence develops by intercalary growth of the internode below the first formed primary branch primordium and the last vegetative leaf. The main rachis never ends in a spikelet. We infer that in *U. plantaginea* the origin of the second and following primary branches are from meristems that form de novo along this elongating internode. These meristems are similar to the adventitious buds described by Rauh (1937). There are three features supporting the adventitious character of these kinds of meristems (cf. Rauh, 1937): (1) they arise on an elongating internode, (2) they arise basipetaly, and (3) the correlation between the development of meristems that form de novo and the incomplete development of the main axis. *Urochloa decumbens* has a similar inflorescence structure as *U. plantaginea*, and its main axis also shows the same kind of intercalary growth with adventitious buds (Stür, 1986). Although adventitious buds are well known in other angiosperm families (Rauh, 1937), these two species of *Urochloa* are the only records of adventitious buds in inflorescences of Poaceae.

Concerning homologies, the main axis of *U. plantaginea*'s inflorescence is homologous to the first basal internode in the main axis of *P. maximum*'s inflorescence. The most distal primary branch of *U. plantaginea* is homologous to the first proximal primary branch in *P. maximum*. The second and following primary branches (in basipetal order) of *U. plantaginea* are not homologous to the first most distal primary branch in the same inflorescence, nor are they homologous to any structures in the inflorescence of *P. maximum*.

Regarding the direction of initiation of branch primordia, *P. maximum* shows acropetal initiation at every branch degree, both in the inflorescence as a whole as well as along each level of branching (for instance: secondary branches are acropetal on each primary branch and also along the whole inflorescence). Initiation of the primary branches of *U. plantaginea* is basipetal and amphipetal in the secondary ones along the primary branches, but the initiation of the secondary branches is basipetal when considering the entire inflorescence (the secondary branches appear first on the distal primary branch).

Spikelet development—The degree of the branches on which spikelets arise differs between both species: in *P. maximum* spikelets are initiated on first-, second-, third-, fourth- or fifth-order branches, while in *U. plantaginea* spikelets are differentiated on primary, secondary, or less frequently, tertiary branches. In both species, the direction of spikelet differentiation is basipetal, both along the whole inflorescence, and also in the branches on which they arise. This basipetal differentiation of spikelets implies that the branch maturity needed to form spikelets is not related to the timing of differentiation of branches. In *P. maximum*, the last formed branches (whatever degree of ramification) are the first to produce spikelets. In *U. plantaginea*, the amphipetal differentiation of secondary branches does not correlate with the basipetal differentiation of spikelets on the secondary branches of the same primary branch. Besides, the differentiation of spikelets is basipetal along the inflorescence (it begins in the older, apical primary branch). In contrast, Stür (1986) reported an amphipetal differentiation of spikelets in *Urochloa decumbens* (sub *Brachiaria decumbens*), and also amphipetal differentiation of secondary branches.

Floral development—Although the distal floret of *P. maximum* and *U. plantaginea* has the same growing pattern, three related differences can be observed between both species in the development and sex expression of the proximal floret: (1) developmental changes determining sex expression, (2) the different size of the floret meristem, and (3) the timing of elongation of the glumes.

Panicum maximum has bifloral spikelets in which the proximal floret is male and the distal one is hermaphroditic (Zuloaga and Morrone, 1995). Both florets start their development as hermaphroditic primordial, but only the distal ripens as hermaphroditic. The arrest of the gynoecium primordium in the proximal floret determines the formation of a male proximal floret. This phenomenon was also observed in some members of the tribe Andropogoneae, as in *Zea mays* L. (Sundberg and Orr, 1996), *Heteropogon contortus* (L.) P. Beauv. ex Roem and Schult. (LeRoux and Kellogg, 1999), *Tripsacum dactyloides* L. (Orr et al., 2001), and in one species of the tribe Paniceae, *Panicum repens* L. (LeRoux and Kellogg, 1999). Concerning the floral development of Andropogoneae, LeRoux and Kellogg (1999) concluded that cell death in a subepidermal core of the gynoecium primordium leads to the arrest of gynoecium growth and the formation of a male floret; they hypothesized that this mechanism of sex expression may be shared among the subfamily Panicoideae (LeRoux and Kellogg, 1999). The arrested gynoecium in *P. maximum* is new evidence for that hypothesis. However, there are differences in the pattern of cell death in the arrested gynoecium of the male floret between *Panicum* and members of Andropogoneae. While in Andropogoneae, cell death occurs in a core of subepidermal cells of

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Figs. 29–37. Continued. florets at the pollen mother cell stage and the proximal floret with aborting gynoecium. (b) Anthers of the distal floret develop up to the 1-celled pollen stage, while anthers of the proximal floret remain at the pollen mother cell or tetrad-microspore stage. (c) Anthers of the proximal floret reach the 1-celled pollen stage, while anthers of the distal floret are still at the 1-celled pollen stage. (d) Pollen maturation (3-celled stage) and anthesis occur first at the distal hermaphroditic floret. (e) Pollen maturation and anthesis of the proximal floret occur while the caryopsis of the distal floret begins its development. The asterisk represents the aborted gynoecium. Bar = 100 μm.

the gynoecium primordium, in *P. repens* dead cells appear in an epidermal ring at the base of the gynoecium primordium, and in *P. maximum*, death of both epidermal and subepidermal cells occurs in a subapical, transverse plate one cell thick. In *P. maximum*, dead cells also retain their cell walls, as has been reported for the Andropogoneae and *P. repens* (LeRoux and Kellogg, 1999), but cell death occurs earlier in *P. maximum* than in *P. repens* (before the gynoecial ridges appear) and the Andropogoneae. Therefore, not only does the pattern of cell death vary in location, but it also varies in timing, which is not as subtle as LeRoux and Kellogg (1999) suggested.

Species of *Urochloa* have bifloral spikelets in which the distal floret is always hermaphroditic and the proximal one can be male or neutral (Morrone and Zuloaga, 1992). *Urochloa plantaginea* is an example of the last case. Both florets develop as hermaphroditic primordia, but when the thecae are clearly differentiated and the gynoecium ridge is just arising, the proximal floret ceases its growth resulting in a sterile anthecium. This pattern of development of the proximal floret could be shared by other species of *Urochloa*, although it is not common to other members of the PCK clade (*Eriochloa montevidensis*, R. Reinheimer, unpublished data).

The proximal and distal floret in *P. maximum* clearly differ not only in gynoecium development (because the proximal gynoecium aborts), but they also differ subtly in pollen development. Six stages of compared pollen development between the proximal and distal floret can be distinguished: (1) anthers of both florets begin to develop at the same time until the pollen mother cell (PMC) forms; (2) anthers of the proximal floret arrest their development at the PMC, while anthers in the distal floret undergo meiosis and reach the 1-celled pollen stage; (3) anthers of the proximal floret restart development, undergo meiosis, and reach the 1-celled pollen stage, while the pollen in the distal floret is arrested at the 1-celled stage; (4) pollen in the distal floret undergoes mitosis and reaches the 3-cell stage earlier than the proximal floret; (5) anthesis takes place first in the distal floret of the spikelet; (6) finally, anthesis occurs in the proximal floret. The arrest of pollen development at the PMC in the proximal floret (stage 2) is simultaneous with the abortion of the gynoecium primordium, suggesting a relationship between these two developmental events. Perhaps the genetic control that aborts the gynoecium primordium is also involved in the general delay of floret development (particularly anther and pollen development) and is related to the basipetal maturation of the spikelet's florets.

In maize, Irish and Nelson (1993) found that stamens and gynoecia with regular development are larger than those that will be aborted. Le Roux and Kellogg (1999) did not find this size difference in floral organs of Andropogoneae. Although observations in *P. maximum* and *U. plantaginea* agree with those of Le Roux and Kellogg (1999), there is a relationship between floral meristem size and sex expression in florets in both species. In *P. maximum*, the proximal meristem (male floret) is about 50% smaller than the distal meristem (hermaphroditic floret). In *U. plantaginea*, the difference in size between the proximal and distal meristems is even larger than in *P. maximum*, the meristem of the aborted, proximal floret being less than the 30% the size of the distal one. Therefore, sex expression of florets seems to be already determined as early as the differentiation of floret meristems.

Irish and Nelson (1993) and Irish et al. (1994), studying the floral development in *Z. mays*, related timing of the elongation of the glume with sex expression of the florets. In the maize tassel, glumes elongate and envelop florets when floral organ primordia differentiate, before abortion of the gynoecium. In the ear, florets are enclosed by glumes after abortion of the stamens. These authors suggested that sex determination genes in maize, and possibly in some Andropogoneae, as suggested by LeRoux and Kellogg (1999), influence elongation of the glumes. The elongation of glumes in *P. maximum* and *U. plantaginea* agrees with the hypothesis of Le Roux and Kellogg (1999): the fact that glumes cover the distal floret earlier in *U. plantaginea* than they do in *P. maximum* correlates with the earlier sex expression in *U. plantaginea* (particularly in the proximal floret).

Taxonomical consequences—Brown (1977) excluded *P. maximum* from *Panicum* and mentioned the convenience of transferring this species to the genus *Brachiaria* because of the presence of the phosphoenol pyruvate carboxykinase (PCK) subtype of C_4 photosyntesis and rugose upper anthecium. Later, Webster (1987) suggested that *P. maximum* should be transferred to the genus *Urochloa* due to a similar upper anthecium orientation and photosynthetic subtype. *Panicum maximum* was related, in the phylogenetic analyses of Zuloaga et al. (2000), Giussani et al. (2001), and Aliscioni et al. (2003) to the *Urochloa* clade. Consequently, these authors treated the species as *Urochloa maxima*, following Webster's concept (1987). Recently, Simon and Jacobs (2003) questioned the transfer of *P. maximum* to *Urochloa*, mainly because of the difference in the degree of branching of the inflorescence and because the latest cladistic analyses of Paniceae (Zuloaga et al., 2000; Giussani et al., 2001; Aliscioni et al., 2003) support more the segregation of *P. maximum* from *Panicum* than its inclusion in *Urochloa*. Therefore, these authors considered subgenus *Megathyrsus* Pilger at a generic level, including two species: *M. maximus* (= *Panicum maximum*) and *M. infestus* (= *P. infestus*). Our new findings on inflorescence development in *P. maximum* and *U. plantaginea* also support segregation of *P. maximum* from *Urochloa*, not only for the higher degree of branching, as Simon and Jacobs (2003) stressed, but also due to the different pattern of initiation of primary inflorescence branches, direction of branch differentiation, and phyllotaxis (Table 1)—differences that establish a gap between both developmental patterns. We also suspect that the lack of monophyly of *Urochloa* (Guissani et al., 2001) could be supported also by developmental features of the inflorescence. The structural differences in the inflorescence between the group *U. mutica*-*U. plantaginea* and *U. acuminata* (R. Reinheimer et al., unpublished data), could involve not only minor changes of phyllotaxis and number of orthostichies, but also a deeper change in the pattern of development of the main axis and primary branches, as the one observed between *P. maximum* and *U. plantaginea*.

Among the developmental features analyzed here the following ones cannot be discerned in mature inflorescences and could be potential sources of new morphological characteristics to be used in future cladistic analysis: (1) the direction of branch differentiation on both the entire inflorescence and each branching level; (2) development of adventitious buds; (3) primary branch initiation (apical vs. intercalary elongation); (4) direction of spikelet differentiation on both the entire inflorescence and each branching level; (5) direction of glume, lemma, and palea initiation; (6) position of the lower glume (in some cases); (7) size of the floret meristem; (8) pattern of distal floret development; (9) pattern of gynoecium abortion; and

(10) differential pollen development between proximal and distal floret; (11) glume elongation. *Panicum maximum* and *U. plantaginea* share developmental features 4, 5, and 8, and differ by characters 1, 2, 3, 6, 7, 9, 10, and 11. Among the developmental events related to floret sex expression, some seem to precede sex expression (as size of floret meristem), some seem to be simultaneous with sex expression (as gynoecium abortion and pollen development delay), and some others seem to follow determination of sex (as glume elongation and basipetal floret maturation at anthesis).

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