

Comparison of Upper Floret Development in Bisexual and Male Spikelets of *Thuarea involuta* (Gramineae) with Scanning Electron Microscopy

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ABSTRACT: The inflorescences of *Thuarea involuta* (Forst.) R. Br. ex R. & S. are composed of 3-7 spikelets in a spatheate spike. The spikelets have two florets. All of them are male, except that the basal spikelet has a bisexual upper floret and a male lower floret. A comparative study on the upper florets of the bisexual and male spikelet by SEM showed that the arrest of the development of gynoceium causes the maleness of the upper florets.

KEY WORDS: Development, SEM, Spikelet, *Thuarea involuta*, Gramineae.

INTRODUCTION

Greyson (1994) has discussed four important crops as model research subjects and found that the developments of the inflorescences and flowers of the large and variable family Gramineae (or Poaceae) have been insufficiently studied. Although the information about the development of flowers and inflorescence of other grasses is available (Sachs, 1972; Clifford, 1987 and Watson, 1990), such reports are scarce, especially when dealing with the development of sex expression. Some subject of sex determination has been summarized previously (Irish and Nelson, 1989; Dellaporta and Calderon-Urrea, 1993). Of the monocots, maize (*Zea mays* L.) is the best-studied species (Cheng *et al.*, 1983; Bommineni and Greyson, 1987, 1990; Irish, 1993; Veit *et al.*, 1993; Irish *et al.*, 1994; Dellaporta and Calderon-Urrea, 1993, 1994). *Elaeis guineensis* Jacq. (Van Heel *et al.* 1987), *Asparagus officinalis* L. (Lazarte and Palser, 1979; Caporali, *et al.*, 1994) and *Buchloe dactyloides* (Yin and Quinn, 1995) have also been studied. A few authors studied the dicotyledonous species (Durand and Durand, 1991; Tucker, 1992; Sherry *et al.* 1993; Yonemor *et al.*, 1993; Diggle, 1994; Hormaza and Polito, 1996 and Maier *et al.*, 1997), especially the species *Silene latifolia* (Hardenack *et al.* 1994; Grant *et al.*, 1994; Farbos *et al.* 1997).

The species *Thuarea involuta* is a widespread maritime grass, adapted with the false-fruit for dispersal by sea-water (Nieuwenhuis-UexkÜll, 1902), and grows on sandbeaches and coral gravel in Taiwan (Hsu, 1978). It is an excellent sand-binder and a good fodder

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(Gilliland, 1971; Lazarides, 1980). It is physically small and prostrate, growing rapidly, not requiring large space and special care in its culture (Figs. 1A, B). In addition, it has a relatively long flowering time (May to December in Taiwan), hence can be a potentially attractive model system for studying wild-grass flower development and sex expression. In this preliminary study, we have studied the development of bisexual and male spikelets in different parts of the same inflorescence by using scanning electron microscopy.

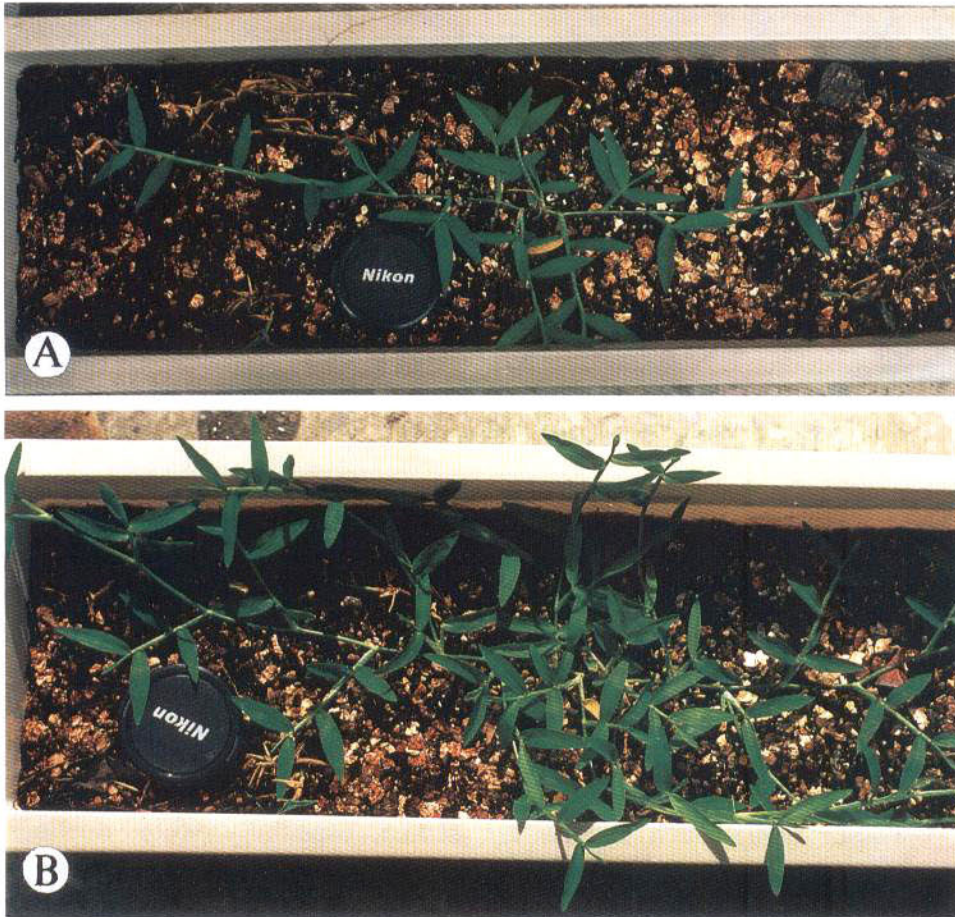


Fig. 1. The culture of *Thuarea involuta*. A. One individual plant cultivated in pot, note that the many branched and creeping stem. B. The same plant rapidly propagates after two weeks.

MATERIALS AND METHODS

Plants of *Thuarea involuta* were collected from Yenliao Park and Kenting National Park and cultivated in the green houses of the department of Biology, National Chen-Kung University. Inflorescences of different sizes were fixed in FAA (Formalin: acetic acid: 50 % ethanol=1:1:18). After fixation for 24 hours they were twice washed with 50 % ethanol with a 20 minute interval, and then stored in ethanol 70% for later study. For the SEM the material was dissected, the desired parts were selected, dehydrated in an alcohol and acetone series, dried to the critical point, and coated with platinum. The specimens were studied and photographed at 25 kV with a Hitachi S-2500 SM scanning electron microscope.

RESULTS

The inflorescence of *Thuarea involuta* is characterized by having a spathe enclosing a spike with a basal bisexual spikelet and 2-6 male spikelets above it (Figs. 3D, E). All spikelets are 2-flowered (Fig. 2). All florets are male (Figs. 2, 3A), except the upper floret of the basal spikelet is bisexual (Figs. 3B, 6B).

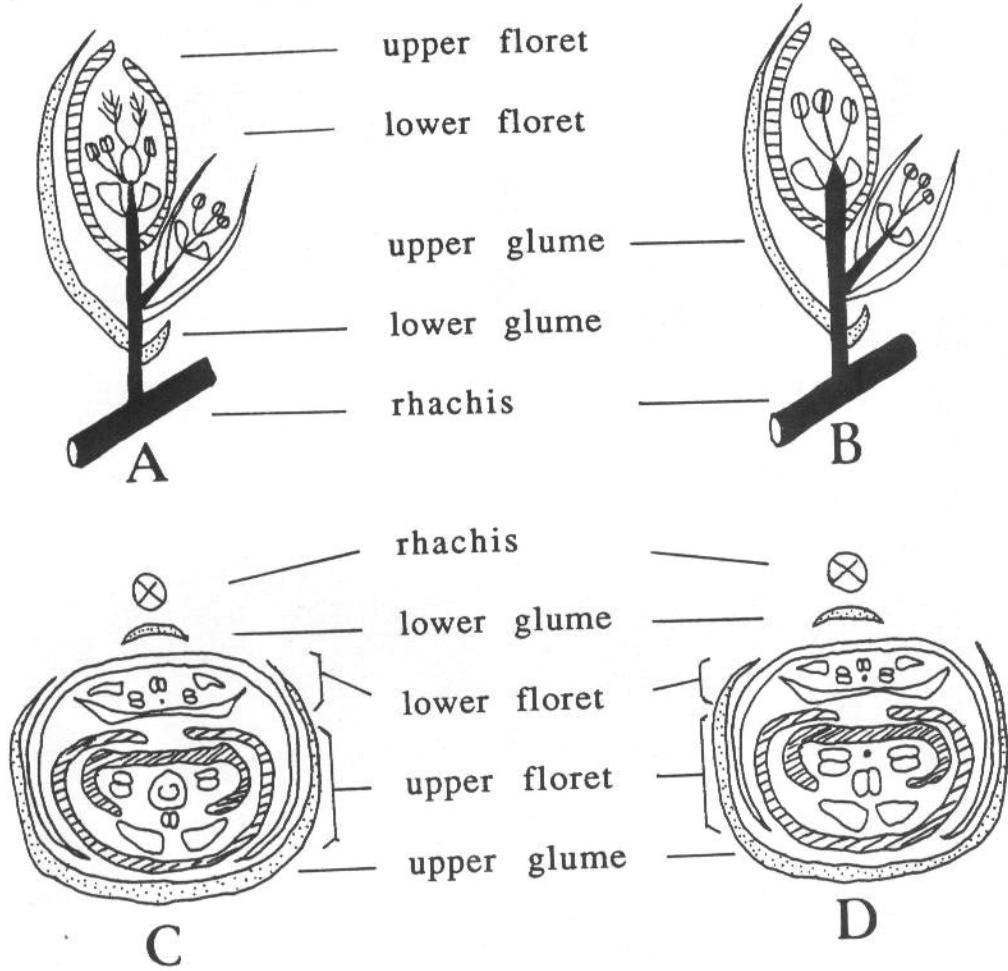


Fig. 2. Diagram of florets in spikelets of *Thuarea involuta*. A. Longitudinal section of bisexual spikelet. B. Longitudinal section of male spikelet. C. Cross section of bisexual spikelet. D. Cross section of male spikelet.

At the vegetative phase, the shoot apex enclosed by two opposite leaf primordia (Fig. 4A). As the distichous pattern of leaf initiation proceeds, the apical meristem of the shoot enlarges in both width and height, while at the reproductive phase the transition apex elongates rapidly (Fig. 4B). From the base of this inflorescence meristem a row of 4-6 primordia of spikelets is acropetally initiated (Figs. 4C-F).

Each spikelet primordium initiates two glumes and two anthoecia (Fig. 4F). The lower anthoecium develops more slowly than the upper one. In an early stage two lodicules, three stamens and a gynoecium are initiated in the upper floret of both spikelet types (Figs. 5A-D). When the stamens are well-developed and differentiated into filament and anther, the further development in the male and bisexual spikelet becomes different (Fig. 5E). The stamens are

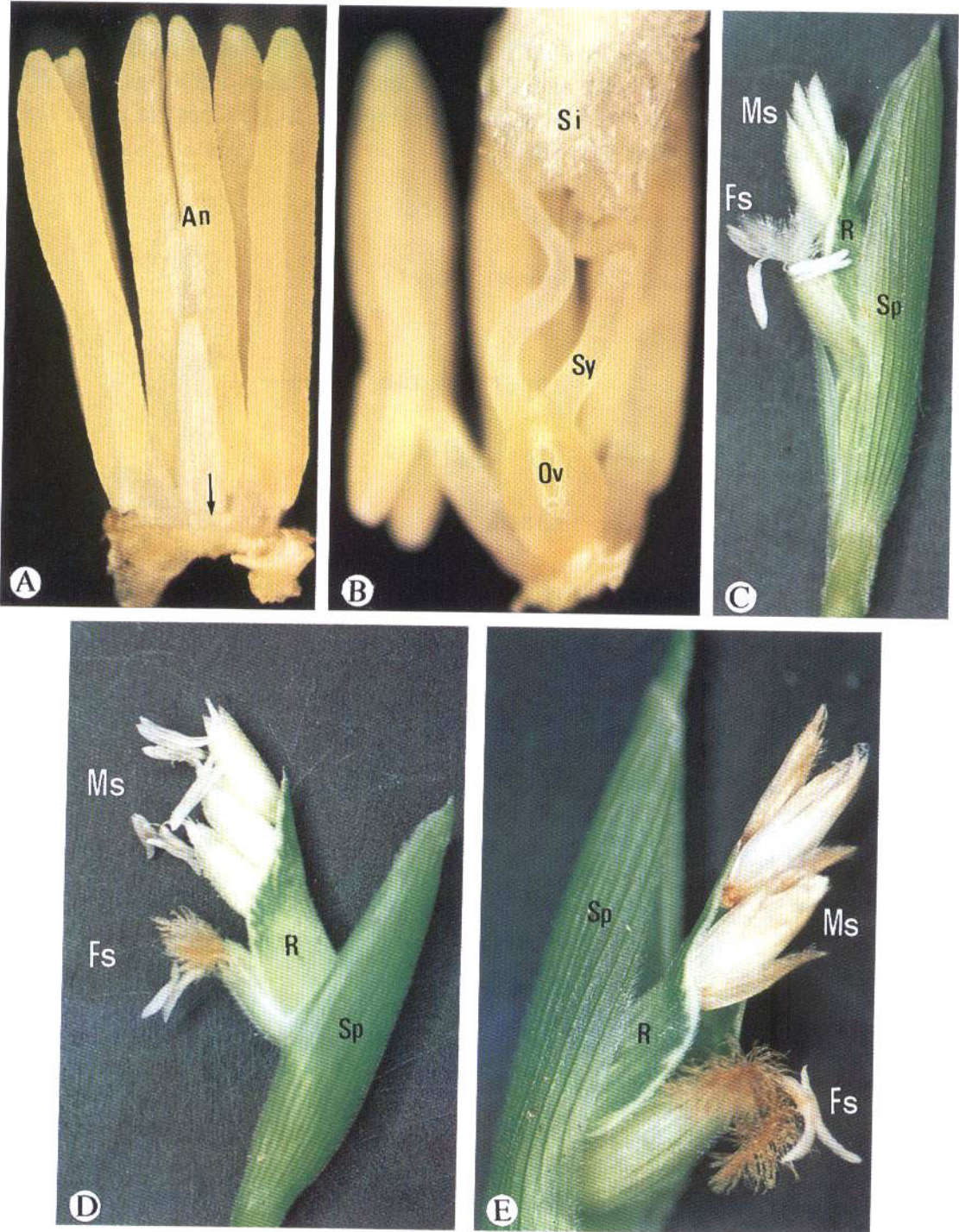


Fig. 3. The flowers, spikelets and the inflorescence of *Thuarea involuta*. A. Flower of upper floret of male spikelet, showing three stamen (An) and vestige of the gynoecium (arrow). B. Flower of upper floret of bisexual spikelet, showing the stamens and the gynoecium with ovary (Ov), style (Sy) and stigma (Si). C. the andromonoecious inflorescence contain two kinds of spikelets on a rhachis (R) in a spathe (Sp), the bisexual spikelet (Fs) at the base of rhachis and the male spikelets (Ms) above it, note that the bisexual spikelet opening first. D. the inflorescence at anthesis of all flowers. E. The inflorescence after anthesis, note the enlarged bisexual spikelet and the wilting male spikelets.

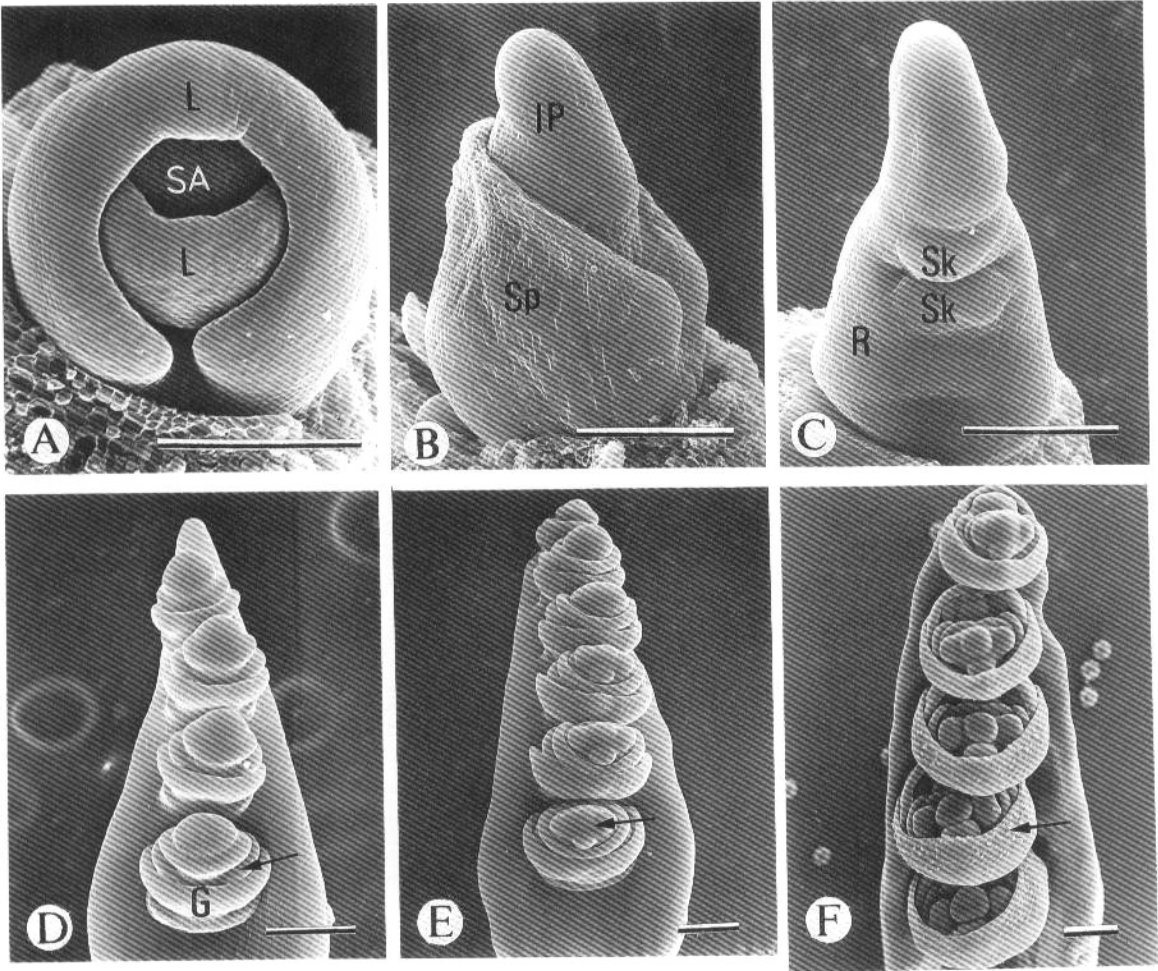


Fig. 4. SEM views of the initiation and development of inflorescence and flower of *Thuarea involuta*. (bar=100 μ m). A. The vegetative phase of the shoot apex (SA) enclosed by two opposite leaf primordia (L). B. The productive phase of the shoot apex elongated as the inflorescence primordium (IP), note the enveloping spathe (Sp). C. Spikelet primordia (Sk) acropetally initiated on one side of the rhachis (R). D. Glume (G) and lemma (arrow) initiation first appear at lower spikelet. E. Upper floret initiation (arrow) begins near the lower spikelet. F. Floret developments, note that some upper floret (arrow) is larger than the lower one.

slightly smaller in the bisexual spikelet than that in the male spikelet (Fig. 5F). The gynoecial ridge overgrows the flower apex leaving a small stylar canal (Fig. 5F), and continues to form the ovary, style, and stigma (Fig. 6A). Contrary the development of the gynoecium in the male spikelet stops, the stamens grow and mature (Figs. 5F, 6B). At anthesis the stamens of the upper floret in both spikelet types are nearly with the same shape and size (Figs. 6A, B).

DISCUSSION

According to the modes of sexuality of flowering plants (Dellaporta and Calderon-Urrea, 1993) and the descriptions in several Floras (Gilliland, 1971; Hsu, 1978; Lazarides, 1980; Koyama, 1987), *Thuarea involuta* could be described as a monoecious or andromonoecious

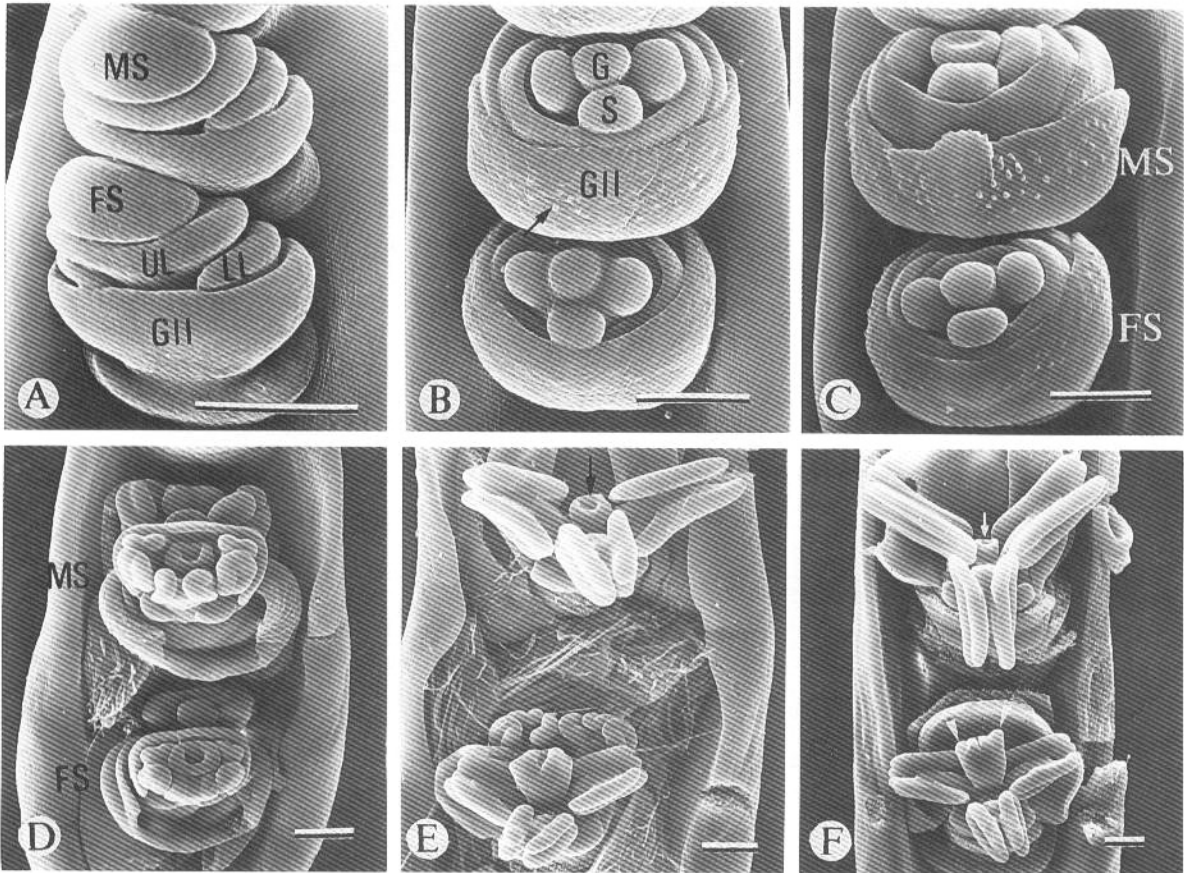


Fig. 5. SEM views of bisexual and male spikelet of *Thuarea involuta* for comparing the development of upper floret (bar=100 μ m). A. Glume (GII) and lemma (UL, LL) initiation of bisexual spikelet (FS) and male (MS) spikelet. B. Upper floret initiates in each spikelet with stamen primordia (S) and gynoecium primordium (G), note that the hair primordia (arrow) first appear on upper glume (GII) of male spikelet. C. The male spikelet (MS) precedes the bisexual spikelet (FS). D. Bisexual and male spikelet development, note that the male (MS) one is slightly larger than the bisexual one (FS). E. Upper floret development, note the termination of gynoecium (arrow) of male spikelet. F. Upper floret development, note the continually growth of the gynoecium of bisexual spikelet (arrowhead), and the arrested gynoecium (arrow) of male spikelet.

grass, when the upper floret of the basal spikelet is female or bisexual. Three features of unisexuality included in grass breeding system features were distinguished by Watson (1990): only unisexual florets, monoecious, and dioecious. *Thuarea* was cited in category "only unisexual florets" by Watson (1990), but our observations (Figs. 3A-B, 6B) do not match any one definition of his system. We prefer to follow the system of Dellaporta and Calderon-Urrea (1993) for clarity, and *Thuarea involuta* in Taiwan should be an andromonoecious grass.

The events occurring during inflorescence and flower development of *Thuarea involuta* are summarized in Table 1., adapted the stage system from Latting (1972). The crucial moment in sex differentiation is in stage 7 (Table 1, Fig. 5E), when the anthers are fully lobed and the stamens are differentiated.

Two major types of development of unisexuality in flowering plants are distinguishable based on whether or not the unisexuality is caused by the reduction or abortion of sex organ

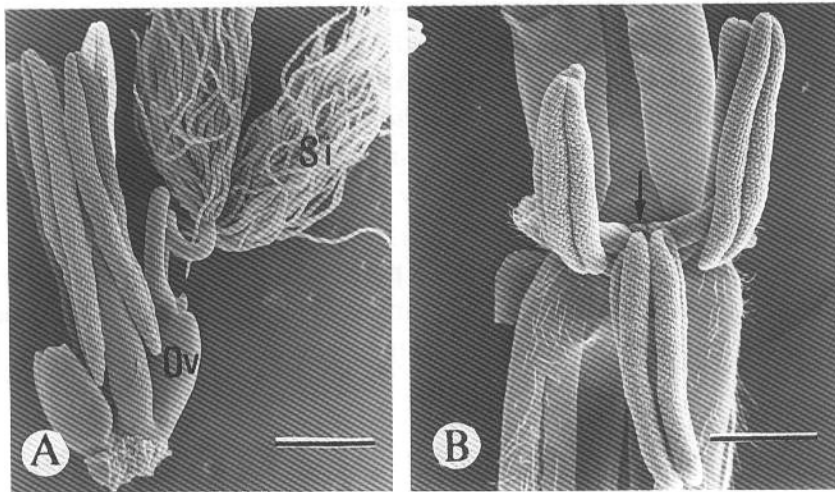


Fig. 6. SEM views of florets of bisexual and male spikelet of *Thuarea involuta*. A. Upper floret of bisexual spikelet before anthesis, note plumose stigma (Si), ovary (Ov) and cuticle on anther surface (bar=500 μ m). B. Face view of male upper floret of male spikelet, with the arrested pistil (arrow) at the center (bar=500 μ m).

Table 1. Stage system for inflorescence development of *Thuarea involuta* (condensed and adapted partially from Latting, 1972).

Stage of development	Stage index	Fig(s).
Vegetative phase	0	4A
Elongation: predouble ridge	1	4B
Double ridge: first morphological change	2	4C
Spikelet primordia	3	
Differentiation of spikelet primordia as shown by appearance of glume primordia and budding of spikelet apex	4	4D
Differentiation of florets, indicated by appearance of lemma primordia and floret apices	5	4D 4E
Differentiation of stamens, from first budding to lobed anthers; palea and lodicule primordia appear at this stage	6	4E 4F 5D
Glumes half enclosing spikelet, anthers fully lobed, ovaries in-folding	7	5E

primordia. The common type involves the arrest of performed sexual organs in bisexual primordia, while the others lack any vestiges of organs of opposite sex (Dellaporta and Caldero-Urrea, 1993; Caporali *et al.* 1994). Despite the quite difference in inflorescence pattern and unisexuality, however, sexual development of male florets by gynoeical abortion in spikelets is similar in *Thuarea involuta*, *Zea mays* and *Spinifex littoreus* (Kuoh *et al.*, unpublished data).

Watson, 1990; Grant *et al.*, 1994). However, there is a notable tendency towards increasing the dominance of male structures in Panicoideae, whose spikelets usually have male to sterile lower florets (Clayton and Renvoize, 1986). All florets of the inflorescences of *Thuarea involuta* are staminate, only a pistil is found in the upper floret of the lowermost spikelet (Fig. 3D). After fertilization, the upper (male) part of rachis bends over after male spikelets have wilted. (Fig. 3E; Lazarides, 1980). It is probably an advantage of this sex arrangement to the formation of the capsule.

We believe that *Thuarea involuta* can be a potentially attractive model system to study the regulation of sex expression, there by proving the hypotheses regarding the mechanisms of sex determination, and to examine the influence of andromonoecism on the false-fruit formation.

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芻蓄草兩性及雄性小穗的上位小花發育之比較

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摘 要

芻蓄草花序由 3-7 個小穗組成，於一佛焰苞內排成穗狀。除了基部之一小穗為兩性的上位小花和雄性的下位小花組成之外，所有小穗之兩小花均為雄性。經由掃描式電子顯微鏡技術比較各小穗之上位小花，結果顯示雌蕊發育受阻是造成上位小花雄性之主因。

關鍵詞：掃描式電子顯微鏡，小穗，發育，芻蓄草，禾本科。