

Reproductive Morphological Changes in Ruzi Grass (*Brachiaria ruziziensis* Germain and Everard).

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ABSTRACT

The morphological changes occurring during reproductive development in Ruzi grass (*Brachiaria ruziziensis* Germain and Everard), from initiation to emergence of the inflorescence from the flag leaf are described and presented in a series of photographs taken using Scanning Electron Microscopy (SEM). Five stages are described, with stages 3 and 4 being divided into two substages representing "early" and "advanced" development *ie.* (1) vegetative stage, (2) early raceme initiation and "double ridge" stage, (3a) early floret initiation stage, (3b) advanced floret initiation stage, (4a) early floret differentiation stage, (4b) advanced floret differentiation stage and (5) inflorescence exertion stage. The time from floral initiation to first raceme emergence above the flag leaf (stages 2-5) was approximately 22 days.

Additional index words: morphological changes, Scanning Electron Microscopy (SEM), growth stages, floret initiation, floret differentiation.

INTRODUCTION

Seed production is the culmination of a number of development phases which are influenced largely by daylength and temperature (Humphreys and Riveros, 1986). According to Cooper (1960) the initiation and development of grass inflorescences can be divided into three stages *viz.* (1) floral induction, during which apical meristems become capable of responding to flowering stimuli, (2) floral initiation, in which visible changes in the inflorescence occur in response to environmental conditions, and (3) floral development of the meristem tissue with accompanying elongation of the stem internodes.

A flowering stimulus arises in the leaves of both short-day plants and long-day plants under favourable daylength conditions, and is transported to the meristems where it causes a vegetative apex to become reproductive (Wareing and Phillips, 1986). The transition from a vegetative to a floral apex is marked by an increase in length, a cessation of leaf primordia initiation, and the development of floral primordia in the leaf axils.

In many grasses, the visible onset of this reproductive stage is usually determined by using the "double ridge" identification. These so-called "double ridges" arise from a double structure composed of leaf and bud primordia which grows rapidly. However, in physiological terms, this event reflects a complete change in the relationship of leaf and bud. Whereas in the vegetative condition the bud remains dormant, often for prolonged periods, the reverse takes place in the reproductive stage. The development of leaves is inhibited and the reproductive buds develop further (Langer, 1972). The formation of seeds follows the differentiation of inflorescences on the terminal apices of the shoots or tillers, which formerly budded off leaf initials.

In addition, axillary buds located within the leaf bases expand and provide a further reservoir of sites for inflorescence differentiation.

Morphological changes in the apex of temperate grasses, and development and visible changes occurring during reproductive development, have been studied by a number of workers; for example, Sharman (1947) with *Agropyron* spp; Evans and Grover (1940) with perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.) and cocksfoot (*Dactylis glomerata* L.); and Hill (1971) with ryegrass, timothy and prairie grass (*Bromus willdenowii* Kunth). By comparison, only limited studies of these changes have been made in tropical grasses, possibly the best of which is by Humphreys and Riveros (1986), who described six stages of morphological change in *Brachiaria decumbens* Stapf: *viz.* stage 1 : Vegetative, stage 2 : Transition, stage 3 : Raceme Initiation (RI), stage 4 : Spikelet Initiation (SI), stage 5 : Spikelet Differentiation (SD), stage 6 : Inflorescence Exertion (IE).

The objective of the present study was to describe the morphological changes occurring from vegetative to reproductive development and to follow the sequence of development until the inflorescence emerged from the flag leaf of Ruzi grass (*Brachiaria ruziziensis* Germain and Everard), a tropical species utilised widely in many tropical countries.

MATERIALS AND METHODS

Ruzi grass seeds obtained from Thailand were germinated using the top of paper method (ISTA, 1996) in a controlled environment cabinet which provided a temperature of 30°/20° (day/night), 70% relative humidity and 12 hours of light. After 10 days, 50 germinated

seedlings were then transplanted into individual plastic pots (15 cm diameter and 30 cm deep) containing a uniform potting mixture (ponga fibre, pumice, 3-4 months osmicote plus N:P:K - 15:10.8:10.8 and trace elements) and grown under controlled temperature conditions of $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in a glasshouse at the Seed Technology Centre, Massey University, New Zealand (latitude 40°S , longitude 170°E), under natural decreasing daylength (14.5-10.5 h) from 10 January to 10 April, 1993.

Thirty days after transplanting the seedlings the first basal tillers of five individual plants were removed at ground level for dissection. The leaves and leaf sheaths were removed, using a razor blade and needle and working under a binocular microscope to facilitate the operation. The investigation started from the top node to the lowest node in each tiller.

All samples of apices dissected were kept in FAA solution (Formalin 5%, Acetic acid 5% and Alcohol 90%) until photographs were taken, using Scanning Electron Microscopy (SEM) to pictorially present the sequence of floral development.

The transition from the vegetative to the reproductive stage was determined by looking for the first visible sign of the onset of reproductive growth, the "double ridge". Five plants were dissected every 2-3 weeks and examined under the binocular microscope. However, as the plants approached the transition stage, dissections (each of which took 15-30 minutes) were made every 3-5 days.

The series of floral developments used to describe the sequence of the changeover from vegetative to reproductive development was based on those used by Humphreys and Riveros (1986). However, in the present study, stages 2 and 3 as described by Humphreys and Riveros (1986) were combined into stage 2 because of the close proximity in time and position in the apex and the associated difficulty in differentiation. Also stages 3 and 4 were each subdivided into two sub-stages described as "early floret initiation" and "advanced floret initiation" in stage 3, and "early floret differentiation" and "advanced floret differentiation" in stage 4, which differentiated the developmental changes more precisely. As a consequence this paper presents five stages of morphological development but with stage 3 and 4 each having two sub-stages.

RESULTS

The development stages are shown in Plates 1-4 as follows:

Stage 1: Vegetative stage (Plate 1)

This refers to seedlings or new tillers from established plants which have been previously defoliated and have passed through a vegetative phase in which leaf differentiation and basal shoot production increase the density of growing points. The vegetative apex is characterised by a simple dome-like structure which has leaf primordia initiating on alternate sides. At the beginning of this stage, the vegetative apex is usually situated at ground level before the node is developed, and is enclosed

within the leaf sheath.

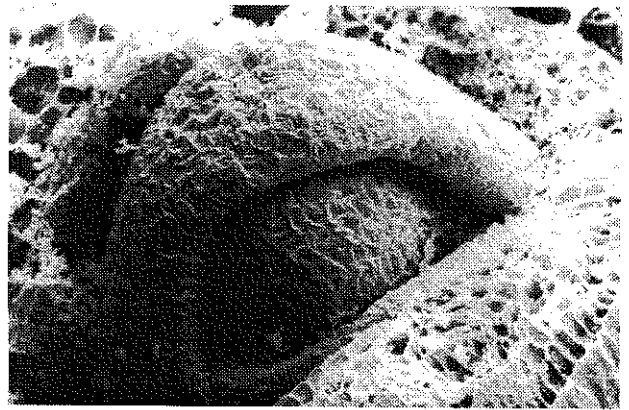


Plate 1. Vegetative apex with leaf primordia, Stage 1, SEM x 650.

Stage 2: The appearance of early raceme initiation and the "double ridge" stage (Plate 2)

The transition from the vegetative to the reproductive phase is commonly observed in the structure of the shoot apical meristem, described as the "double ridge" stage. As shown in Plate 2, early raceme initiation was evident along with the double ridge, and hence it was decided to combine these two stages into a single stage 2.

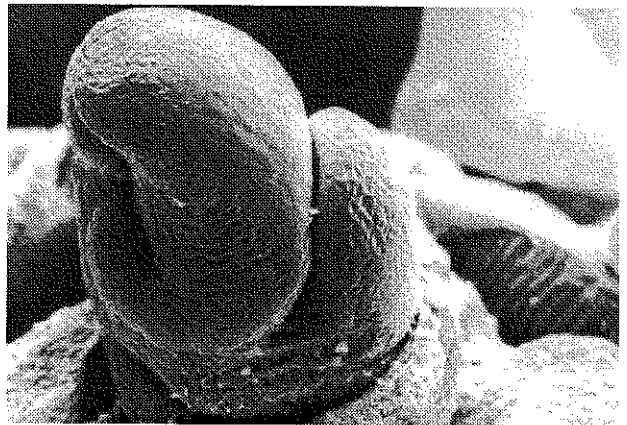


Plate 2. The transition from a vegetative to a floral apex showing the "double ridge" at the base and early raceme initiation, Stage 2, SEM x 250.

Stage 3: Floret Initiation (FI) stage (Plates 3.1 and 3.2)

Florets emerge first as ridges in the middle region of each raceme, and develop both acropetally and basipetally. This pattern of emergence helps to explain why anthesis usually commences in the middle region of the uppermost raceme. The number of racemes is always completed during this stage, and varies from 1 to 8. The original apex then degenerates.

This stage was divided into two sub-stages according

to floret appearance as follows:

3a. Early floret Initiation

This sub-stage starts when a raceme clearly shows florets in the middle part of the raceme. These appear as two parallel vertical lines, as shown in Plate 3.1.



Plate 3.1 Floret initiation (FI), Stage 3a, early floret initiation, SEM x 137.

3b. Advanced Floret Initiation

This sub-stage is apparent when the florets increase in size and fully occupy the whole raceme (Plate 3.2).

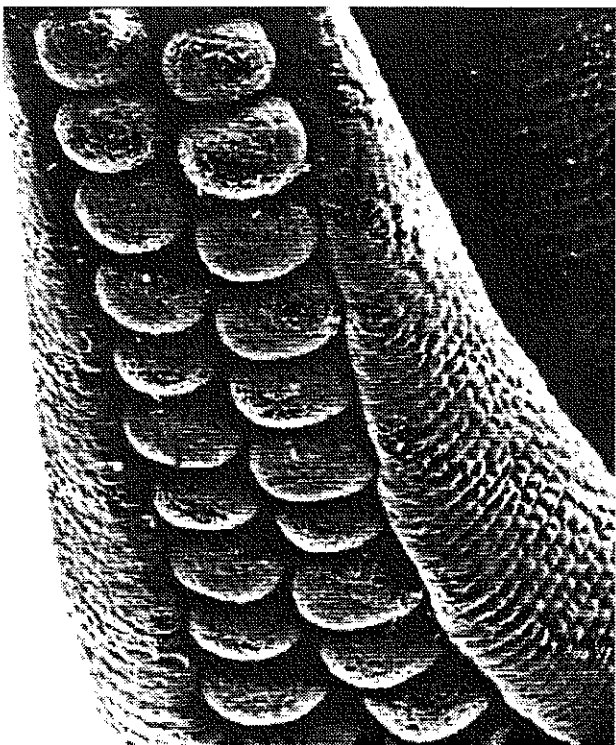


Plate 3.2 Floret initiation (FI), Stage 3b, advanced floret initiation, SEM x 180.

**Stage 4: Floret Differentiation (FD) stage
(Plates 4.1 and 4.2)**

This event occurs when ridges appear on the most advanced florets in the middle region of the uppermost raceme. Floret numbers range from 7-50 per raceme and the average is about 33 florets per raceme. This stage was also divided into two sub-stages as follows:

4a. Early Floret Differentiation

The first appearance which identifies the starting point of early floret differentiation is the development of glumes as shown in a close-up of a floret in Plate 4.1. Lemma also develop at the latest phase of early floret differentiation.

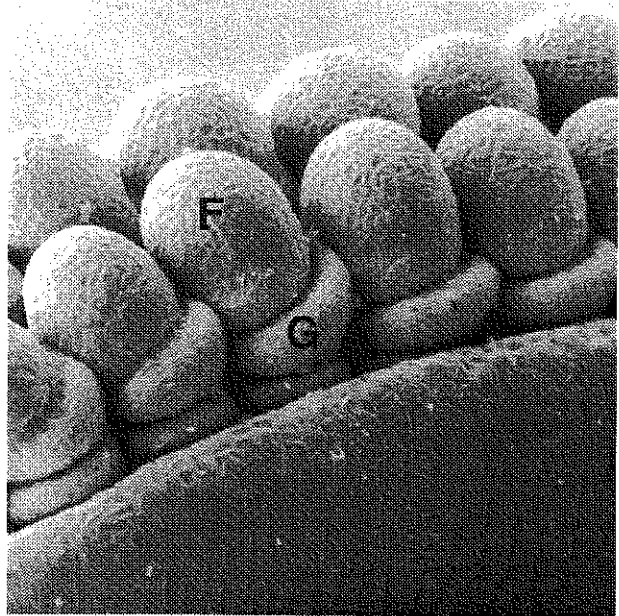


Plate 4.1 Floret differentiation (FD), Stage 4a, early floret differentiation, (F-floret, G-glume), SEM x 240.

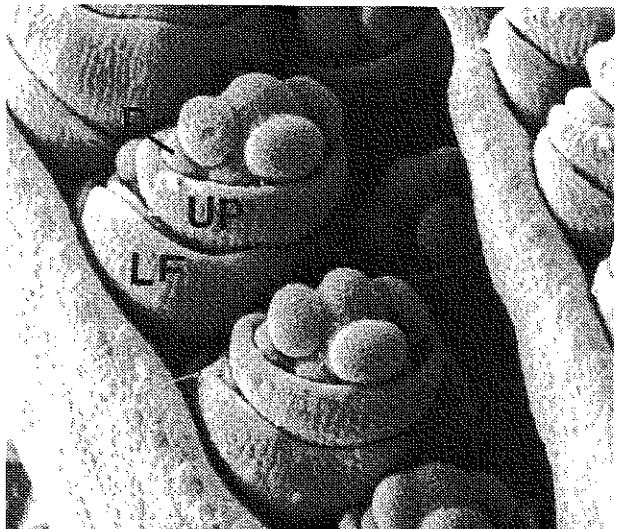


Plate 4.2 (a) Floret differentiation (FD), Stage 4b, advanced floret differentiation showing (a) palea (P), upper floret (UP) and lower floret (LF) - SEM x 160:.

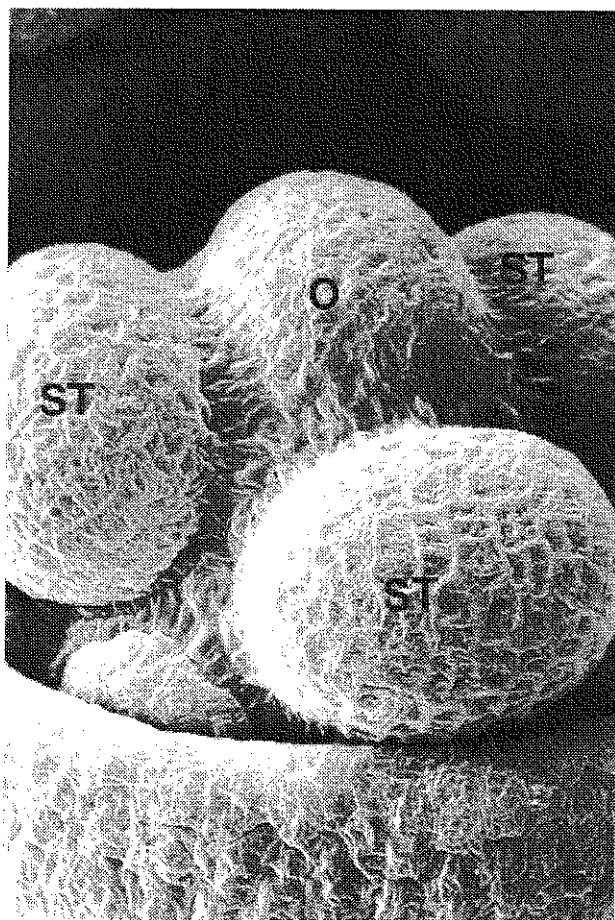


Plate 4.2 (b) ovary (O) and stamen (ST) - SEM x 430.

4b Advanced Floret Differentiation

This sub-stage presented in Plate 4.2 (close-up of florets) shows the further development of the floret, the appearance of the palea (P), the differentiation of the top of the floret into stamens and an ovary in the upper fertile floret, and stamens only in the lower fertile floret. In the final phase the ovary and stamens are enclosed by the lemma and palea. This sub-stage reflects a similar pattern to that described by Humphreys and Riveros (1986).

Stage 5: Inflorescence Exertion (IE) stage

The stage when an inflorescence is fully exerted above the flag leaf.

DISCUSSION

The stages of morphological change in this study are based on those described by Humphreys and Riveros (1986) who reported that the visible onset of reproduction takes place rapidly in *Brachiaria decumbens* and it is common to discover apices that are either vegetative or exhibit raceme initials (RI) even at some later development stage. Therefore, it was considered more appropriate to combine their stages 2 and 3, and thereby reduce the total number to 5 rather than 6 stages. However, stages 3 and 4 in this study were then each divided into two sub-stages which

served to differentiate the development changes more precisely.

Unfortunately, the exact time between each morphological stage was not determined in this study. However, the time from floral initiation to first anthesis was approximately 29 days. Hopefully this study has contributed to our knowledge of the sequence of reproductive development of a lesser-known tropical grass which is making a significant and increasing contribution to forage and animal production in Thailand.

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REFERENCES

1. Cooper, J.P. 1960. The use of controlled life-cycles in the forage grasses and legumes. *Herbage Abstracts* 30 (2) : 71-79.
2. Evans, M.W. and Grover, F.O. 1940. Developmental morphology of the growing point of the shoot and the inflorescence in grasses. *Journal of Agricultural Research* 61 (7) : 481-521.
3. Hill, M.J. 1971. A study of seed production in "Grasslands Kahu" timothy (*Phleum pratense* L.) and prairie grass (*Bromus uniloides* H.B.K.). Unpublished PhD thesis, Massey University, Palmerston North, New Zealand.
4. Humphreys, L.R. and Riveros, F. 1986. Tropical Grass Seed Production. Food and Agriculture Organisation of the United Nations, Rome, pp. 203.
5. International Seed Testing Association. 1996. International Rules for Seed Testing. Supplement to *Seed Science and Technology* 24 : 1-335.
6. Langer, R.H.M. 1972. How Grasses Grow. Edward Arnold Limited, London.
7. Sharman, B.C. 1947. The biology and developmental morphology of the shoot apex in the gramineae. *New Phytologist* 46: 20-34.
8. Wareing, P.F. and Phillips, I.D.J. 1986. Growth and Differentiation. Pergamon Press, Oxford, Great Britain.