CHAPTER 2
FLOWER DEVELOPMENT WITH REFERENCE TO INFLORESCENCE DEVELOPMENT

ABSTRACT

Development of hermaphrodite flowers of 'Kent' was examined. Petal and sepal primordia are first to differentiate from the base of the apical dome of the flower initial, followed acropetally by primordia of the staminodes, the fertile anther and ovary. Signs of nectar gland primordia are only visible later in the development of the flower. There are no signs of adnation between the different floral parts, and the style, undergoing rapid elongation just before anthesis, ends in a single stigma. The fertile anther develops faster than any of the other floral parts, while the four to nine staminodes remain inconspicuous.

2.1 INTRODUCTION

In this study, the emphasis was on the ontogeny of the perfect (hermaphrodite) flower in order to determine at what stage of ovary development stomata start developing. Flower ontogeny is, however, an integral part of inflorescence development and is therefore discussed in more detail, mainly based on the paper by Robbertse *et al.* (2001). Figures of the latter paper are supplied in the appendix to make it more convenient for the reader to follow. Robbertse *et al.* (2001) did not include flower ontogeny.

Growth of mango branches is rhythmic or episodic and occurs in growth flushes. These flushes can be either vegetative or reproductive. Each flush starts in a resting bud (terminal or lateral), which consists of an apical dome (meristem) and a number of leaf primordia enveloped by protective bud scales (Mallik, 1957 and Robbertse *et al.* 2001).

Flushing involves two distinctly different components. The first event, common for vegetative and reproductive flushes involves the activation of the resting bud. Bud activation can be either triggered by phytohormones produced by the roots during active root growth or by pruning to activate lateral buds. The second component can be described as induction/ differentiation (Pimental *et al.,* 1984; Robbertse *et al.,* 2001). Prevailing conditions during the induction/differentiation period will determine whether the differentiating bud will become a vegetative or reproductive shoot. Temperature is the most important factor where night temperature below 15 ºC and day temperature below 20 ºC will induce inflorescence development (Davenport and Núñez-Elisea, 1997 and Robbertse *et al.,* 2001).

The primary mango inflorescence is monopodial, which bears secondary and tertiary axes in bract axils. Each secondary inflorescent axis bears opposite bracts subtending sympodial tertiary inflorescence axes. All branches, primary, secondary and tertiary are determinate, ending in a flower (Davenport and Núñez-Elisea, 1997 and Robbertse *et al.,* 2001) (Appendix Fig. A6).

Night/day temperatures below 15/25 ºC is crucial from mid April to July for the first five stages of floral bud differentiation to complete, taking about 40 to 60 days. Any change in the temperature regime during this period can have an influence on the morphology of the developing shoots.

Joubert (1995) divided early floral bud development into 5 developmental stages. Figure 1A (appendix) shows a transverse section and figure 1C (appendix), a SEM micrograph of a resting stage 1 bud and figure 1B (appendix) a section of an activated stage 1 bud.

The resting inflorescence bud has a flattened apical part with bract and leaf primordia, situated more or less at the same level as the apical dome.

Axillary bud meristems in the bract axils appear to be aborted while no prominent bud meristems are present in the leaf primordia axils (Appendix Fig. 1A). The elongation of the terminal bud axis (primary inflorescence axis) is the first microscopic sign of bud activation (Appendix Fig. 1B). Thereafter the appearance of bud meristems (Stage 2) in the axils of the preformed leaf primordia follows (Appendix Fig. 2A, B and C). These bud meristems are the precursors of the secondary inflorescence axes bearing the flowers (Appendix Fig. 4 and A5).

During stages 2 to 4 the terminal meristem continue to initiate new leaf primordia at its secondary axillary axis meristem (Appendix Fig. A3 and A4), before ending in a terminal flower bud primordia. The preformed and neoformed secondary axis meristems in turn produce their own leaf (bract) primordia, each subtending the terminal flowers of the sympodial, tertiary inflorescence axis (Appendix Fig. 4B and 5B), stage 5. Stage 5 depicts bud break and is followed by further axes elongation, differentiation of flower buds, unfolding of the inflorescence and anthesis. After bud development has progressed up to stage 5, the fate of the bud to become an inflorescence has been finalised since primordia of all the essential parts have been initiated (Robbertse *et al.,* 2001).

The inflorescence branches terminate in dichasia, consisting of a central (first order) perfect flower followed by lateral staminate or perfect flowers and third order staminate flowers (Appendix Fig. 6). In most instances, the apical portion of the inflorescence has the highest percentage of perfect flowers due to less third order flowers in comparison with the middle and basal part with more third order flowers (Pimental *et al.,* 1984).

The calyx of both staminate and hermaphrodite flowers has five free deciduous, ovate-oblong, concave, yellowish green to light green and densely pubescent sepals. The corolla, inserted at the base of a fleshy

disk, consists of five pale yellow petals, twice as long as the sepals with five red tinted ridges on the ventral sides. As the petals mature, they become yellowish to pinkish in colour. Each flower has only one functional stamen, opposite to the dorsal side of the ovary, and four to nine sterile stamens (staminodes). Perfect flowers contain one sessile ovary (Juliano and Cuevas, 1932 and Pimental *et al.,* 1984).

In spite of the amount of literature available on the mango inflorescence, very little information is available on flower ontogeny and will therefore this chapter reports on a study of the flower ontogeny, mainly to determine at which stage stomata start developing on the ovary.

2.2 MATERIALS AND METHODS

Flower buds and ovaries were obtained from fully bearing 9-year-old 'Tommy Atkins' (TA') mango (*Mangifera indica* L.) trees grafted onto 'Sabre' seedling rootstocks, from commercial blocks at Bavaria Estate, Hoedspruit (24°22'32"S, 30°53'26"E). Perfect flowers were obtained from the apical portion of the inflorescence as mentioned by Pimental *et al.* (1984).

Material was fixed in 2.5 % glutaraldehyde 0.1 M $NaPO_4$ buffer (pH 7.4), followed by three rinses (10 minutes each) with the same buffer. Postfixation was done with 1 % OsO_4 for two hours and were removed with three rinses (10 minutes each) of distilled water. Material was dehydrated in a graded ethanol series, followed by critical point drying in a Polaroid critical point dryer. Dried samples were coated with gold, using a Polaron E5200C sputter coater, for conductivity. Specimens were viewed with a JOEL-840 scanning electron microscope (SEM), operated at 5 kV. Images were recorded digitally.

The material for resin embedding was fixed in paraformaldehyde (4% formaldehyde in 0.15 M phosphate buffer). Following fixation in paraformaldehyde and dehydration in a graded ethanol series (Sass, 1966). Sections of 0.5 µm were cut, using an ultramicrotome (Ultracut E, Reichert, Vienna, Austria) and preparations were stained with Toluidine blue. Preparations were viewed under a Leitz Biomed microscope and photographs were taken with an Olympus Camedia C-4000 Zoom digital camera.

2.3 RESULTS AND DISCUSSION

The flower bud primordia of the mango are subtended by bud scales (Fig. 2.1B and 2.2A), protecting them from the environment. Initiation and differentiation of the different organs are readily distinguishable. The axillary bud meristem starts by increasing in width (Fig. 2.1A), allowing space for the different primordia of the flower parts to initiate and develop.

The whorl of five sepals are already differentiated on the rim of the receptacle, while the other organs are still rather undifferentiated. The single fertile stamen is already distinguishable by the characteristic two-lobed anther while only small domes from which the staminodes and carpel will develop are visible (Fig 2.1A). All the primordia are separate without any adnation.

In a further stage of development, the elongating, arcing petals are starting to enclose the other juvenile flower parts (Fig. 2.1B). The fertile anther lobes are in an advanced stage of development, while, only the meristematic domes of the carpel and the staminodes are apparent. Primordia of the nectar glands are not yet distinguishable.

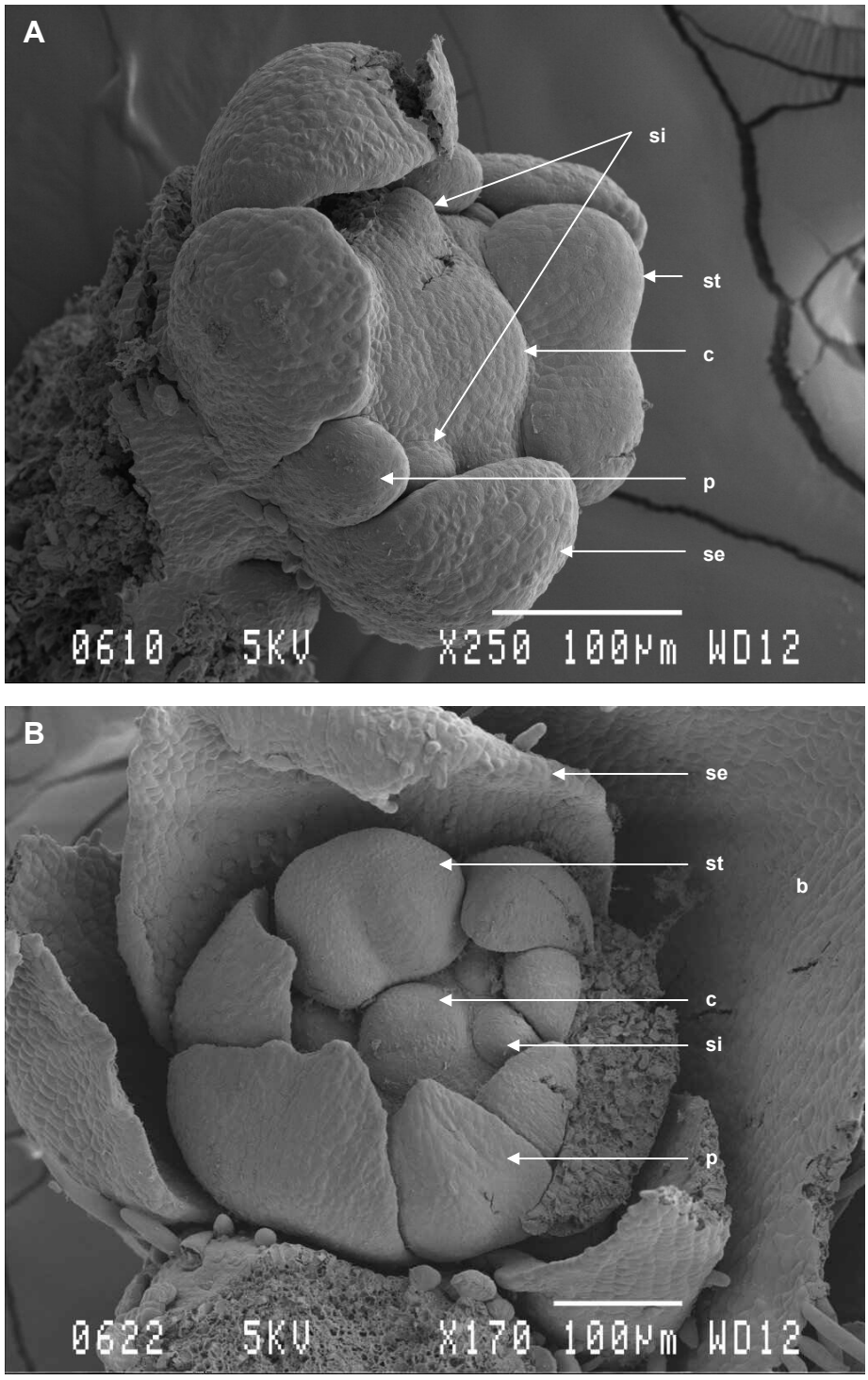

Figure 2.1 (A) Oblique view of a hermaphrodite 'Kent' flower bud primordium. Sepal (se), stamen (st), carpel (c), petal (p) and staminode primordia are visible. (B) Polar view of a more advanced stage of (A). b – bud scale.

The carpel in (Fig. 2.2A) started to differentiate, showing the depression of its single locule. The staminodes are visible as small stubs and the petals are starting to grow over, covering the different flower organs.

Figure 2.2B shows the carpel opening getting narrower as the carpel enlarges. First signs of nectar gland primordia are situated adjacent to the staminodes on the rim of the flower bud. Four larger staminode primordia are alternated by five smaller staminode primordia. An indentation caused by the removed fertile anther is visible on the ovary surface.

Style starts to elongate on top of the ovary that is still showing the unfused margins of carpel (Fig. 2.3A). The fusion of the carpel margins progress in an acropetal direction. The indentation caused by the fertile anther on the ovary is also visible in this figure. Nectar gland development caught up with the development of the rest of the organs. The filament of the fertile anther (removed) is visible, situated on the dorsal side of the carpel. This is consistent in all the flowers and flower buds investigated.

The four lobes of the fertile anther is visible in figure 2.3B, dominating the smaller two lobed staminodes surrounding the carpel. The style is still elongating. The fissure is closing acropetally by the concrescence of the adjacent tissue (Fig. 2.3B - arrow).

An excised carpel is showed in figure 2.4A. The style straightened and ends in a relative unspecialised stigma. The development of the floral organs of the hermaphrodite flower of the 'Kent' mango is, therefore acropetal.

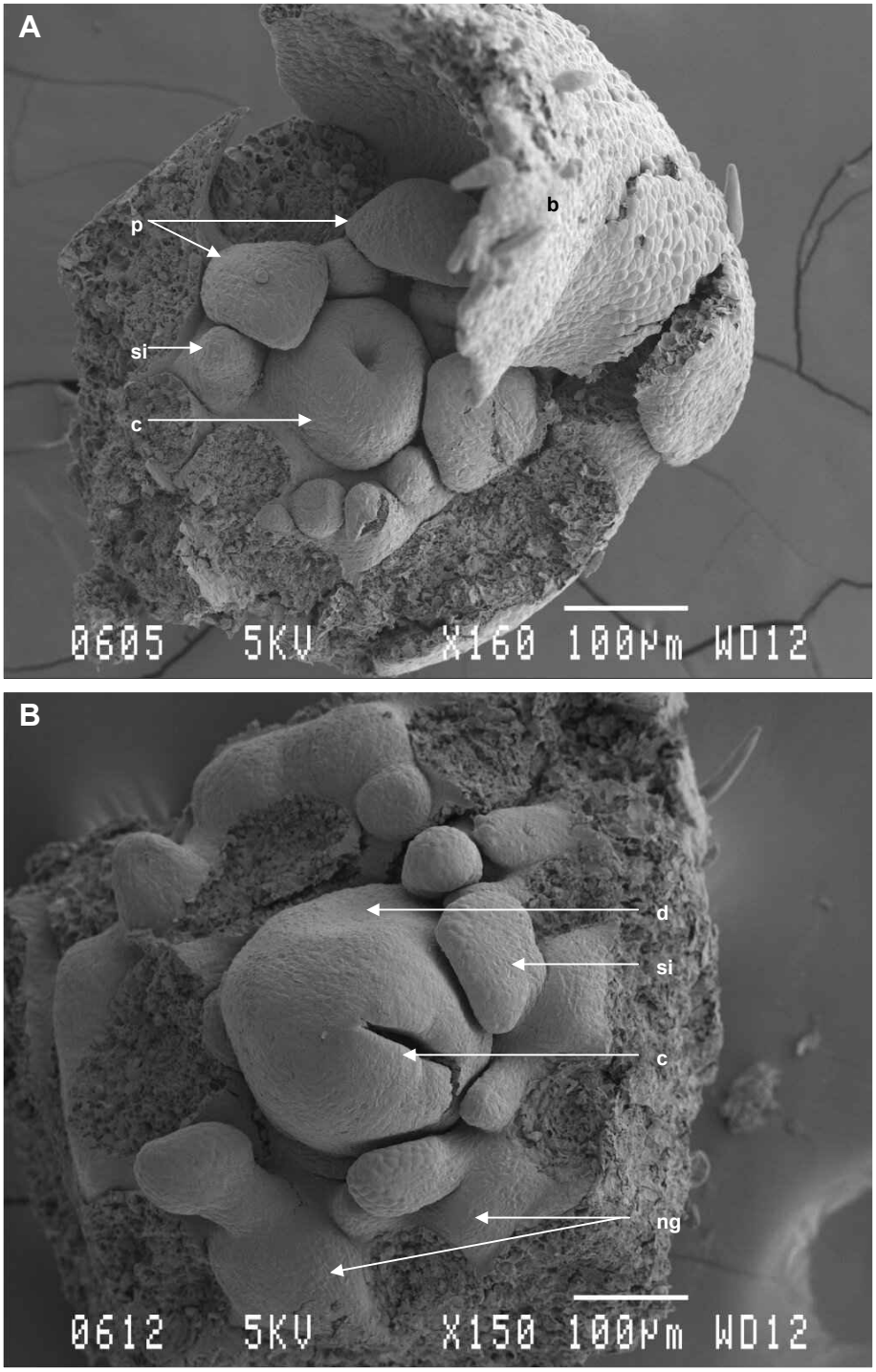

Figure 2.2 Flower buds with petals and sepals removed. (A) Differentiating carpel (c) with indentation of locule. Enlarging petal (p) and staminode primordia (si) visible. (B) Showing folded carpel. Further developed staminodes (si) with filament and first indications of nectar gland primordia (ng) are visible. d – indentation, b – bud scale.

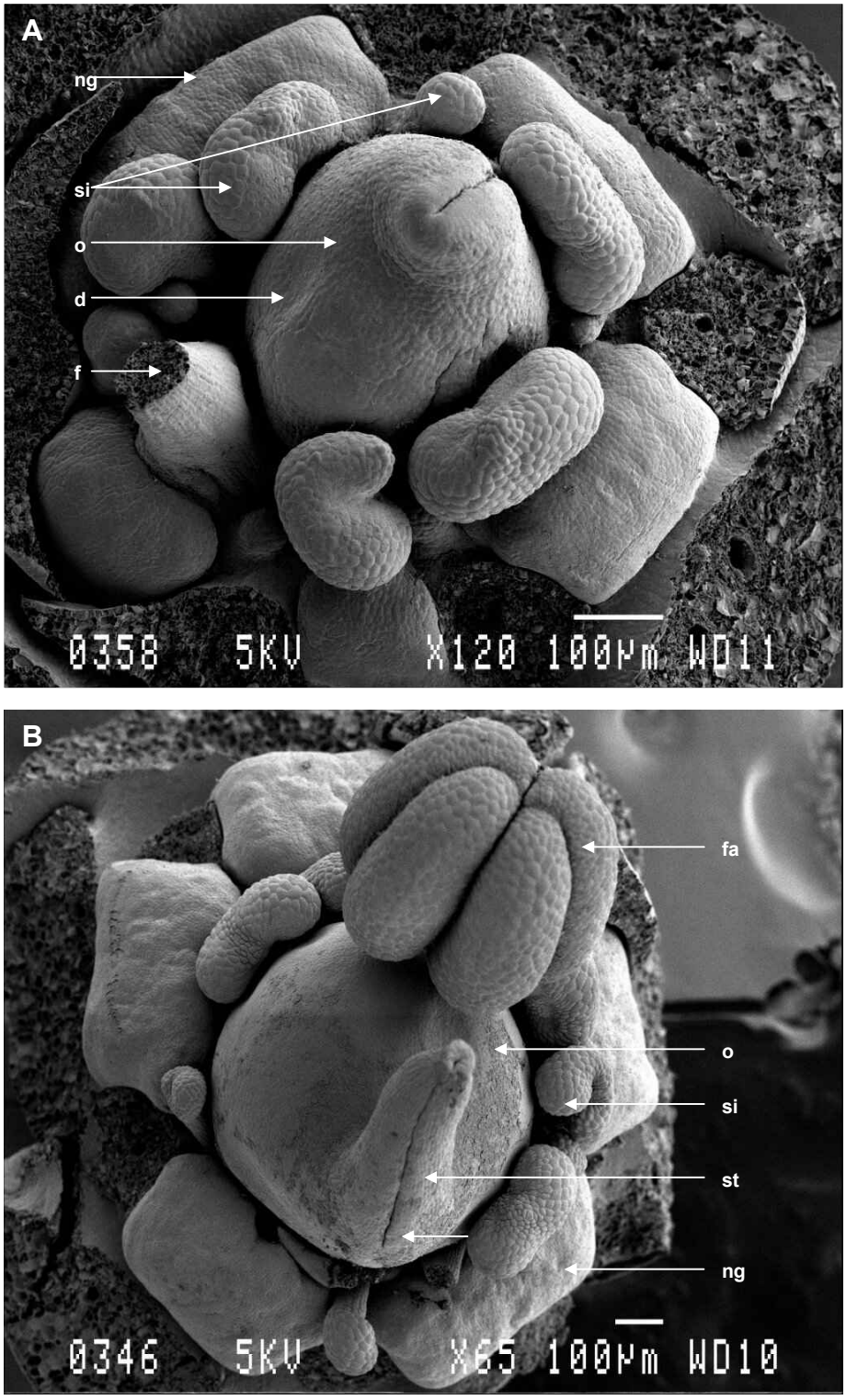

Figure 2.3 Flower buds with petals and sepals removed. (A) Style starts to elongate from ovary (o). Filament (f) of fertile stamen (anther removed) and nectar glands (ng) are visible. (B) More advanced stage of (A), showing fertile anther (fa) and elongated style (st). d – indentation.

Figure 2.4B shows part of a flower at anthesis. The nectar glands took on a strange reticulate appearance. Five nectar glands are situated on the rim of the receptacle. Clearly, the staminodes lost their integrity, shrivelled and died off.

2.4.1 Stomatal development

Stomata in dicotyledons may originate by cell division resulting in an oblique wall in the epidermis. The smaller cell resulting from this division functions as the guard cell mother cell or meristemoid (Cutter, 1978). Meristemoids were visible on the ovary surface prior to anthesis (Fig. 2.5A). They appeared as enlarged, spherical cells of which the cytoplasm stained dark blue with Toluidine blue (Fig. 2.5A). Figure 2.5B shows two guard cells of the developing stoma with the nuclei visible in the two cells. Subsidiary cells are absent. Guard cells took on their characteristic bean-shaped form at the time of anthesis (Fig. 2.5C), but seemed to be non-functional, as the schizogenic stomatal opening was still sealed with cutin and wax (arrow and Fig. 2.5E). Figure 2.5D shows a stoma of a fruitlet shortly after anthesis, clearly functional due to the opening visible between the two adjacent guard cells. The stomatal guard cells on mango fruit, therefore develops directly from a single differentiated cell in the epidermis. The absence of subsidiary cells means that their stomata correspond with the anomocytic type at anthesis (Cutter, 1978).

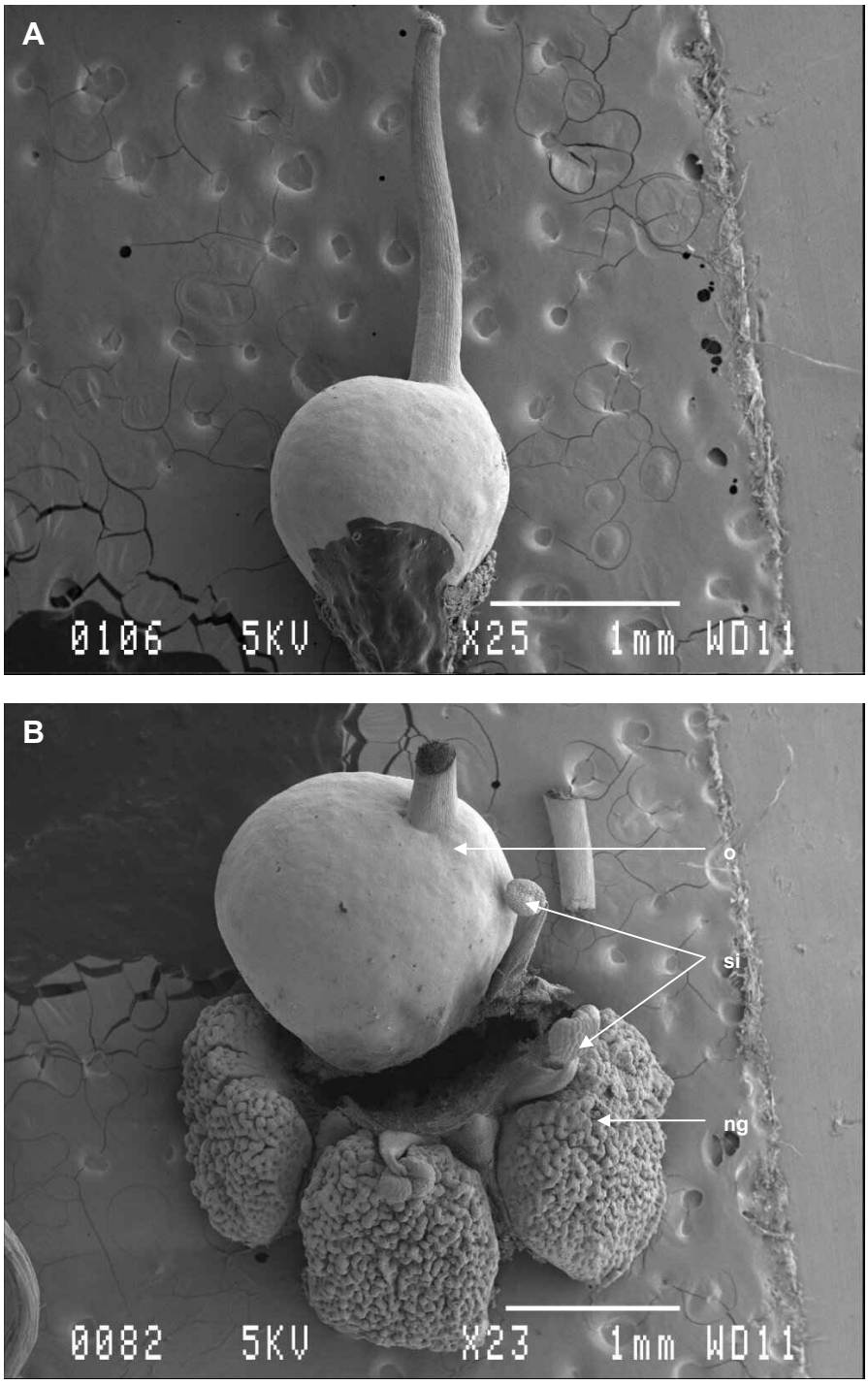

Figure 2.4 (A) Excised pistil prior to anthesis. (B) Showing a flower during anthesis. Staminodes (si) and nectar glands (ng) are visible.

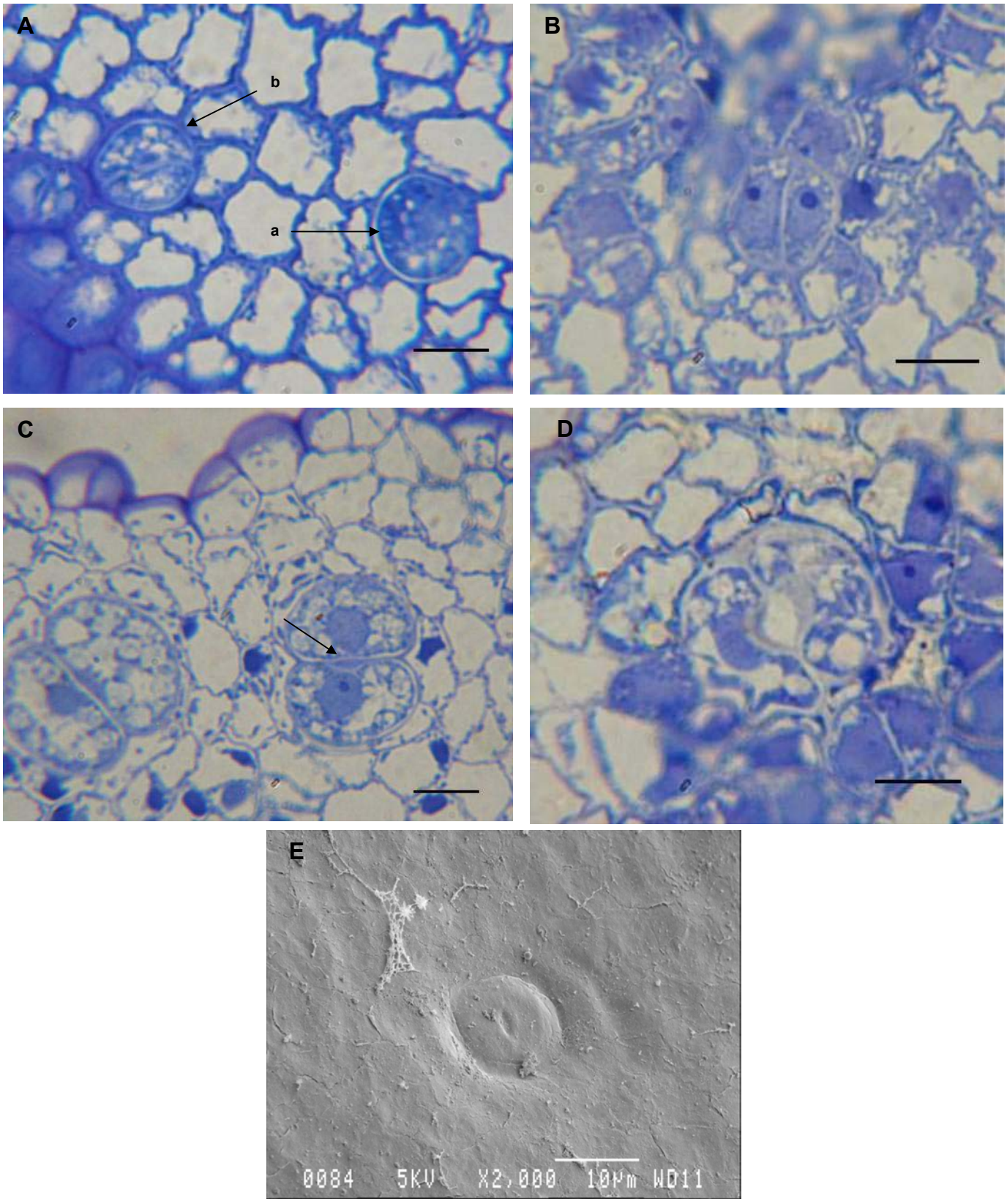

Figure 2.5 Paradermal sections of mango ovary surfaces of different ages. (A) Meristemoid (a) and early development of guard cells (b). (B) Nucleoli visible in cells just after division. (C) Later stage of development of guard cells. (D) Functional stoma shortly after anthesis. (E) Same stage as C, stomatal opening still covered with cutin, therefore not functional yet. Scale = 10µm

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