

**A COMPARATIVE DEVELOPMENTAL STUDY OF FLOWERS AND FRUITS  
IN *CITRULLUS LANATUS* (CUCURBITACEAE)**

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**The Faculty of Graduate Studies**

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**The University of Guelph**

**by**

**HANNAH BONTLE LECHA**

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**for the degree of**

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## **ABSTRACT**

### **A COMPARATIVE DEVELOPMENTAL STUDY OF FLOWERS AND FRUITS IN *CITRULLUS LANATUS* (CUCURBITACEAE)**

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University of Guelph, 2000

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This thesis presents the results of a comparative study on floral development, and fruit morphology and anatomy in the cultivated (var. *lanatus*) and wild (var. *citroides*) watermelon. The order of initiation and development of floral organs in the staminate and pistillate flower is the same for both varieties. There are no rudimentary carpels in the staminate flower. In the pistillate flower, the anthers fail to develop to maturity and remain as staminodes. A layer of wax covers the fruit epicarp of both varieties. In the cultivated watermelon fruit, stomata occur on the surface of the epicarp. In contrast, the stomata in the wild watermelon fruit are sunken in depressions on the epicarp. The bands of sclereids comprising the outer-mesocarp tissue are more extensive, broader and more lignified in the wild watermelon than in the cultivated one. The differences observed in stomatal morphology and rind structure suggest that structural features may play a role in enhancing the keeping quality of watermelon fruits.

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## CHAPTER 1

### GENERAL INTRODUCTION AND LITERATURE REVIEW

#### 1.1 INTRODUCTION

*Citrullus*, a member of the Cucurbitaceae, is a genus of great economic importance largely due to one species, *Citrullus lanatus*. *C. lanatus* (Thunb.) Matsum. and Nakai consists of both the cultivated and wild forms of watermelon. The cultivated watermelon belongs to the var. *lanatus*, whereas the wild forms belong to var. *citroides* (Bailey) Mansf. (Bailey, 1930; Robinson and Decker-Walters, 1997). Although the cultivated watermelon is feral in warm parts of the world, it is thought to be native only in the dry sandy areas of southern Africa (Whitaker and Bemis, 1976). In contrast to the domesticated watermelon, which is cultivated throughout the world for consumption of its sweet flesh, the wild forms are locally important in Africa. In Botswana for instance, the wild form known as "Tsamma" or "Tsama" melon (*C. lanatus* var. *citroides*) plays a major role in the economy of the Bushmen of the Kalahari Desert. Tsamma melon is also of added interest to the government of Botswana because it has been identified as one of the indigenous plants with potential for commercial exploitation in arid areas (Arnold et al., 1985; Taylor, 1985). The general location of Botswana is shown in Figure 1.

Tsamma melon, which is characterized by a white firm flesh and a high water content, occurs in two biochemical forms; one with bitter fruits containing cucurbitacin E -glucoside (elaterind) and a bland one without elaterind (Meeuse, 1962; Whitaker and Bemis, 1976; Renew, 1968). The Bushmen use the non-bitter form as a critical source of food and water during the 9-month period when the Kalahari Desert is devoid of surface water (Macrone, 1937; Lee, 1979; Mills, 1980; Arnold et al. 1985; Taylor, 1985; Knight, 1995). Similarly, wildlife in the region uses the fruit for food and water (Mills, 1980; Knight, 1995).

The cultivated watermelon and Tsamma melon differ in a number of morphological traits, the most prominent being fruit characteristics. One of the most outstanding differences is in the keeping quality of the fruits when fully matured.

Tsamma melon is able to withstand both low and high temperatures without experiencing any chilling injury or decay. As a result, the fruit is able to remain intact and fleshy for more than a year after the parent plant has died (Macrone, 1937; Lee, 1979; Mills, 1980; Arnold *et al.*, 1985; Knight, 1995). Botha (1982) found that it remained sound and maintained more than 95% water on a fresh mass basis seven months after abscission from the parent plant. In contrast, the cultivated watermelon fruit can only withstand moderate temperatures for a short period of time. For satisfactory storage, a period of 2 weeks at a temperature of 15°C is recommended (Robinson and Decker-Walters, 1997).

Although the use and remarkable keeping quality of the Tsamma melon is widely documented in the literature (Macrone, 1937; Lee, 1979; Mills, 1980; Arnold *et al.*, 1985; Knight, 1995), the mechanism by which it is achieved is not known. Previous studies have intensively documented the adaptation of plants to extreme conditions (e.g. Maximov, 1929; Levitt, 1980). However, those studies focused on leaves and roots, and neglected fruits. Botha (1982) investigated the mode of water conservation in Tsamma melon without reaching any definite conclusions. He hypothesized that the Tsamma melon avoided water loss by permanently closing its stomata with some "material". However, several questions were left pending. For instance, he did not determine nor did he speculate on the nature of the supposed "material". Although form and function must be studied together in order to understand structural modifications, Botha's approach did not incorporate detailed morphological and anatomical studies. Moreover, the methodologies used in the study were not documented. This is the first comparative study to document anatomical and morphological development of Tsamma melon fruit, and the domesticated watermelon.

## **1.2 LITERATURE REVIEW**

### **1. 2.1 Ecology**

Tsamma is an annual herb that grows along the ground in irregular patches (Mills, 1980). It is thought to be native only in the sandy areas of southern Africa (Mills, 1980, Botha, 1982). In Botswana, it occurs predominantly in the sandy areas

of the Kalahari Desert (Fig. 2). (Renew, 1968; Mills, 1980; Botha; 1982; Knight, 1995).

The Kalahari Desert is semi-arid and characterized by sandy soil thrown into permanent sand dunes (Mills, 1980; Knight, 1995). It experiences irregular summer rainfall (Knight, 1995) and high daily and seasonal temperatures ranging from 32° to 38°C (Botha, 1982). Natural surface water is found typically in the hard bottom pans for short periods during the summer rainfall period (Lecha, personal observation). For the rest of the year, the Kalahari is devoid of surface water (Knight, 1995). It is during this dry period that the Tsamma melon plays a major role in the ecology of the Kalahari Desert. In the Kalahari Desert, the year can be divided into 3 seasons: the hot-wet season, January -April; the cold-dry season, May-August; and the hot-dry season, September-December (Knight, 1995). Of these three seasons, only the hot-wet season receives enough rainfall to stimulate germination of the Tsamma seeds. At the start of the rainy season, Tsamma seeds germinate and complete their life cycle, leaving the fruits to survive alone during the following drought period. Owing to this ephemeral behaviour, the growth and occurrence of Tsamma melon in the field is seasonal. The Tsamma germinate in January-February, and set the first fruit in February. The fruit reaches maturity in April-May (Knight, 1995) and survives through the cold-dry season into the hot-dry season (Lee, 1979).

The Tsamma melon has a high water content ranging from 89% (Knight, 1995) to 96% (Mills, 1980; Botha, 1982; Arnold et al., 1985), and is therefore a good source of water during the dry season. Although three species of Cucurbitaceae occur in the Kalahari Desert (Tsamma melon, *C. lanatus* var. *citroides*; wild cucumber, *Cucumis africanus* L.; and gemsbok cucumber, *Acanthosicyos naudinianus* (Sond.) C. Jeffrey), the Tsamma melon is the most common and important of these species in the region (Knight, 1995). A wide range of animals, ranging from rodents to ungulates and carnivores consumes the melons. Larger animals use the Tsamma melon to a greater extent in areas without artificial water supplies (Knight, 1995). The persistence of the fruits into the hot-dry season facilitates consumption by wild animals, and as a result, the seeds are mainly dispersed by animals. The Tsamma melon also plays an important role in

the diet of the Bushmen of the Kalahari Desert. The Bushmen eat the Tsamma melon raw, or cooked in the form of a stew (Renew, 1975).

### **1.2.2 Genetic resource**

Since var. *citroides* (Tsamma melon) has been proposed as the wild progenitor of the cultivated forms of watermelon (Shimotsuma, 1963; Fursa, 1972; Botha, 1982; Navot and Zamir, 1987), it has great potential to serve as a genetic resource in the breeding of cultivated watermelon. Studies have shown that var. *citroides* has the highest rate of polymorphism in the genus (Navot and Zamir, 1987; Jarret *et al.*, 1997) and can thus contribute important genetic traits for crop improvement. It also exhibits high levels of resistance to a number of diseases such as fusarium wilt (Martyn and Netzer, 1991), gummy stem blight (Sowell, 1975) and anthracnose race 2 (Sowell *et al.*, 1980). Fusarium wilt resistance displayed by cultivars such as "Conqueror" was obtained from var. *citroides* (Robinson and Decker-Walters, 1997).

### **1.2.3 Storage Properties**

As stated previously, the Tsamma fruit has a remarkable keeping quality when fully mature. The fruit matures in the cold-dry season, and is able to remain intact and fleshy for more than a year. After ripening, the fruits are collected and eaten through the cold-dry season into the hot-dry season without decaying or losing their water content. Botha (1982) found that the fruits maintained 95% water content on a fresh mass basis seven months after abscission. It is important to note that this long duration of storage is achieved throughout both the cold and hot-dry seasons. In winter, temperatures can drop as low as 0°C; during the hot-dry season, they range from 32-38 °C. Despite exposure to these extreme conditions, no incidences of chilling injury or decay have been documented. Instead, Tsamma exhibits high levels of resistance to a number of diseases, as stated previously.

The behaviour of the cultivated watermelon during storage contrasts to that reported for Tsamma melon. The cultivated watermelon fruit is sensitive to both low and high temperatures. Fruits subjected to lower temperatures are susceptible to chilling injury (Picha, 1986; Risse *et al.*, 1990), which consequently causes fruit decay (Risse *et al.*, 1990). Brownish stains on the surface of the watermelon

indicate chilling injury. Watermelon is reported to be more susceptible to chilling injury when stored below 10°C (Picha, 1986; FAO, 1988; Risse *et al.*, 1990). Higher temperatures are also unfavourable since they activate diseases (Robinson and Decker-Walters, 1997) and ultimately cause fruit decay (Risse *et al.*, 1990). Watermelon is thought to be more susceptible to decay when stored at 21°C or higher. Prolonged storage has also been found to enhance fruit decay (Risse *et al.*, 1990).

In addition to fruit decay, temperature and prolonged storage also affect the texture and quality of the flesh of the cultivated fruit. In some cultivars, ripeness increases with temperature and storage whereas others remain firm without showing any signs of over ripening (Risse *et al.*, 1990). The thickness of the rind is also affected by prolonged storage. For instance, Risso *et al.* (1990) found that in some cultivars, the thickness of the rind decreased with storage.

#### **1.2.4 Physiology**

Most plants inhabiting dry environments circumvent severe water loss by utilizing the Crassulacean acid metabolism (CAM) photosynthetic pathway (Levitt, 1980; Moore *et al.*, 1995). CAM plants have a photosynthetic pathway that is temporally separated and thus, open their stomata during the night and close them during the daytime. This mechanism ensures that the stomata only open at night when the temperature is lower and humidity much higher than during the day, thus preventing water loss. Given the environmental conditions under which Tsamma melon occurs and the hydration that it exhibits, one would expect it to utilize the CAM pathway. However, this is not the case. When conducting transpiration experiments, Botha (1982) found that Tsamma melon does not utilize the CAM pathway, but is a typical C<sub>3</sub> plant with stomata open during the day and closed at night, a mechanism that is less effective in minimizing water loss by transpiration.

The Tsamma melon loses water very rapidly immediately after abscission (Botha, 1982). However, the transpiration rate gradually decreases after sometime, approaching zero approximately 12 weeks after abscission. The weight of the fruit also exhibited the same pattern as the transpiration rate, remaining fairly constant after approximately 12 weeks from abscission.

This observation led Botha to conclude that water loss stopped completely 12 weeks after abscission.

### **1.2.5 Morphology**

*C. lanatus* is an annual vine that spreads along the ground in patches. It exhibits the general cucurbit shoot morphology. The leaves are alternate, with 3-5 lobes. The tendrils are branched, and borne in the axil of the leaves. Both Tsamma melon and cultivated watermelon are monoecious, bearing staminate and pistillate flowers in the same plant. The flowers occur singly, and opposite the branched tendril in the axil of each leaf (Rosa, 1925) (Fig. 3 and 4). Pistillate flowers occupy every seventh node of the plant, whereas staminate ones occupy the intervening nodes (Rosa, 1925; Whitaker and Davis, 1962). Pistillate flowers bear an inferior ovary, which develops directly into a fruit after pollination.

Because the fruit develops from the ovary, floral developmental studies lead to understanding the nature and timing of the fruit that will be formed. This view is supported by previous studies on other cucurbits. In squash (*Cucurbita*), for instance, fruit shape and stripe pattern are evident in the immature ovary (Robinson and Decker-Walters, 1997). Despite the apparent link between floral development and ultimate fruit characters, floral developmental studies are lacking in *Citrullus*.

In contrast to the cultivated watermelon fruit, which is large and round to oblong, the tsamma fruit is small and round in shape. A Tsamma fruit has an average diameter of 15 cm (Taylor, 1985) and an average mass of 1.15 kg (Mills, 1980). The fruits of most watermelon cultivars, on the other hand, weigh 4- 25 kg. The only exception is the icebox cultivar, which weigh about 1 kg (Robinson and Decker-Walters, 1997). The tsamma melon rind is light green with faint stripes. The flesh is white, and firm. Flesh taste can be either bitter or bland (Meeuse, 1962; Whitaker and Bemis, 1976).

## **5. Anatomy**

Although documentation of the utilization of Tsamma melon dates as far back as David Livingstone and De Candolle's era (Barber, 1909; Whitaker and Davis, 1962; Shimotsuma, 1963), the anatomy of the fruit has not been studied. The few anatomical studies documented on *C. lanatus* have been on the cultivated

watermelon (*C. lanatus* var. *lanatus*). (Barber, 1909; Whitaker and Davis, 1962; Lal et al., 1977).

Barber (1909) gave a generalized description of fruit histology of most Cucurbitaceae fruits, including cultivated watermelon, *C. vulgaris* (Syn. *C. lanatus*). This description still serves as the definitive work on the fruit anatomy of *Citrullus*. In their monographic work on cucurbits, Whitaker and Davis (1962) gave a generalized description of cultivated Cucurbitaceae. However, their report was based on Barber's (1909) work, and did not include information provided by Lal et al (1977) on the pericarp anatomy and fruit characters of some cultivars of watermelon (var. *lanatus*). The study by Lal et al (1977) showed that anatomical features played a major role in determining transportation and storage properties of watermelon. For instance, fruits characterized by thin outer mesocarp cell walls were prone to rupturing during storage and transportation.

When investigating the mode of water conservation in Tsamma melon, Botha (1982) gave a passing reference to the surface anatomy of the fruit. He reported that the pericarp was covered by stomata. He also documented that the stomata were plugged by some material after fruit abscission and attributed this to the apparent cessation of water loss in the fruit.

Adaptive mechanisms to xeric environments have been widely documented in the literature (e.g. Maximov, 1929; Levitt, 1980). Cuticular modification, such as the deposition of wax on the cuticle (Denna, 1970) has been associated with reduced cuticular transpiration in plants. Stomatal modifications, on the other hand, have been associated with reduced stomatal transpiration. Wax deposition in the stoma, in particular, has been found to be effective in reducing stomatal transpiration (Jeffree et al., 1971; Rentschler, 1974). Jeffree et al. (1971), when investigating interactions of waxes with the water vapour pathway through the stomata of Sitka spruce (*Picea sitchensis*) leaves, found that the presence of wax in the antechambers of the stomata reduced the rate of transpiration by two thirds. Similarly, Rentschler (1974) observed that stomatal plugging by wax reduced water loss considerably in *Brassica napus* leaves. Removal of the wax with chloroform resulted in an increase in water loss, supporting the theory that wax plugs in the stomata minimize stomatal transpiration. In fruits, general wax deposition has been documented for apple (*Malus*) (Hall, 1966), grape (*Vitis vinifera*) (Possingham, et

al., 1967), and Wax gourd (*Benincasa hispida*) (Morton, 1971). The latter is the most relevant to this study since this genus is closely related to *Citrullus* (Fursa, 1972). The wax gourd has a high water content (ca 96%) similar to that reported for Tsamma melon. Moreover, it can also be stored for as long as a year (Morton, 1971). The wax gourd fruit has a thick, waxy cuticle that is thought to prevent water loss and pathogen infection (Morton, 1971; Robinson and Decker-Walters, 1997). According to Robinson and Decker-Walters, (1997) the waxy bloom develops on mature fruits and continues to develop even after the fruit is harvested. The development of the wax bloom is thought to be stimulated by high temperatures (Robinson and Decker-Walters, 1997).

### **Thesis goal and Objectives**

Although the utilization of Tsamma fruit is widely documented, the adaptive mechanism for its keeping quality and water conservation has received very little attention from researchers. The objective of this thesis is to investigate how the Tsamma fruit achieves its remarkable keeping quality. Since these adaptive features appear to be lacking in the cultivated watermelon, studying the Tsamma melon and cultivated watermelon fruit comparatively might reveal structural differences associated with fruit keeping quality. This objective was addressed by means of a comparative developmental study of Tsamma melon and the cultivated watermelon.

Plants inhabiting extreme environmental conditions often possess adaptive features that allow them to survive in these conditions. Consequently, morphological, anatomical and physiological adaptations have been documented for plants inhabiting extreme environmental conditions. For instance, structural modifications of the epidermis such as cuticular wax, and stomatal modifications, have been widely associated with hot and dry environments (Maximov, 1929; Levitt, 1980). The possession of peculiar morphological and anatomical features might be one reason why the Tsamma melon is able to remain intact and fleshy for more than a year. Since previous studies have shown that fruit characters in Cucurbitaceae are initiated before pollination (Robinson and Decker-Walters, 1997), it was necessary to carry out a comparative developmental study from the time of floral ontogeny to fruit maturity.

Although the watermelon has been studied with respect to its cultivation as an economically important crop, there have been no recent developmental or anatomical studies of fruit development. Despite the advent of modern techniques with high resolution (Scanning Electron Microscopy (SEM), Laser Scanning Confocal Microscopy (LCSM) and Transmission electron Microscopy (TEM), Barber's (1909) study still serves as the definitive work in the fruit anatomy of *Citrullus*. This thesis is the first complete and detailed developmental study of the genus *Citrullus* from seed germination to fruit maturity.

In addition to answering questions related to morphological and structural modification through developmental studies, there are other interesting questions that can be answered by studying morphological development. Cucurbits display a unique diversity in sex expression (Whitaker and Davis, 1962). However, the development of this diversity is not well understood. Documenting floral development in *Citrullus* will thus contribute to the knowledge of sex expression in cucurbits.

#### Thesis goal

The main goal of this thesis is to investigate how the Tsamma fruit differs from the domesticated watermelon in terms of initiation, development, morphology and anatomy, and has been divided into two major chapters.

Chapter 2 will describe, document and compare floral initiation and development in Tsamma melon (*Citrullus lanatus* var. *citroides*) with that of "Sugar Baby" (*C. lanatus* var. *lanatus*), and Chapter 3 will establish whether there are any anatomical differences between the Tsamma and cultivated watermelon ("Sugar Baby") fruits that may account for their differences in keeping quality.

## **PLATE 1**

**Fig. 1.** Outline map of Africa showing the general location of Botswana.

1



■ Botswana

## **PLATE 2**

**Fig. 2.** Outline map of Botswana showing the general locality of the Tsamma melon: the Kalahari Desert.

2



## **PLATE 3**

**Figs. 3-6.** Plant habit of *C. lanatus*: University of Guelph Cambridge Research Station, Cambridge, On, Canada.

**Figs. 3 and 5.** Overview of the plant habit of "Sugar Baby" and Tsamma melon in the field.

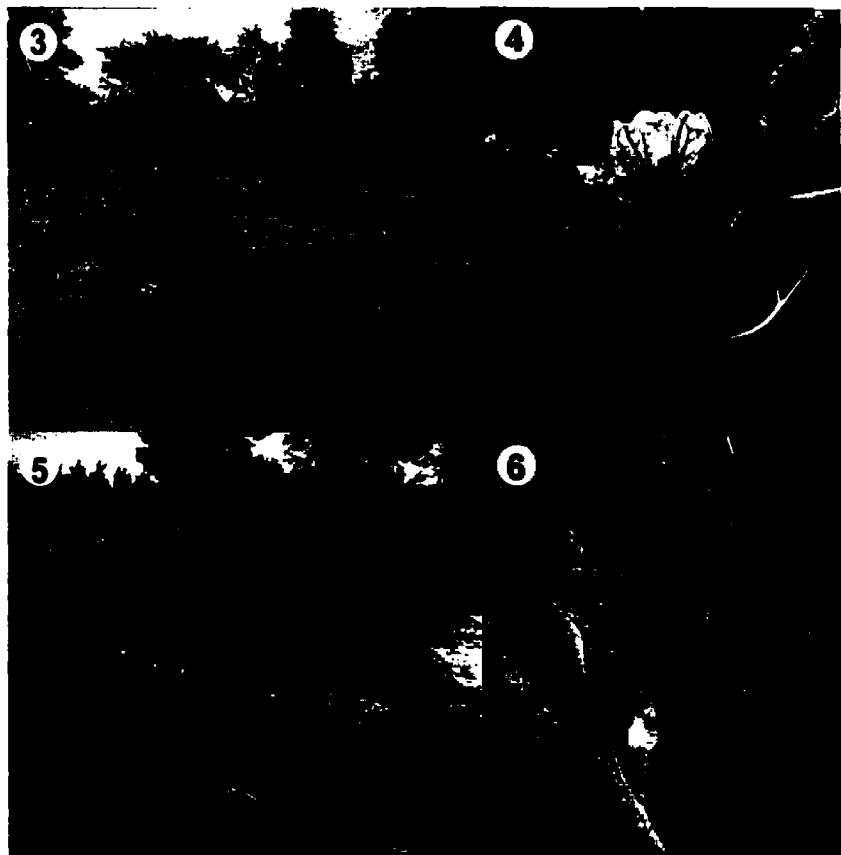
**Fig. 3.** "Sugar Baby"

**Fig. 5.** Tsamma melon

**Figs. 4 and 6.** Close up of "Sugar Baby" and Tsamma melon habit showing the position of the flower (Fl) relative to the leaf (L) and tendril (Td). Flower is at the axil of the leaf, opposite the tendril.

**Fig. 4.** "Sugar Baby"

**Fig. 6.** Tsamma melon



## CHAPTER 2

### FLORAL DEVELOPMENT IN *CITRULLUS LANATUS*: VAR. *LANATUS* AND *CITROIDES*

#### 2.1 INTRODUCTION

*Citrullus* is a small genus of four species, with a distribution ranging from the Mediterranean to India and Ceylon and throughout the greater part of Africa (Meeuse, 1962). *C. lanatus* is the only species of economic importance, and encompasses both the cultivated (var. *lanatus*) and wild forms (var. *citroides*) of watermelon (Robinson and Decker-Walters, 1997; Shimotsuma, 1963). The two botanical varieties have similar vegetative characters (Bailey, 1930), and differ mainly in fruit characters. For instance, the wild form known as Tsamma melon (var. *citroides*) can survive for months in the desert (Knight, 1995, Botha, 1982) after abscission from the parent plant whereas the cultivated forms can barely survive for a month even with refrigeration facilities.

Previous studies on cucurbits have shown that mature fruit shape and stripe pattern are evident in the immature ovary (Robinson and Decker – Walters, 1997). These findings suggest that floral developmental studies of the pistillate flower could shed some light on the differences exhibited by fruits at maturity. However, floral and fruit developmental studies are lacking in *C. lanatus*. In fact, not a single thorough floral developmental study has been conducted on the genus. Thus, statements made about *Citrullus* have been generalizations based on studies of other members of the Cucurbitaceae such as *Cucumis* and *Cucurbita* (Whitaker and Davis, 1962). The only known study of floral biology of var. *lanatus* (cultivated watermelon) lacks illustrations of the developmental pathways (Katrodia et al., 1974).

Studies of floral development are widely documented for other monoecious, economically important cucurbits such as *Cucumis* (Judson, 1928; Judson, 1929; Nitsch, et al., 1952; Atsmon and Galun, 1960; Galun et al., 1963; Goffinet, 1990) and *Cucurbita* (Nitsch, et al., 1952; Pereira, 1968; Scheerens et al., 1987). These studies have revealed that a diversity of sex expression exists within the

monoecious cucurbits. In *Cucumis*, all flowers are bisexual early during their ontogeny, and separate into functional sexes when development of one of the reproductive organs is inhibited. In contrast, in *Cucurbita* only the pistillate flower is bisexual during early developmental stages. Because the floral development of *Citrullus* has not been investigated, it is not known how staminate and pistillate flowers are achieved in the genus.

### Objectives

The objectives of this study are threefold; 1) to examine and document floral organogenesis in *C. lanatus*, 2) to compare the floral development of pistillate flowers in var. *lanatus* (cultivated watermelon) and var. *citroides* (Tsamma melon) to determine if there are any differences in development that may eventually influence the keeping quality of mature fruits, and 3) to critically examine floral development in staminate and pistillate flowers to determine whether or not the floral primordium in *C. lanatus* is bisexual during the early stages of ontogeny.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Seed Germination

Seeds of *Citrullus lanatus* var. *citroides* were obtained from the National Plant Germplasm System in the USDA: S-9 Plant Germplasm collection, Griffin, Georgia, USA. Two accessions: PI 532669 148 and PI 542114 were used in this study. The accessions were collected from Botswana under the name var. *citroides*. The two accessions are from different localities; the former accession was collected from Central Kalahari Game Reserve (CKGR) whereas the latter was collected from Moremi Game Reserve (MGR). The cultivated watermelon, *Citrullus lanatus* var. *lanatus* cv. "Sugar Baby" was selected for comparison with Tsamma melon. It was chosen for its relatively small sized fruit and short life cycle. Seeds of "Sugar Baby" were purchased from Stokes' and Ontario seed companies. Seeds of both varieties were pre-soaked in water for 8 hours and germinated in Petri dishes containing a single sheet of Whatman filter paper. Each Petri dish contained 8 seeds. The Petri dishes were placed in a growth chamber (16 h/day) at 27°C. *C. lanatus* var. *citroides* seeds were further treated with 100ppm Streptomycin to prevent any

bacterial infection that may be activated in the soil (Dr Jarret, USDA; personal communication). In the first germination trial of Tsamma melon, 12 out of 50 seeds germinated. The second germination trial involved soaking them in water overnight before incubation in the growth chamber, and resulted in the germination of 36 out of 50 seeds. "Sugar Baby" seeds had a germination percentage of 89%.

### **2.2.2 Transplanting**

All the material used in this study was started from seed in the greenhouse, and then transplanted out into the field when the danger of frost had passed. After germination, seedlings of both varieties were transplanted into 4-inch pots containing a 3:1 substrate of Pro-Mix to Turface and moved into the greenhouse. The seedlings were fertilized with a 20:20:20 fertilizer 2 weeks after being transplanted into pots. 108 seedlings (70 var. *lanatus*, 38 var. *citroides*) were transplanted into the field at the University of Guelph Cambridge Research Farm when they were a month old. The seedlings were kept for a week outside the greenhouse to harden prior to transplanting. In the field, each variety was planted in single row plots with a row-to-row distance of 2m, a plant-to-plant distance of 1.5m and a 6m distance between the two varieties.

### **2.2.3 Sampling Protocol and Techniques**

Flowering shoots were excised from the parent plant and immediately fixed in FAA (1:1:9:9 of 10% formalin: acetic acid: 95% ethanol: distilled water) to be examined later in the laboratory. Since *C. lanatus* has indeterminate axes, a flowering shoot had a wide range of developmental stages. For each variety, more than 50 flowering shoots were sampled for developmental and histological studies. The floral developmental study was conducted using epi-illumination light microscopy, scanning electron microscopy and paraffin serial sectioning.

#### **2.2.3.1 Epi-illumination Light Microscopy**

For observations with epi-illumination light microscopy, the fixed material were stained with 0.1% nigrosin before and after dissection and mounted on a custom-made sample holder. The floral apices were viewed using a Zeiss photomicroscope III fitted with Leitz Ultropak dipping cones (Charlton et al., 1989), photographed with

a digital camera (Sony 750) using Northern Eclipse and imported into Adobe Photoshop version 5.0.

#### **2.2.3.2 Scanning Electron Microscopy (SEM)**

Some of the specimens examined under epi-illumination light microscopy were also viewed using SEM. The floral apices were dehydrated in a graded ethanol series, critical point dried, mounted on metal stubs, coated with 600 Angstroms of gold – palladium in a Hummer V sputter coater, and examined using a Scanning Electron Microscope (Hitachi S-570 L). The images captured were scanned from negatives using a Hewlett Packard ScanJet 4c/T and imported into Adobe Photoshop version 5.0.

#### **2.2.3.3 Histology**

For paraffin serial sectioning, floral apices were dehydrated in a tertiary butyl alcohol series and embedded in Paraplast. Longitudinal sections of apices were cut 10 $\mu\text{m}$  thick with a rotary microtome, mounted on Fisher Brand Extra Frost microscope slides, stained with safranin – alcian green, and mounted permanently in Permount mounting media (Johansen, 1940). Slides were viewed with a Zeiss photomicroscope III, photographed with a digital camera, (Sony 750) and imported into Adobe Photoshop.

### **2.3 RESULTS**

There were no differences observed in terms of the initiation, order and development of floral organs between Tsamma melon and "Sugar Baby". The only difference observed involved the variability in number of stamens and carpels in "Sugar Baby". Since all aspects of floral development for the two varieties are similar, results will be reported for only one variety, Tsamma melon. For "Sugar Baby", only the variability in number of stamens and carpels will be reported.

### **2.3.1 Organography**

*Citrullus lanatus* is an annual vine that grows along the ground in a weed-like manner (Fig. 3 and 4). The leaves are simple, alternate and lobed. The tendrils are branched, and occur at the axil of the leaf. The species bears both staminate and pistillate flowers on the same plant.

For both varieties, the plants display a very skewed sex ratio: with about 7-9 males to 1 female flower along the vine. The unisexual flowers occur singly in the axil of a leaf, and opposite the tendril (Fig. 5 and 6). In both sexes, the calyx is made up of five small green sepals, and the corolla consists of five yellow petal lobes, which are subtended by a hypanthium.

There are no rudiments of the pistil on the male flower. The androecium, which is attached to the hypanthium, consists of three stamens: two large bi-thecal ones, and a small mono-thecal one. The male flower has a tri-lobed nectary situated at the base of the flower.

The pistillate flower is bisexual early in ontogeny, but development of the androecium is arrested, leaving three vestigial stamens (staminodes). The pistillate flower has an inferior ovary located below the hypanthium. The gynoecium is made up of three free separate carpels with anatropous pendulous ovules, a short 3-parted columnar style and three stigmatic lobes. A nectary ring surrounds the base of the stigma-style complex. The general arrangement of floral organs in both the staminate and pistillate flower is the same for the two varieties (Fig 7 and 8).

### **2.3.2 Organogenesis**

The initiation, order and development of organs in the staminate and pistillate flowers of var. *citroides* and *lanatus* were investigated. In order to study floral development, progressively younger flowers were examined and then the developmental sequence was reconstructed.

#### **2.3.2.1 *C. lanatus* var.*citroides* (Tamma melon)**

##### ***Staminate flower***

The initiation of the floral meristem from the axillary meristem occurs at a very early stage of shoot development. Immediately after initiation in the axil of a

leaf, the axillary meristem elongates distally on one end, initiating a bract primordium (Fig. 9). After this stage, the axillary meristem increases in size and bifurcates into vegetative and reproductive primordia (Fig. 10). Then the vegetative primordium further bifurcates into tendril and axillary bud primordia (Fig. 11). At the time of initiation, the flower bud primordium can be distinguished by its dome shape (Fig. 12). As growth progresses, the floral apex becomes larger and flattened. After becoming flattened, the first sepal (M) is initiated in the median adaxial position, on the edge of the floral apex (Fig. 13). By this time, trichomes can already be seen on the initiating sepal primordia. The subsequent sepals are initiated in a unidirectional order. The two lateral sepals (L) appear to be initiated simultaneously (Fig. 14). The two abaxial sepals are initiated last, successively. The fourth sepal (Ab1) is initiated first, (Fig. 15), followed by the fifth one (Ab2) adjacent to the leaf lobe (Fig. 16). Elongation of the primordium below the base of the sepals results in a concave depression, the earliest stage of hypanthium (sometimes referred to as floral tube (Judd, et al., 1999)) development (Fig. 18). As the sepals continue to develop, their lobes arch over the centre of the flower and enclose the petals (Fig. 17). The sepals continue to increase in size until maturity, when the lobes curl away from the centre of the flower (not shown).

The petal primordia initiate following the sepals in antesepalous positions. The primordia may be initiated before the fifth sepal primordium has completely developed, or immediately after all the sepals have been initiated. The first petal primordium is initiated between the first and one of the two lateral sepal primordia (Fig. 16), and the rest are initiated in rapid succession (Fig. 18) making it difficult to determine the order of initiation. As the petal primordia enlarge, a depression develops due to the upgrowth of the hypanthial wall (Fig. 19). At about the time of stamen initiation, the petal lobes begin to increase in size. Eventually the lobes curve inwards with their tips meeting at the centre, above the cavity (Fig. 20).

Stamen primordia are initiated following the sepals on the rim of the hypanthial wall. Stamen initiation begins with the smaller mono-thecal stamen primordium on the abaxial side of the floral apex, followed by the bi-thecal stamen to the left, and then the second bi-thecal stamen (Fig. 21 and 22). At maturity, each of the larger bi-thecal stamens is adaxial in position, and each stamen lobe is alternate with a petal primordium. The smaller stamen is alternate with a petal, and

opposite a sepal. As development progresses, the stamens elongate and expand laterally to utilize all the space in the upper rim of the hypanthium (Fig. 23). Following this increase in size, each stamen differentiates into three regions: a broad filament, connective tissue and thecae (Fig. 24). As growth continues, the thecae of the stamens bend towards the centre of the hypanthium, and their connective tissues fit closely together. As the connective tissues of the three stamens come into contact, the anther is folded away from the centre of the flower. At this time, two thecae are evident on the two larger stamens. After this stage, the thecae elongate, and fold. The double stamens fold three times, whereas the smaller one folds one and half-way round (Fig. 25). Further dissection of the stamens demonstrated that the larger stamens have double microsporangia and the smaller one has a single microsporangium (not shown). As development progresses, further folding and twisting of the thecae leads to the mature sigmoid shape of the stamens (Fig. 26). A nectary is formed at the base of the male flower after the stamen primordia have differentiated into elaborate filament, connective tissue and thecae (Fig. 27). The nectary primordium initiates as a small round mass of tissue between the bases of the filaments (Fig. 28, 29 and 30). The primordium flattens and spreads between the bases of the three filaments, forming a tri-lobed gland-like structure at the base of the mature flower (Fig. 31). The adaxial surface of the nectary is perforated with numerous nectariferous pores (Fig. 32).

#### *Pistillate flower*

Distinguishing between the staminate and pistillate flower prior to the formation of the ovary is difficult. This difficulty is further confounded by the skewed sex ratio reported earlier, making it very difficult to capture very early stages of carpel primordium initiation. Initiation and development of sepals and petals in the pistillate flower are identical to that reported for the staminate flower (Fig. 13-18). The stamen primordia also initiate in the same order and sequence as in the male flower (Fig. 21 and 22), except that further development of the primordia is arrested. Consequently, the stamen primordia fail to differentiate into elaborate thecae, resulting in the formation of three staminodes restricted to the rim of the hypanthium (Fig. 35).

Initiation of carpel primordia occurs immediately after stamen primordia initiation. The carpel primordia initiate as three blunt protuberances near the base of the hypanthium, below and internal to the staminodes (Fig. 33). As growth progresses, each of the three carpel primordia become "horse-shoe" shaped (Fig. 34) and extends down towards the base of the developing ovary facing towards the centre of the flower (Fig. 35 and 36). At this stage, the staminode primordia are very conspicuous. The staminodes enlarge slightly, developing a short filament with traces of thecae, but fail to elongate and twist (Fig. 37).

Once the "horse-shoe" shaped carpel primordium reaches the centre of the ovary, the margins of each lobe become folded towards the centre of the ovary (Fig. 37 and 38), expand tangentially and differentiate into the placenta (Fig. 47). The margins of the individual carpels do not fuse. They remain separated by a septal partition (Fig. 37, and 48), which is parenchymatous in nature. The style starts to differentiate after the placentae have been formed. As the style elongates, it pushes the folded distal portion of the carpel primordium above the hypanthium rim and initiates stigma differentiation (Fig. 39). At this point, stamen development is arrested, and the stigmatic lobes occupy the centre of the flower. When the folded upper portions of the carpel primordia reach the hypanthium rim, they emerge between the three staminodes and curve away from the centre of the flower towards the hypanthium wall to form three distinct stigmatic lobes (Fig. 39 and 40). Further elongation of the style occurs after the stigmatic lobes have extended fully above the three staminodes (Fig 41), resulting in a short column of tissue characterized by three slits at the stigma-style juncture (Fig. 42). A ring of nectary tissue is formed at the base of the style. The nectary primordium is initiated around the style and alternate to the three staminodes (Fig. 43). With further development, the nectary tissue broadens and extends within the base of the staminodes (Fig. 44). Staminal hairs are also apparent on the periphery of the nectary ring. At maturity, the nectary tissue appears as a conspicuous ring surrounding the style (Fig. 45). As in the staminate flower, numerous nectariferous pores perforate this nectary tissue (Fig. 46). Ovule development begins shortly after the differentiation of the stigma-style complex. The ovules are initiated as small bumps on the edges of each placenta (Fig. 47 and 48). Placentation is parietal (Fig. 48). The ovules are pendulous, anatropous (Fig 49) and bi-tegmic at maturity (Fig.

50). A cross section through the ovary shows that there are no specialized locules in the carpels; rather the developing ovules are embedded in parenchyma tissue (Fig. 48).

### 2.3.2.2 Variability in number of stamens and carpels in "Sugar Baby" (var. *lanatus*).

#### *Variability in number of stamens*

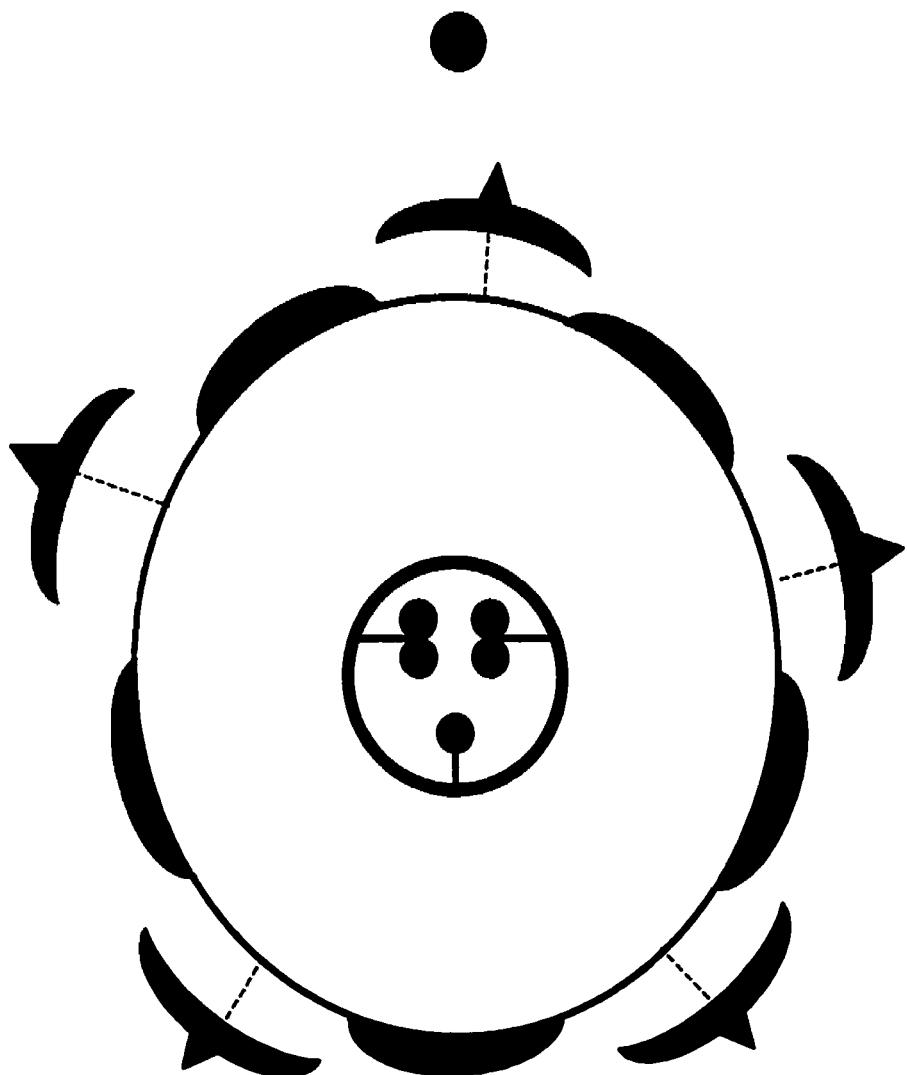
Out of more than 50 shoot apices that were examined for floral development of the staminate flower, two developing flowers had four stamens, instead of the usual three. The flowers were found on the same plant along with other three-staminate flowers. Of the four stamens, two are bi-thecal whereas the other two are mono-thecal (Fig. 52). The fourth stamen primordium is initiated between the bi-thecal stamens, and opposite the smaller single one (Fig. 51). This fourth stamen appears to be slightly smaller than the other mono-thecal one. The initiation and order of development of organs in these flowers could not be determined because younger stages were not encountered.

#### *Variability in number of carpels*

Out of more than 50 shoot apices that were examined for floral development of the pistillate flower, two developing flowers were encountered with an "abnormal" number of carpels. The flowers examined had two large carpels, instead of the "normal" three reported for *Tsamma* melon (Fig. 53 and 54). The number of staminodes in either case did not change, however. The initiation and order of development of organs in these flowers could not be determined because younger stages were not encountered.

**PLATE 4**

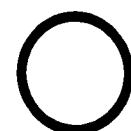
**Figs. 7.** Floral diagram of the staminate flower of *C. lanatus*.



= sepals



= petals



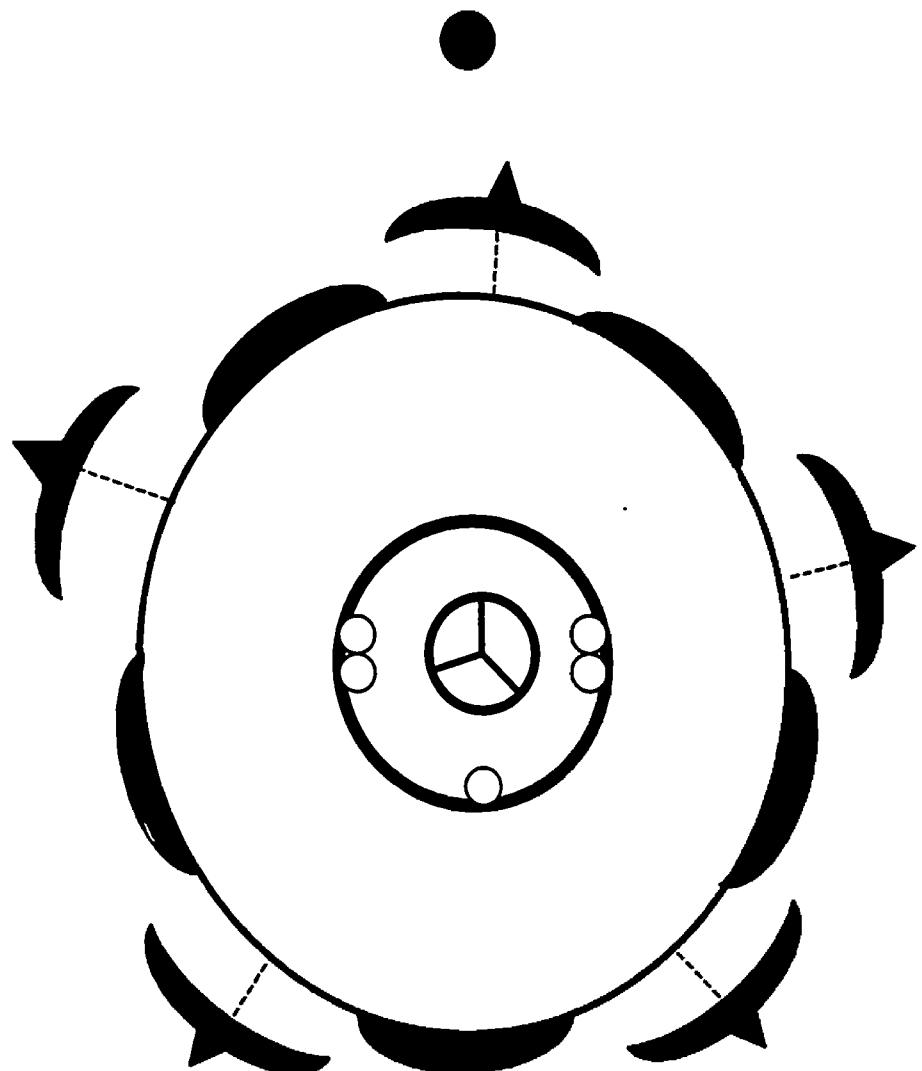
= hypanthium



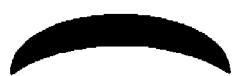
= stamens

## **PLATE 5**

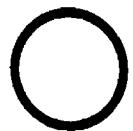
**Fig. 8.** Floral diagram of the pistillate flower of *C. lanatus*.



= sepals



= petals



= hypanthium



= staminodes



= gynoecium

## **PLATE 6**

**Figs. 9 – 12.** Scanning electron micrographs showing the developing staminate floral primordium in var. *citroides*.

**Fig. 9.** Top view of axillary meristem (V) showing bract (B) initiation. Scale bar = 60  $\mu\text{m}$ .

**Fig. 10.** Top view of axillary meristem in the early stage of dividing into vegetative (V) and reproductive (pF) axes. Note the bract (B). Scale bar = 60  $\mu\text{m}$ .

**Fig. 11.** Third stage of axillary meristem divergence showing the differentiated tendril primordium (Td), axillary bud (Axb) and floral primordium (F) just about to separate. Scale bar = 60  $\mu\text{m}$ .

**Fig. 12.** Top view of developing floral primordium (F) after diverging from the vegetative meristem. Bract (B) has slightly enlarged. Scale bar = 60  $\mu\text{m}$ .



## **PLATE 7**

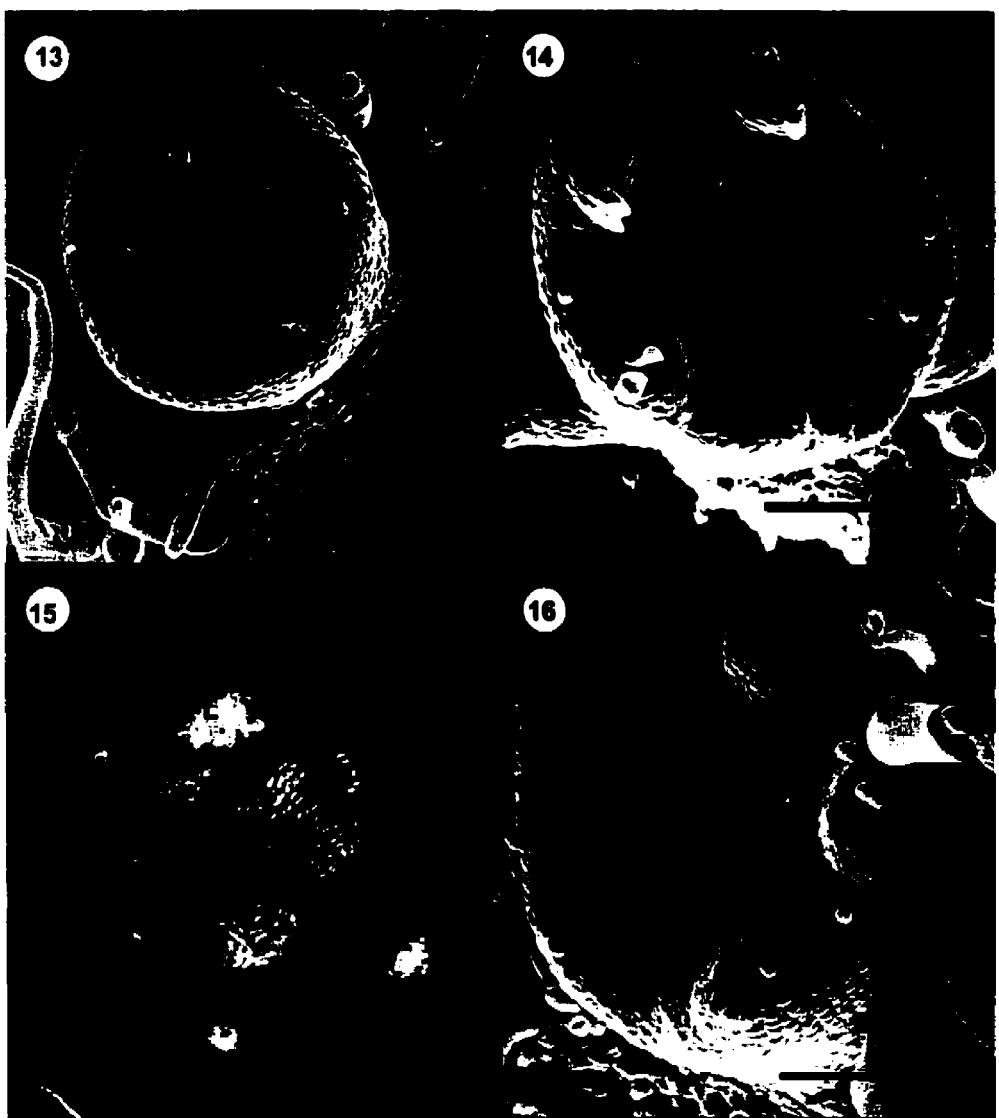
**Figs. 13-16.** Scanning electron micrographs of early staminate floral development in var. *citroides* showing sepal and petal initiation.

**Fig. 13.** Adaxial view of floral primordium showing initiation of the first sepal (M). Scale bar = 75  $\mu\text{m}$ .

**Fig. 14.** Adaxial view of developing floral primordium showing development of the two lateral sepal primordia (L). Scale bar = 100  $\mu\text{m}$ .

**Fig. 15.** Floral primordium showing initiation of one of the two abaxial sepal primordia (Ab1). Scale bar = 1 mm.

**Fig. 16.** Floral primordia showing the development of the last sepal primordium (Ab2), completing the unidirectional pattern of initiation. Note the initiation of the first petal primordia (C1) between the first (M) and one of the second sepal primordia (L) to initiate. Scale bar = 100  $\mu\text{m}$ .



## **PLATE 8**

**Figs. 17-18.** Scanning electron micrographs showing further petal development in var. *citroides*.

**Fig. 17.** Further development of the flower showing the sepals (K) beginning to enclose the petals (C). Scale bar = 75  $\mu\text{m}$ .

**Fig. 18.** Floral primordium showing initiation of remaining petal primordia (C). Note the presence of the developing hypanthium (H). Scale bar = 100  $\mu\text{m}$ .



## **PLATE 9**

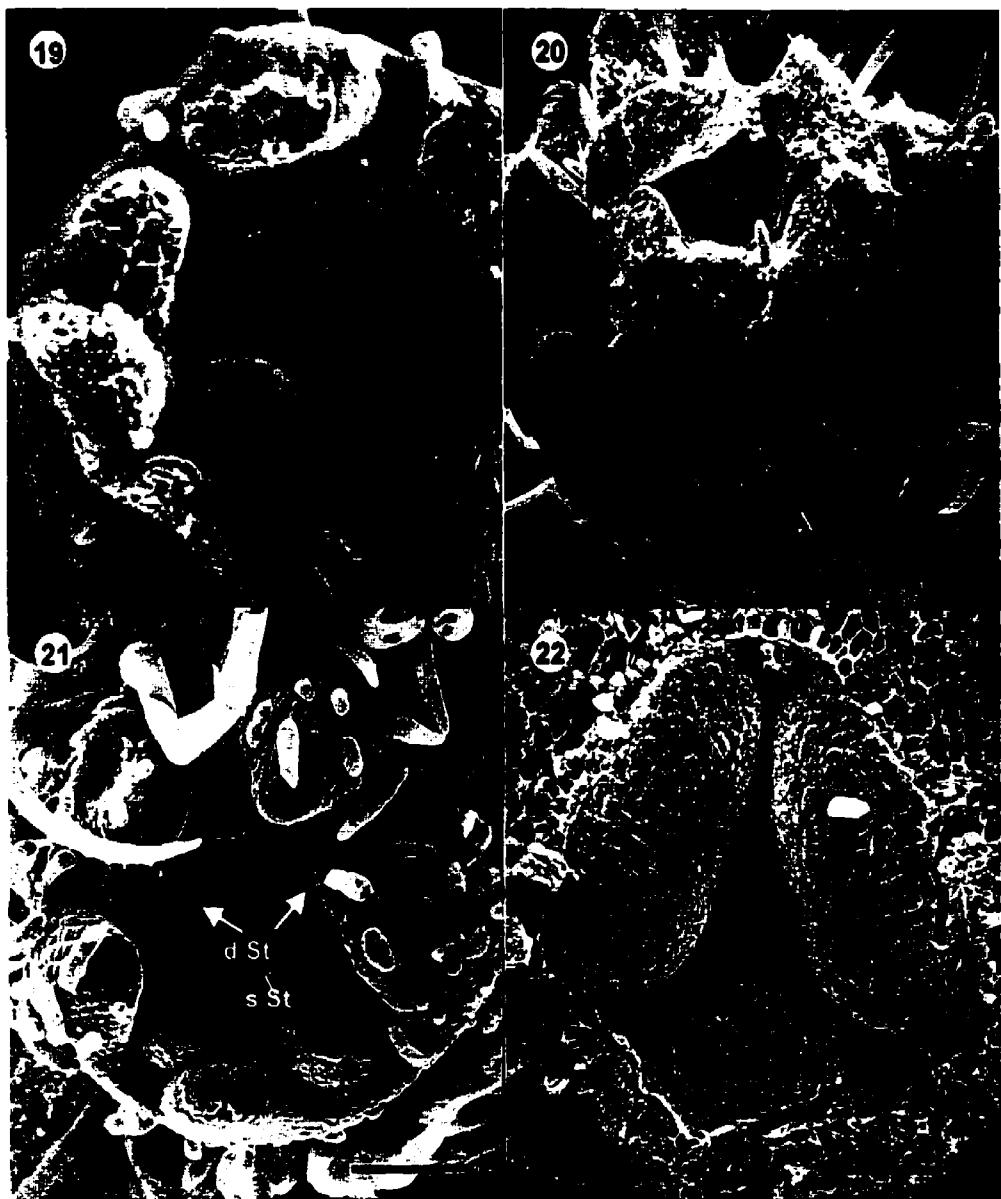
**Figs. 19-22.** Scanning electron micrographs of male floral primordia (var. *citroides*)

**Fig 19.** Male flower showing late stage of petal primordia (C) with the petal lobes arching over the centre of the flower. Scale bar = 176 µm.

**Fig 20.** Same stage as shown in Fig. 19. Sepals and petals removed to show the depression (Cav) where stamen primordia will initiate. Scale bar = 75 µm.

**Fig. 21.** Early stage of stamen primordia development. Note the two bi – thecal stamen (d st) and the single mono – thecal one (s St), and their position relative to the sepals (K) and petals (C). Scale bar = 100 µm.

**Fig. 22.** Same stage as in Fig. 21: Sepals and petals have been removed to show the attachment of the stamen primordia to the hypanthium wall (H). Scale bar = 75 µm.



## **PLATE 10**

**Figs. 23-26.** Scanning electron micrographs of male flowers of var. *citroides* showing development of the stamen primordia. Sepals and petals have been removed.

**Fig. 23.** Note the elongated and expanded stamen primordia (d St and s St). Scale bar = 100 µm.

**Fig 24.** Later stage than in Fig. 23. Stamen primordia differentiating into filament (Fi), connective tissue (Ct) and thecae (T). Scale bar = 150 µm.

**Fig 25.** Later stage than in Fig. 24 showing the folded anthers. The double stamens have folded three times (F1, F2, and F3), and the single stamen one and half-way around (F1, F1/2). Scale bar = 176 µm.

**Fig 26.** Top view of male flower showing stamens at the mature sigmoid state. Sepals and petals have been removed. Scale bar = 38 mm.



## **PLATE 11**

**Figs. 27-28.** Longitudinal paraffin sections through developing male flower of var. *citroides*.

**Fig. 27.** Lower portion of male flower showing developed stamens with elaborate filament (Fi), connective tissue (Ct) and thecae (T), but with no sign of inhibited carpel primordia (arrow). Scale bar = 250  $\mu\text{m}$ .

**Fig 28.** Later stage than the one shown in Fig. 27, showing the initiation of the nectary primordium (arrow) as one single mass of tissue. Note the filament (Fi) and microsporangia (Mi). Scale bar = 200  $\mu\text{m}$ .

**Figs. 29-30.** Epi-illumination micrographs of male flowers of var. *citroides*.

**Fig. 29.** Longitudinal dissection of male flower at the same stage as shown in Fig. 28. Note the developing nectary primordium (NP) at the base of the flower. Scale bar = .7 mm.

**Fig. 30.** Same stage as shown in Fig. 29 with the stamens (r St) removed to show the overview of the nectary primordium (arrow). Scale bar = .7 mm.

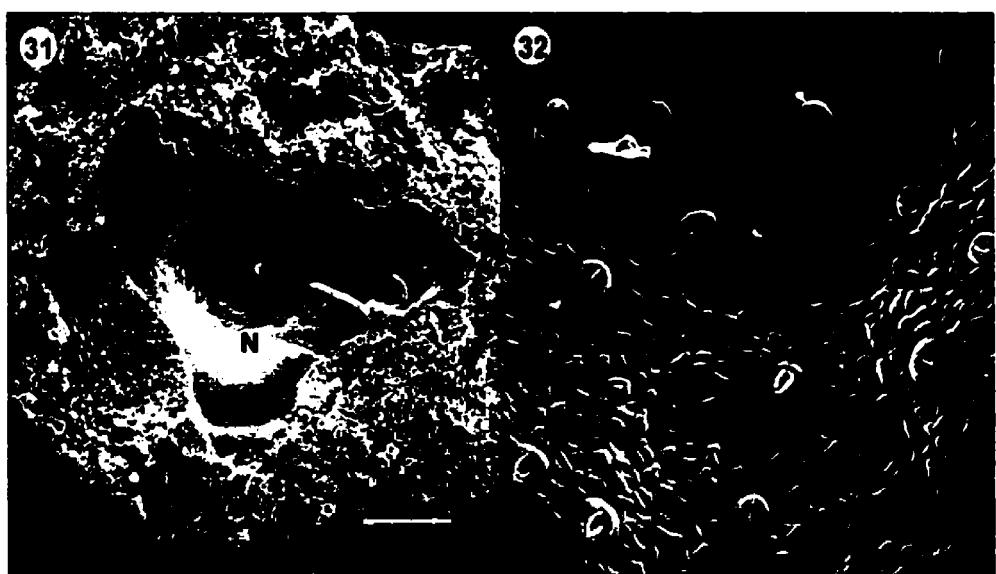


## **PLATE 12**

**Fig. 31-32.** Scanning electron micrographs of male flower nectary tissue in var. *citroides*.

**Fig. 31.** Base of the male flower showing the tri-lobed nectary tissue (N). Stamens have been removed (r St). Scale bar = 60 mm.

**Figs. 32.** Close up of nectary tissue in Fig. 31 showing nectariferous pores (arrows). Scale bar = 60  $\mu$ m.



## PLATE 13

Figs. 33-38. Epi-illumination and scanning electron micrographs showing early and later stages of carpel development in var. *citroides*. Sepals and petals have been removed.

**Fig. 33.** Epi-illumination: Flower viewed from above showing initiation of carpel primordia (arrows) at the base of the staminodes (Std). Sepals, petals and two staminodes and one carpel primordium have been removed. Scale bar = .5 mm.

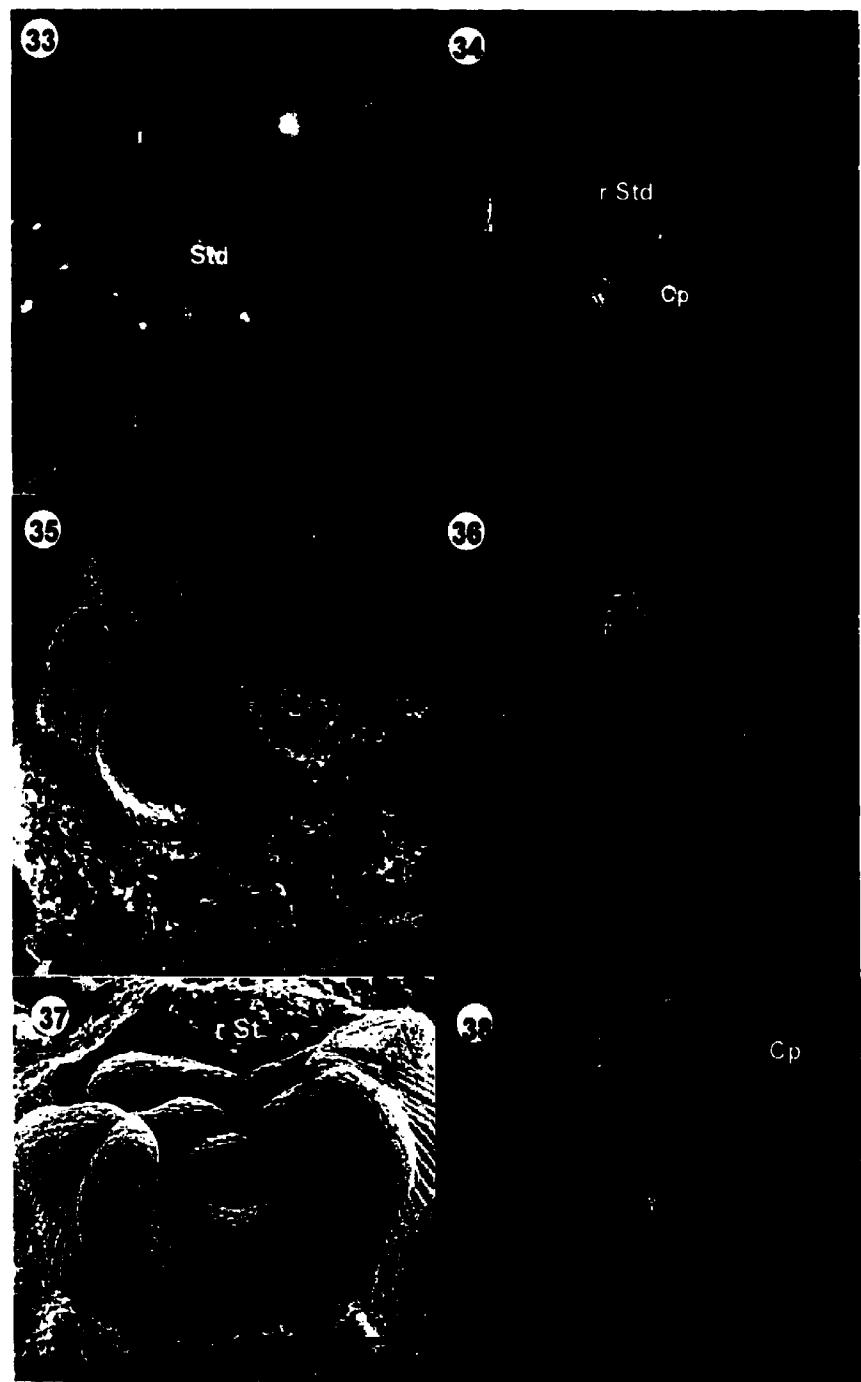
**Fig. 34.** Epi-illumination: Later stage than in Fig. 33 showing the carpel primordia (Cp) becoming "horse-shoe" shaped. Two staminodes have been completely removed, leaving a portion of only one staminode (r Std). Scale bar = .7 mm.

**Fig. 35.** Carpel primordia (arrow) elongating, as the hypanthial wall extends up. Note three staminodes (Std) arching over the carpel primordia. Scale bar = 120  $\mu$ m.

**Fig. 36.** Same stage as in Fig. 35: Longitudinal dissection through ovary showing elongating "horse-shoe" (arrow) carpel primordium. Two other carpel primodia and staminodes have been removed. Scale bar = 1 mm.

**Fig. 37.** SEM preparation: Later stage of carpel development showing each carpel primordium (Cp) folding towards the centre of the ovary. Carpels are displacing the staminodes (Std) and occupying the central part of the flower. Scale bar = 120  $\mu$ m.

**Fig. 38.** Epi – illumination: Same stage as above with sepals, petals and two staminodes removed to show the folding carpel primordia (Cp). Note the septal ridge (arrow) between individual carpels. Staminodes have been removed. Scale bar = 1 mm.



## **PLATE 14**

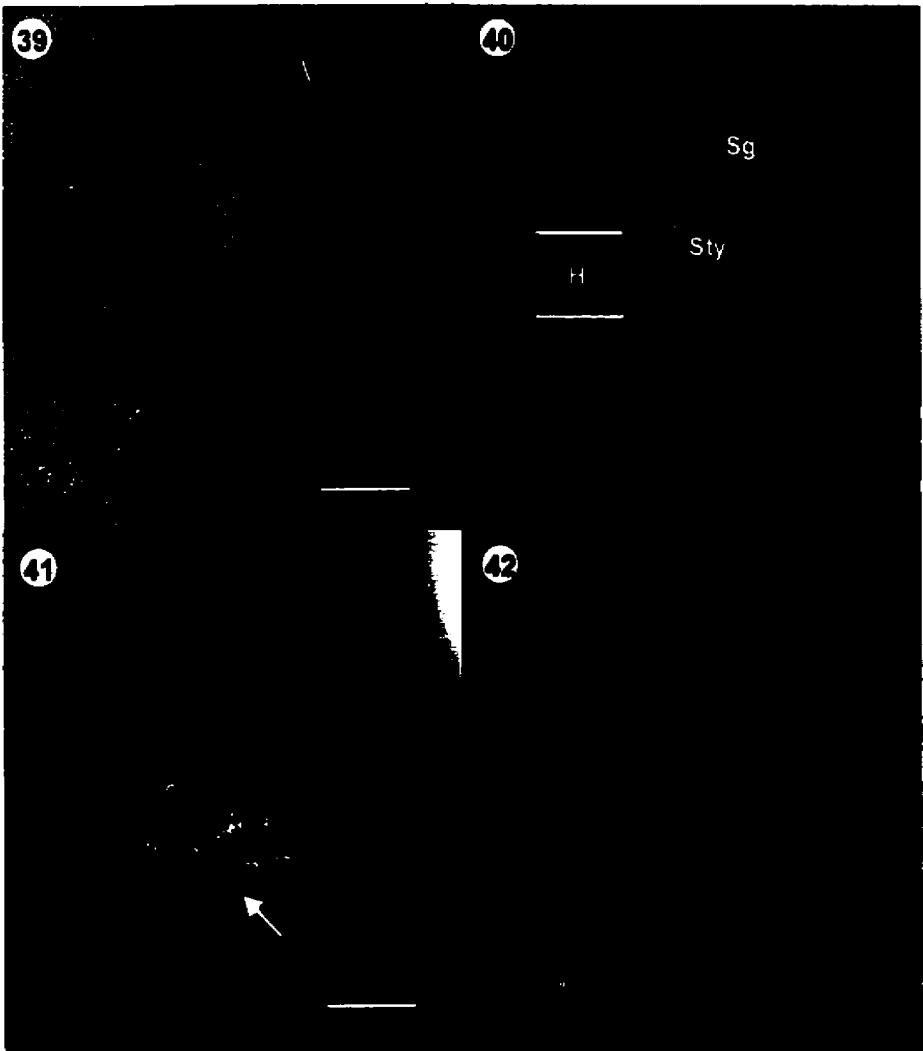
**Figs. 39-42.** Epi-illumination micrographs showing later stages of carpel development in var. *citroides*.

**Fig. 39.** Side view of ovary at stage when upper portions of carpels are differentiating into stigmatic lobes (Sg). Scale bar = .5 mm.

**Fig. 40.** Longitudinal dissection of female flower showing early differentiation of stigma (Sg)-style (Sty) complex. Hypanthium (H) also evident at this stage. Scale bar = .5 mm.

**Fig. 41.** A dissected female flower viewed from above. Note the fully developed stigmatic lobes (Sg) and less conspicuous staminode (arrow). Scale bar = .5 mm.

**Fig. 42.** Longitudinal dissection of female flower at the same stage as shown in Fig. 41, showing the fully developed columnar style (Sty) and nectary tissue (N), at the base of the style. Scale bar = .5 mm.



## PLATE 15

**Figs. 43-46.** Scanning electron micrographs showing female flower nectary tissue of var. *citroides*.

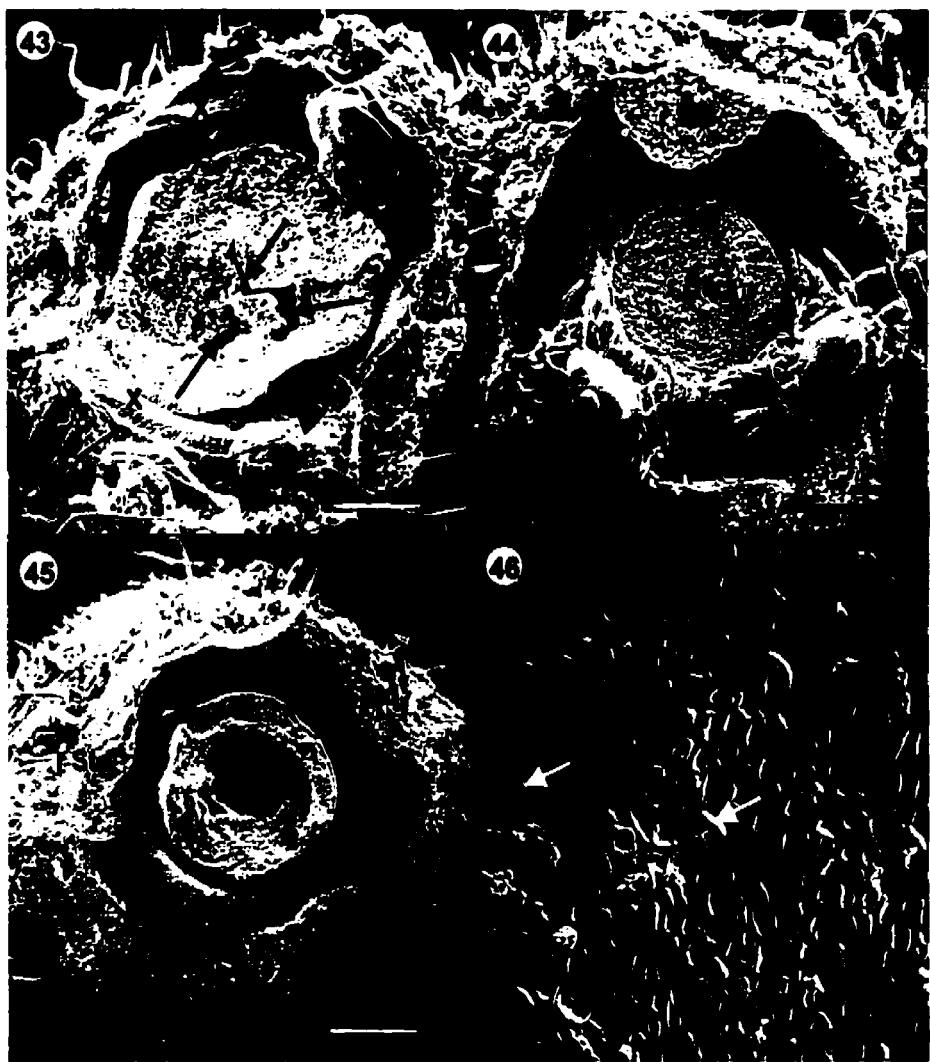
**Fig. 43.** Dissection of female flower showing early stage of nectary development. Nectary tissue (X) initiates between the three staminodes. Two staminodes (r Std) have been removed to show the thin layer of nectary tissue (arrow-head) at the base of the staminodes. Note three slits (arrows) corresponding to the three stigmatic lobes on the remnants of the style.

Scale bar = 30 mm.

**Fig. 44.** Later stage than in Fig. 43 showing further development of the nectary tissue. One staminode (r Std) has been removed to show the nectary extending within the staminodes (arrow). Note the hairs (arrow-head) surrounding the staminodes. Sepals, petals, stigma and style (r Sty) have been removed. Scale bar = 86 mm.

**Fig. 45.** Dissection of female flower at anthesis: style (r Sty) and staminodes (r Std) have been completely removed to display the nectary ring at the base of the style. Scale bar = 75 mm.

**Fig. 46.** Close-up of nectary ring in Fig. 45 showing nectariferous pores (arrows). Scale bar = 50 µm.



## **PLATE 16**

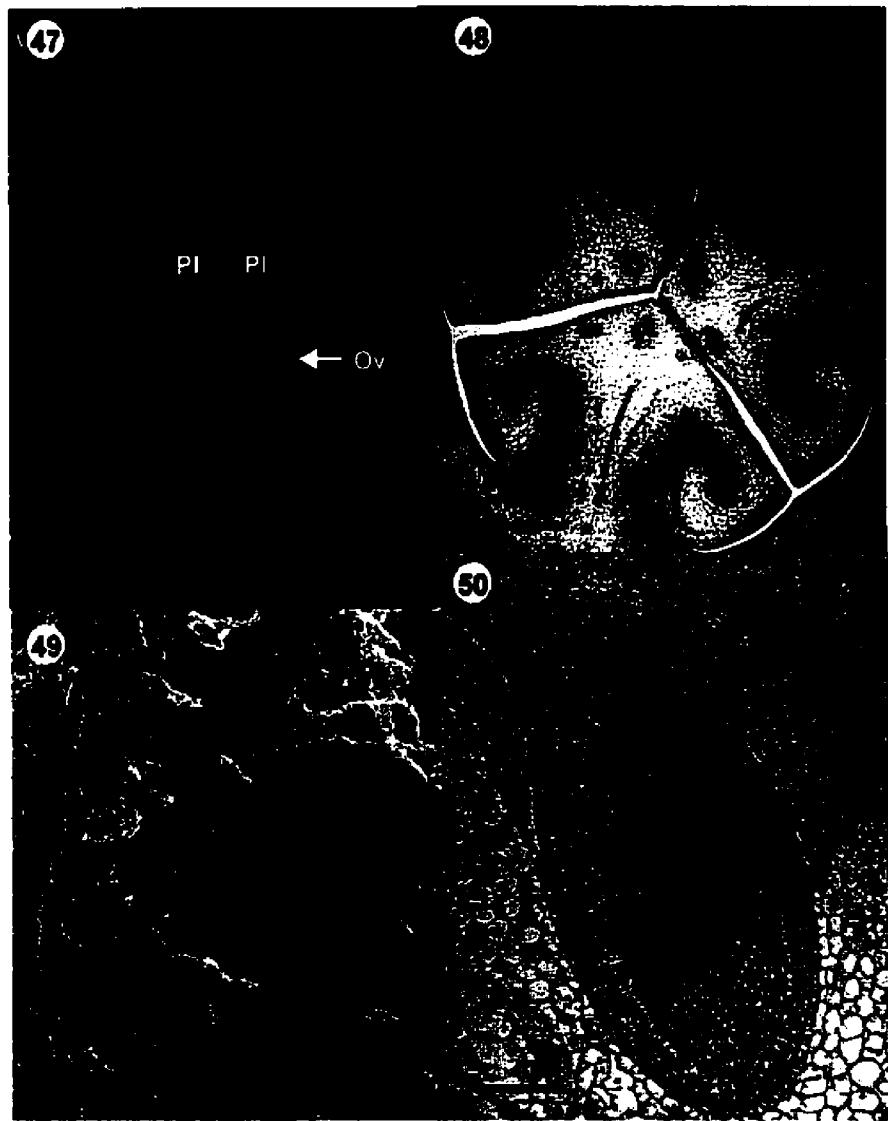
**Figs. 47-50.** Longitudinal and cross section of ovary showing ovule initiation and position in var. *citroides*.

**Fig. 47.** Epi-illumination micrograph: Longitudinal section through the ovary showing abaxial view of one carpel with ovules (Ov) initiating on the placentae (Pl). Scale bar = .7 mm.

**Fig. 48.** Transverse paraffin sections through the developing carpel primordia at same stage as shown above: Ovule primordium (Ov) initiating on the placenta (Pl). Note the parietal placentation and partition (x) between individual carpels. Scale bar = 250  $\mu$ m.

**Fig. 49.** Scanning electron micrograph: Transverse section of ovary showing later stage of ovule development. Note the pendulous and anatropous position of the ovule (Ov) on the placenta (Pl). Scale bar = 720  $\mu$ m.

**Fig. 50.** Transverse paraffin section through the ovary showing the bitegmic ovule (Ov). Note the outer (O<sub>i</sub>) and (I<sub>i</sub>) integuments of the ovule. Scale bar = 150  $\mu$ m.



50

## **PLATE 17**

**Fig. 51-52.** Epi-illumination micrographs of staminate flower of "Sugar Baby" (var *lanatus*). Sepals and petals have been removed.

**Fig. 51.** Dissected male flower showing four stamen primordia: two bi-thecal (d St) and two mono-thecal (s St). Scale bar = 1 mm.

**Fig. 52.** Later stage than above. Stamen primordia have elongated and expanded. The last single stamen primordium (s St2) to initiate appears slightly smaller. Scale bar = 1 mm.

51

52

s St

d St d St

s St

s St2

d St  
s St

d St

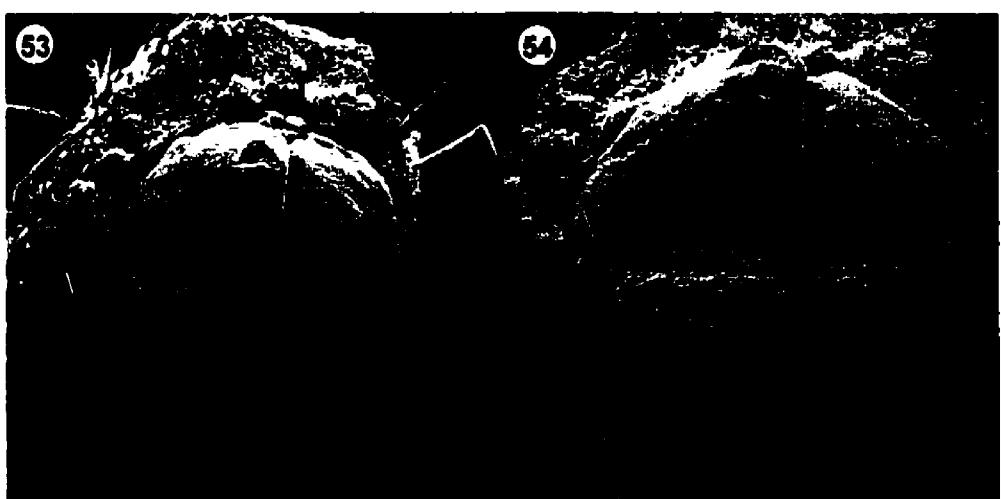
52

PLATE 18

**Fig. 53-54.** Scanning electron micrographs of female flower of var *lanatus*.

**Fig. 53.** Dissected female flower showing two large stigmas (Sg), instead of three. Note staminodes (Std) below the stigmatic lobes. Scale bar = 60 mm.

**Fig. 54.** Close-up view of Fig. 53 captured at a different angle to show the staminodes (Std) below the stigma (Sg). Scale bar = .38 mm.



## **2.4 DISCUSSION**

Results of this study have shown that the development of organs in the pistillate and staminate flower is the same in both var. *lanatus* (Sugar Baby) and var. *citroides* (Tsamma melon). This study is the first to present floral development in the genus *Citrullus*.

### **Floral development of the pistillate flower in var. *citroides* and *lanatus***

The initiation of primordia, sequence and development of the pistillate flower in Tsamma melon and "Sugar Baby" were identical except for rare incidences when two or four carpels, instead of three were formed in "Sugar Baby". The sepals were the first organs to form in a unidirectional pattern, followed by the petals and the staminodes. Meristematic upgrowth below the bases of the developing sepal and petal primordia resulted in the formation of a hypanthium. The carpel primordia initiated last, and were alternate and equidistant to the three staminodes. Both the stamen and carpel primordia were initiated on the hypanthial wall. Except for the phyllotactic pattern of sepal initiation, the floral development pathway in *C. lanatus* appears similar to that described for *Cucumis* (Judson, 1929; Goffinet, 1990). Goffinet's (1990) work on *C. sativus* shows that sepals arise in a 2/5 phyllotactic pattern. In contrast, in *C. lanatus* sepals arise in a unidirectional pattern starting from the abaxial to the adaxial side of the floral primordium.

There is a controversy regarding the interpretation of the hypanthium and the nature of the wall of the inferior ovary. According to Kaplan (1967), the wall of an inferior ovary can be interpreted as either appendicular or receptacular in nature. Sattler (1974), on the other hand claims that the formation of the ovary wall of an inferior ovary cannot be determined empirically, and therefore the ovary should be considered acarpellate. The nature of the wall of the inferior ovary was not considered in this study. In this thesis, the word "hypanthium" was used to describe the floral tube formed above the base of the ovary (joining the perianth members and the ovary) without any attempt to interpret its nature.

At anthesis, the ovary has three carpels, a tri-lobed style and three stigmatic lobes. A ring of nectary tissue develops at the base of the style, within the three

staminodes. This is the first study to report the presence of a nectary ring at the base of the style in *Citrullus*. This finding came as a surprise because the character is widely used in taxonomic keys. For instance, in the "key to different genera of cucurbits occurring in southern Africa", the absence or presence of a nectary ring is used to distinguish between species. Specifically, Meeuse (1962) quotes that *Citrullus* lacks a nectary ring at the base of the style.

In both varieties, the female flower is bisexual during the early stages of ontogeny, but becomes unisexual following the inhibition of stamen development shortly after carpel primordia have initiated. This finding agrees with those of Judson (1929), Nitsch *et al.*, 1952, Atsmon and Galun (1960), Goffinet (1990) in *Cucumis*. The identical development of the female flower in both var. *lanatus* and var. *citroides* suggests that differences observed in keeping quality of mature fruits likely occur after, and not before pollination.

#### Floral development of the staminate flower in var. *citroides* and *lanatus*

The initiation of primordia, sequence and development of the staminate flower in Tsamma melon and Sugar Baby were identical except for rare incidences when four stamens, instead of three were formed in Sugar Baby. The presence of four stamens is not unique to Sugar Baby. The presence of four stamens in species with typically three staminate flowers has been reported in *Cucumis sativus*, *Lagenaria vulgaris* (Chakravarty, 1958) and *Citrullus rheumii* (De Winter, 1990).

Similar to in the pistillate flower, the sepals are the first organs to form in a unidirectional pattern, followed by the petals and then the stamens. The stamen primordia are initiated on the hypanthial wall. Unlike the pistillate flower where the staminode primordia are initiated very early during the course of floral development, no traces of carpel primordia were observed either during the early stages or late stages of floral ontogeny. The next structure to form in the development of the staminate flower was a round mass of tissue, which has been referred to as a nectary primordium in the results section. This primordium arose at a very late stage of floral development; when the stamen primordia had already differentiated into distinct filaments and thecae. The order and sequence of initiation of organs in

the staminate flower suggests that the male flower in *C. lanatus* is not bisexual at any stage of development.

The results of this study do not agree with floral developmental studies conducted on *Cucumis*. Various researchers (Goffinet, 1990; Galun et al., 1963, Atsmon and Galun, 1960 Nitsch, et al., 1952, Judson, 1929, Judson, 1928) have demonstrated that the male flower in *Cucumis* is bisexual during ontogeny. In *Cucumis sativus*, the primordia of the pistillodium initiated immediately after the staminode primordia as three separate entities, and maintained their integrity as floral development progressed (Atsmon and Galun, 1960; Goffinet, 1990). Likewise, the nectary primordium initiated as three upgrowths on the basal abaxial flank of a carpel lobe.

In this study, there were no separate carpel primordia observed initiating below the stamens in the male flower. Also, the only primordia observed did not initiate as three independent structures: rather, it initiated as one structure and became tri-lobed at maturity. It appears that the pressure exerted by the expanding and twisting of anthers, and the limited space between the three stamens caused the nectary to attain the tri-winged shape at maturity.

Based on the timing of initiation, and the nature of the initial nectary primordium, I am inclined to conclude that the male flower in *C. lanatus* is never bisexual at any stage of development. The meristem of the male flower is fully used by the formation of the stamens. If the male floral meristem were potentially bisexual, I would expect the carpel primordia to be initiated immediately after the stamen primordia (Atsmon and Galun, 1960; Goffinet, 1990), not very late when all the floral parts had matured. Since the results of this study have shown that the tri-lobed gland-like structure in the male flower of *C. lanatus* does not originate as carpel primordium, I recommend that the organ be interpreted as a nectary, and not as a pistillodium.

## CHAPTER 3

### FRUIT MICRO-MORPHOLOGY AND ANATOMY OF *CITRULLUS LANATUS*: VAR. *LANATUS* AND VAR. *CITROIDES*

#### 3.1 INTRODUCTION

Watermelon (*Citrullus lanatus*) is one of the oldest crops known to man. It is native to southern Africa (Meeuse, 1962; Whitaker and Bemis, 1976), and introduced elsewhere for commercial cultivation. The species consists of both the cultivated (var. *lanatus*) and wild forms (var. *citroides*) of watermelon. Unlike the domesticated forms, which are commercially grown for consumption, the wild forms are utilised at a local scale in Africa. In Botswana, the native people (Bushmen) use the wild form known as "Tsamma" melon (var. *citroides*) as a source of food and water (Macrone, 1937; Botha, 1982).

The cultivated watermelon and Tsamma melon differ strikingly in keeping quality. Tsamma melon is able to withstand both low and high temperatures without experiencing any chilling injury or decay. As a result, the fruit is able to remain intact and fleshy for more than a year after the parent plant has died (Macrone, 1937; Lee, 1979; Mills, 1980; Arnold *et al.*, 1985; Knight, 1995). In contrast, the cultivated fruit is sensitive to both low and high temperatures. It can only withstand moderate temperatures for a short period of time. For satisfactory storage, a period of 2 weeks at a temperature of 15°C is recommended (Robinson and Decker-Walters, 1997).

Although the use and remarkable keeping quality of the Tsamma melon is widely documented in the literature (Macrone, 1937; Lee, 1979; Mills, 1980; Arnold *et al.*, 1985; Knight, 1995), the reason for this keeping quality is not known. Previous studies that have documented the adaptation of plants to extreme conditions have focused on leaves and roots, and neglected fruits (e.g. Maximov, 1929; Levitt, 1980).

Barber (1909) gave a generalized description of fruit histology of most Cucurbitaceae fruits, including the cultivated watermelon, *C. vulgaris* (Syn. *C. lanatus*). This description still serves as the definitive work on the fruit anatomy of

*Citrullus*. She divided the pericarp of all cucurbit fruits into six distinct tissues: epicarp, hypodermis, outer-mesocarp, middle-mesocarp, inner-mesocarp and endocarp. In watermelon, the first four tissues make up the rind, and the last two the edible flesh. Lai *et al.* (1977) documented a comparative and illustrative account of pericarp anatomy and fruit characters of some cultivars of watermelon (var. *lanatus*). Their study showed that anatomical features played a major role in determining transportation and storage properties of watermelon. They found that rind features, particularly the outer mesocarp tissue, influences the keeping quality of the fruit. Fruits characterized by thin outer mesocarp cell walls were found to be prone to rupturing during storage and transportation.

Epicuticular waxes have been found to play a major role in minimizing water loss through the cuticle in different plant organs (Kerstiens, 1996). In addition to functioning as a barrier to water loss, epicuticular waxes also act as a mechanical barrier against penetration by fungal hyphae and insect mouthparts. Consequently, plant organs lacking epicuticular wax are more prone to fungal infection, than those having epicuticular wax. Craenen *et al* (1997) found that banana and plantain leaves lacking epicuticular wax were more susceptible to a fungal infection causing banana leaf spot compared to bananas with epicuticular wax. When investigating the mechanism of disease resistance in cotton leaves, Ashraf and Zafar (1999) also found that all the resistant lines had considerably higher epicuticular wax content, than the susceptible ones.

When investigating the mode of water conservation in Tsamma melon, Botha (1982) gave a passing reference to the surface anatomy of the fruit. He documented that the stomata were plugged by some material after fruit abscission and attributed this to the apparent cessation of water loss in the fruit. However, he did not determine or speculate on the nature of the "plug". Botha's work, which is to date the only investigation related to the keeping quality of Tsamma melon, did not focus on morphological and anatomical studies but was strictly physiological, and hence the morphological findings that he documented were incidental to the main focus of his work.

## Objective

Given the differences in keeping quality between Tsamma melon (var. *citroides*) and the cultivated watermelon (var. *Ianatus*), the objective of this portion of the thesis was to examine and compare the rind morphology and anatomy of Tsamma melon with the cultivated watermelon in order to identify structural features associated with keeping quality. The differentiation of the rind tissue was also investigated to determine whether structural differences are initiated at fruit set or at maturity. This objective was achieved by comparing Tsamma melon with the cv. Sugar Baby.

## 3.2 MATERIALS AND METHODS

Fruit material used in this chapter was grown from seed at Cambridge Research Station of the University of Guelph (Cambridge, Ontario, Canada). For seedling establishment and growth, see Methods and Materials Chapter 2.

Fruits of both var. *Ianatus* ("Sugar Baby") and *citroides* (Tsamma melon) were harvested from anthesis to maturity at different stages of development based on ovule and seed development. Fruits were picked randomly from different plants, to obtain representative samples, and then transported to the laboratory. Extra care was taken to prevent damage to the fruit surface. At the laboratory, fruit samples were prepared for both scanning electron and light microscopy studies.

### 3.2.1. Scanning Electron Microscopy (SEM)

Fruit surface was examined using both Cryo -SEM (Low -Temperature SEM) and conventional SEM. When using Cryo-SEM, conventional preparations such as fixation, dehydration, drying, and metal coating are substituted by freezing to allow for observation of the specimen in its native form. This technique is very useful for preserving surface structures such as wax. A minimum sample of two fruits was taken at each sampling time. 5 x 5 mm cubes of rind tissue were excised from the middle section of the fruit and processed for examination. 30 fruits were sampled. Some fruits were stored for a month in a cold room to allow for the examination of the surface after storage.

### 3.2.1.1 Low – temperature Scanning Electron Microscopy

Fresh tissue was used for observation with low – temperature scanning electron microscopy to preserve surface features in their natural state. The samples were placed on a specimen holder, immediately plunged into liquid nitrogen and cryo-transferred under vacuum in the pre-chamber of the cryo-system. The frozen samples were then sublimated by raising the temperature of the stage in the pre-chamber to – 180°C. The specimens were then sputter coated with gold – palladium in the pre – chamber and transferred to the cryo – stage in a Scanning Electron Microscope (Hitachi S-570 L) for observation. The images captured were scanned from negatives using a Hewlett Packard ScanJet 4c/T and imported into Adobe Photoshop version 5.0.

### 3.2.1.2 Conventional Scanning Electron Microscopy (SEM)

After the surface features of the rind were ascertained with low – temperature scanning electron microscopy, more fruits were screened further for stomatal morphology using conventional electron scanning microscopy. Rind tissue samples were dehydrated in a graded ethanol series, critical point dried, mounted on metal stubs, coated with 600 Angstroms of gold – palladium in a Hummer V sputter coater, and examined using a Scanning Electron Microscope (Hitachi S-570 L). The images captured were scanned from negatives using a Hewlett Packard ScanJet 4c/T and imported into Adobe Photoshop version 5.0.

## 3.2.2 Light microscopy

### 3.2.2.1 Developmental staging of rind structure

Field observations showed that the size of the ovary at the time of pollination was very variable, both between and within varieties, therefore eliminating the use of size as a reliable marker. Gillaspy *et al* (1993) divided the stages of fruit development from gynoecia of higher plants into three distinct phases. These phases were used to compensate for the differences in fruit size during fruit set. The first phase includes the development of the ovary, fertilization and subsequent fruit set. The second phase involves fruit growth as a result of cell division. This stage is characterized by seed formation and early embryo development. In the

third phase, cell expansion (enlargement) is the main contributor to fruit growth. This stage is characterized by embryo maturation in the fruit. Since these three stages are coordinated with ovule and seed development within the fruit, they facilitate sampling of fruits at the same developmental stages based on ovule and seed development. For stage 1, fruits were assessed at anthesis (when the two integuments were still intact) and after fertilization (when the inner integument of the ovule had started degenerating). For stage 2, fruits were sampled during early seed development, when the seed had already assumed the bi-polar shape. Stage 3 was characterized by seed coat formation in the seed. These criteria allowed for comparison of fruits from the two varieties irrespective of their differences in size at pollination and maturity. Fruit assessment was conducted at stages from anthesis up to fruit maturity.

### **3.2.2.2 Histology**

Initially, sectioning of the rind was attempted with free-hand sections, but it did not produce good sections. Because of the differential thickness of cell walls from epicarp towards the inner most tissues, it was difficult to obtain relatively thin free-hand sections. Relatively thin free-hand sections obtained were stained with Sudan IV for cuticle morphology. For paraffin serial sectioning, pieces of rind tissue were excised from the middle section of the fruit, then dehydrated in a tertiary butyl alcohol series and embedded in Paraplast. Cross sections of the rind were cut 8-15 $\mu$ m with a rotary microtome (10  $\mu$ m), mounted on Fisher Brand Extra Frost microscope slides, stained with safranin-alcian green, and mounted permanently in Permount mounting media (Johansen, 1940). Slides were viewed with a Zeiss photomicroscope III, photographed with a digital camera, Sony 750 and imported into Adobe Photoshop version 5.0.

## **3.3 RESULTS**

The fruits of the two varieties have the same type of general construction. However, there are individual diagnostic differences visible both at the morphological and anatomical level. The fruit is a pepo, and develops from an inferior ovary. In both cultivars, the ovary is completely covered with hairs (Fig. 55

and 56). Mature fruits of "Sugar Baby" are round to oblong, ranging from 25-30cm long and 20 cm in diameter. The exocarp has a green background, mottled with a relatively thin band of dark green stripes (Fig. 57). Fruit flesh is reddish-pink. Mature Tsamma melon fruits are round and range from 15-20cm in diameter. The exocarp has a green to dark-green background, mottled with longitudinal irregular bands of light green (Fig. 58). Fruit flesh is white.

### 3.3.1 Fruit surface-morphology and anatomy in var. *lanatus* (Sugar Baby) and *citroides* (Tsamma melon).

#### 3.3.1.1 Fruit surface morphology

##### *Trichomes*

In both varieties, the ovary was densely covered by glandular and covering trichomes. The trichomes were denser in Tsamma melon (Fig.55 and 60) than in "Sugar Baby" (Fig. 56 and 59). In "Sugar Baby", the stripe pattern background could be seen through the trichomes. The covering trichomes are very long, multi-cellular and tapered at the ends (Fig. 59 and 60). The glandular hairs have a short stalk and a terminal multi-cellular secretory head (Fig. 61 and 62). Normally the trichomes do not persist to fruit maturity. In "Sugar Baby", the hairs fall off immediately after fertilization leaving a smooth fruit behind (Fig. 63 and 65). In Tsamma melon, the hairs persist for sometime after fertilization (Fig. 64 and 66), to an extent that some glandular hairs are still visible in fruits approaching maturity (Fig. 58).

##### *Stomata*

Stomata were present on the fruit of both varieties from anthesis up to maturity. In both varieties, the stomata occur singly or in groups, with the latter being common. The stomata in the two varieties differ, however, in terms of position in the epicarp. These differences in position are visible during fruit set and at maturity. In "Sugar Baby", the stomata occur at the surface of the epicarp beginning from the onset of fruit set (Fig. 67 and 69) to fruit maturity (Fig 71 and 73). In Tsamma melon, the stomata occur on the surface of the epicarp during

anthesis and fruit set (Fig. 68 and 70) when the ovary is still covered by hairs. The stomata become sunken in the epicarp with fruit development and maturity (Fig. 72 and 74). The stomata begin to sink into the epicarp as the hairs start falling off. The transition process begins with the delimitation of a pronounced boundary around the guard cells proper (Fig. 75). Following the delimitation of this boundary, the fringes of the guard cells collapse (Fig. 76) then the guard cells sink in the epicarp (Fig. 77 and 78), becoming deeply sunken with fruit development (Fig 72 and 74).

#### *Epicuticular Wax*

A layer of epicuticular wax covers the surface of both varieties. This layer becomes apparent after most of the trichomes have fallen off the fruit. The epicuticular wax occurs in the form of individual wax particles of different shapes during the early stages of fruit development (Fig. 79 and 80). As fruit development progresses, the individual wax particles increase, fuse together and agglomerate to form a layer of amorphous wax on the fruit. At fruit maturity, the amorphous wax bloom covered the entire fruit, including the stomatal complex (Fig. 81 and 82). Examination of fruit surface one month after abscission revealed a tremendous increase in wax deposition on the entire fruit in the two varieties. In "Sugar Baby", the amorphous wax had accumulated and formed an elliptical border around the guard cells, leaving the stomatal pore slightly open (Fig. 83). In Tsamma melon, the bloom completely occluded the guard cells and the stomatal pore (Fig. 84). In some specimens of Tsamma melon observed, the entire stomatal complex was buried under the wax bloom (Fig. 85).

The conventional SEM revealed peculiar cracking of epicuticular waxes on mature fruit samples of "Sugar Baby". The wax cracked along the middle lamellae of the epicarp cells and separated into blocks (Fig. 86), resulting in the whole layer of wax peeling off to expose the cuticle (Fig. 87) and stomata (Fig. 88). The wax appeared as if it had not adhered firmly to the cuticle matrix. It also appeared very dry and brittle. In Tsamma melon, the wax showed evidence of flaking (Fig. 89), but did not peel off. The epicuticular wax appeared more stable, as if it had adhered to the surface well enough to form an integral part of the fruit surface.

### **3.3.2 Rind anatomy and development**

#### **3.3.2.1 Rind anatomy**

The general histological features of the mature rind in both varieties are similar to previous descriptions for other watermelon cultivars (Barber, 1909, Lal et al., 1977). The rind is composed of four tissues, which starting from the outside are: epicarp, hypodermis, outer-mesocarp and middle-mesocarp (Fig. 90 and 91).

##### *Epicarp*

Except for the position of stomata on the epicarp, there are no striking differences between the epicarp of the two varieties. The epicarp is made up of one layer of epidermal cells, which are rectangular in transverse section and highly cutinized (Fig. 92, 93, 94 and 95). The cuticle is laid down on both the radial and outer tangential walls of the cells (Fig. 94 and 95). The cuticle appears to be slightly thicker in Tsamma melon than in "Sugar Baby", but no measurements were taken. The epicarp is perforated with stomata in both varieties. In "Sugar Baby", the stomata occur at the surface of the epicarp cells (Fig. 96). The stomata have small guard cells, surrounded by one pair of subsidiary cells. Both the subsidiary and guard cells are not sunken, but positioned level with other epicarp cells. In Tsamma melon, the stomata are deeply sunken in the epicarp (Fig. 97). The stomatal complex is made up of two guard cells and three pairs of subsidiary cells. It appears as if these cells act together in concert to sink the guard cells below the surface of the epicarp.

##### *Hypodermis*

There are no significant differences between the hypodermal tissues of the two varieties. The hypodermis forms the green tissue of the rind beneath the epicarp (Fig. 90, 91, 98 and 99). It is made up of compactly packed chlorenchyma cells. The number of layers in this tissue ranges from 10-12 cells in both varieties. The outermost cells in transverse section are more cuboidal than the inner ones

and often interspersed with small intercellular spaces. In contrast, the innermost cells are smaller, tangentially elongated and lack intercellular spaces.

#### *Outer-mesocarp*

This is the most distinct tissue in the rind since it contains more than one cell type. It consists of bands of brachysclereids separated from each other by parenchyma cells referred to as a "gap" (Fig 90 and 91). The size and shape of cells in this tissue is highly variable. The sclereids around the gap consist of regularly arranged rectangular cells (Fig. 100 and 101). In contrast, the sclereids away from the gaps are irregularly arranged and isodiametric (Fig. 102 and Fig. 103). Cells in the outer-most layer (towards the hypodermis) are often small, highly lignified with very small lumens compared to ones located towards the inner-mesocarp, which are usually highly pitted. The band of brachysclereids is broader and more extensive in Tsamma melon (Fig. 90) than in "Sugar Baby" (Fig. 91). The sclereids are relatively large, and more lignified than in "Sugar Baby" (Fig. 103).

#### *Middle-mesocarp*

This layer constitutes the thickest portion of the rind. It consists of thick walled parenchyma cells interspersed with intercellular spaces (Fig. 104-107). Cells in this tissue are generally small and compact increasing in size towards the flesh of the fruit. The cells towards the outer-mesocarp appear to be in a transitory stage towards becoming lignified. This layer often contains small vascular bundles with reduced phloem tissue (not shown).

#### 3.3.2.2 Rind development

Developmental stages leading to the mature rind structure reported in the previous section were documented. Despite differences in rind anatomy, the fruit of the two varieties undergoes the same developmental process. The following description of rind development applies to both "Sugar Baby" and Tsamma melon, unless specified.

At the stage of anthesis and fruit set, there are no distinct histological zones in the fruit tissue (Fig. 108 and 109). The ovary wall makes up the fruit tissue. The

ovary wall tissue, which is devoid of vascular bundles, will eventually form the rind of the fruit. The epicarp is conspicuous with a single layer of epidermal cells. Numerous stomata can be seen on the epicarp at this stage. In "Sugar Baby", the stomata occur on the surface of the epicarp, and consist of one pair of subsidiary cells (Fig. 110). In Tsamma melon, the stomata are raised above the surface of the epicarp (Fig. 111). The stomatal complex has three pairs of subsidiary cells, which subtend the guard cells above the surface of the epicarp. The hypodermis has not yet differentiated at this stage.

The second stage of rind development is marked by rapid cell division restricted to cells in the hypodermis proper and the layer beneath it (Fig. 112 and 113), marking the earliest differentiation of the hypodermis tissue. The appearance of hypodermal tissue was used as a marker for this stage. At this stage, the stomata in Tsamma melon are slightly raised. The cells in the hypodermal layer and those below it appear irregular, and stain densely. Cell division at this stage results in an increase in fruit size as evidenced by the distance between the epicarp and the boundary of the placenta. Fertilised ovules at different developmental stages can be seen in this phase.

The third stage of rind development involves the differentiation of the rind into distinct layers. At this stage, the two polar vascular bundles in the seed have differentiated, and the seed coat has become distinct. Cell division has ceased in the layer below the epicarp. The hypodermis layer has fully differentiated and is distinct from other layers (Fig. 114 and 115). The stomata appear slightly sunken in Tsamma melon. The cell contents in a group of cells below the hypodermis lyse, and the cell walls become lignified marking the first stage of differentiation of the outer-mesocarp layer in the rind (Fig. 114 and 115). As the fruit increases in size, more cells become lignified and approach each other to form bands of brachysclereids below the hypodermis (Fig. 116 and 117). The stomata in Tsamma melon appear deeply sunken in the epicarp at this time. At maturity, the groups of sclereids come very close together to form bands of brachysclereids below the hypodermis (Fig. 90, 91, 100 and 101). The bands are separated by small gaps of cells that remain parenchymatous. The last layer to differentiate in the rind is the middle-mesocarp, positioned immediately below the outer-mesocarp. The cells in this layer appear to be in an intermediate stage between the outer-mesocarp and

inner-mesocarp tissue. The cells are larger than those of the hypodermis layer, with thickened parenchymatous cell walls (Fig. 104 and 105)

## **PLATE 19**

**Fig. 55-56.** Macro-photographs of the female flower in "Sugar Baby" (var. *lanatus*) and Tsamma melon (var. *citroides*).

**Fig. 55.** "Sugar Baby": Ovary (Ov) is slightly covered by hairs (arrow-head). Note the stripe pattern in the background.

**Fig. 56.** Tsamma melon: Ovary (Ov) densely covered by hairs (arrow-head).

55

56

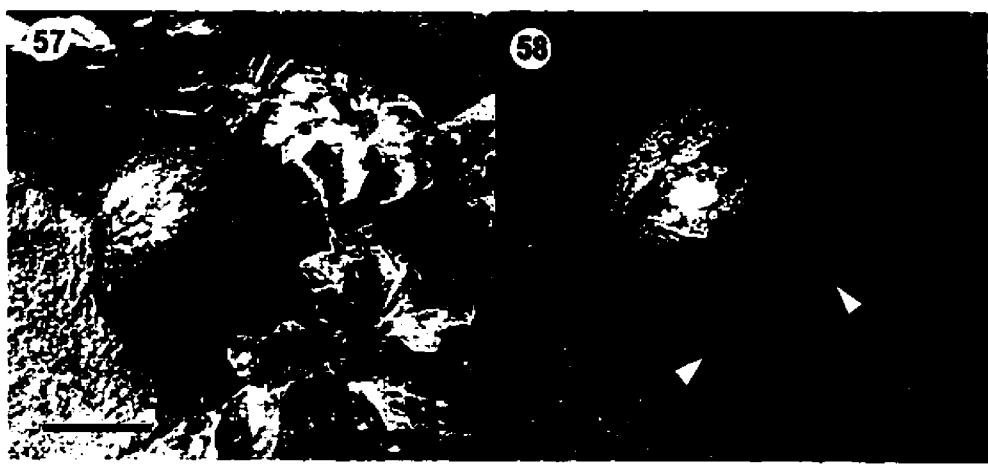


## **PLATE 20**

**Fig. 57-58.** Photographs of "Sugar Baby" and Tsamma melon fruits showing the stripe pattern on the exocarp.

**Fig. 57.** "Sugar Baby": note the green background, mottled with dark green stripes. Scale bar = 1 mm.

**Fig. 58.** Tsamma melon: note the bright green background mottled with longitudinal irregular light green bands. Some trichomes (arrow-heads) can be seen in the background. Scale bar = .5 mm.



## **PLATE 21**

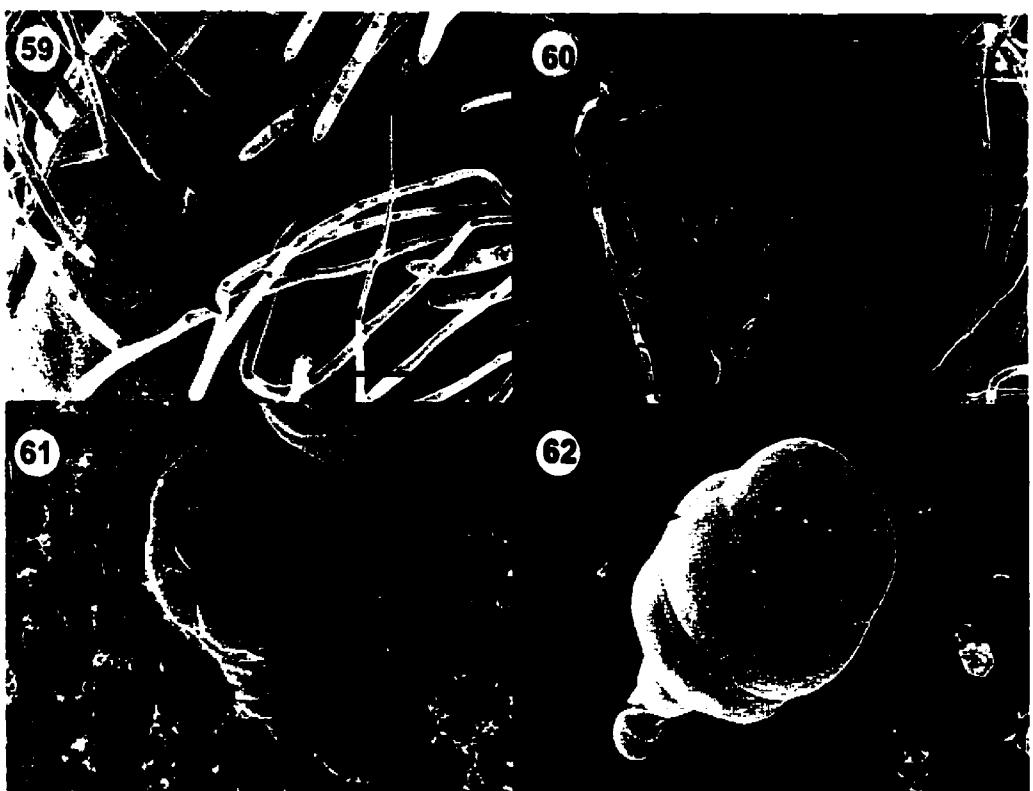
**Fig. 59-62.** Scanning electron micrographs of trichomes on the ovary of "Sugar Baby" and Tsamma melon.

**Fig. 59.** "Sugar Baby": Overview of trichomes before anthesis. Note the glandular (Gt) and covering (Et) trichomes. Scale bar = 600  $\mu\text{m}$ .

**Fig. 60.** Tsamma melon: Overview of trichomes just before anthesis. Note the dense Covering (Et) trichomes. Scale bar = 600  $\mu\text{m}$ .

**Fig. 61.** "Sugar Baby": Close-up view of a glandular trichome. Note the short stalk (St) and secretory head (Sh). Scale bar = 30  $\mu\text{m}$ .

**Fig. 62.** Tsamma melon: Close-up view of a glandular trichome. Note the short stalk (St) and secretory head (Sh). Covering trichomes were manually removed to reveal glandular trichomes. Scale bar = 30  $\mu\text{m}$ .



## **PLATE 22**

**Fig. 63-66.** Photographs of young fruits of "Sugar Baby" and Tsamma melon.

**Fig. 63.** "Sugar Baby": one-week-old fruit. Note the absence of trichomes on the fruit.

**Fig. 64.** Tsamma melon. One week old. Fruit densely covered by trichomes (arrow).

**Fig. 65.** "Sugar Baby": Older fruit than in Fig. 63 showing the absence of trichomes.

**Fig. 66.** Tsamma melon: Older fruit than in Fig. 64. Trichomes (arrow) have not yet fallen off the fruit.



## **PLATE 23**

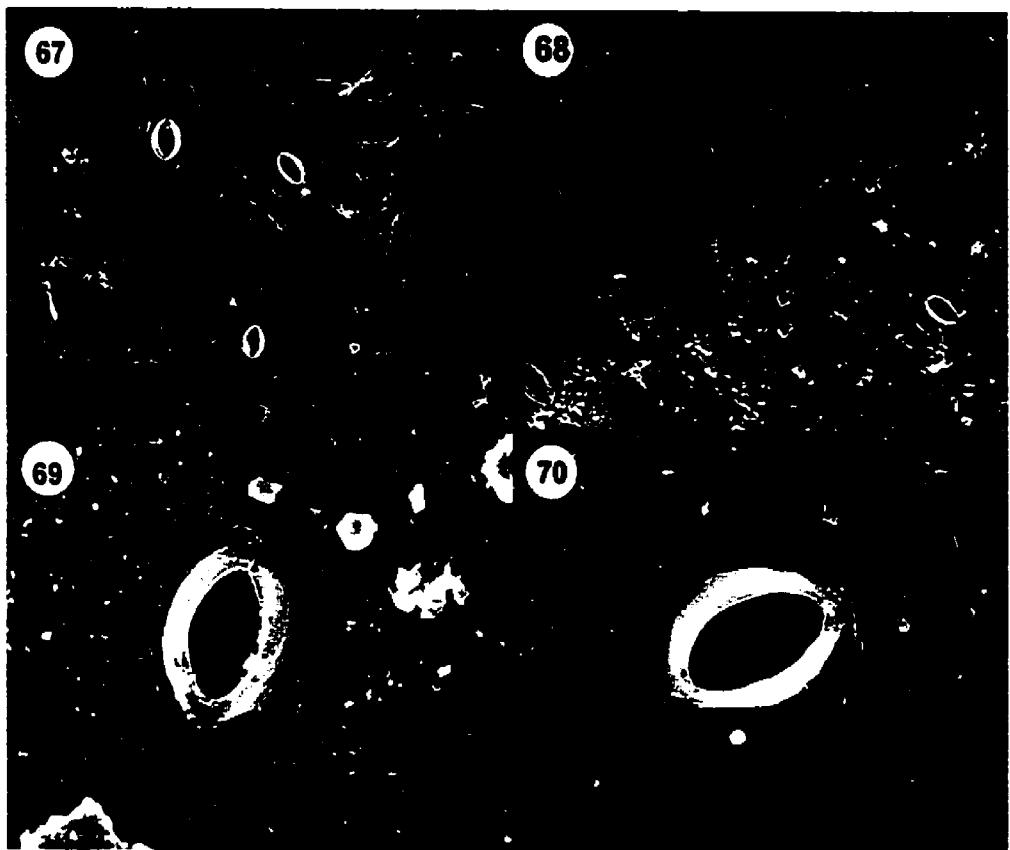
**Fig. 67-70.** Scanning electron micrographs of stomata on the fruit surface of "Sugar Baby" and Tsamma melon at fruit set.

**Fig. 67.** "Sugar Baby": Overview of stomata. Scale bar = 50  $\mu\text{m}$ .

**Fig. 68.** Tsamma melon: Overview of stomata. Scale bar = 50  $\mu\text{m}$ .

**Fig. 69.** "Sugar Baby": Close-up of Fig. 67. Stomata occur on the surface of the epicarp. Scale bar = 9  $\mu\text{m}$ .

**Fig. 70.** Tsamma melon: Close-up of Fig. 68. Stomata occur on the surface of the epicarp. Scale bar = 9  $\mu\text{m}$ .



## **PLATE 24**

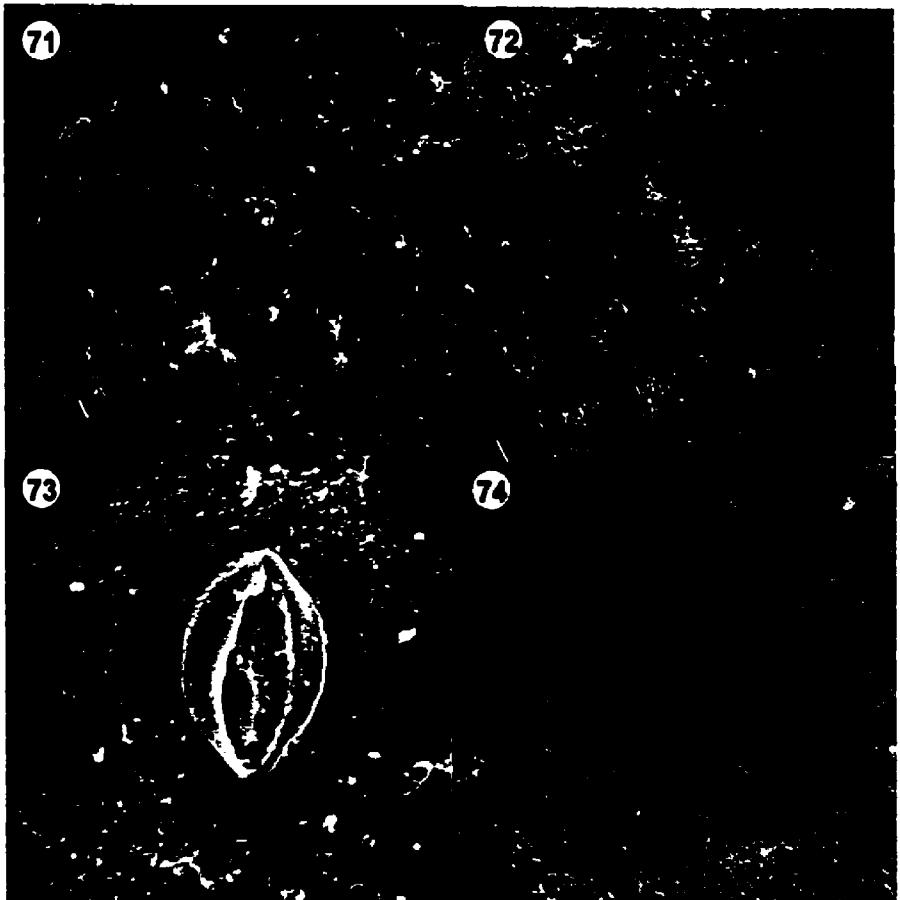
**Fig. 71-74.** Scanning electron micrographs of stomata in mature fruits of "Sugar Baby" and Tsamma melon.

**Fig. 71.** "Sugar Baby": Overview of fruit surface showing stomata situated on the surface of the epicarp. Scale bar = 120  $\mu\text{m}$ .

**Fig. 72.** Tsamma melon: Overview of fruit surface showing stomata sunken in the epicarp. Scale bar = 200  $\mu\text{m}$ .

**Fig. 73.** "Sugar Baby": Close-up of fruit surface showing stomata. Note the stomatal complex on the surface of the epicarp. Scale bar = 10  $\mu\text{m}$ .

**Fig. 74.** Tsamma melon: Close-up of fruit surface showing stomata. The stomatal complex is deeply sunken in the epicarp. Scale bar = 7.5  $\mu\text{m}$ .



## PLATE 25

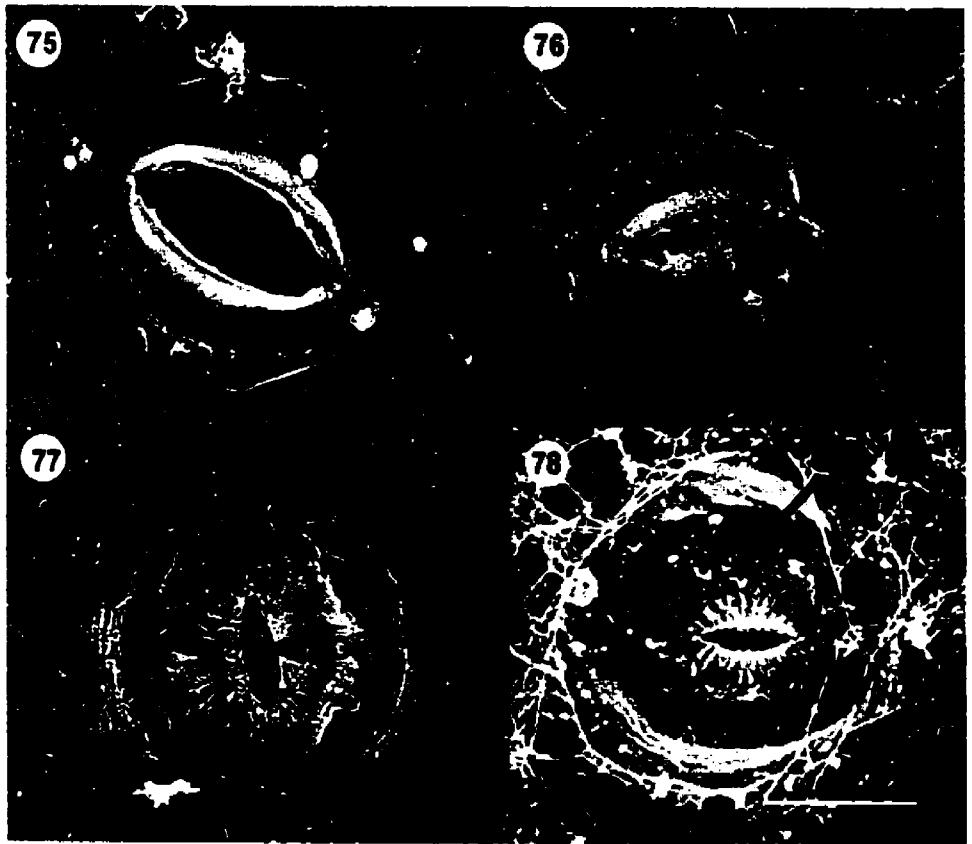
**Fig. 75-78.** Scanning electron micrographs of stomata in var. *citroides* showing transition from the raised to the sunken state.

**Fig. 75.** Close up of stomata. Note the slightly raised fringes (FGc) of the open guard cells, and the pronounced delimitation of the boundary (arrow head) of the guard cells proper (Gc). Scale bar = 9  $\mu\text{m}$ .

**Fig. 76.** Second stage of the transition process: the fringes of the guard cells (FGc) are beginning to collapse below the surface of the epicarp. Scale bar = 12  $\mu\text{m}$ .

**Fig. 77.** Third stage of the transition process: the fringes of the guard cells (FGc) have collapsed below the epicarp, The guard cells (arrows) are beginning to sink into the epicarp. Scale bar = 6  $\mu\text{m}$ .

**Fig. 78.** Later stage than in Fig 77: Guard cells (Gc) are situated slightly below the surface of the epicarp. Scale bar = 6  $\mu\text{m}$ .



## **PLATE 26**

**Figs. 79-82.** Cryo-scanning electron micrographs of epicuticular wax in "Sugar Baby" and Tsamma melon fruit.

**Fig. 79-80.** Epicuticular wax of "Sugar Baby" and Tsamma melon during the early stages of fruit development.

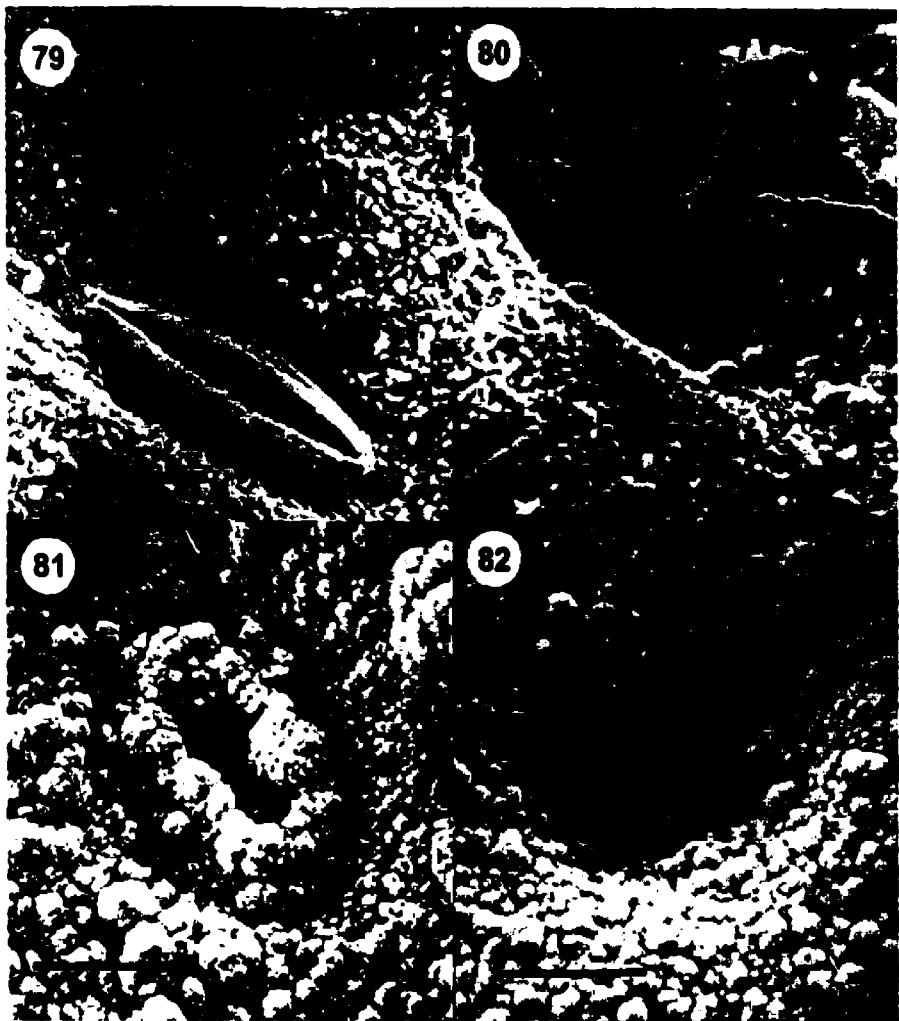
**Fig. 79.** "Sugar Baby": Note the scattered individual crystals of wax. Scale bar = 15  $\mu\text{m}$ .

**Fig. 80.** Tsamma melon: Note the scattered individual crystals of wax. Scale bar = 15  $\mu\text{m}$ .

**Fig. 81-82.** Epicuticular wax of "Sugar Baby" and Tsamma melon at maturity. Individual wax crystals have agglomerated to form amorphous wax.

**Fig. 81.** "Sugar Baby": Note the wax bloom surrounding the stomatal complex. Scale bar = 12  $\mu\text{m}$ .

**Fig. 82.** Tsamma melon: Note the amorphous wax bloom around the sunken stomata. Scale bar = 12  $\mu\text{m}$ .



## **PLATE 27**

**Fig. 83-84.** Cryo-Scanning electron micrographs of amorphous wax on the surface of "Sugar Baby" and Tsamma melon fruits examined one month after storage.

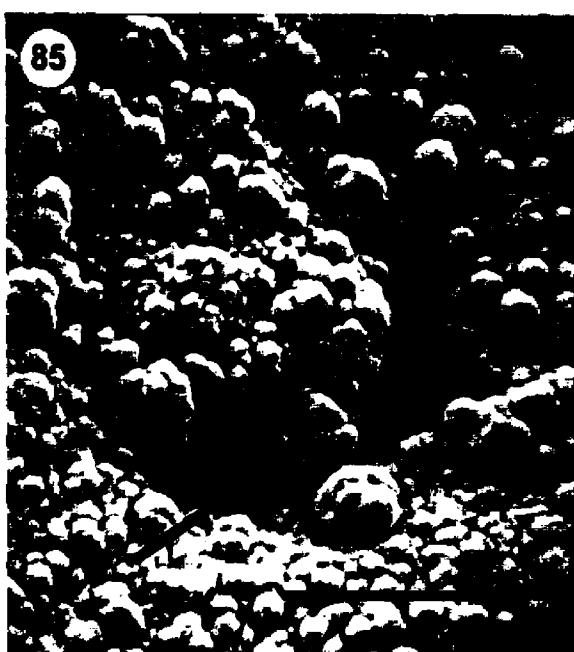
**Fig. 83.** "Sugar Baby": Note the elliptical border formed by wax around the guard cells. The stomatal pore is slightly open (arrow). Scale bar = 12  $\mu\text{m}$ .

**Fig. 84.** Tsamma melon: Wax bloom had completely occluded the entire stomatal complex (arrow). Scale bar = 12  $\mu\text{m}$ .



## **PLATE 28**

**Fig. 85.** Cryo-Scanning electron micrograph of epicuticular wax on *Tsamma* melon fruit after one-month storage. The stomatal complex (arrow) is buried under the wax bloom. Scale bar = 12  $\mu\text{m}$ .



## **PLATE 29**

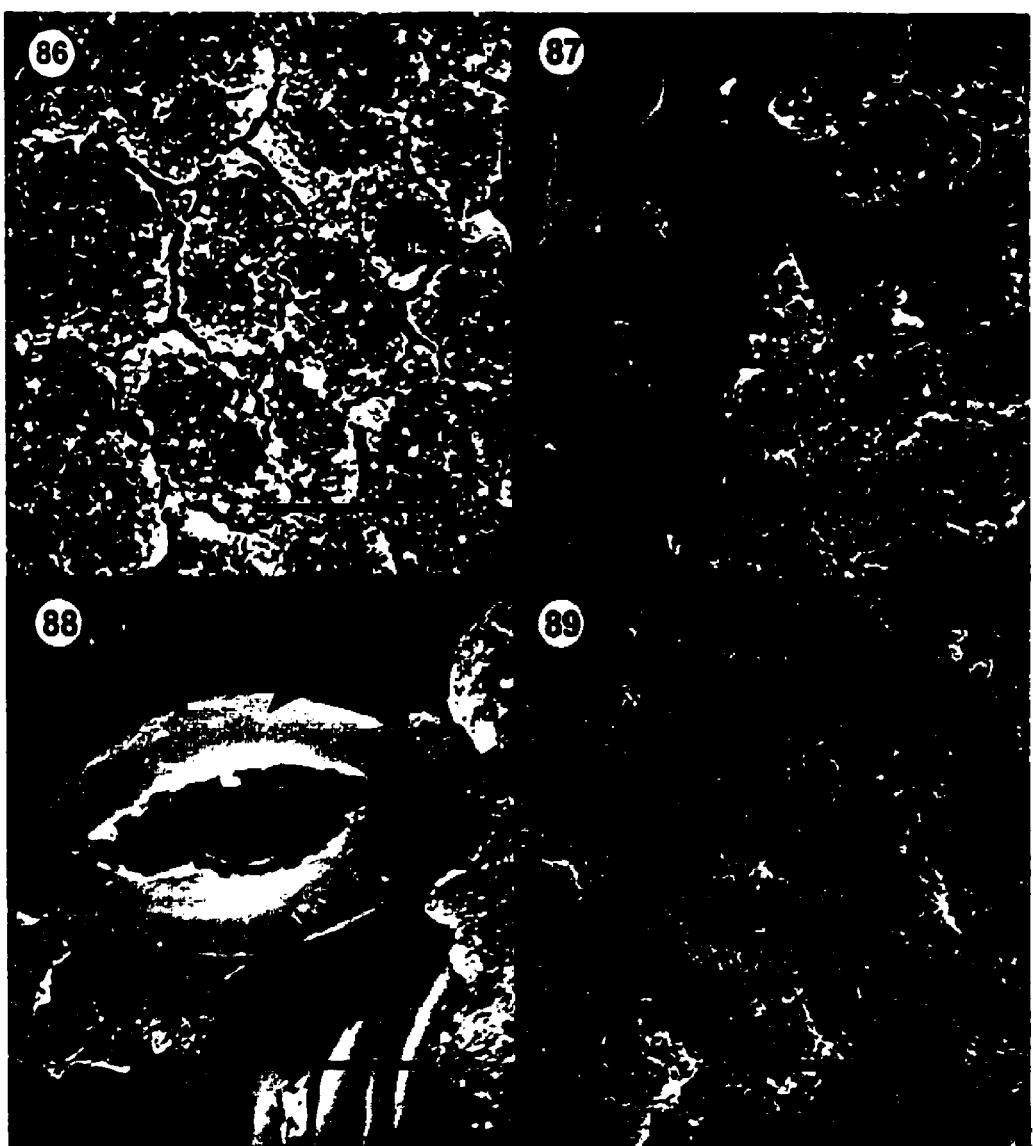
**Fig. 86-89.** Scanning electron micrographs of epicuticular wax in "Sugar Baby" and Tsamma melon showing cracking caused by tissue processing.

**Fig. 86.** "Sugar Baby": Overview of the cracked epicuticular wax. Cracking is localized to the middle lamellae (arrows) of the epicarp cells. Wax appears hard and brittle. Scale bar = 30  $\mu\text{m}$ .

**Fig. 87.** "Sugar Baby": Some of the wax has peeled off to expose the surface epicarp cells (Epi). Scale bar = 30  $\mu\text{m}$ .

**Fig. 88.** "Sugar Baby": Close-up of cracked surface. Wax around stoma (arrow) has peeled off. Scale bar = 30  $\mu\text{m}$ .

**Fig. 89.** Scanning electron micrograph of epicuticular wax showing the response to tissue processing in Tsamma melon. Note evidence of flaking in the wax (arrows). Scale bar = 7.5  $\mu\text{m}$ .

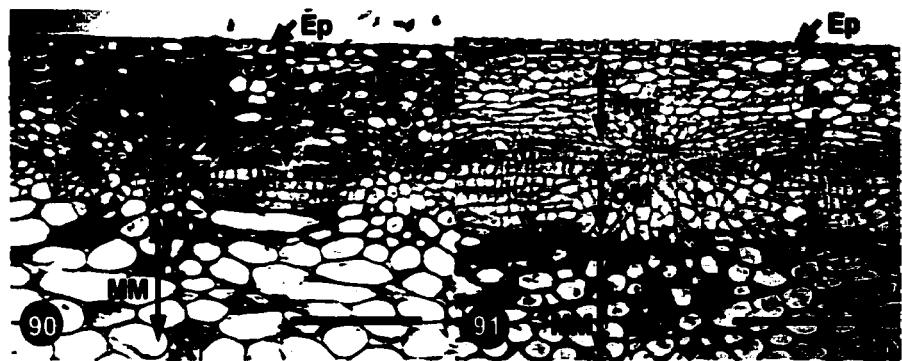


## **PLATE 30**

**Fig. 90-91.** Transverse section of rind tissue from mature fruits of "Sugar Baby" and Tsamma melon showing the epicarp (Ep), hypodermis (Hyp), outer-mesocarp (OM) and middle-mesocarp (MM). Note the parenchyma (Pa) gap separating the bands of brachysclereids. Scale bars = 250 µm.

**Fig. 90.** "Sugar Baby": Note the relatively thin band of brachysclereids.

**Fig. 91.** Tsamma melon: Note the broad and extensive bands of brachysclereids.



## **PLATE 31**

**Figs. 92-95.** Transverse sections of the rind of "Sugar Baby" and Tsamma melon.

**Fig. 92-93.** Transverse sections of the rind of "Sugar Baby" and Tsamma melon showing the epicarp layer. Note the rectangular epidermal cells (Ep). The cuticle (Ct) is also visible. Scale bars = 50  $\mu\text{m}$ .

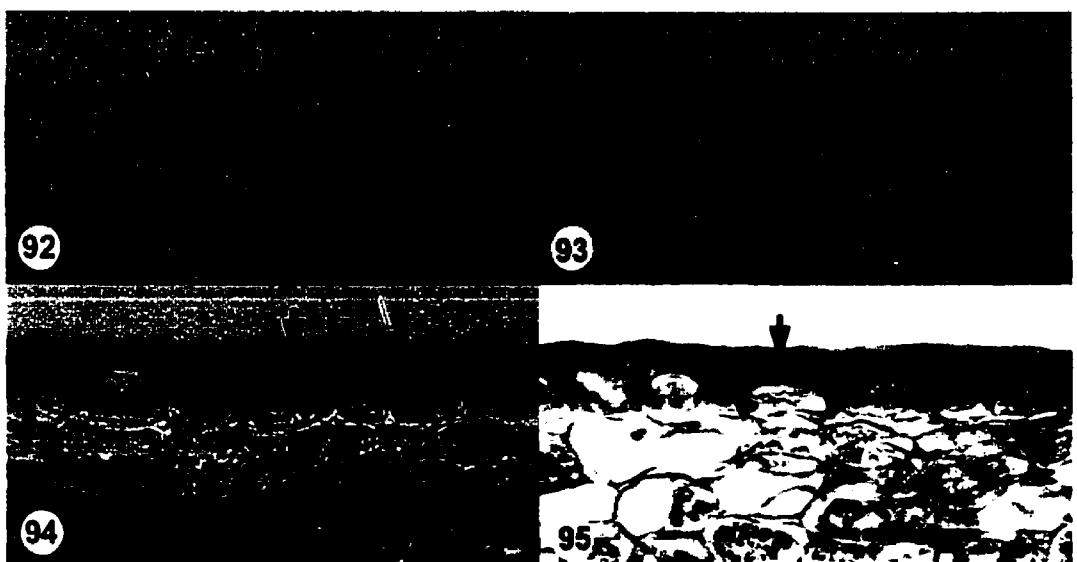
**Fig. 92. "Sugar Baby"**

**Fig. 93. Tsamma melon**

**Fig. 94-95.** Free hand-sections of the rind showing details of the cuticle. Cuticle is laid down on both the tangential (arrows) and radial walls (arrow heads) of the epicarp cells. Sections were stained with Sudan IV. Scale bars = 50  $\mu\text{m}$ .

**Fig. 94. "Sugar Baby"**

**Fig. 95. Tsamma melon**



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## **PLATE 32**

**Fig. 96-99.** Transverse sections of the rind of "Sugar Baby" and Tsamma melon showing stomata and hypodermis tissue.

**Figs. 96- 97.** Transverse sections from the rind showing stomata. Scale bars = 50  $\mu\text{m}$ .

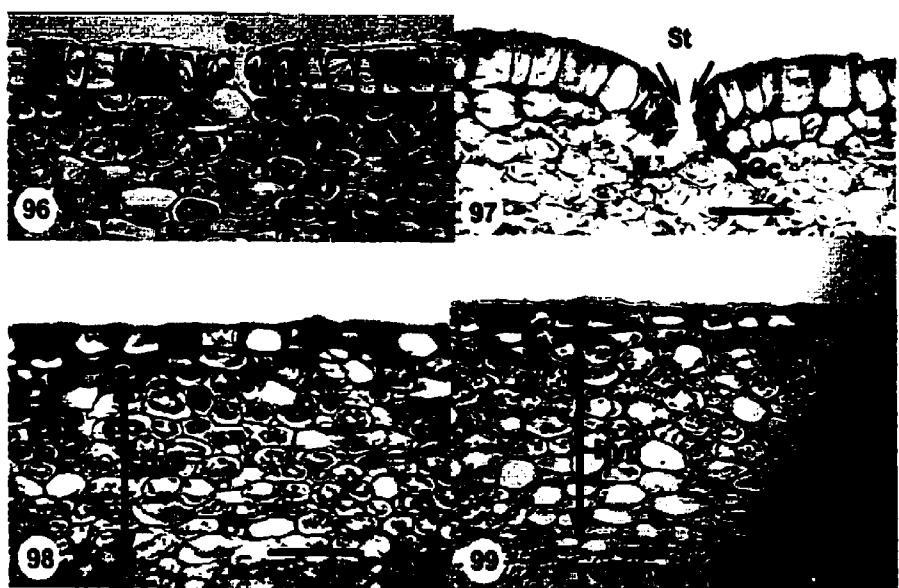
**Fig. 96.** "Sugar Baby": Stomata (St) have one pair of subsidiary cells (Sc) and small guard cells (Gc). Stomata situated at the same level with other epicarp cells.

**Fig. 97.** Tsamma melon: Note the three subsidiary cells ( $\pi$ ) and the sunken guard cells (Gc). Subsidiary cells appear to be acting together (downward arrows) to sink guard cells below surface of epicarp.

**Fig. 98-99.** Transverse sections from the rind of "Sugar Baby" and Tsamma melon showing the hypodermis tissue (Hyp). Scale bars = 75  $\mu\text{m}$ .

**Fig. 98.** "Sugar Baby"

**Fig. 99.** Tsamma melon.



## PLATE 33

**Fig. 100-103.** Close-up of rind transverse sections in Fig. 90 and 91 showing the outer-mesocarp tissue

**Fig. 100-101.** Close-up view of the outer-mesocarp showing details of the outer - mesocarp tissue associated with the parenchyma gap (arrow). Note the regularly arranged rectangular sclereids. Scale bars = 50  $\mu\text{m}$ .

**Fig. 100.** "Sugar Baby": Note the relatively thin band of brachysclereids.

**Fig. 101.** Tsamma melon: Band of brachysclereids broader than in "Sugar Baby".

**Fig. 102-103.** Close-up view of Fig. 90 and 91 showing the details of the outer - mesocarp tissue away from the parenchyma gap. Cells in the first layer are highly lignified (Hlc) with small lumens. Note the pitted cells (arrow heads). Scale bars = 50  $\mu\text{m}$ .

**Fig. 102.** "Sugar Baby"

**Fig. 103.** Tsamma melon



## **PLATE 34**

**Fig. 104-107.** Close-up of the rind transverse section in Fig. 90 and 91 showing the middle-mesocarp tissue.

**Fig. 104-105.** Transverse sections from the rind showing overview of the middle-mesocarp. Cells in the outermost layer (Ts) appear to be in the process of becoming lignified. Scale bars = 250 µm.

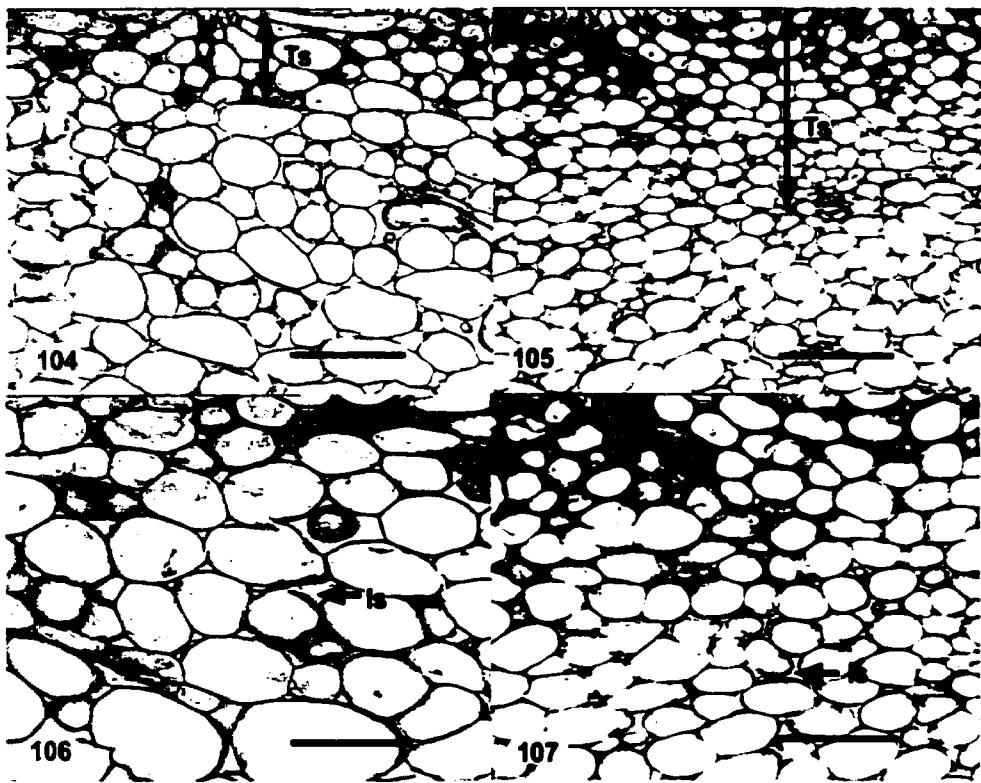
**Fig. 104.** "Sugar Baby": Cells relatively larger in size compared to in Tsamma melon.

**Fig. 105.** Tsamma melon

**Fig. 106-107.** Close-up view of 104 and 105 showing details of the middle-mesocarp tissue. Note the intercellular spaces (Is) between the cells. Scale bars = 100 µm.

**Fig. 106.** "Sugar Baby"

**Fig. 107.** Tsamma melon



## **PLATE 35**

**Fig. 108-111.** Transverse sections through ovary of "Sugar Baby" and Tsamma melon.

**Fig. 108-109.** Overview of ovary showing the histologically undifferentiated tissue of the ovary wall (OW). Note the vascular bundles (Vb). Scale bars = 250  $\mu\text{m}$ .

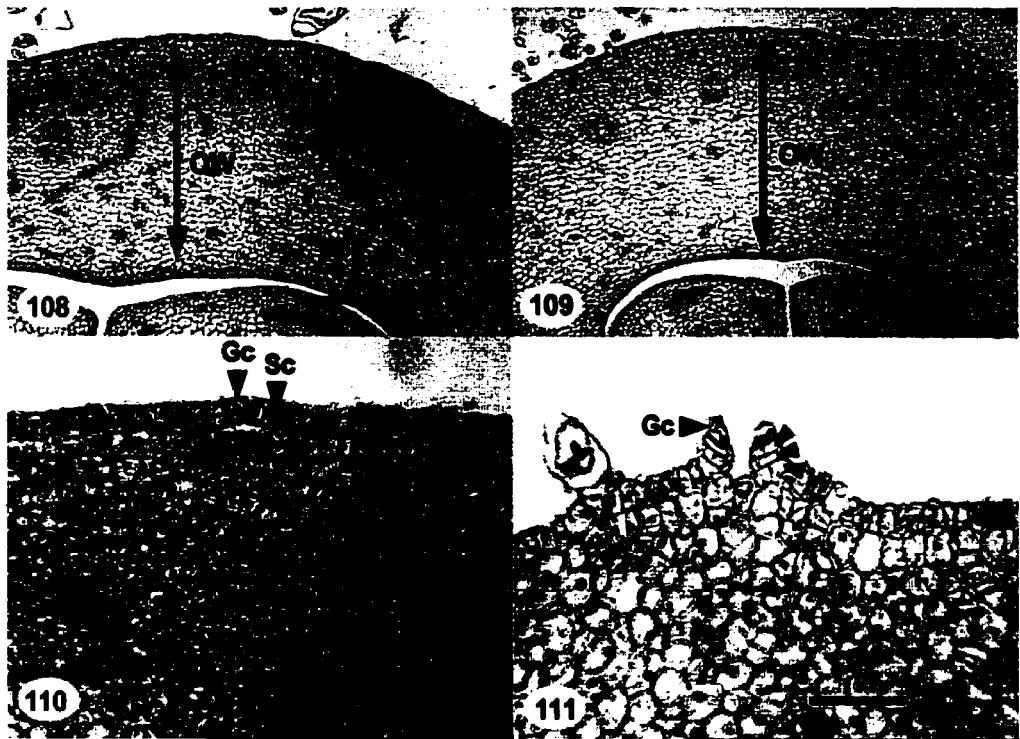
**Fig. 108.** "Sugar Baby"

**Fig. 109.** Tsamma melon

**Fig. 110-111.** Close-up view of Fig. 108 and 109, respectively, showing location of stomata on the epicarp. Scale bars = 50  $\mu\text{m}$ .

**Fig. 110.** "Sugar Baby": Subsidiary cells (Sc) and Guard cells (Gc) are situated on the surface of the epicarp.

**Fig. 111.** Tsamma melon: stomata raised above the surface of the epicarp. Note the three subsidiary cells (arrow heads) and elevated guard cells (Gc).



## PLATE 36

**Fig. 112-115.** Transverse sections from the rind of "Sugar Baby" and Tsamma melon showing early differentiation of the hypodermis and outer-mesocarp tissue.

**Fig. 112-113.** Early stage of hypodermis differentiation. Hypodermis initials (Hi) appear to be actively dividing. Scale bars = 50  $\mu\text{m}$ .

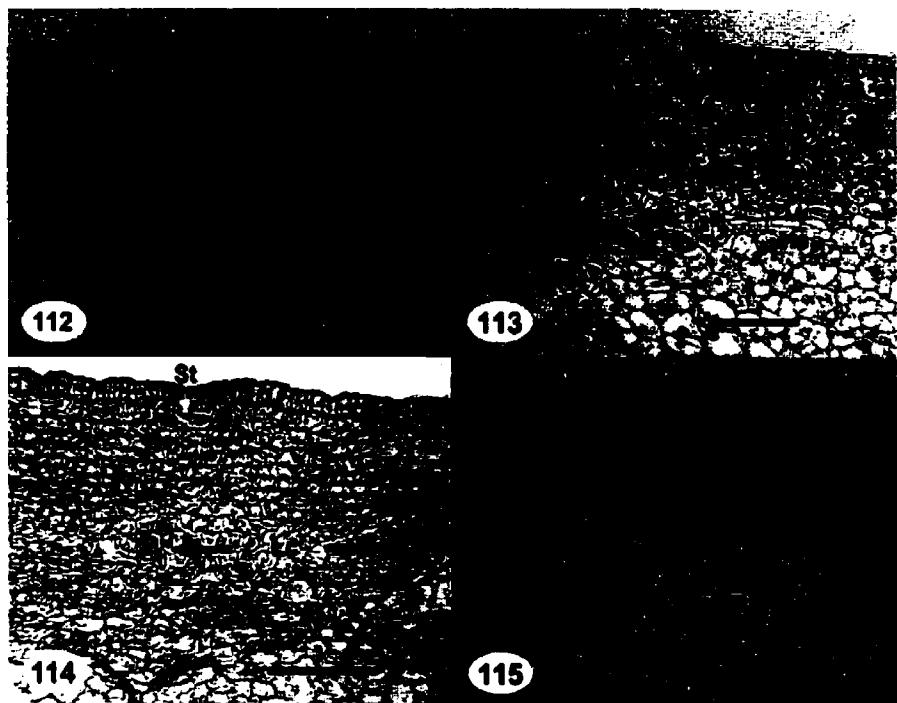
**Fig. 112.** "Sugar Baby": Stomata (St) still at same level with other epicarp cells.

**Fig. 113.** Tsamma melon: Stomata (St) slightly raised above the epicarp

**Fig. 114-115.** Early differentiation of the outer-mesocarp tissue. Hypodermis layer has already differentiated. Note the groups of cells (Lc) beginning to become lignified. Scale bars = 75  $\mu\text{m}$ .

**Fig. 114.** "Sugar Baby": stomata still on surface of epicarp.

**Fig. 115.** Tsamma melon: Note the slightly sunken stomata (St).



## PLATE 37

**Fig. 116-117.** Transverse sections from the rind showing further differentiation of the outer-mesocarp tissue. More groups of cells have become lignified and expanded towards the inner-mesocarp. Note small distance between groups of lignified cells compared to Fig. 114 and 115. Scale bars = 100  $\mu\text{m}$ .

**Fig. 116.** "Sugar Baby": Stomata (St) still on surface of epicarp.

**Fig. 117.** Tsamma melon: stomata (St) deeply sunken.



### **3.4 DISCUSSION**

This chapter examined and compared three aspects of the rind of "Sugar Baby" (*Citrullus lanatus* var. *lanatus*) and Tsamma melon (*C. lanatus* var. *citroides*). The first subsection compared surface features of the rind. The second subsection compared the anatomy of the mature rind. The third and final section compared the development and differentiation of the rind from anthesis to fruit maturity. Despite the differences in macro-morphological features such as flesh and exocarp colour, results from these studies show that both "Sugar Baby" and Tsamma melon share a number of general features. The two varieties differ, however, in stomatal morphology and the extent of the outer- mesocarp tissue. These differences may shed some light on the mechanisms of prolonged keeping ability in wildtype Tsamma melon fruit compared to cultivated watermelon ("Sugar Baby").

#### **Surface features**

The surface of the rind in both varieties shares a number of common features. Immature fruits are densely covered by glandular and covering trichomes. Generally the trichomes were denser in Tsamma melon than in "Sugar Baby". The trichome types are similar to those documented for other cultivars in *Citrullus lanatus* (Thanki, 1989) and *Citrullus colocynthis* (Inamdar et al., 1990). The higher density and persistence of hairs on Tsamma melon might reflect the xerophytic nature of the plant.

The most striking difference between the two varieties involves the position of the stomata in the epicarp. In "Sugar Baby" the stomata occur at the surface of the epicarp from anthesis to fruit maturity. In Tsamma melon, the stomatal complex is raised above the epicarp at anthesis and fruit set, and becomes deeply sunken with fruit growth. This study is the first to document the transition of stomatal position in fruits. These changes in stomatal positioning appeared to be correlated with the presence of trichomes on the fruit. The stomata were raised when the ovary and immature fruit were still densely covered by hairs, and gradually sunk into the epicarp when the hairs started falling off. This correlation between the presence and absence of trichomes, and changes in stomatal morphology suggests that there is a trade-off between minimising water loss and maximizing

gaseous exchange. It appears as if the fruit alters the morphology of the stomata to maximize gaseous exchange when trichomes are present, and to minimise transpiration when the fruit surface is more vulnerable (i.e. trichomes absent) and exposed to external influences.

The presence of sunken stomata is widely documented in leaves (Meidner and Mansfield, 1968; Moore *et al.*, 1995; Soliman and Khedr, 1997; Oran *et al.*, 1998), but is apparently uncommon in fruits. Inamdar *et al* (1990) reviewed stomatal types in Cucurbitaceae leaves, but did not include fruits. An intensive literature review failed to produce previous studies of sunken stomata in fruits. Hence, this is the first documentation of sunken stomata in cucurbit fruits.

The fruits of both varieties were covered by a layer of epicuticular wax. The wax deposits changed from a crystalline to an amorphous type with fruit development. Fruit development was also accompanied by an increase in wax deposition. The transition from crystalline to amorphous wax, as well as an increase in wax deposition with maturity has been reported for "Valencia" orange (Albrigo, 1972; Storey and Treeby, 1994), *Citrullus lanatus* cv. Charleston Gray (Corey *et al.*, (1988), and mango, *Mangifera indica* L. cv. Kensington Pride (Bally-Ian, 1999). Examination of the fruit surface one-month after abscission revealed a tremendous increase in wax deposition on the entire fruit in the two cultivars. In "Sugar Baby", the wax deposits accumulated around the stomata, without blocking the stomatal pore. Because of the sunken nature of the stomata in Tsamma melon, the wax completely occluded the entire stomatal complex, and blocked the stomatal pore. When investigating the mode of water conservation in the Tsamma fruit, Botha (1982) reported that the stomata appeared to be clogged by some "material" sometimes after abscission, but never determined the nature of the material. This study has demonstrated that the "material" reported by Botha is most likely to be epicuticular wax, hence making his conclusion that stomatal plugs were responsible for cessation of water loss in the fruits more plausible.

Another striking difference between "Sugar Baby" and Tsamma melon is the way that epicuticular waxes responded when processed for conventional SEM. The epicuticular waxes in mature fruits of "Sugar Baby" cracked and peeled off the cuticle matrix, whereas in Tsamma melon the wax flaked off without peeling. In "Sugar Baby", the epicuticular wax appeared not to bond strongly onto the middle

lamellae, as opposed to Tsamma melon where the wax appeared to have adhered closely to the cuticle matrix. Also, the wax layer appeared to be hard and brittle in "Sugar Baby", compared to the seemingly soft, stable and robust one in Tsamma melon. Storey and Price (1999) reported similar fracturing of epicuticular wax in mature d' agen plums (*Prunus domestica*). They also found that water loss and collapse of dermal tissues were restricted to the area around fractures. The different response exhibited by epicuticular waxes of the two varieties when subjected to mechanical stress implies that the waxes differ in terms of structure and arrangement on the cuticle matrix. Variation in epicuticular wax composition and structure has been reported for apple cultivars (Belding et al., 1998).

#### *Rind development and anatomy*

Comparison of cultivars or varieties that set different sizes of mature fruit makes comparative fruit anatomy studies difficult since fruit size cannot be used as a comparative marker. In order to compensate for this difficulty, Gillaspy et al., (1993) used three stages of fruit development as comparative markers. The comparative criteria revealed a correlation between the differentiation of the fruit rind and seed development in the fruit. This correlation indicates that seed developmental staging is a useful and more reliable marker than fruit size when comparing cultivars that set different sizes of fruits.

Rind anatomy of the two varieties observed in this study at maturity is remarkably similar to each other and to the general features of other watermelon fruits (Barber, 1909; Lal et al., 1977). The rind is made up of four layers of tissue: epicarp, hypodermis, outer-mesocarp and middle-mesocarp. Differences were observed, however, in stomatal anatomy and the nature of the outer-mesocarp tissue.

Despite the differences in fruit size at fruit set and maturity in the two cultivars, the differentiation of the rind into the four distinct layers is identical. The first layer to differentiate is the epicarp, followed by the hypodermis, outer - mesocarp and finally, the middle-mesocarp. Maturation of the outer-mesocarp occurs at fruit maturity, resulting in a layer of tightly packed bands of brachysclereids below the hypodermis. Tapping (thumping) of the fruit is one of the most common ways of determining fruit maturity and ripening in watermelon.

Tapping produces a sharp, ringing sound if the fruit is immature; and a resonant sound when the fruit is mature (Robinson and Decker-Walters, 1997). This difference in sound is likely to be correlated to the differentiation of the outer-mesocarp tissue in the rind. Although it has not been experimentally determined, the bands of brachysclereids might be responsible for the resonate sound produced by thumping the fruit at maturity.

Studies of rind anatomy and development revealed the anatomical details of the stomata, in addition to differences in the developmental pathway of sunken versus non-sunken stomata observed at the surface level. In "Sugar Baby", the stomatal complex has one pair of subsidiary cells situated at the surface of the epicarp. In Tsamma melon the stomatal complex consists of one pair of guard cells and three pairs of subsidiary cells that aid in sinking the guard cells below the surface of the epicarp. It is important to note that the nature of sunken stomata in Tsamma melon appears to be rather unique, and does not conform to any stomatal type documented in the literature.

Structural differences were also observed in the outer-mesocarp tissue of the two varieties. The band of brachysclereids in the outer-mesocarp tissue was extensive and broader in Tsamma melon than in "Sugar Baby". Also, the sclereids appeared to be highly lignified in Tsamma melon than in "Sugar Baby". Variations in the thickness of sclereids that make up the outer-mesocarp tissue have been documented in other watermelon cultivars (Lal et al., 1977; Sugiyama et al., 1999). Based on the function of sclereids as a supporting tissue (Esau, 1977), the outer-mesocarp may be responsible for providing rigidity to the fruit rind.

#### *Structural features associated with keeping quality of watermelon fruits.*

This study is the first to combine surface features and rind structure studies in the investigation of structural features determining storage properties of watermelon fruits. The differences exhibited by "Sugar Baby" and Tsamma melon in rind anatomy and surface morphology indicate that there are a number of features likely to be associated with the keeping quality of watermelon fruits.

Previous studies have reported a correlation between rind tissue structure and the resistance to cracking following mechanical shocks. Lal et al (1977) compared five cultivars of watermelon, and their study showed that the crack-prone

cultivar, "Japanese Yellow Melon" had weakly differentiated sclereids in the outer-mesocarp tissue. When investigating the relationship between rind hardness and rind tissue structure, Sugiyama et al (1999) also found that the sclereids in the outer-mesocarp tissue of the crack-resistant cultivar, "Africa 22857" were extensively developed and highly lignified compared to those of the crack-prone cultivar, "Beni Kodama". Tsamma melon displayed more extensive bands of brachysclereids in the outer-mesocarp tissue than Sugar Baby, suggesting that the outer-mesocarp may also play a role in enhancing the keeping quality of the fruit by making the fruit rind rigid and less prone to cracking.

The presence of epicuticular wax is another feature that is likely to enhance the keeping quality of watermelon fruits. The wax layer acts as a mechanical barrier against external influences such as pathogen entry and injury resulting from mechanical stress. By acting as a mechanical barrier, epicuticular wax is likely to minimize chances of infection and fruit decay in watermelon fruit, thereby enhancing their keeping quality. The role of epicuticular waxes in enhancing disease resistance is widely documented for leaves (Blakeman and Sztejnberg, 1973; Ortiz and Vuylsteke, 1994; Ashraf and Zafar, 1997; Craenen et al., 1997; Shepherd et al., 1999). Craenen et al. (1997) found that the presence of epicuticular wax conferred resistance to *Mycosphaerella fijensis* on banana and plantain leaves. In wheat, the presence of wax on leaf surfaces has been found to reduce the accumulation of moisture on leaves, therefore retarding the establishment and germination of spores (Blakeman and Sztejnberg, 1973; Ortiz and Vuylsteke, 1994). It is possible that the epicuticular waxes in the Tsamma fruit also function in a similar way to enhance disease resistance, hence resulting in better keeping quality.

The observation that only the epicuticular waxes on "Sugar Baby", and not Tsamma melon cracked when processed for conventional SEM, despite being exposed to the same treatment implies that the physical arrangement and molecular organization of wax also play a role in enhances keeping quality. Epicuticular wax in Tsamma melon appeared to be very stable suggesting that a robust wax structure is required to withstand high temperatures and prevent cracking. The role of epicuticular wax in reflecting excessive light radiation in dry and hot environments has been experimentally determined for wheat leaves

(Johnson *et al.*, 1983). The robust wax layer in Tsamma fruit may also increase reflectance of heat radiation, especially since the fruit must withstand high desert temperatures. In addition to increasing heat reflectance, the robust wax structure in Tsamma melon is also likely to play a role in preventing cracking. Epicuticular wax cracking and wax removal has been found to promote chilling injury in grapefruit. Nordby and McDonald (1991) reported high incidences of chilling injury in de-waxed grapefruits, compared to non-dewaxed fruits suggesting that removal of wax exposes the cuticle to the cold temperature. Similarly, the peeling of wax after cracking might be responsible for promoting chilling injury in watermelon fruits, hence reducing the shelf life of the fruits.

The differences in stomatal morphology between Tsamma melon and "Sugar Baby" suggest that sunken stomata play a role in enhancing the keeping quality of watermelon fruits. The presence of sunken stomata is considered to be an adaptive response to dry and hot environments, and has been shown to minimise uncontrolled water loss through the stomata in leaves by providing an air space with comparatively high water vapour immediately after the stomatal pore (Meidner and Mansfield, 1968, Moore *et al.*, 1995). Based on previous studies of sunken stomata and their function in leaves, sunken stomata are also likely to minimize uncontrolled water loss through the stomata of the Tsamma fruit. Since it has been shown that a number of pathogens (bacteria and fungi) gain entry into plant organs through the stomata (Wilmer, 1983), sunken stomata are also likely to reduce chances of injury and infection in Tsamma fruit. In fruits where the stomata are highly exposed to external influences like in "Sugar Baby", pathogens and insect mouthparts can more readily enter the stomatal pores, hence making the fruit more susceptible to diseases.

Sunken stomata may also enhance the keeping quality of fruits by facilitating stomatal wax "plugs". Previous studies on leaves have shown that wax plugs in the stomata minimize stomatal transpiration drastically (Jeffree *et al.*, 1971; Rentschler, 1974). In particular, Jeffree *et al* (1971) noted that the presence of wax in the antechambers of the stomata reduced the rate of transpiration by two thirds. When conducting transpiration experiments on Tsamma fruit following abscission, Botha (1982) noted that transpiration reduced gradually and ceased 12 months after abscission, at which time the stomata were completely clogged by some material.

This apparent correlation between transpiration, fruit maturity and stomatal "plugging" suggests that waxes completely "seal" off the fruit after abscission when the need for gaseous exchange is no longer critical, thereby eliminating water loss. By "sealing" off the fruit, the wax plugs also form an effective barrier against all external influences that may shorten the life span of the fruit. In watermelon, characteristics associated with rind tissue are heritable. Sugiyama *et al* (1999) crossed a crack-prone resistant cultivar, "Beni Kodama" with a crack-resistant cultivar, "Africa 22857" and found that the progeny produced had a rind structure similar to the crack-resistant cultivar. Studies involving epicuticular waxes in leaves have also shown that epicuticular wax properties are heritable (Craenen *et al.*, 1997). Since both Tsamma melon and "Sugar Baby" were grown under identical conditions, it might be concluded that surface and structural features unique to Tsamma melon are genetically determined, and not just phenotypic. If indeed the features are genotypic, they can be introduced into watermelon cultivars to enhance their transportation and storage properties.

## CHAPTER 4

### SUMMARY AND CONCLUSIONS

The primary goal of this thesis was to clarify and understand the differences between the flowers and fruits of cultivated and wild watermelons. The particular goal was to understand why the wild watermelon fruit has a much better keeping quality compared to the cultivated watermelon fruit. The goal was achieved by two major objectives addressed in two chapters. The first objective was to compare floral development of pistillate flowers in *Citrullus lanatus* var. *lanatus* ("Sugar Baby") and var. *citroides* (Tsamma melon) to ascertain whether or not differences in keeping quality are initiated during the early stages of floral development. Floral development of the staminate flower was also examined to determine whether or not the floral meristem in *C. lanatus* is bisexual during the early stages of ontogeny. The second goal was to compare the surface morphology and anatomy of the rind in var. *lanatus* and *citroides* in an attempt to identify structural features that may influence keeping quality in Tsamma melon.

#### Floral development in *Citrullus lanatus* (var. *lanatus* and *citroides*).

The developmental sequence of floral parts in the staminate and pistillate flower is similar in both var. *lanatus* and *citroides*. In the pistillate flower, the sepals were the first organs to form in a unidirectional phyllotactic pattern, followed by the petals and stamens. The anthers failed to develop to maturity, but remained as staminodes. The carpel primordia initiated next, alternate and equidistant to the three staminodes. The nectary ring formed last at the base of the style. The identical development of the pistillate flower in the two varieties suggest that differences observed in keeping quality of mature fruits likely occur after, and not before pollination.

In the staminate flower, like in the pistillate flower, the sepals are the first organs to form, followed by the petals and then the stamens. The anthers develop elaborate connective tissues, filaments and thecae. The nectary arises last at a very late stage of floral development. There are no rudimentary carpels in the

staminate flower. The order and initiation of organs in the staminate flower suggests that the male flower in *C. lanatus* is not bisexual during ontogeny. This was the first report of nectary tissue in both staminate and pistillate flowers in *Citrullus*.

Fruit surface-morphology and anatomy in var. *lanatus* ("Sugar Baby") and *citroides* (Tsamma melon).

This study examined and compared fruit-surface morphology and rind anatomy in "Sugar Baby" and Tsamma melon. The results showed that the two botanical varieties share a number of general features, but differ in stomatal morphology and anatomy, and in the extent of the outer-mesocarp tissue. The differences observed might be responsible for the enhanced keeping quality reported for Tsamma melon, compared to "Sugar Baby"

In "Sugar Baby", the stomatal complex has one pair of subsidiary cells, and is situated at the surface of the epicarp during anthesis to fruit maturity. In Tsamma melon, the stomatal complex, which consists of three pairs of subsidiary cells is raised above the epicarp during anthesis, and become sunken with fruit growth. This study is the first to document the development of sunken stomata in fruits, and the first to document this phenomenon in Tsamma melon fruit. The nature of sunken stomata in the Tsamma fruit appears to be unique, since it does not conform to any stomatal type documented in the literature.

At maturity, a layer of amorphous wax covers the fruits of both varieties. Wax deposition increased after abscission in the both varieties. Because of the sunken nature of the stomata in Tsamma melon, the wax bloom occluded the entire stomatal complex and blocked the stomatal pore. A previous study by Botha (1982) reported stomatal plugging in Tsamma fruit, but did not determine the nature of the "material" clogging the stomata. The epicuticular waxes of the two varieties responded differently when processed for conventional SEM, suggesting variability in wax composition. Rind anatomy of the two varieties is remarkably similar. The rind consists of four layers of tissue: epicarp, hypodermis, outer-mesocarp and middle-mesocarp. Development studies of the rind have shown that differentiation is identical in the two varieties. The first layer to differentiate is the epicarp, followed

by the hypodermis, outer-mesocarp and finally, the middle-mesocarp. The outer-mesocarp is the most distinct tissue in the rind, and is made up of tightly packed bands of brachysclereids. The band of sclereids is more extensive and broader in Tsamma melon than in "Sugar Baby". Also, the sclereids appeared to be highly lignified in Tsamma melon than in "Sugar Baby".

#### Structural features associated with keeping quality of watermelon fruits.

Rind anatomy, and particularly the nature of the outer-mesocarp tissue have been associated with crack resistance. The outer-mesocarp tissue is likely to enhance keeping quality by minimizing cracking following mechanical shocks. Epicuticular waxes are likely to enhance keeping quality by 1) minimising chances of infection, injury caused by mechanical stress and chilling injury, and 2) increasing the reflectance of heat radiation that may promote fruit decay. Although the composition of wax was not analyzed in this study, the composition of wax (physical arrangement and molecular organization) is another feature that may play a role in prolonging the storage period of watermelon fruits. Sunken stomata are likely to enhance keeping quality of watermelon fruits by 1) minimising uncontrolled water loss through the cuticle 2) reducing chances of infection and injury and 3) facilitating stomatal plugs which completely seal off the fruit after abscission, thereby keeping all external influences at bay.

To conclude, results of this study suggest that rind anatomy, epicuticular wax, wax structure and sunken stomata may act together in enhancing the keeping quality of Tsamma fruit. Sunken stomata, in particular appear to play a more significant role by facilitating wax "plugs" that completely seal off the fruit after abscission.

#### Significance of study

This is the first study to describe and document floral development in the genus *Citrullus*. Results from this study may be useful for systematic purposes. The current circumscription of species in *Citrullus* has been based mostly on mature fruit characters, seed anatomy and differences in cucurbitacin compounds. Floral

developmental characters maybe useful as additional markers in the circumscription of species in this genus. Since the current key to southern African Cucurbitaceae considers female flowers of *Citrullus* lacking a nectary, it is recommended that the key be upgraded to include the presence of a nectary as an additional marker in the identification of *Citrullus* species. Despite the diverse sex expression that exists within the Cucurbitaceae, little is known about the different modes of sex expression. Results from this study will thus contribute to the knowledge of sex expression in cucurbits.

Tsamma melon (var. *citroides*) is important for two reasons: 1) it has potential to serve as a genetic resource in the breeding of cultivated watermelon and 2) it has been identified as a potential arid land crop in Botswana. However, information regarding its morphology, anatomy and physiology is lacking in the literature. Most comparative studies conducted on watermelon have focussed on the cultivated watermelon. This study is the first to compare a wild type watermelon and the cultivated watermelon. The information obtained from this study will thus contribute knowledge to the biology of wild watermelon. Results from this study may also be useful for watermelon crop improvement purposes. The susceptibility of watermelon to both low and high temperatures, as well as decay during storage and transportation are serious problems in the watermelon industry. This problem can be alleviated by further crop research. If it can be experimentally determined that structural features unique to Tsamma melon are heritable, these characters could be introduced into watermelon cultivars to enhance fruit storage, resistance to pathogens and surface disorders such as chilling injury.

#### Future Recommendations

Further studies are needed to fully ascertain the role played by structural features in enhancing the keeping quality of watermelon fruits. The different response exhibited by epicuticular wax in the two varieties is indicative of differences in wax properties. Future studies should investigate the composition of wax in the two varieties and characterize them, to further explore the possible role of epicuticular wax in enhancing keeping quality. Previous studies have shown that most incidents of fruit decay originate from the stem end (Risse *et al.*, 1990)

suggesting that the stem end facilitates the entry of pathogens into the fruit. Knowledge of the nature of the abscission zone in watermelon is needed to elucidate the role of abscission in the storage quality of watermelon. To gain more insight on the differences in storage properties between the Tsamma fruit and cultivated watermelon fruit further, future studies should investigate the storage properties of the two varieties under controlled conditions. For instance, the fruits could be subjected to controlled temperatures and then evaluated for chilling injury and decay.

### General Conclusions

The main goal of this thesis was to investigate how the Tsamma fruit differs from the domesticated watermelon fruit in development, morphology and anatomy, in an attempt to identify structural features associated with the keeping of watermelon fruits. The identical development of the pistillate flower in both Tsamma melon (var. *citroides*) and the domesticated watermelon var. *lanatus*) suggests that differences observed in the keeping quality of mature fruits are likely to be initiated after, and not before fertilization. Differences exhibited by the two varieties in terms of stomatal morphology and the nature of the outer-mesocarp tissue suggest that morphological and structural features may play a role in enhancing the keeping quality of watermelon fruits. Further studies are needed to ascertain the role of morphological and structural features in enhancing keeping quality of watermelon fruits.

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