Studies on Bali Salak Cultivars

(*Salacca zalacca* var*. amboinensis*)

(Arecaceae)

Thesis submitted by
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ABSTRACT

Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) (Arecaceae) commonly grow in Bali and other areas of the Indonesian archipelago and are of considerable value as a trade commodity because of their edible fruits. The thirteen cultivars that are currently recognised in Bali are distinguished from each other on the basis of fruit colour and taste. The research reported in this thesis documents the variability in Bali salak cultivars and provides a method for their reliable identification prior to fruiting. This involved analysis of vegetative anatomical and morphological characters as well as reproductive characters of all 13 cultivars.

Analysis of variance (ANOVA) was used to analyse the quantitative features to determine which characters differed significantly between cultivars. Multivariate techniques of cluster analysis and multidimensional scaling (MDS) were used to delimit and test groups of cultivars. Means for 17 of the 42 quantitative characters differed significantly ($p \leq 0.05$) and nine of the qualitative characters showed distinct variation between the cultivars. Cluster analysis of quantitative and qualitative characters established that the 13 cultivars grouped into four distinct clusters. MDS indicated that plant height, leaf length, middle leaflet length, female flower length, adaxial cell length, periclinal cell wall pattern, flesh taste, presence of fruits and seeds, which had square correlation values of more than 0.7, are reliable discriminators of cultivars. This study showed that anatomical and morphological characters and reproductive characters can be used as diagnostic tools to identify the 13 Bali salak cultivars. However, based on the multivariate analysis, it is proposed that this 13 be reduced to eight. The eight cultivars recognised in this study are Muani, Bingin, Putih, Nyuh, Maong, Boni, Gula and Biasa. Keys and descriptions of each cultivar are provided and recommendations are made for future research in *Salacca zalacca* var*. amboinensis*.

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GLOSSERY OF TERMS

abaxial – the side of an organ that faces away from the axis that bears it, for example, the under surface of the leaf.

adaxial – the side of an organ that faces toward the axis that bears it, for example, the upper side of the leaf.

amphistomatic – stomata on both upper and lower epidermis**.**

anisocytic – stomata surrounded by three unequally sized cells.

anomocytic – the cells surrounding each stomata are not recognizably different from the remaining epidermal cells.

aperture – a specialized region of a pollen grain wall, that is thinner than the remainder of the wall and generally differs in ornamentation and/or in structure.

cyclocytic – stomata have a ring of subsidiary cells of more or less equal size.

diacytic – stomata surrounded by two subsidiary cells.

disulcate – referring to pollen structure with two furrow like apertura.

echinate – referring to exine structure which bears spinelike sculpturing elements.

helicocytic – stomata are surrounded by a helix of four or more cells.

hypostomatic – stomata only on the lower epidermis**.**

inaperturate – referring to pollen structure without aperture.

meridionosulcate – a pollen grain with an encircling sulcus.

monosulcate – referring to pollen structure with one furrow like aperture.

paracytic – one or two subsidiary cells enclosing the guard cell length at right angles to the longitudinal axis of the guard cells.

plicate leaves – a situation in which the leaflets remain fused, as seen in seedling leaves.

polyembryony – referring to the development of more than one embryo from a single egg or ovule.

reticulate – referring to exine structure which consists of a network enclosing small, often in irregular spaces.

scabrate/granulate – a general term for sculpturing elements of exine which is less than 1 µm diameter and varying in shape.

sporoderm – the entire wall of a pollen grain or spore.

staurocytic – stomata surrounded by three subsidiary cells.

subsidiary cell – epidermal cells surrounding the guard cells.

tetracytic – stomata surrounded by four subsidiary cells.

vivipary – a situation that occurs when the embryo breaks through the seed coat (and defies natural growth inhibitors) to begin growing, sometimes while the fruit is still attached to the parent plant.

CHAPTER 1

General introduction

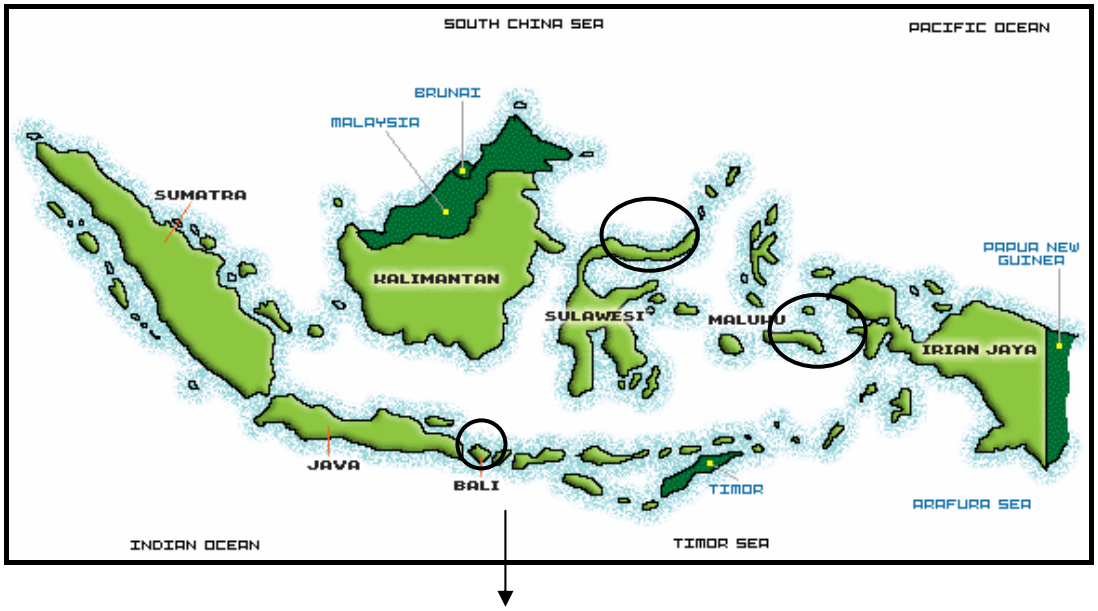
1.1 Background

Bali salak (*Salacca zalacca* var*. amboinensis* (Becc.) Mogea) is a member of the family Arecaceae, and of the genus *Salacca* (Uhl and Dransfield 1987). The genus is widespread in South East Asia occurring in in Burma, Thailand, Malayan Peninsula, Sumatra, Java, Bali, Borneo, and the Southern part of Philippines (Mogea 1980). The cultivar of Bali salak commonly grows in Bali and other areas of the Indonesian archipelago such as North Sulawesi and Ambon (Figure 1.1). The fruit is of considerable economic importance, principally for Balinese farmers because of its potential as an export commodity for Indonesia (Wijana 1997).

There were more than 12 million Bali salak plants growing in Bali in 1993, with more than 95% of these grown in the Karangasem district (Table 1.1) (Oka 1995). The production centre of Bali salak in Bali is Sibetan village. Approximately 30,777 tonnes of Bali salak were produced in 1993, more than 30,000 tonnes of which were produced in Karangasem regency (Table 1.2) (Oka 1995).

Thirteen cultivars are found in Sibetan village, and the identification of these cultivars is based on market perception of the taste of its fruits and other fruit features, which are variable. For example, the Nanas cultivar tastes like pineapple, while Nangka tastes like jackfruit (Figure 1.2). The cultivar Boni/Barak has red stripes on its flesh (Figure 1.3) (Oka 1995): the 13 cultivars are listed in Table 1.3.

A. Indonesian Achiphelago



B. Map of Bali island

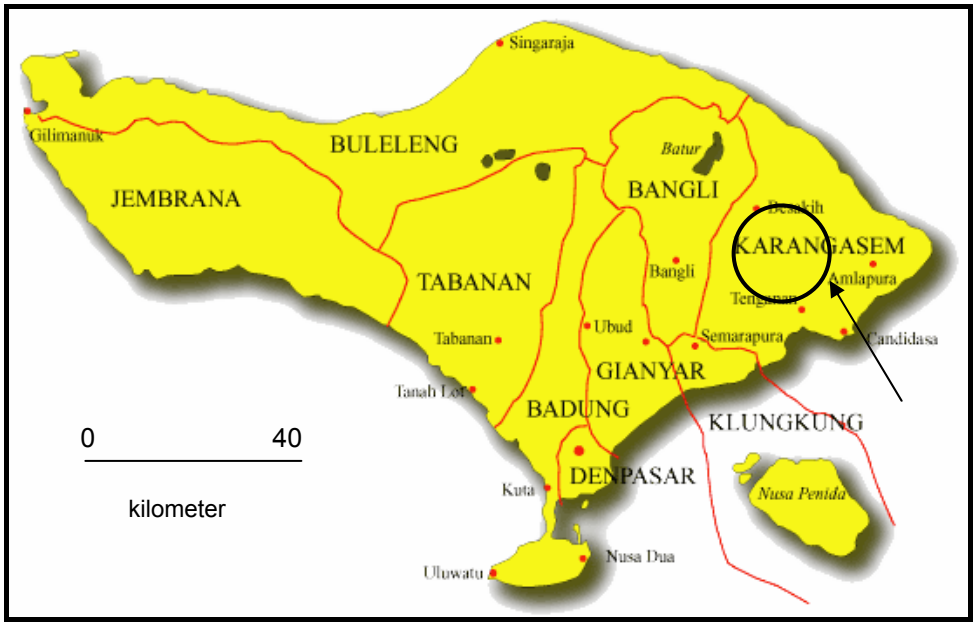


Figure 1.1 Maps showing Bali Island in the Indonesian archipelago: Bali, North Sulawesi and Ambon are designated by circles in Map A. The circle in map B shows the area from which samples were taken.

Figure 1.2. Fruits of Bali salak, showing brown scales and yellowish flesh of Nanas cultivar.

Figure 1.3. Fruits of Bali salak, showing red flesh of Boni cultivar (A) and golden yellow scales of Putih cultivar (B).

Table 1.1 The number of Bali salak plants grown in eight Bali districts from 1989 to 1993 (Oka 1995).

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Table 1.2 The annual production of Bali salak fruits (tonnes) from 1989 to 1993, in eight Bali districts (Oka 1995).

Table 1.3 Fruit characteristics of the Bali salak cultivars grown in Sibetan village, Karangasem, Bali based on Wijana (1997) and Karang Asem Tourism Government Office (2000).

Numb.	Cultivar	Characteristics of fruit
1	Gondok	Round fruits, brown skin and yellowish thick flesh; sweet, small kernels.
2	Nanas	Round fruits, brown skin, similar to Gondok, tastes like pineapple, with thick and juicy white flesh.
3	Gula	Similar fruit to Nanas; dark brown skin, white flesh, very sweet.
4	Nangka	Big yellowish-brown fruit, yellowish flesh smells like jackfruit, thick, juicy and sweat flesh.
5	Selem/Injin	Skin and fruit similar to Nanas; black stripes in flesh.
6	Embadan/Raja	Big yellowish- brown fruits, similar to Nangka but more juicy flesh.
7	Boni/Barak/ Getih	Similar to other salaks, black skin on the tips, red flesh, sweet and fresh.
8	Bingin	Curly leaves, rarely produces fruits.
9	Maong	Similar to other cultivar fruits, white spots on the skin.
10	Nyuh/Kelapa	Big yellowish-brown fruits, fruit and flesh similar to Nangka, tastes like coconut.
11	Penyalin/Sepet	Similar to Nyuh, astringent to taste.
12	Putih/Toris	Golden yellow scales, sweet flesh.
13	Muani	Mantained as ‘a male parent’, never produces fruits.

Although a number of research studies have been conducted using anatomical and morphological features (Utami 1989; Darmadi 2001), there has been no significant study that comprehensively distinguishes each cultivar of Bali salak based on their leaf epidermal and morphological features. Utami (1989) found the average stomatal index (the number of stomata per unit area divided by the total number of stomata and epidermal cells per unit area) of Bali salak cultivars to be 35.7%. But, did not specify on which cultivar this figure was based.

More recent studies by Darmadi (2001) established that the epidermal cell shape of Bali salak cultivars is rectangular and the arrangement of the stomata is paracytic i.e., stomata surrounded by two or more subsidiary cells which are parallel and adjacent to the guard cells. The research also found that the stomatal index of the cultivars varies from 17.6% to 37.9%. However, these studies also did not stipulate to which cultivars these two values apply. Furthermore, Darmadi (2001) established that 13 cultivars of Bali salak could be divided into eight groups based on the morphological features. These groups are “Biasa”, “Maong”, “Gula pasir”, “Nyuh”, “Putih”, “Boni”, “Injin” and “Pada”. Note, the cultivar “Pada” is not grown in the field, only in pots. However, the study was mainly based on fruit features, and as few specimens were examined, this study was not representative of the cultivars, due to limited replications.

Detailed studies of the cultivars based on vegetative anatomical and morphological characters and reproductive characters have not been conducted. Therefore, this study contributes to a broad understanding of morphological variability in the existing *Salacca* cultivars which may be utilized in cultivar identification.

1.2 *Salacca*

1.2.1 Distribution

The genus *Salacca* is known as “salak” in Indonesian, “snake fruit” in English, and “sala” in Thai. In Indonesia, salak is widely cultivated in the lowlands throughout the islands (Figure 1.4). The plant is also widely grown throughout South East Asia, Myanmar, Thailand, Malaysia, and the Philippines (Mogea 1980; Schuiling and Mogea 1992). Salak plants have been introduced into New Guinea, Queensland (Australia), Ponape Island (Caroline Archipelago) and the Fiji Islands (Schuiling and Mogea 1992).

Figure 1.4 The Bali salak plant (*Salacca zalacca* var. a*mboinensis*).

1.2.2 Ecology

Salacca are plants of the undergrowth of primary tropical rain forest. Many species thrive in swampy valleys where they form dense spiny thickets (Uhl and Dransfield (1987). *Salacca* is a tropical palm, which thrives in sandy fertile soil. The plants optimally grow in an environment at temperatures between 22^{o}C and 32^{o}C, high humidity, and at least 1700 mm rain per annum with only a short dry season (Chay-Prove and Goebel 2000).

1.2.3 Generic description of *Salacca*

The genus *Salacca* is composed of spiny dioecious or andromonocious plants with small to rather robust habit, acaulescent or with short stems, usually clustered; height ranges from 1 – 12 m. Stems are short, 8 - 15 cm long and erect, rather narrow, 15 - 25 cm wide, mostly subterranean, internodes congested, with leaf traces inserted almost horizontally. Petioles are channeled on the adaxial surface near the base (Figure 1.5 A). Petiole and leaf rachis are armed with numerous long spines. Leaves are small to robust and range from 4 – 7 m long. The pinnate leaf has leaflets 0.40 – 0.73 m long and the terminal leaflets are bifid (Figure 1.5 B & C). Leaflets are reduplicated each with a prominent abaxial midrib and a smaller lateral leaf. Inflorescences are usually short or, sometimes spicate, or with one or two orders of crowded or spreading branches. Fruits are in tight somewhat globose bunches, 2.5 – 10 cm long, 5 – 7 cm wide (Figure 1.5 D) (Uhl and Dransfield 1987; Darmadi 2001; Hodel 1997; Lestari and Ebert 2002).

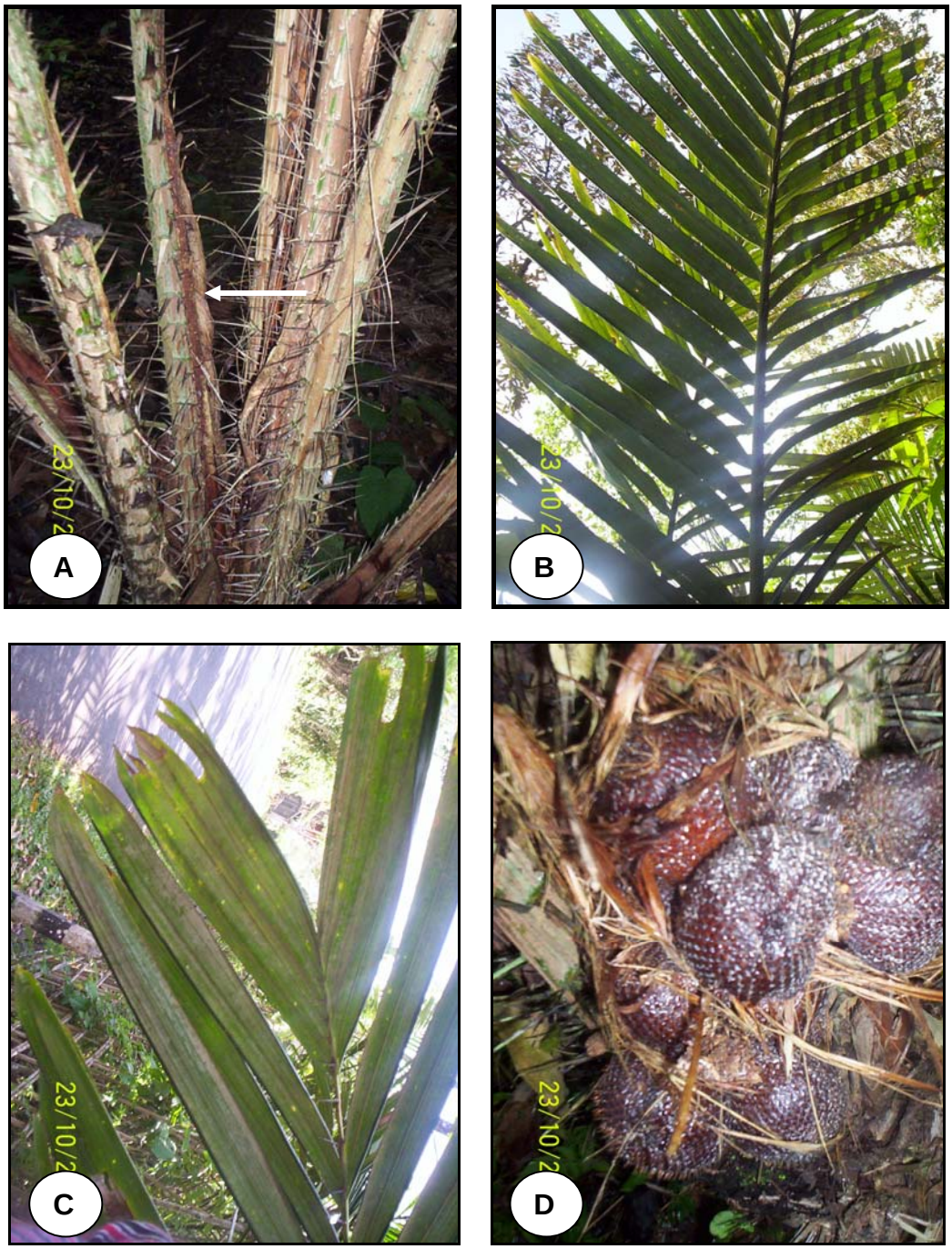

Figure 1.5 Bali salak plant (*Salacca zalacca* var. a*mboinensis*). A, the adaxial surface of the petioles showing a channel or groove near the base. B, the leaf with a pinnate shape. C, a bifid form of terminal leaflets. D, fruits in a tight globose bunches.

1.2.4 Classification

The following classification is based on the taxonomy of Cronquist (1981) modified by Uhl and Dransfield (1987).

Division	:	Spermatophyta
Sub Division	:	Angiospermae
Class	:	Monocotyledoneae
Subclass	:	Arecidae
Order	:	Arecales
Family	:	Arecaceae (Palmae)
Sub family	:	Calamoideae
Genus	:	*Salacca* Reinw.
Species	:	*Salacca zalacca* (Gaertn.) Voss

According to Govaerts and Dransfield (2005), *Salacca* consists of 20 species (Table 1.4), four of which have been developed as horticultural plants in different areas: *Salacca wallichiana* (Thailand), *Salacca glabrescens* (Malaysia), *Salacca sumatrana* (Sumatra island), and *Salacca zalacca* (Java, Bali, Sulawesi and Maluku/Ambon) (Mogea 1982; Schuiling and Mogea 1992). *S. zalacca* has also been developed in North Queensland, Australia (Chay-Prove and Goebel 2000).

Salacca zalacca, has a recognised variety *S. zalacca* var. *amboinensis.* This variety is different with the Javanese forms (*Salacca zalacca* var. *zalacca*) (Mogea 1982). For further discussion see Chapter 5 under taxonomy.

Table 1.4 The species names and the areas of distribution of *Salacca* species throughout South East Asia. Adapted from Mogea (1981b) and Govaerts and Dransfield (2005).

Num.	Species name	Distribution area
1	*Salacca affinis* Griff.	Sumatra, Malaysia, Borneo
2	*Salacca clemensiana* Becc.	Philippines (Mindanao), Borneo
3	*Salacca dolicholepis* Burr.	Sabah (Borneo)
4	*Salacca dransfieldiana* Mogea	South Borneo, West Borneo
5	*Salacca flabellata* Furt.	Malayan peninsula
6	*Salacca glabrescens* Griff.	Thailand, Malayan peninsula
7	*Salacca graciliflora* Mogea	Malayan peninsula (Johor)
8	*Salacca lophospata* J. Dransf. & Mogea	Sabah (Borneo)
9	*Salacca magnifica* Mogea	Sarawak (Borno)
10	*Salacca minuta* Mogea	Malayan paninsula (Johor)
11	*Salacca multiflora* Mogea	Malayan paninsula (Trengganu)
12	*Salacca ramosiana* Mogea	Borneo, Philippines (Sulu island)
13	*Salacca rupicola* J.Dransf.	Sarawak (Borneo)
14	*Salacca sarawakensis* Mogea	Sarawak (Borneo)
15	*Salacca secunda* Griff.	Malaysia
16	*Salacca stolonifera* Hodel	Thailand
17	*Salacca sumatrana* Becc.	North Sumatra
18	*Salacca vermicularis* Becc.	Borneo
19	*Salacca wallichiana* Mart.	Myanmar, Thailand, Malayan peninsula
20	*Salacca zalacca* (Gaertn.) Voss	Java, Madura, Bali, North Sulawesi, Ambon
21	*Salacca zalacca* var. *amboinensis* (Becc.) Mogea	Bali, North Sulawesi, Ambon

1.3 Research aims

Studies were carried out on 13 Bali salak cultivars. The aims of the present studies were:

1. to analyze and compare leaf epidermal characters
2. to evaluate and compare vegetative and reproductive characters
3. to conduct multivariate analyses of 13 Bali salak cultivars using vegetative anatomical and morphological data, as well as reproductive data.
4. to identify morphological and leaf epidermal characters that could be used to distinguish these 13 Bali salak cultivars.

1.4 Significance of the research

This research utilised a novel set of data based on vegetative, reproductive and leaf epidermal information. Analysis of this data was to:

1. allow inference of the patterns of morphological variation in Bali salak cultivars
2. enhance our ability to identify *Salacca* cultivars using vegetative, reproductive, and leaf epidermal materials
3. improve opportunities for particular cultivar plantation development of this important species in Bali

1.5 Scope of the research

In order to analyse leaf epidermal characteristics of the Bali salak cultivars grown in Sibetan village, Karangasem district, 13 cultivars were investigated. Leaf epidermal and morphological observations and analyses of the relevant data are presented in chapter 2 and 3 respectively. Discrimination among 13 Bali salak cultivars was determined using multivariate analyses, based on vegetative anatomical and morphological characters, as well as reproductive characters. This work is

presented in chapter 4. Identification keys were developed using characteristics of each cultivar based on vegetative anatomical and morphological characters, and reproductive features. These keys have potential for widespread use in plantations by Balinese farmers. This part is documented in chapter 5. Summary and conclusion are presented in chapter 6.

CHAPTER 2

Analysis of Leaf Epidermal Characters of Bali Salak Cultivars

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2.1 Introduction

Micromorphological characters of leaf surfaces can assist in taxonomic identification and classification because of their high structural diversity (Wilkinson 1979; Clifford and Watson 1977; Metcalfe and Chalk 1979; Stace 1984). Extensive research on plant diversity based on leaf epidermal characteristics has shown significant patterns of variation among and within the dicotyledons and monocotyledons (Cutler 1982). Additionally, more recent studies have documented epidermal leaf features as useful taxonomic tools in identifying various angiosperms (Olowekudejo and Sheteolu 1988; Yukawa *et al*. 1992; Olowokudejo 1993; Kong, 2001). In this chapter the variety of techniques that have been used in epidermal studies of various plants will be discussed, as only a few studies (Utami 1989; Darmadi 2001) involved Bali salak cultivars.

Olowekudejo and Sheteolu (1988) demonstrated that leaf epidermal characters were valuable in identifying species within the genus *Ocimum* (Lamiaceae). These characters included the frequency and size of stomata, cell shape and size, and thickness of the cell walls. In addition, five of the six species examined showed inconspicuous anticlinal walls: the positions of the walls being obscured by the cuticular fold, while in one species the wall was indicated by a broad shallow groove.

Yukawa *et al*. (1992) used epidermal features in identifying 153 species of the genus *Dendrobium* (Orchidaceae), and found that the shape and size of the outer stomatal ledge was useful in identifying the species. Based on these characters, the 153 species investigated were divisible into three groups. The first three groups of species were quite distinct from each other; however, for the results for third group, were variable and indicative of heterogeneity in the characteristics measured, i.e. they were poorly resolved.

Comparative epidermal morphology of eight West African species of *Jatropha* (Euphorbiaceae) exhibited variations in stomatal size both within and between taxa. The stomata of *J. atacorensis* were generally the smallest among the species investigated. *J. gossypiifolia* had the largest stomata. Other characteristics of the epidermis, including cell size and periclinal cell walls, also varied and had taxonomic significance in identification of species of this genus (Olowokudejo 1993).

Gogoi *et al*. (2002) in a study of epidermal characters for 12 species of Zingiberaceae, found that the stomatal index varied among the species examined. It was highest in *Kaempfera galanga* and lowest in *Elettaria cardamomum*. Epidermal cell walls varied from straight or straight to curved between the 12 species investigated.

Diverse epidermal characters have also been used for identifying other plant genera, species or groups within *Albizia* (Ogundipe and Akinrinlade 1998), *Crotalaria* (Parveen *et al*. 2000), *Cassia* (Kotresa and Seetharam 2000), *Pinus* (Chaturvedi 1998; Whang and Pak 2001), *Eugenia* (Van Wyk *et al*. 1982; Haron and Moore 1996), *Sterculia* (Hussin and Sani 1998), as well as for documenting within species variation in *Populus ciliata* (Sharma *et al*. 2001), *Vigna unguiculata* (L.) Walp. (Ghimiray and Das 1996), and the hybrid *Hebe franciscana* (Heenan 1994).

2.2 Epidermal cells

2.2.1 Cell size

According to Stace (1965a), the size of the epidermal cells cannot be assumed to be a reliable taxonomic feature. This is due to variability in the epidermal cell size, which may be correlated with the age of the leaf, genetic variation, and the environment. However, many taxonomists use this characteristic to differentiate closely related species. Thus, it is important to characterize the variability within species if this feature is to be utilised as a character.

Epidermal cells may differ significantly from species to species, principally when seen in surface view (Cutler 1982). The variation may be in cell size and wall thickness, or only in the distribution of stomata on each surface (Cutler 1982). For example, in a study of *Jatropa.* Olowakudejo (1993) found that the cells vary in size both within and between the species; the adaxial cells of the genus are generally bigger in size than those on the abaxial surface.

2.2.2 Cell shape

The majority of the dicotyledons and many monocoyledons tend to have epidermal cells that are irregular in shape, with straight or sinuous anticlinal walls (Cutler 1982). Wilkinson (1979) established eight basic patterns of anticlinal walls (flanges) as seen in surface view (Figure 2.1).

Christophel and Rowett (1996) in their study of Lauraceae used the general categories of angular, rounded, undulate, sinuous and irregular rounded for the cell shapes examined, with regard to anticlinal wall patterns.

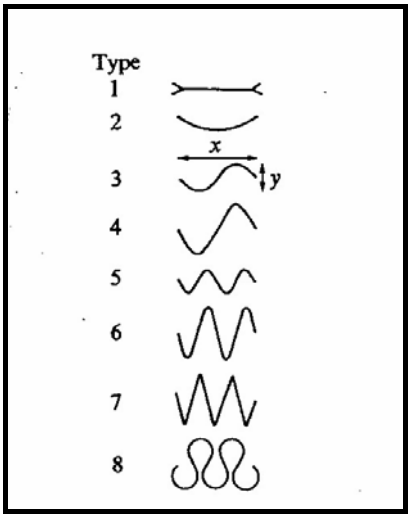

Figure 2.1 Eight basic patterns of anticlinal cell walls as seen in surface view of leaf epidermis. Types 1) straight, 2) curved, 3) loose, wide U-shaped curves of shallow amplitude, 4) loose V-shaped curves of deep amplitude, 5) tight with frequent U-shaped curves of shallow amplitude, 6) tight, acutely angled, U-shaped curves of deep amplitude, 7) tight, sharply angled, V-shaped curves of deep amplitude, 8) tight, deep convolutions of omega (Ω) shape (Wilkinson 1979; Stace 1965a).

Kong (2001) who studied sixteen species within the family Chlorantaceae observed polygonal or irregular cell forms. The anticlinal cell walls exhibited variations in different species or between the adaxial and abaxial epidermis of the same species. This characteristic has significant taxonomic value in differentiating the species. The patterns were straight to arched, undulate, or sinuous anticlinal walls. Four species investigated had straight to arched walls. Whereas, slightly undulate walls occurred in nine species and very sinuous walls were found in three species.

Whang and Pak (2001) found the shape of the anticlinal walls of the epidermal cells of *Pinus*, provided some evidences for subdividing the genus. The anticlinal wall shapes in all species of subgenus *Strobus* are straight, serrate, sinuous, and very sinuous; but there is less variability in subgenus *Pinus*, where anticlinal walls are straight and/or sinuous.

Dilcher and Zack (1968) found a similar pattern of wall undulation in sun and shade leaves of *Fagus grandifolia,* but not in *Quercus alba* or *Quercus rubra*. The authors concluded that possible environmental effects must be considered before undulation of the anticlinal cell walls can be considered a useful character to delimit species.

2.3 Stomata

2.3.1 Stomatal distribution

Stomatal distribution patterns are considered to have taxonomic significance among some plants. In monocotyledons, which have parallel venation, the stomata are oriented parallel to the longer axis of the lamina (Rajagopal 1979). Patil and Patil's (1987) study of the genus *Chlorophytum* indicated that the stomatal distribution pattern was such that the longer axis of the stomata were parallel to the longer axis of the lamina and they were restricted to the area between two adjacent veins. The vein surfaces were devoid of stomata except in *Chlorophytum tuberosum* where fewer stomata occurred.

On the basis of presence and absence of stomata, leaves are differentiated into three types (Wilkinson 1979):

(i) amphistomatic: the stomata occur on both sides of leaf surfaces,

(ii) hypostomatic: stomata are almost exclusively on the abaxial leaf surface,

(ii) epistomatic: stomata are present on the adaxial leaf surface only, principally this is observed in floating leaves.

In most gymnosperms and evergreen angiosperms, stomata tend to be on the lower/abaxial leaf surface only; in other angiosperms the distribution varies from species to species (Cutler 1982).

2.3.2 Stomatal structure

A stoma generally consists of an elliptical pore in the epidermis of leaves, herbaceous stems and floral parts, enclosed by two specialized kidney-shaped epidermal cells, the guard cells (Figure 2.2) (Wilkinson 1979). The elevation of the cuticular membrane which rises from the guard cells is known as the outer stomatal ledge or rim (Figure 2.3); (Wilkinson 1979). Transverse sections of leaf epidermis can show some of the characteristics that are to be seen in surface view. The sections reveal the extent to which the stomata are sunken into, or raised above the leaf surfaces (Wilkinson 1979)

Generally in dicotyledons, the guard cells are immediately surrounded by one or more subsidiary cells. These cells sometimes are not distinguishable from other epidermal cells; however in some species they are morphologically distinctive because of their shape, size and orientation in relation to the guard cells (Wilkinson 1979).

On the basis of the shape and arrangement of subsidiary cells, various types of stomata have been described. Stomatal types and their characteristics are presented in Table 2.1.

2.3.3 Guard cells

Wilkinson (1979) considered that the outline of the pair of guard cells as seen in surface view is usually constant at the species level and is sometimes characteristic of a genus. According to Christophel and Rowett (1996) the range of variation that may be possible is: 1) broad, 2) round, 3) broadly elliptical, 4) narrowly elliptical, and 5) angular.

Figure 2.2 Diagram of stomatal apparatus in surface view (Wilkinson, 1979).

Figure 2.3 Diagramatic representation of the stomatal apparatus from transverse section (Wilkinson, 1979).

Table 2.1 Types and characteristics of stomata found in various plants. Adapted from Dilcher (1974) and Wilkinson (1979**).**

Type of stomata	Characteristics	Examples
Anomocytic	The cells surrounding each stomata are not recognizably different from the remaining epidermal cells.	Ranunculaceae
Diacytic	Stomata surrounded by two subsidiary cells	*Justicia*, *Dianthus*
Paracytic	Stomata have one or two subsidiary cells adjacent to the guard cells.	*Juncus*, *Sorghum* and C*arex*
Tetracytic	Stomata surrounded by four subsidiary cells	*Tradescantia*
Anisocytic	Stomata surrounded by three cells of different sizes	*Plumbago*
Cyclocytic	Stomata have a ring of subsidiary cells of more or less equal size	Piperaceae, Apocynaceae
Staurocytic	Stomata surrounded by three to five subsidiary cells with anticlinal walls arranged crosswise to the guard cells.	Marcgraviaceae
Helicocytic	Stomata surrounded by a spiral of four or more cells.	Malvaceae, Asteraceae

Christophel and Rowett (1996) considered that stomatal patterns can provide an excellent basis for the identification of species within the family Lauraceae. The cuticular ledges of guard cells in different genera Lauraceae differ significantly in appearance. A large and almost butterfly shape was found in some species of *Cryptocarya*. Whereas, slightly less broad and kidney-shaped cells occurred in some of the other species of the genus. Cuticular ledges were found to be relatively straight and thin in *Beilschemiedia*, but consistently double in *Endiandra.*

Wilkinson (1979) established that stomatal size shows a much wider range in some taxa than in others, so it can sometimes be a useful diagnostic character when dealing with taxa in which the size ranges are restricted. However, plants growing in

shade, humid environments and moist soil conditions tend to have smaller stomata, while plants growing in full sunlight and drier conditions seem to produce larger stomata. Nevertheless, stomata are less easily influenced by these factors than are ordinary epidermal cells (Wilkinson 1979).

2.4 Epidermal studies on some Palms

Tomlinson (1990) described the general construction of the palm leaf blade as a plate-like sheet of tissue supported by the midrib of the leaf segments. There is a longitudinal vein between the blades, which reflects the sequence of their initial differentiation within lamina tissue. The transverse vein connects with some of the longitudinal veins and forms an important component of the blade. The most distinctive cells in palms, which may be thick-walled and thickly cutinised, lie above the larger rib.

Furthermore, Tomlinson (1990) found that cells on the adaxial leaf surface of palm leaves, which lack of stomata, form a uniform sheet of cells. The cells are commonly rectangular, with the long axis parallel to the veins. In most arecoid palms, however, the epidermal cells are rhomboid. A more obvious feature is the development of a small sinuous outline to the anticlinal cell walls.

Stomata are generally most abundant on the abaxial leaf surface of the palm leaves, but in some genera are equally numerous on both surfaces. For example, *Butia*, *Phoenix*, *Sabal* and some other coryphoid palms have an equal abundance of stomata on both leaf surfaces (Tomlinsion 1990). The stomata typically are confined to relatively narrow bands which lie above or below veins or fibrous strands. Within stomatal bands, the stomata are arranged in indistinct files, the cells in the stomatal files sometimes being shorter and wider than those elsewhere (Tomlinson 1990).

Banson and Velasco (1982) reported that the stomata always occur in intercostal regions of coconut palms (*Cocos nucifera*). The densities of these stomata are considered characteristic among the coconut leaf varieties.

The length of palm stomata as measured by the length of the guard cells differs considerably. The guard cells of most Lepidocaryoid palms are small (15–25 µm) with conspicuous ledges, while guard cells of the arecoid, chamaedoroid, and iriarteroid palms are larger (20–35 µm) and have inconspicuous ledges. The size of the guard cells is usually correlated with that of the epidermal cells overall, so the differences in guard cell size can be used to identify palm species with small or large epidermal cells (Tomlinson 1990).

Research by Ghose and Battacharya (1996) on coconuts in the Sundarbans area of West Bengal, India indicated that epidermal characteristics of leaves could be regarded as useful characters for identification and selection. The authors found that the length of epidermal cells, length of guard cells, stomatal density, and stomatal index vary significantly among the cultivars. The highest frequency of stomata expressed as the number of stomata per unit area and the largest guard cells occur in the dwarf cultivars Malayan dwarf yellow and Chowghat dwarf orange, respectively. Another characteristic which varied significantly among the cultivars was the length of epidermal cells (Ghose and Battacharya 1996).

2.5 The results of preliminary studies on Bali salak cultivars

Preliminary studies established that five characters: cell shape, adaxial cell length, adaxial cell width, stomatal density, and stomatal index, were significantly different among three cultivars Muani, Nyuh, and Putih examined under light microscopy (LM); (Gari 2003, unpublished data).

Additionally, the multivariate analysis of the cultivars using ten replicates per cultivar indicated the existence of a main group consisting of the nine cultivars Boni, Nangka, Penyalin, Maong, Nyuh, Bingin, Selem, Putih and Embad. Four cultivars (Muani, Gula, Gondok, and Nanas) were distinctly separate from this group of nine. The separation was supported by the following characters adaxial cell length, adaxial cell width, guard cell width, abaxial stomatal density, and stomatal index, which had a principal component loading of more than 0.7 (Gari, 2003 unpublished data).

Further LM observations were conducted to provide more replicates. In addition, scanning electron microscopy (SEM) was used to amplify the results of LM and also permitted analysis of additional leaf epidermal characters for discriminating between cultivars, which could not be detected by LM.

2.6 Specific Aims

The specific aims of this section of the study were:

i. to quantify variability of leaf epidermal characters within and among Bali salak cultivars based on observations made under a light microscope.
ii. to quantify variability of leaf epidermal characters within and among Bali salak cultivars based on scanning electron microscope observations.

2.7 Methods

2.7.1 Plant materials and sampling sites

Samples were collected from plantations at Sibetan village, Karangasem, Bali, Indonesia during January – February 2003. The village is the centre of production of Bali salak in Bali. In 1994, there were more than 12 million plants growing in Bali,

with more than 95% of these grown in the Karangasem District, principally in Sibetan Village (Oka 1995). The village covering, an area of 11.25 km^2, is at an altitude of 600 – 1000 m above sea level and characterized by a tropical climate with temperatures varying between 24^oC - 31^oC. January - February is the peak season for Bali salak fruit production. Collection of leaves was made during the fruiting season to ensure that the identity of the salak cultivars studied was determined directly from the fruits. Thirty leaf samples for the epidermal analyses were taken from at least five different sites, distributed throughout the village. However, for the rare cultivars Boni, Putih, and Bingin, it was necessary to collect the 30 individuals samples from more than five different locations due to low numbers of individual plants present at each site. Leaf samples taken for epidermal measurements were immediately placed in silica gel to desiccate the leaf tissue and to eliminate fungal growth. The location of the sampling site is shown in Figure 1.1.

2.7.2 Sampling leaves for microscopy

Selective sampling was carried out from mature plants. Thirty leaf samples were collected from 12 of the 13 cultivars. However, for Bingin only nine samples were available. Therefore, the total number of plants sampled was 369 (Figure 2.4). The third leaf from the base was taken to ensure comparable stages of maturity of the fronds (Figure 2.5). For consistency of data analysis, the samples for epidermal peels were taken from the central portion of middle leaflets (Figure 2.6).

Figure 2.4 Sampling procedure used for analysing leaf epidermal characters of 13 Bali salak cultivars.

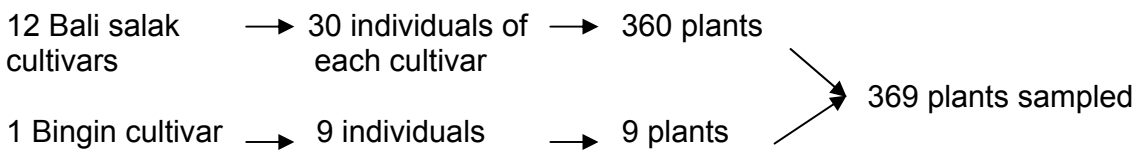

Figure 2.5 The position of leaf sampled for the leaf epidermal observation. The arrowhead shows the position of leaf sampled at the third leaf from the base.

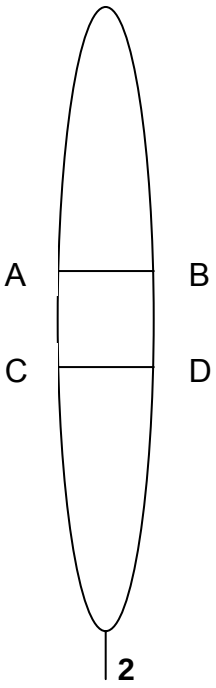

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Figure 2.6 The position of the leaflets sampled from the fronds for leaf epidermal analysis. Diagram 1 shows the position of the leaflet sampled from the fronds, as indicated by the arrowhead. Diagram 2 shows where samples were taken from lamina (A–B and C–D).

2.7.3 Scanning electron microscopy (SEM)

The use of SEM was required to amplify the results of the light microscope (LM) observations of the preliminary study (Gari 2003, unpublished data). The studies suggested that qualitative leaf epidermal characters, such as cell surface structures and stomatal structures did not differ significantly between the 13 cultivars when examined using LM. The higher resolving power of SEM was used to determine if there were differences that could not be detected with the LM.

To facilitate examination of the leaf epidermal structures various techniques were investigated to clean the wax from the leaf surfaces. Small (1 cm x 1 cm) pieces of the leaf were cut and immersed in de-waxing chemicals (chloroform, 1:3 mixture of methanol and chloroform, and detergent) for 1, 2, 3, and 4 days. The use of the methanol and chloroform mixture (1:3) for a period of three days in combination with gently brushing the surface using a fine brush produced the best results for photo imaging. The others methods did not produce clear images under SEM.

The clean samples were then mounted onto the SEM stubs by double-sided adhesive tape, critical point dried, sputter-coated with gold/palladium and scanned using a Philips XL-20 scanning electron microscope. Two replicates of each of the 13 cultivars were scanned, giving a total of 26 samples/stubs.

Table 2.2 Characters used for analysing leaf epidermal features of Bali salak cultivars using electron microscopy.

Number	Character
1	periclinal cell wall pattern
2	anticlinal cell wall pattern
3	stomatal structures
4	guard cell position

The characters observed under SEM are shown in Table 2.2. The terminology used for the observations of SEM of the leaf epidermis in this study was adapted from the study of the Lauraceae (Christophel and Rowett 1996).

2.7.4 Light microscopy

2.7.4.1 Preparation of leaf epidermal peels.

There are a number of approaches for obtaining epidermal peels from a leaf. Initially, three techniques were investigated to determine the most appropriate method of obtaining epidermal peels from Bali salak cultivars. These were the methods of Artschwager (1930), Ramm and Nayar (1974), and Hilu and Randall (1984). All epidermal imprints were observed under a compound Olympus™ microscope.

a. Method of Artschwager (1930)

Small segments of lamina/dried leaflet (1 cm^2) were cut. Tissue samples are placed in test tubes and 2 ml of concentrated nitric acid was added together with a few crystals of potassium chlorate ($KClO_2$). The mixture was boiled and then poured slowly into distilled water to cool the mixture and to halt the maceration process (Artschwager 1930). The sample was stained in a mixture of safranine and alcian blue (0.5% aqueous solution) for three hours, then rinsed in absolute ethyl alcohol for 30 minutes, followed by a second rinse for additional 30 minutes and mounted in glyserin (Illic 1985).

b. Method of Ramm and Nayar (1974)

Lamina samples (1 cm^2) were boiled in 2 ml 5-10% cupric sulphate for two minutes, then 4 ml concentrated HCl was added and the solutions were boiled for another 2 minutes. The lamina was stained in safranine (0.5% aqueous solution) overnight at

room temperature. Samples were then dehydrated in an ethyl alcohol-xylol series and mounted in glycerin.

c. Method of Hilu and Randall (1984)

The leaf surface was simply coated with a layer of clear, non-coloured nail polish, and then the hardened nail polish was peeled off after two hours and mounted in glycerin at a microscope slide under a cover slip.

d. Method used in this study

Based on the results of the pilot study, it was determined that Artschwager's (1930) method was the most appropriate technique of the three (Figure 2.7) and was adopted in this study. Boiling the leaf in a mixture of nitric acid and of potassium chlorate resulted in the acid rapidly digesting the inner tissues including the vascular fibres. The two cuticular layers separated from each surface, resulting in clear views of the epidermal cells.

However, it was necessary to modify the method of Artschwager (1930) because boiling the leaf in 100% concentrated nitric acid also resulted in the disintegration of the epidermal layers. Therefore, nitric acid concentrations of 80%, 70% and 60% were investigated (Table 2.3). The use of 70% nitric acid produced optimal results of thin epidermal layers, while surface layers still rapidly dissolved using 80% and 100% nitric acid. Boiling leaves in 60% nitric acid produced a thick epidermal layer and the epidermal cells could not be clearly observed under a microscope.

An experiment was carried out to compare the length of staining time required using two stain reagents: safranine, and mixture of safranine and alcian blue. The time was varied over periods of 2, 3, 4, 6 hours, and over night (16 hours). Three hours staining gave optimal results. Each of the stains i.e. safranine alone or mixture

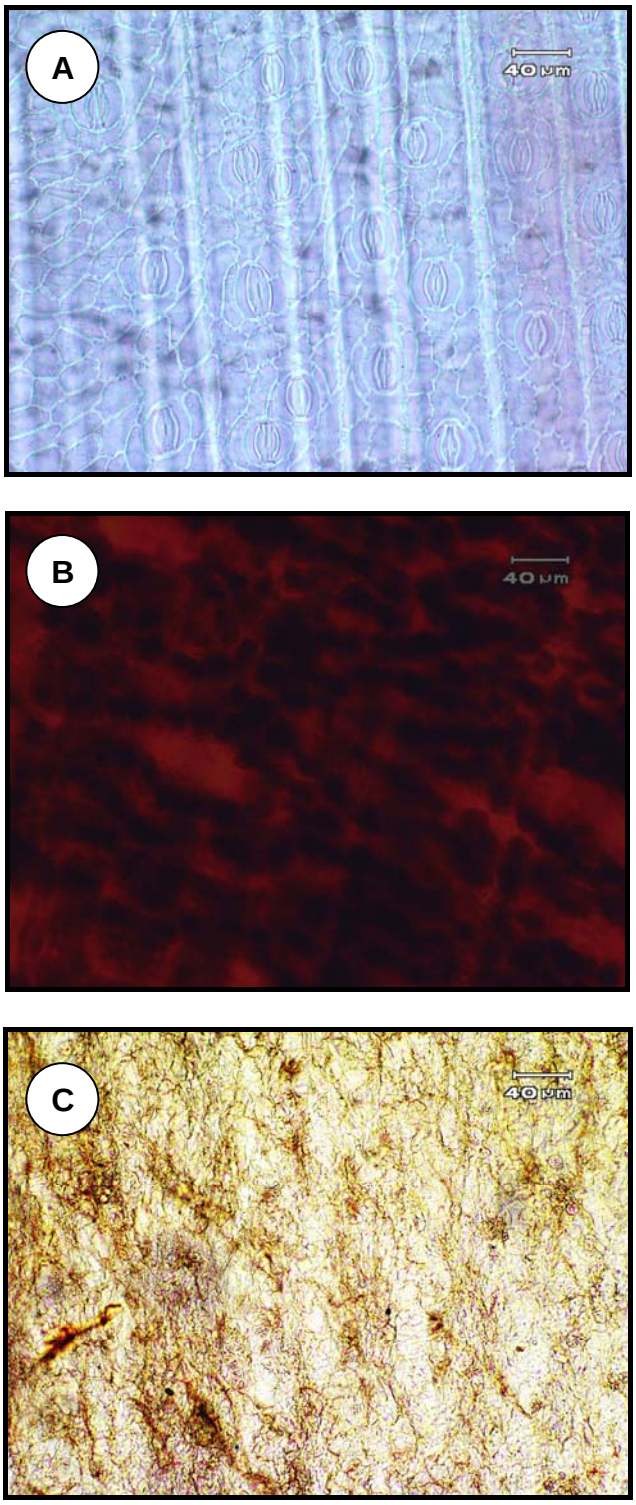


Figure 2.7 Epidermal peels of Bali salak cultivars using light microscopy produced from three different methods: (A) Artschwager (1930), (B) Ram and Nayer (1974) and (C) Hilu and Randall (1984). The pictures were taken at magnification X 200.

safranine and alcian blue resulted in clearly differentiated tissues and the later was adopted because it resulted in clear photographic images.

Ghose and Davis (1973) found that epidermal peels from adaxial and abaxial surfaces of *Cocos nucifera* (Arecaceae) leaves showed differences in pattern such that adaxial-abaxial surfaces could be identified without difficulties.

The methods of Ram and Nayyar (1973) and Hilu and Randall (1984) did not produce appropriate results for studying epidermal peels of Bali salak leaves, and were not used for reasons outlined bellow:

Table 2.3 Results of boiling leaves of Bali salak cultivars in various concentrations of nitric acid.

Nitric acid concentration	Result
Nitric acid 100%	dissolved epidermal layer
Nitric acid 80%	dissolved epidermal layer
Nitric acid 70%	clear epidermal imprint
Nitric acid 60%	thick epidermal imprint

Ram and Nayyar (1973)

The epidermal coat obtained from this method had a thick tissue layer. A range of boiling times was used in an attempt to produce thinner epidermal layers and the time was extended from 4 minutes to 6, 8, 10, and 15 minutes respectively. However, in spite of the extended time epidermal layers were still thick and were difficult to observe under the microscope.

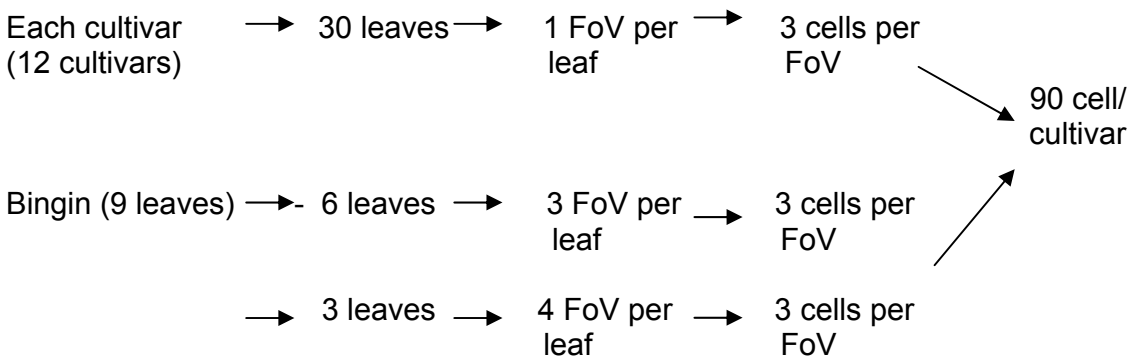
Hilu and Randall (1984)

When nail polish was used, the epidermal imprint could not be easily removed from the leaf surfaces. This might be due to the fact that the leaves of Bali salak cultivars used in this study had been dried.

2.7.4.2 Observation of leaf epidermal characters

One slide was made for each of the 30 leaf samples and one representative field of view was selected from each slide. Three cells were selected randomly from within each field and length and width were measured. Thus, the total number of cells observed per cultivar was 90 (n = 30) (Figure 2.8). For Bingin cultivar only nine leaf samples were found (n = 9). In order to obtain 30 fields of views for this cultivar, three fields of views were taken from each of six samples and four fields of views were taken from each of the three samples and 3 cells were measured per field of view (Figure 2.8). The means of length and width of epidermal cells were then determined for each leaf sample, and subsequently a mean was determined for each cultivar. The resulting 30 means were used in statistical analysis (ANOVA).

Figure 2.8 Sampling data used for analysing leaf epidermal of 13 Bali salak cultivars (FoV = field of view). Measurement per leaf was based on three cells and per cultivar was based on 30 leaves.



Each field of view of the epidermal surface used to measure quantitative features was also used to determine qualitative features. The characters and measurements used in this study are shown in Table 2.4. The terminology used for both qualitative and quantitative character measurements of LM observations in this study was adopted from the study of the Lauraceae (Cristophel and Rowett 1996).

Table 2.4 Characters and measurements used for analysing leaf epidermal features of Bali salak cultivars under the light microscope.

Character	Character	Character
1. stomatal occurrence	7. abaxial cell width (µm)	13. thickening on anticlinal walls
2. adaxial cell shape	8. stomatal shape	14. pattern of anticlinal cells
3. abaxial cell shape	9. guard cell length (µm)	15. thickening on guard cells or outer stomatal ledge
4. adaxial cell length µm)	10. guard cell width (µm)	16. stomatal orientation
5. adaxial cell width (µm)	11. stomatal density (number)	17. ratio adaxial cell length to width
6. abaxial cell length (µm)	12. stomatal index (number)	18. ratio abaxial cell length to width

Stomata and epidermal cells were counted in three fields of views per sample to estimate stomatal density and stomatal index, and means were calculated. The stomatal index (SI) was calculated according to the formula given by Salisbury (1927): **I = S / S + E x 100%,**

where S = number of stomata per unit area, and E = number of epidermal cells per unit area.

All light microscopy observations were carried out using a compound Olympus microscope. The size of the cells and guard cells were recorded with a calibrated eyepiece micrometer at magnification X 400, which provided a field of views of

0.12 mm^2. Frequency data were converted to stomata per mm^2. Images were taken using a high power microscope equipped with a digital camera DP 12 system.

2.7.4.3 Paraffin embedding sections

Three cultivars, Bingin, Gondok, and Nyuh which showed appreciable differences in the positions of the guard cells relative to the epidermal cell surfaces under SEM observations, were investigated following the technique of Winsor (1994). Three leaf samples per cultivar were fixed in FAA for four days, due to the hardness of their structure. The leaves were then transferred to a graded alcohol series for dehydration: initially two changes in 70% alcohol for one hour, then in 80% and 90% each for one hour, in 95% for 3 hours, three changes in absolute alcohol for 3 hours, 2 changes in xylene for 3 hours, and lastly two changes in paraffin for 3 hours. The infiltrated samples were then transferred into a flat bottomed embedding capsule. The embedding capsules were filled with paraffin and the lid closed tightly. The sample was allowed to polymerise at room temperature for 24 hours, then sectioned using a feather disposable blade of a Heidelberg rotary microtome and floated in a hot water bath. The sections were stained with the mixture of safranine and alcian blue. The sections were mounted on the slides using Haup's adhesive, then a drop of DPX was used to mount a cover glass on the section (Illic 1985).

2.8 Data analyses

Data were collected as measurements were made, then entered into a spreadsheet in Microsoft Excel. Each page of data was printed out and carefully proof read to avoid the possibility of data errors. The values of means and standard errors were calculated for all quantitative characters using Microsoft Excel. Data were analysed by univariate statistical tests. Single classification analyses of variance (ANOVA) tests were performed using the SPSS 11.5 package for Windows, to determine

whether there were any significantly different characters among the cultivars (Zar 1999). The 95% confidence level ($p \leq 0.05$) was used to indicate significant differences between the means. Prior to the analysis, normality and homogeneity of variance were tested to meet the ANOVA assumptions.

The LM data and SEM observation data were used to classify the leaf epidermal features of the cultivars. Selected characters from quantitative measurements that showed significant differences based on the results of the ANOVA, and qualitative features of LM and SEM observations that demonstrated distinct variations between cultivars were statistically analysed (Chapter 4).

2.9 Results

The results of SEM and LM observations in this study are described separately. Using LM the qualitative leaf epidermal features of 13 Bali salak cultivars appeared to be identical. Cultivars were indistinguishable using this method (Appendix 2.1). Summary of statistical results from analysis of variance (ANOVA) of 10 quantitave leaf epidermal characters of 13 Bali salak cultivars. and scatter plots of 6 characters that showed significant differences ($p \leq 0.05$) based on ANOVA are shown in Appendix 2.2 and 2.3 respectively.

2.9.1 Scanning electron microscopy (SEM)

A summary of leaf epidermal features of Bali salak cultivars under SEM is presented in Table 2.5, and the representative images are illustrated in Figures 2.9, 2.10, and 2.11. The adaxial epidermis of all Bali salak leaves had curved to undulate anticlinal-cell wall patterns with ridges (Figure 2.9A & B). However, these features were more suited for observation under LM. The cultivars of Gula, Boni, Bingin, Maong, and Muani showed flat outer periclinal cell walls and the anticlinal cells showed thick ridges (Figure 2.9B). The epidermis consisted of regularly curved

square to rectangular cells with depressions on their surfaces (Figure 2.9B). The remaining cultivars, Selem, Embad, Nangka, Penyalin, Nanas, Gondok, Nyuh, and Putih had periclinal cell walls that were convex with shallow depressions on the surfaces (Figure 2.9D-F). Periclinal cell walls were found to be smooth or with cuticular folds, usually covered with particles of epicuticular wax (Figure 2.9C).

Table 2.5 The leaf epidermal features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) under SEM.

Character / Cultivar	Periclinal cell wall	Guard cell position
Gula	flat	level
Boni	flat	level
Bingin	flat	raised
Maong	flat	level
Muani	flat	sunken
Nangka	convex	sunken
Penyalin	convex	level
Selem	convex	level
Nanas	convex	level
Gondok	convex	level
Embad	convex	sunken
Nyuh	convex	sunken
Putih	convex	level

The abaxial leaf features of all cultivars investigated showed sculptured abaxial surfaces (Figure 2.10A-F). The characteristic of abaxial cells, such as anticlinal and periclinal cell walls, and subsidiary cells which surround the stomata, were difficult to determine due to the thick epicuticular folds and wax ornamentations that covered the epidermal surfaces (Figure 2.10A-F).

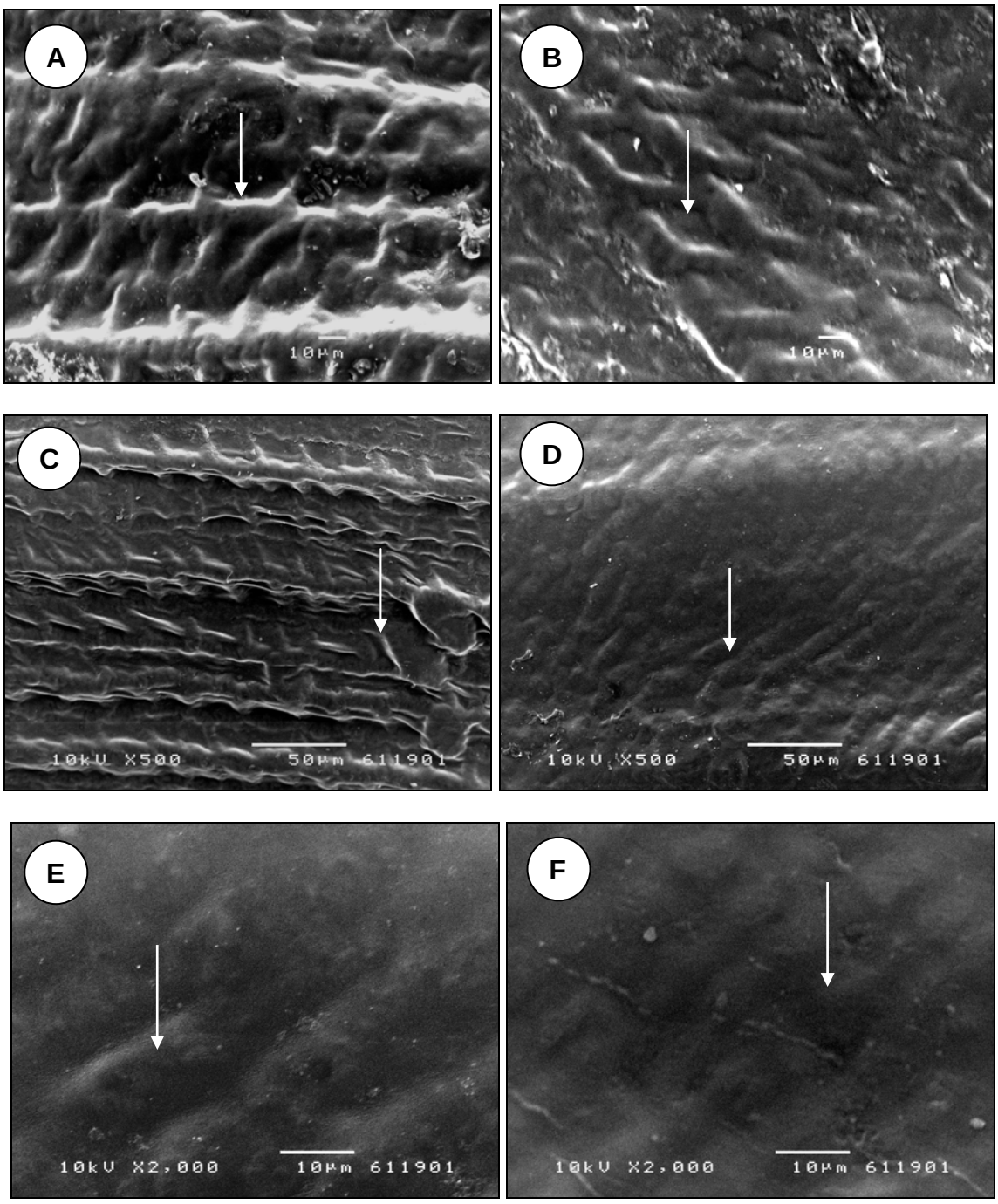


Figure 2.9 Scanning electron micrographs of adaxial leaf surfaces of Bali salak cultivars. Gula (A) showing curved to undulate anticlinal cell wall patterns, with thick ridges and Muani (B) showing depressions on the periclinal cell wall surfaces. Bingin (C) displaying epidermal surface with undulate anticlinal cell walls with cuticular folds (indicated with an arrow). Nanas (D) and (E), and Selem (F) showing convex periclinal cell walls with shallow depressions on their surfaces.

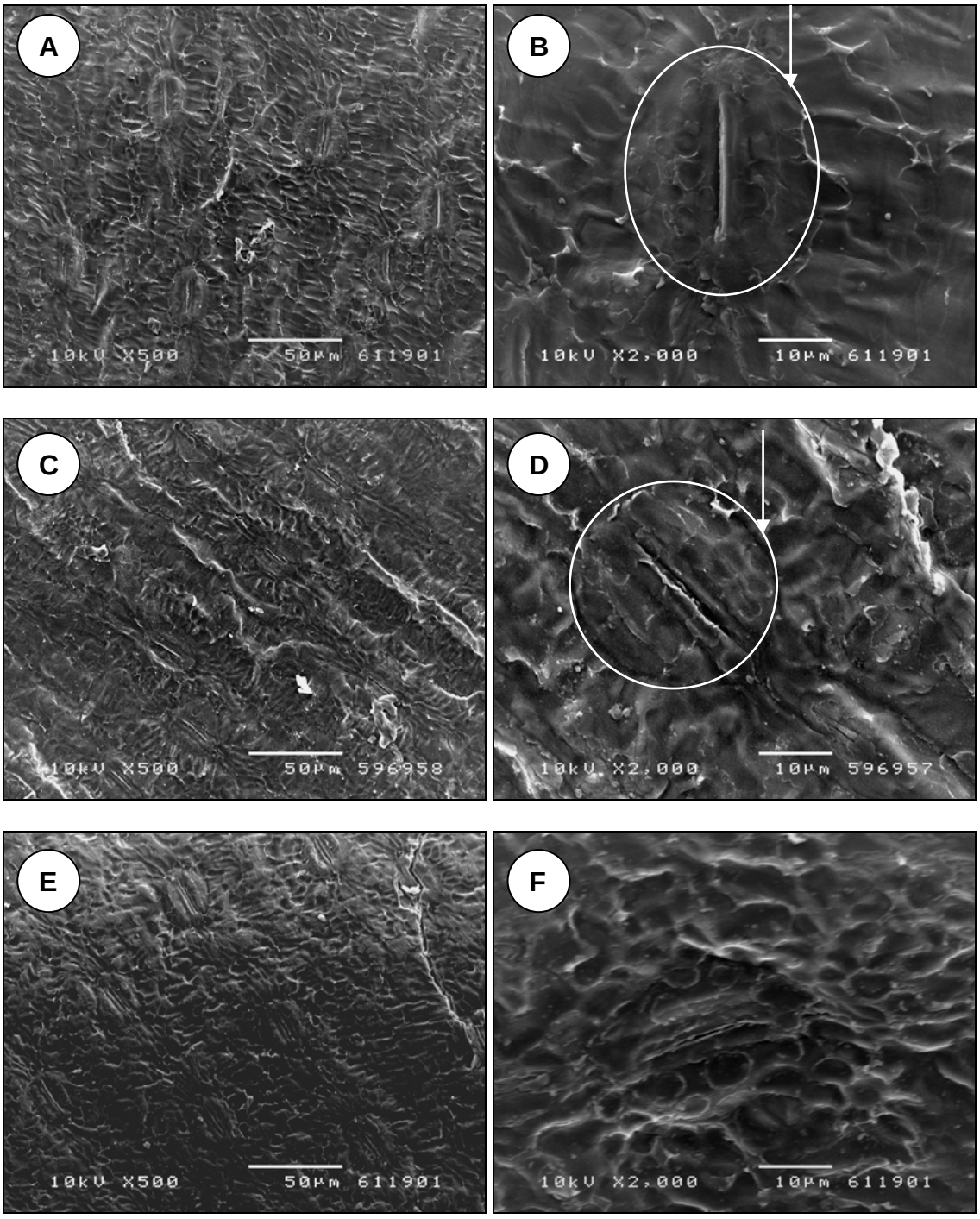


Figure 2.10 Scanning electron micrographs of abaxial leaf surfaces of Bali salak cultivars. Nanas (A & B), Muani (C & D), and Nangka (E & F) all displaying sculptured abaxial surfaces with curved cuticular folds. Stomata were either elliptical or round, as indicated by the outline in Figure B, & D respectively.

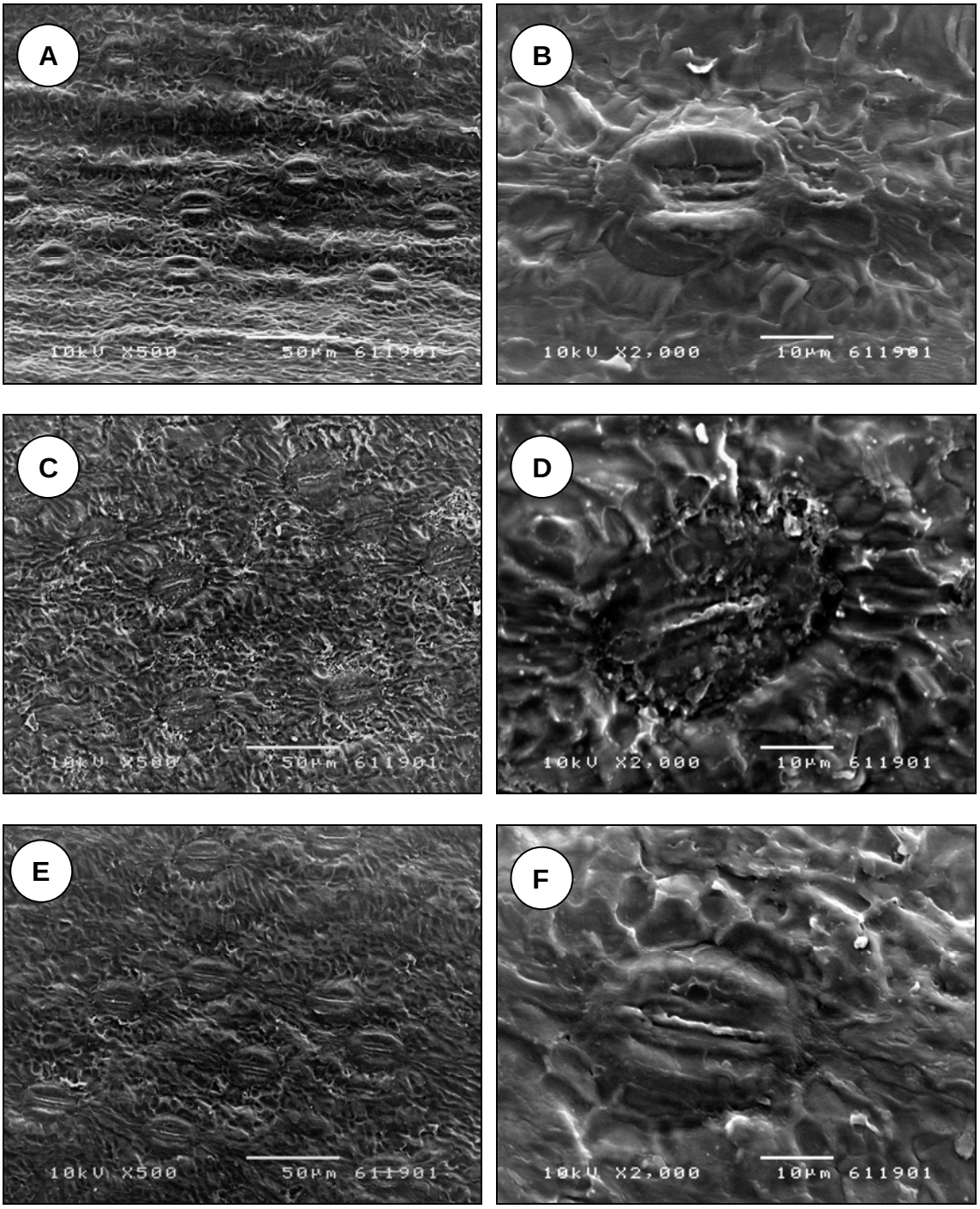


Figure 2.11 Scanning electron micrographs of abaxial leaf surfaces of Bali salak cultivars showing variation in guard cell position. Bingin (A & B) showing epidermal surface with raised guard cells. Nyuh (C & D) showing epidermal surface with sunken guard cells. Nanas (E & F) showing epidermal surface with the guard cells at the same level as the surrounding epidermal cells.

Stomata examined in all 13 cultivars were elliptical or round in shape (2.10 B and D). The outlines of the pair of guard cells were usually broadly elliptical as seen in surface view (Figure 2.10C). The outer stomatal ledges or rims in all cultivars were conspicuous, but the structure was poorly developed as the poral walls were thin and not raised (Figure 2.10 B,D,F). As indicated in Table 2.5, only one cultivar Bingin, possessed guard cells raised above the leaf surface (Figure 2.11 A&B), and four cultivars had sunken guard cells as exemplified by cultivar Nyuh (Figure 2.11 C&D). In the remaining eight cultivars (Table 2.5), the guard cell was level with the surrounding epidermal cells (Figure 2.11 E), and the epidermal cells immediately surrounding the stomatal complex were somewhat depressed when viewed under SEM (Figure 2.11F).

2.9.2 Light microscopy (LM)

2.9.2.1 Cell shapes, anticlinal cell wall patterns, thickening on anticlinal cell walls, stomatal shape, and stomatal occurence.

Characteristics of leaf epidermal cells of 13 Bali salak cultivars as seen under LM for both abaxial and adaxial leaf surfaces are presented in Figures 2.12 A-F, 2.13 A-F and Appendix 2.1. Adaxial epidermal cells were rectangular to rhomboid shaped. These occurred in all the 13 cultivars investigated (Figure 2.12 A,C,E). The abaxial cells of the surface, were usually rectangular (Figure 2.12 B,D,F). Similar features were also shown by all cultivars with respect to anticlinal cell walls. All cultivars examined showed undulation and ridged anticlinal cell wall patterns, with thickening on their anticlinal walls (2.12 A–F).

All 13 cultivars examined were amphistomatic which stomata were found on both adaxial and abaxial surfaces, however, the stomata were rare on the adaxial surface or they were not seen in some fields of views (Figure 2.12 A-F).

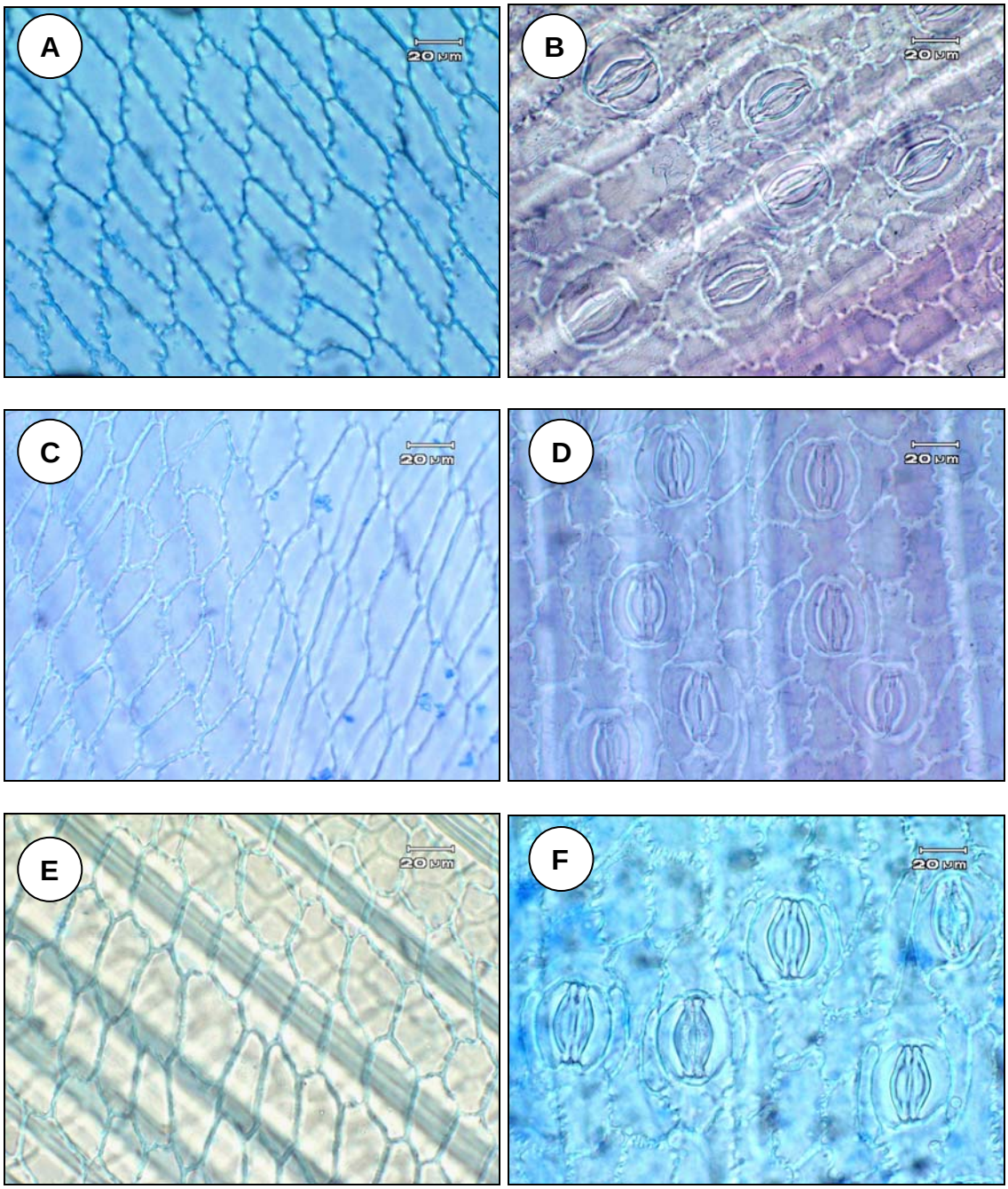


Figure 2.12 Light microscope images of leaf epidermal surfaces of Bali salak cultivars. The images show some field of views of adaxial leaf surfaces are without stomata but numerous stomata on abaxial surfaces. (A & B) Muani, (C & D), Bingin, and (E & F) Nanas. Magnification x 400.

A-C-E Adaxial leaf epidermal surface without stomata showing rectangular to rhomboid cell shapes with undulation and ridged anticlinal cell wall patterns.

B-D-F Abaxial leaf epidermal surface with stomata showing rectangular cell shapes, with undulation and ridged anticlinal cell wall patterns.

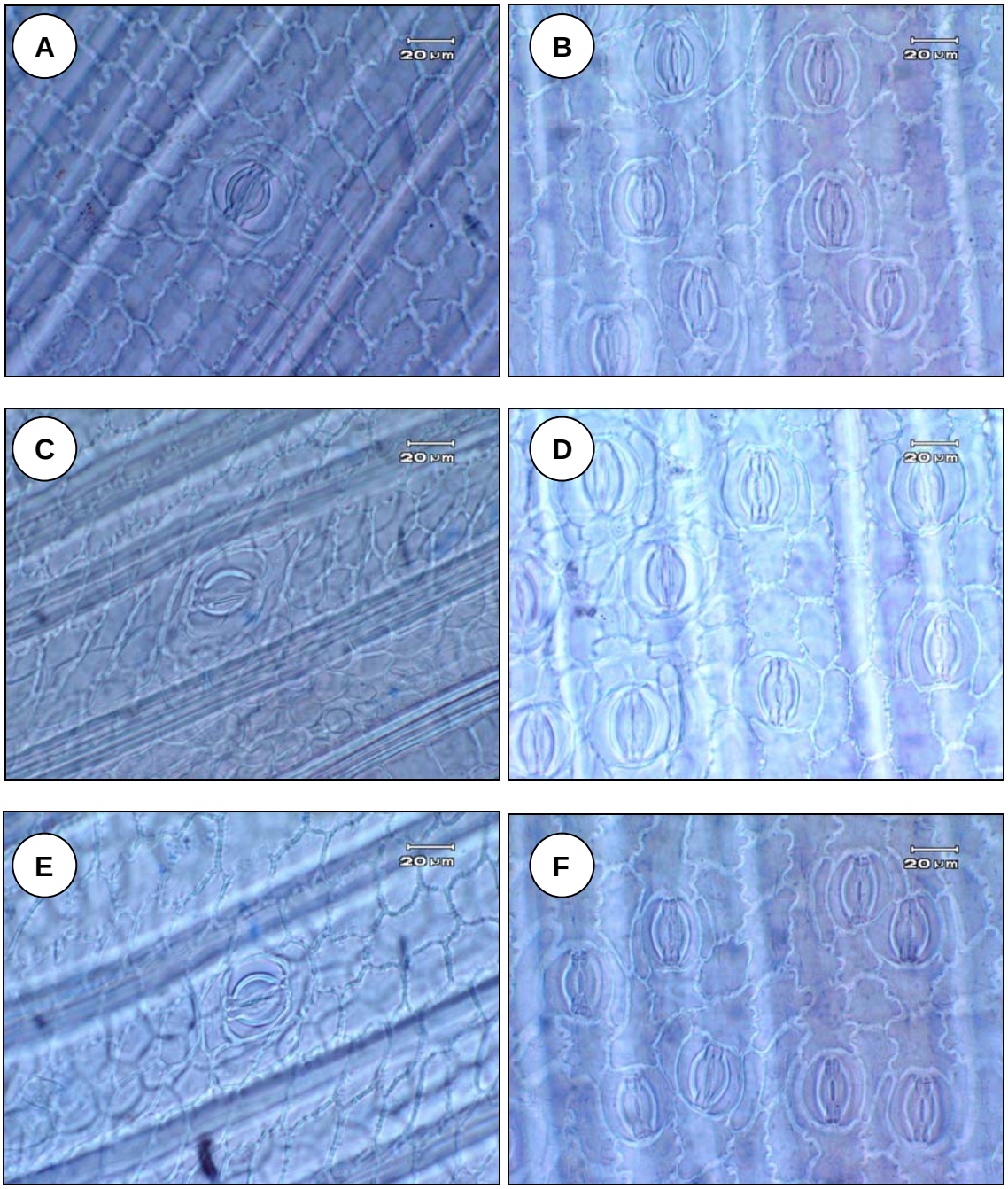


Figure 2.13 Light microscope images of leaf epidermal surfaces of Bali salak cultivars showing that stomata were rare on the adaxial surface (A,C,E) and much more frequent on the abaxial surface (B,D,F). The cultivars are Boni (A & B), Bingin (C & D), and Gondok (E & F). Stomata display paracytic types with the thickening on their outer stomatal ledges or rims. Magnification x 400.

A - C - E The adaxial surfaces showing one stomata in one field of view

B - D - F The abaxial surfaces showing more dense stomata than on the adaxial leaf surfaces.

On the abaxial surfaces the stomata were dispersed randomly over the whole area and aligned parallel to the veins. However, the occurrence of stomata per field of view on both surfaces of the leaves was somewhat different. Figure 2.13 A-F illustrates that as the same magnification, stomata on the abaxial leaf epidermal surface were more frequent than those on the adaxial surfaces. However, the comparison of the number and size of stomata were not done due to the small number of stomata on the adaxial epidermal surface. With respect to stomatal type, all cultivars examined displayed paracytic stomata, in which the stomata were enclosed by two subsidiary cells, which were adjacent to and parallel with the guard cells (Figure 2.13 B,D,F). All cultivars investigated also showed the thickening on the outer stomatal ledge or rims of the guard cells (Figure 2.13 B,D,F).

2.9.2.2 ANOVA

From the analysis of variance (ANOVA), it can be seen that 6 characters from 10 quantitative variables examined in this analysis were significantly different ($p \leq 0.05$) among the 13 Bali salak cultivars (Appendix 2.2). These characters were adaxial cell length, abaxial cell length, abaxial cell width, ratio of adaxial cell length to cell width, stomatal density, and stomatal index. However, four other characters, adaxial cell width, guard cell length, guard cell width, and ratio of adaxial cell length to width did not show any significantly different characters (Table 2.6).

2.9.2.3 Cell length, cell width, ratio of cell length to width

Adaxial cells

The length of adaxial cells varied significantly ($p \leq 0.05$) among the 13 cultivars investigated (Appendix 2.2). From the mean values (Table 2.7), it can be seen that the Muani cultivar had the longest adaxial cells (51.1 ± 0.7 µm), and Nangka the shortest adaxial cells (37.1 ± 0.7 µm). Muani possessed the widest adaxial cells

(23.9 ± 0.7 µm). The narrowest adaxial cell was found in the Nanas (22.1 ± 0.6 µm) (Table 2.7). However, the adaxial cell width was not significantly different ($p \leq 0.05$) between the cultivars investigated. The ratio of adaxial cell length to width differed significantly ($p \leq 0.05$) among cultivars (Appendix 2.2). Muani had the highest value of ratio cell length to width (2.1 ± 0.2 µm) (Table 2.7), Conversely, four cultivars, Boni, Bingin, Embad, and Gondok, had the lowest ratio values among the cultivars, each of which showed the same ratio values of 1.6 (Table 2.7).

Table 2.6 Statistical results from ANOVA ($p \leq 0.05$) of 10 quantitative measurements of leaf epidermal features of 13 Bali salak cultivars.

Num.	character	Significant ($p \leq 0.05$)
1	Adaxial cell length	significant
2	Adaxial cell width	-
3	Abaxial cell length	significant
4	Abaxial cell width	significant
5	Guard cell length	-
6	Guard cell width	-
7	Stomatal density	significant
8	Stomatal index	significant
9	Ratio abaxial cell length to width	-
10	Ratio adaxial cell length to width	significant

Abaxial cells

The length and width of abaxial cells varied significantly ($p \leq 0.05$) among the 13 cultivars examined (Appendix 2.2). The mean values of abaxial cell length varied from 38.4 ± 0.7 µm in Gondok to 44.2 ± 0.8 µm in Maong (Table 2.7), while the abaxial cell width ranged from 22.8 ± 0.7 µm in Nyuh to 28.7 ± 0.5 µm in Muani (Table 2.7). However, the ratio of abaxial cell length to width did not show any significant differences ($p \leq 0.05$); (Appendix 2.2).

Table 2.7 The leaf epidermal features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on quantitative measurements. The table shows mean values and standard errors of cell length (l), width (w), and the ratio of l:w (R), for cells sampled from the adaxial (Ad) and abaxial (Ab) leaf surfaces. Extreme mean values are indicated by bold figures. Measurements represent 90 observations from 30 leaves of each of 12 cultivars and 9 leaves of Bingin cultivar.

Character / **Cultivar**	**Ad. cell l. (µm)**	**Ad. cell w. (µm)**	**R (adaxial)**	**Ab. cell l. (µm)**	**Ab. Cell w. (µm)**	**R (abaxial)**
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
GULA	39.1 ± 0.8	23.1 ± 0.4	1.7 ± 0.1	42.8 ± 1.1	27.8 ± 0.7	1.5 ± 0.2
BONI	37.7 ± 1.2	23.4 ± 0.7	**1.6 ± 0.09**	41.5 ± 0.5	24.9 ± 0.7	1.7 ± 0.08
BINGIN	40.6 ± 1.1	22.1 ± 0.7	**1.6 ± 0.07**	41.7 ± 1.1	26.1 ± 0.5	1.6 ± 0.1
SELEM	38.5 ± 0.5	21.7 ± 0.4	1.8 ± 0.1	40.7 ± 0.8	24.8 ± 0.6	1.6 ± 0.05
EMBAD	39.5 ± 0.6	23.6 ± 0.9	**1.6 ± 0.1**	41.4 ± 0.8	23.5 ± 0.6	1.8 ± 0.1
NANGKA	**37.1 ± 0.7**	21.4 ± 0.6	1.7 ± 0.1	43.1 ± 0.3	25.1 ± 0.5	1.7 ± 0.1
PENYALIN	39.7 ± 0.4	23.4 ± 0.5	1.7 ± 0.3	39.8 ± 0.8	26.4 ± 0.4	1.5 ± 0.02
MAONG	39.9 ± 0.4	23.4 ± 0.5	1.7 ± 0.2	**44.2 ± 0.8**	25.6 ± 0.4	1.7 ± 0.04
NANAS	44.7 ± 1.5	22.1 ± 0.6	2 ± 0.1	39.3 ± 1.5	27.6 ± 1.2	1.4 ± 0.07
GONDOK	39.7 ± 0.5	21.7 ± 0.7	**1.6 ± 0.08**	**38.4 ± 0.7**	23.2 ± 0.9	1.7 ± 0.1
MUANI	**51.1 ± 0.7**	23.9 ± 0.7	**2.1 ± 0.2**	43.7 ± 1.2	**28.7 ± 0.5**	1.5 ± 0.2
NYUH	40.7 ± 1.2	23.2 ± 0.9	1.8 ± 0.1	40.9 ± 0.9	**22.8 ± 0.7**	1.8 ± 0.08
PUTIH	41 ± 0.7	22.7 ± 0.7	1.8 ± 0.2	40.3 ± 0.9	23.1 ± 1.1	1.7 ± 0.1

Table 2.8 The stomatal features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on quantitative measurements. The table shows mean values and standard errors of length and width of guard cells, stomatal density, and stomatal index. Extreme mean values are indicated by bold figures. Measurements represent 90 observations from 30 leaves of each of 12 cultivars and 9 leaves of Bingin cultivar.

Character / Cultivar	Guard cell length (µm)	Guard cell width (µm)	Stomatal density (number per mm^2)	Stomatal index (%)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
GULA	34.1 ± 0.3	10.3 ± 0.4	126.1 ± 1.8	14.4 ± 0.5
BONI	37.3 ± 1.2	9.9 ± 0.1	137.1 ± 1.7	15.6 ± 0.7
BINGIN	37.3 ± 0.3	10.1 ± 0.2	132.8 ± 0.9	13.2 ± 0.9
SELEM	38.6 ± 0.7	10.3 ± 0.2	149.4 ± 1.7	14.5 ± 0.8
EMBAD	36.2 ± 0.5	9.7 ± 0.2	138.1 ± 1.8	15.1 ± 0.5
NANGKA	35.6 ± 0.1	9.9 ± 0.1	125.8 ± 1.2	15.5 ± 0.9
PENYALIN	37.7 ± 0.2	10.1 ± 0.2	135.2 ± 1.3	15.8 ± 1.3
MAONG	36.3 ± 0.2	9.8 ± 0.1	124.5 ± 0.5	13.8 ± 0.8
NANAS	35.8 ± 0.5	9.9 ± 1	150.8 ± 1.4	17.4 ± 1.1
GONDOK	35 ± 0.9	9.8 ± 0.1	**153.1 ± 1.5**	**18.3 ± 0.4**
MUANI	39.8 ± 0.5	10.9 ± 0.4	**113.4 ± 1.8**	**11.9 ± 0.1**
NYUH	37.1 ± 0.4	10.7 ± 0.5	141.8 ± 0.6	16.6 ± 0.7
PUTIH	36.6 ± 0.2	10.1 ± 0.5	139.6 ± 0.3	15.4 ± 0.9

Three cultivars, Nanas, Gondok and Muani, had longer epidermal cells on the adaxial than the abaxial surfaces. The other six cultivars (Gula, Boni, Selem, Embad, Nangka and Maong) had longer cells on the abaxial than the adaxial surfaces. While, four cultivars (Bingin, Penyalin, Nyuh, Putih) their adaxial and abaxial cell length were overlap (Table 2.7).

2.9.2.4 Guard cells

Guard cell length and width did not show any significant difference among the cultivars (Appendix 2.2). The size was relatively uniform among the 13 cultivars investigated.

2.9.2.5 Stomatal density

In all cultivars stomatal density was higher on the abaxial leaf surface than on the adaxial surface. On the adaxial leaf surfaces, stomata were observed very rarely. If stomata occurred, an average of 1 stoma was found per field of view at magnification x 400 (0.12 mm^2) for all 13 cultivars. Due to the rare occurrence of adaxial stomata, stomatal densities were not determined for the adaxial surface. However, on the abaxial leaf surface, the number of stomata per square millimetre differed significantly ($p \leq 0.05$) among the cultivars. Stomatal density ranged from 113.4 to 153.1 per square mm. Muani cultivar had the lowest stomatal density (113.4 ± 1.8) and the highest stomatal density was found in Gondok cultivar (153.1 ± 1.5); (Table 2.8).

2.9.2.6 Stomatal index

In order to determine the relative frequencies of stomata in relation to epidermal cells, a stomatal index was calculated for all 13 cultivars (Table 2.8). In general, the stomatal index varied significantly ($p \leq 0.05$) among the cultivars (Appendix 2.2). The

highest stomatal index values (18.3 ± 0.4 %) occurred in Gondok cultivar, while the lowest stomatal index was 11.9 ± 0.1 % found in Muani (Table 2.8).

2.9.2.7 Stomatal apparatus in transverse section

Transverse section of leaflets from three cultivars, Nanas, Bingin, and Nyuh investigated in this study are shown in Figure 2.14A-C. The guard cells appeared as two rounded thick-walled cells with a narrow and nearly inconspicuous pore. Outer stomatal ledges or rims were poorly developed as they did not rise from the guard cells, while the epidermal cell surfaces were covered by a cuticular membrane with flange (2.14A,B,C). The guard cells of Nanas were at the same level as the surrounding epidermal cells (2.14A), while in Bingin guard cells were just slightly raised above the leaf surfaces (2.13B), and in Nyuh the guard cells were slightly sunken into the leaf epidermal surface (2.14C).

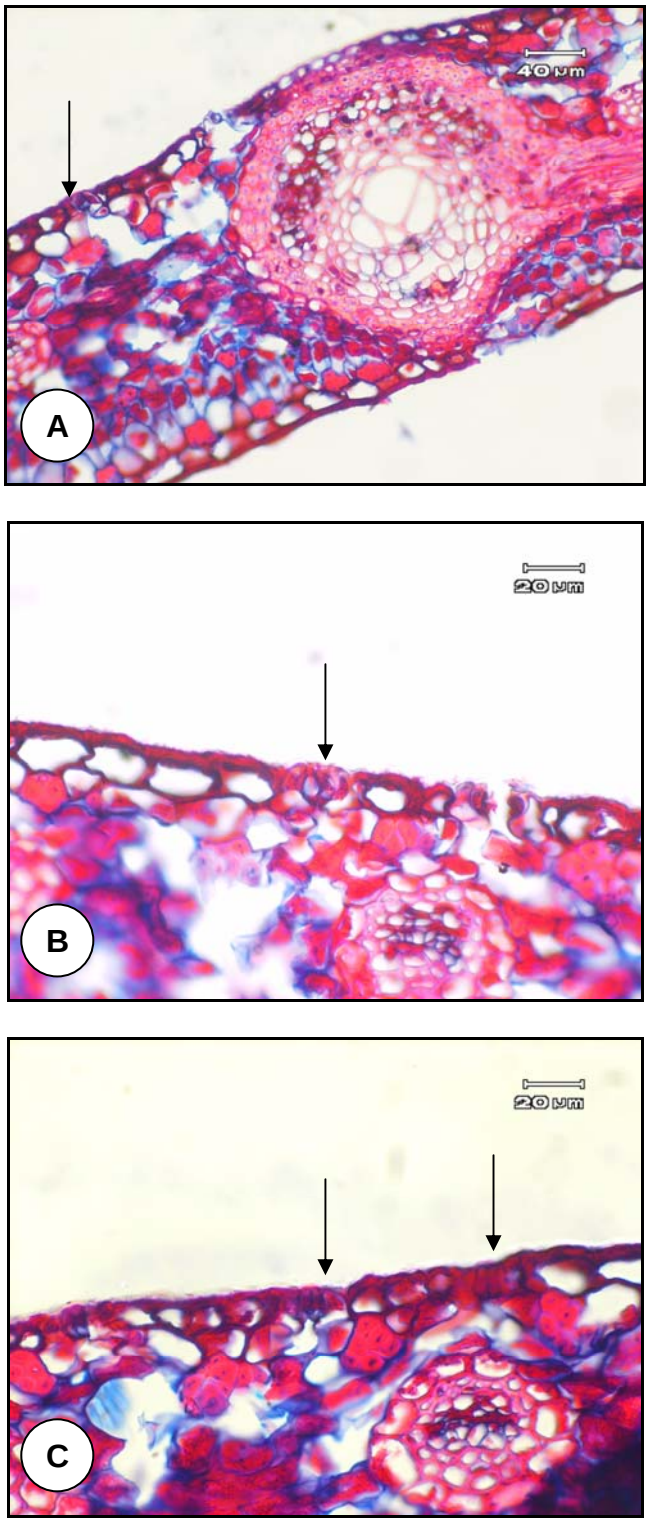


Figure 2.14 Transverse sections of Bali salak lamina showing the positions of the guard cells in relation to the surrounding epidermal cells. The guard cells show positions at the same level as the surrounding epidermal cells in Nanas (A), just slightly raised above the leaf surfaces in Bingin (B), and slightly sunken into the leaf epidermal surface in Nyuh (C).

2.10 Discussion

Results of the study show that there is variability in the leaf epidermal characteristics between the 13 Bali salak cultivars studied under SEM and LM. However, all of the qualitative characters seen under LM were constant among the cultivars. Both SEM and LM showed that the adaxial epidermal cells had rectangular to rhomboid cell shapes, while the abaxial epidermis had a rectangular cell shape. This feature was not recognized under SEM, as thick cuticles covered the abaxial surface. Because all shape was uniform in all 13 cultivars, it cannot be regarded as a useful character for differentiating the cultivars, and may be typical of the species or genus. Tomlinson (1961) reported that the *genus Salacca* had large epidermal cells with sinuous walls, and also found that rectangular and rhomboid of cell shapes occurred in *Salacca affinis* and *Salacca wallichiana*. Ghose and Davis (1973) found that adaxial cells of *Cocos nucifera* were long, rectangular, non-sinuous, and thick walled, tending to hexagonal. Tomlinson (1990) found that the adaxial epidermal cells of palm leaves tend to be irregular in shape, lack stomata and form a uniform sheet of cells. The cells are commonly rectangular, with the long axis parallel to the veins. In most arecoid palms, however, the epidermal cells are rhomboid.

The adaxial cells of all leaf epidermis examined under SEM had curved to undulate anticlinal cell walls with thick ridges. On the abaxial surface, however, a thick cuticle hindered the examination of this characteristic. Similarly, curved to undulate anticlinal cell wall patterns were shown by both adaxial and abaxial leaf epidermis under LM. The variability from curved to undulate anticlinal cell-wall patterns may be the result of the environmental conditions where the species or cultivars grow. Baas (1975) and Steiner (1999) established that the undulation of cell walls was influenced by environmental factors such as latitude, altitude, and combined temperature and precipitation.

Five cultivars, Gula, Boni, Maong, Muani, and Bingin showed flat outer periclinal cell walls and covered with particles of epicuticular wax. The other eight cultivars, had periclinal cell walls that were convex with shallow depressions on the surfaces. However, this finding may need further investigation as only two samples per leaf were scanned from each cultivar.

Under SEM all cultivars investigated showed sculpturing on abaxial surfaces. Anticlinal and periclinal cell walls, and subsidiary cells which surround the stomata, were not visible because of thick epicuticular folds and wax particles. The form of wax was constant in all cultivars. Studies by Juniper (1959) and, Hallam and Chambers (1970) found that the wax may differ under various conditions. The amount increased under in conditions of low soil moisture or air humidity, although the shape and chemical composition did not vary greatly. Species from different areas may show variation in the amount, microstructure (plates or tubes), and chemical composition of wax. In glaucous leaves the amount of wax increases and takes the form of tubes rather than plates (Hallam and Chambers 1970).

The paracytic type of stomata observed in the cultivars generally has been reported by Darmadi (2001). This stomatal shape was also reported in *Salacca affinis* and *Salacca wallichiana* (Tomlinson 1961). Results from the present study, however, did not support the previous epidermal study of Bali salak cultivars, in terms of stomatal occurrence. Samples examined by Darmadi (2001) showed hypostomatic leaves. The present study, however, found that stomata were present on both surfaces. Therefore, from this result, it can be noted that the Bali salak cultivars have an amphistomatic leaf type, stomata occuring on both adaxial and abaxial leaf surfaces. Nevertheless, stomata were found very rarely on the adaxial leaf surfaces compared with the abaxial surfaces. Tomlinson (1990) found that stomata were generally most

abundant on the abaxial leaf surface of palm leaves, however in a number of genera with isobilateral leaves the stomata were equally numerous on both surfaces.

This study shows that the outer stomatal rims of all cultivars examined under SEM were not well developed, as the poral walls were thin and not raised. This confirmed the study of Tomlinson (1990) who found that in most palms the guard cell walls were usually thin, with only slight thickenings below each ledge. However, the position of the guard cells differed obviously between the cultivars when observed under SEM. The guard cells of Bingin cultivar were raised above the leaf surfaces, whereas guard cells of Muani, Nyuh, Nangka, and Embad were sunken into the epidermal surface. The remaining cultivars Gula, Boni, Selem, Penyalin, Maong, Nanas, Gondok, and Putih had guard cells which were at the same level as the surrounding epidermal cells.

Examination by LM indicated that leaf transverse sections of three cultivars Nanas, Bingin, and Nyuh. In Nanas, the guard cells were at the same level surrounding epidermal cells, while in Bingin just slightly raised and in Nyuh slightly sunken with respect to the leaf epidermal surfaces. These differences indicate that the guard cell features provide enough information to clearly distinguish the cultivars. However, these results need further detailed examination, as in this study only three samples of leaves per cultivar were observed. Tomlinson (1990) established that sunken stomata occur in some palms for example, *Corypheae*, *Nypa*, and *Sclerosperma*.

Observations of the leaf epidermal characteristics of Bali salak cultivar under LM generally indicated that qualitative features could not be regarded as useful characters for differentiating the cultivars. Krisnamurthy and Kannabirant (1973) stated that anatomical characters should be considered for identifying taxa, as the epidermal cells and stomatal characters remain constant at the genus level.

However, this pilot study suggests that features of surface micromorphology may have some potential in being able to distinguish between some cultivars.

The results from the analysis of variance (ANOVA) for the quantitative characters showed that six characters from ten quantitative variables examined in this analysis were significantly different ($p \leq 0.05$) among the 13 Bali salak cultivars (Appendix 2.2). These characters were adaxial cell length, abaxial cell length, abaxial cell width, ratio adaxial cell length to cell width, stomatal density, and stomatal index. However, four other characters adaxial cell width, guard cell length, guard cell width, and ratio of adaxial cell length to width did not differ significantly. A number of these results are in agreement with the study of some tall and dwarf cultivars of Coconut (*Cocos nucifera*) in the Sundarbans area of West Bengal, India, in which the length of epidermal cells, stomatal density, and stomatal index were found to vary significantly (Ghose and Bhattacharya 1996).

The length of abaxial cells of Muani was close to the upper limit (44.2 ± 0.8 cf. 43.7 ± 1.2 Maong, Table 2.7). Muani had the lowest mean values of stomatal density and stomatal index (113.4 ± 1.8 cf. 11.9 ± 0.1) (Table 2.8). Similarly, Maong had the second lowest stomatal density and the third lowest mean values of stomatal index. Values for these two characters were 124.5 ± 0.5 and 13.8 ± 0.8 (Table 2.8). This means that these two cultivars generally have larger epidermal cells and lower numbers of stomata per unit area than the other 11 cultivars. This can be explained by an increase in the epidermal cell length and the consequent decrease in stomatal number. As a result, fewer stomata can be observed per unit area. Salisbury (1927) reported that the frequency of stomata was high when the size of the epidermal cells is low; by contrast, the frequency is low when the epidermal cells are large.

Darmadi's (2001) statement that, "in general, the upper epidermis of Bali salak leaves is composed of longer cells than the lower surface", was not fully supported in the present study. Three cultivars, Nanas, Gondok and Muani had longer cells on the adaxial than the abaxial leaf surfaces. However, six cultivars, Gula, Boni, Selem, Embad, Nangka and Maong showed longer cells on the abaxial than the adaxial surfaces. These may be assumed to be reliable characters differentiating the cultivars. Although, Stace (1965a) noted that the variability of the epidermal cell size might be correlated with the age of the leaf, genetic variations and environment, by collecting samples from the similar source or environment and the same stage maturity of the leaves, the variations may be considered to be useful for cultivar identifications.

The present study of 13 cultivars found that the number of stomata per unit area (stomatal density) varied from 113.4 to 153.1 (Table 2.8). Based on ANOVA, this variation was significantly different ($p \leq 0.05$) among the cultivars investigated. However, there has been no previous study reported on the Bali salak cultivars with regard to the stomatal density. The result of this study is consistent with findings of Bavappa (1966) who studied *Areca catechu* Linn. and *A. trianda* Roxb, and of Gose and Bhattacharya (1996) who studied the varieties of Tall and Dwarf coconut (*Cocos nucifera*) and established that stomatal density could be used to identify species and cultivars, respectively.

Stomatal index values ranged from 11.9% to 18.3% (Table 2.8). This observation differs from studies of Bali salak cultivars by Utami (1989) and Darmadi (2001), in which stomatal index values of 35.7% and 17.6% - 37.9% were reported. However, the cultivars examined in their studies were not specified. The research reported here indicates that based on ANOVA (Table 2.6), stomatal index can be regarded as

a dependable character for identification of the 13 Bali salak cultivars studied. This involves the assumption that microclimate is uniform in the area from which the samples of 13 cultivars were taken. These results are consistent with the work of Bavappa (1966) who established that the stomatal index values were reliable for distinguishing between the leaves of *Areca catechu* Linn. and *A. trianda* Roxb. Similarly, Ghose and Bhattacharya (1996) reported that the stomatal index differed significantly among Tall and Dwarf cultivars of Coconut (*Cocos nucifera*). However, some studies have shown that microclimate appears to influence the frequency distribution of stomata on leaf surfaces. This was observed in *Phlomis fruticosa* (Christodoulakis and Fasseas 1991) and 31 species of Boraginaceae (Dasti *et al*. 2003). The possible effects of microclimate on Bali salak cultivars remain to be investigated.

Based on the qualitative observations under SEM and quantitative investigations under LM and SEM in this study, epidermal characteristics of leaves, mainly adaxial cell length, abaxial cell length, abaxial cell width, ratio of adaxial cell length to width, stomatal density, stomatal index, and guard cell positions can be regarded as informative characters for identification of 13 Bali salak cultivars.

CHAPTER 3

Evaluation of Vegetative and Reproductive Characters of Bali Salak Cultivars

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3.1 Introduction

According to Uhl and Dransfield (1987), palms exhibit a wider range of morphological variations, than any other monocotyledonous group. Morphological traits have been analysed in coconuts and these studies have investigated a wide range of characteristics. Arunachalam (1999) reviewed the range of variability of morphological traits in coconut, including variations in habit, leaves, flowers, sex expression, and fruits. Additionally, De Lamothe and Rognon (1977) and Ratnambal *et al*. (1995) reported that Tall and Dwarf were two morphologically contrasting forms of coconuts. Some, but not all Talls have plicate leaves (leaves remain fused as in seedling leaves) and exhibit late flowering. Others may be bi-spatheate (spadix covered by two spathes instead of one), and some may produce secondary spikelets. Dwarfs exhibit other characters including polyembryony, vivipary and pigment variation in leaves.

Morphological variations in the genus *Salacca* have been reported. *Salacca minuta* and *S. graciliflora* are 1 m – 1.5 m tall, while other species such as, *S. zalacca* and *S. affinis* range from 4 m to 8 m and in *S. wallichiana* can reach up to 12 m in height (Mogea 1980, 1984; Hodel 1997). A diversity of leaf forms are also found within the genus. Flabellate leaves are found in *S. flabellata*, *S. dransfieldiana, S. magnifica*, and *S. sarawakensis*. Pinnate leaves occur in some species, such as *S. wallichiana*, *S. affinis, S. stolonifera* and *S. zalacca.* However, *S. clemensiana* has pinnate,

flabellate and an intermediate form of leaves. The intermediate leaves are flabellate at the base, and pinnate from the middle to the apex (Mogea 1980, 1984). The appearance of the leaf surfaces is variable. The adaxial leaf surface is generally smooth, glabrous and glossy green. In *S. dransfieldiana* and in *S. flabellata* the abaxial surface bears a grey-white indumentum. However, in *S. magnifica*, and *S. sarawakensis* both surfaces are glabrous and glossy green (Mogea 1980, 1984).

The variations within the genus *Salacca* indicate that these are important characteristics for establishing differences at the species level. Therefore, it is important to describe the variability within species if some of these features are to be employed as discriminators for taxonomy of the cultivars.

In addition to gross morphology of plant species, palynology can be informative in understanding variation within and between species. The shape, size, aperture type and surface patterning of the pollen grains are regarded as important elements in understanding morphology and taxonomy (More and Webb 1978). In some genera, species, and varieties of *Lycopus*, *Ricinus communis* L. and *Solanum*, distinctive pollen characteristics have been observed (Shaheen 2002; Cavazoz and Moya 2002; Moon and Hong 2003), but in others such as grasses little variation has been documented except in size (Bragg 1969).

As in most monocotyledons, the pollen grains in the Arecaceae are monosulcate, i.e. the pollen has a single long grooved aperture. However, a detailed examination of pollen grains from different members of this family found that variation exists within the family (Mahabale 1966; Sowunmi 1968). Although the majority of species have monosulcate pollen, 17 aperture types and 13 exine types have been described (Harley and Baker 2001). The pollen grains are generally 15 – 75 µm in size, with a smooth or ornamented sporoderm. The pollen structure in different genera is not

entirely homogenous; variations do exist between the species. For example, meridionosulcate grains are found in *Salacca affinis*, while *Salacca secunda* has disulcate pollen grains (Uhl and Dransfield 1987). Variations also occur in pollen surface: in *Areca catechu* the exine sculpture is reticulated, whereas in *Areca obtusifolia* it is spiny/echinate (Mahabale 1966).

The range of vegetative morphological variations and reproductive variations within the genus *Salacca* (Mogea 1980, 1984; Uhl and Dransfield 1987) suggests that as well as differences between species, there may be variability within species. However, little research has been conducted on the variation of vegetative morphological characters and reproductive characters within the Bali salak cultivars. The research reported in this thesis aims to address this lack of information.

3.2 Specific aims

The specific aims of this section of the study were:

i. to quantify variability in vegetative morphological characters within and among Bali salak cultivars.

ii. to quantify variability in reproductive characters within and among Bali salak cultivars.

3.3 Method

3.3.1 Vegetative morphological characters

Data on vegetative morphological characters were compiled during field work in Bali in 2003. Twelve quantitative characters were scored for 369 individuals of 13 cultivars as described in Section 2.7.2 and Figure 2.4. To minimize differences that might be attributable to different developmental stages, mature individuals were sampled and measurements were carried out directly in the field. Mature individuals were those which still bore fruits ready for harvest. The characters investigated in this analysis are summarised in Table 3.1. Height, length and width were recorded in

cm. Spine length was determined by randomly measuring 30 spines at a distance of 50 cm from the base of the second or third petioles. For consistency, spine density was determined by observing a 30 cm length of the second or third petiole. This 30 cm length was measured immediately above the point 50 cm from the base of the petiole.

Table 3.1 The characters used in analysing vegetative features of Bali salak cultivars.

Character	Character
1. plant height (cm)	7. middle leaflet length
2. leaf length (cm)	8. middle leaflet width
3. petiole length	9. apical leaflet length
4. number of leaflets	10. apical leaflet width
5. basal leaflet length	11. spine length
6. basal leaflet width	12. spine density (number / 30 cm of petiole length)

3.3.2 Reproductive features

Twenty fruits and flowers were obtained from each of the 13 cultivars, giving a total of 240 fruits (with the exception of Muani which does not produce fruits) and 260 flowers for further examination.

Flower characters

Fifteen characters were observed using 20 flowers of mature individuals of each of the 13 Bali salak cultivars (Table 3.2). Male and female flowers were sampled from five inflorescences of each cultivar. The length of the style, length of ovary and length of filament were determined. Prior to making the measurements, all dried inflorescences were immersed in 70% alcohol to allow the specimens to swell,

thereby making it easier to pick each flower from the inflorescences and to maximize the flower shapes.

Table 3.2 The 15 characters used for analysing flower features of Bali salak cultivars. Length and width were recorded in mm.

Character	Character	Character
1. Length of male flower	6. Width of calyx, male flower	11. Length of corolla, female flower
2. Width of male flower	7. Length of corolla, male flower	12. Width of corolla, female flower
3. Length of female flower	8. Width of corolla, male flower	13. Length of style plus stigma
4. Width of female flower	9. Length of calyx, female flower	14. Length of ovary
5. Length of calyx, male flower	10. Width of calyx, female flower	15. Length of filament

Pollen characters

Pollen grains were analysed to evaluate the shape, size and exine structure of pollen grains produced by the 13 Bali salak cultivars. To assess pollen fertility, two or three anthers were removed from each flower, transferred to a glass slide, stained with Alexander's stain and a cover-slip was applied. The anthers were gently pressed and pollen observed using a light microscope (LM). Pollen grains which were round and dark pink with Alexander's stain were scored as 'normal' or 'nonaborted', whereas small or shrivelled and yellow pollen grains were scored as 'aborted' or 'sterile' or aborted (Dafni and Firmage 2000). The higher resolving power of scanning electron microscopy (SEM) was also utilized to examine the pollen features of the cultivars. Two or three anthers were removed from each flower and broken open to release pollen grains. The reptured anther was then mounted onto the SEM stubs, critical point dried, sputter-coated with gold/palladium and scanned using a Philips XL-20 scanning electron microscope.

Fruits characters

Six fruit characters: length, width, weight, colour of scales, colour of flesh, and taste of flesh based on local perception were investigated. Weight was determined using a digital balance. Callipers were used to measure the length and width of fruits and seeds.

3.3.3 Data analysis

Data on the quantitative characters are presented as means and standard error. Quantitative data and qualitative data were used to determine the vegetative and reproductive features of the cultivars. Analyses of variance (ANOVA) of each character data set were performed following the methods by Zar (1999) to determine if there were any significant differences among cultivars for vegetative morphology and reproductive features. Those characters found to be significant were utilised in subsequent analyses (Chapter 4).

3.4 Results

The results of vegetative and reproductive observations in this study are described separately below. Appendix 3.1 summarises results of ANOVA for 12 vegetative morphological characters and 15 reproductive characters in 13 Bali salak cultivars. In Appendix 3.2, scatter plots are presented for seven vegetative morphological characters and three reproductive characters that showed significant differences ($p \leq 0.05$) among 13 Bali salak cultivars based on ANOVA.

3.4.1 Vegetative morphology

Summaries of the mean values for 12 vegetative morphological features of 13 Bali salak cultivars are presented in Table 3.3. From Table 3.3, it is evident that seven of the 12 vegetative morphological variables examined in this analysis were

significantly different ($p \leq 0.05$) among the 13 cultivars (Appendix 3.1). These characters were plant height, leaf length, petiole length, leaflet number, middle leaflet length, spine length, and spine density. However, five other characters: basal leaflet length and width, middle leaflet width, and apical leaflet length and width did not show any significant difference (Table 3.3).

Mean values for plant height and leaf length were highest in Muani and lowest in Bingin. Values for these two characters were 589 ± 15.2 cm cf. 428 ± 9.7 cm and 507 ± 10.6 cm cf. 342 ± 27.1 cm in these two cultivars respectively. Putih had the highest number of leaflets (83 ± 1.9). While the lowest number of leaflets (65 ± 0.7) occurred in Bingin (Table 3.3).

There was a significant difference in the spine density ($p \leq 0.05$); (Table 3.3). Boni cultivar had the highest mean values of spine density (102 ± 2.3), while Nyuh had the lowest (54 ± 1.4). The length of the spines differed significantly among the cultivars investigated. Table 3.3 and Figure 3.1 were extremes observed 3.1 ± 0.4 in Muani and 1.9 ± 0.3 in Nyuh. The middle leaflet length was also significantly different ($p \leq 0.05$); Muani possessed the longest (67 ± 3.3 cm), while Gula had the shortest (55 ± 1.2 cm) (Table 3.3).

As regards qualitative characters, a distinctive leaf characteristic occurred in the Bingin cultivar, here the leaflets remained partially fused, as seen in seedling leaves of coconut (*Cocos nicifera*); (Figure 3.2). With respect to spine colour, Putih cultivar was distinguished from the other 12 cultivars because it had yellowish brown spines while they each had dark brown to almost black spines.

Table 3.3 The 12 vegetative features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on field measurements. The table shows mean values and standard errors representing 30 measurements from 30 plants of each of 12 cultivars and 9 plants of Bingin cultivar. Names of seven characters that are significantly different ($p \leq 0.05$) based on the ANOVA are shown in bold.

Character / **Cultivar**	**Plant high (cm)**	**Leaf length (cm)**	**Petiole length (cm)**	**Leaflet number**	**Spine length (cm)**	**Spine density**
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Gula	470 ± 4.7	358 ± 8.2	126 ± 2.7	67 ± 1.1	2.7 ± 0.2	92 ± 1.4
Boni	496 ± 10.7	385 ± 13.8	143 ± 4.9	82 ± 2.2	2.8 ± 0.5	**102 ± 2.3**
Bingin	**428 ± 9.7**	**342 ± 27.1**	**118 ± 7.6**	**65 ± 0.7**	2.9 ± 0.2	89 ± 1.4
Selem	571 ± 10.7	456 ± 14.5	160 ± 4.9	79 ± 1	2.6 ± 0.3	93 ± 2.3
Embadan	550 ± 11.9	463 ± 14.9	199 ± 14	74 ± 1.2	2.7 ± 0.4	94 ± 1.6
Nangka	543 ± 10.7	444 ± 12	146 ± 4.9	78 ± 1.1	2.5 ± 0.2	96 ± 3.2
Penyalin	564 ± 7	463 ± 17.9	**204 ± 7**	72 ± 3.7	2.9 ± 0.1	98 ± 2.1
Maong	529 ± 7.7	452 ± 16.3	167 ± 7.7	75 ± 1.3	2.8 ± 0.2	93 ± 1.8
Nanas	563 ± 9.1	465 ± 11	174 ± 6.7	80 ± 1.2	2.6 ± 0.5	96 ± 2.1
Gondok	540 ± 4.7	445 ± 12.7	155 ± 4.7	81 ± 1.1	2.9 ± 0.2	92 ± 1.2
Muani	**589 ± 15.4**	**507 ± 10.6**	178 ± 2.7	77 ± 1.9	**3.1 ± 0.4**	88 ± 1.7
Nyuh	552 ± 2.9	449 ± 10.1	162 ± 2.9	75 ± 0.3	**1.9 ± 0.3**	**54 ± 1.4**
Putih	566 ± 2.9	454 ± 11.7	126 ± 7.9	**83 ± 1.9**	2.7 ± 0.1	79 ± 0.9

Table 3.3 (Continued) The 12 vegetative features analysed in 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on field measurements. The table shows mean values and standard errors representing 30 measurements from 30 plants of each of 12 cultivars and 9 plants of Bingin cultivar. Character names that are significantly different ($p \leq 0.05$) based on the ANOVA shown in bold.

Character / Cultivar	Basal leaflet length (cm)	Basal leaflet width (cm)	**Middle leaflet length (cm)**	Middle leaflet width (cm)	Apical leaflet length (cm)	Apical leaflet width (cm)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Gula	28 ± 2.3	1.6 ± 0.08	**55 ± 1.2**	3.8 ± 0.8	23 ± 0.9	7.7 ± 1.2
Boni	27 ± 2	1.7 ± 0.4	56 ± 2.2	3.6 ± 0.4	23 ± 3.7	6.9 ± 2.8
Bingin	27 ± 1.9	1.6 ± 1.1	58 ± 3.3	4.1 ± 0.1	23 ± 1.4	7.5 ± 0.4
Selem	28 ± 2	1.6 ± 0.2	56 ± 2.2	4.2 ± 0.4	24 ± 1.2	8.2 ± 0.4
Embadan	27 ± 0.9	1.7 ± 0.8	57 ± 1	3.9 ± 0.1	23 ± 2.8	8.9 ± 0.9
Nangka	28 ± 2	1.5 ± 0.2	58 ± 2.2	4.7 ± 0.2	24 ± 1.2	7.3 ± 2.4
Penyalin	28 ± 1.5	1.6 ± 0.1	62 ± 1.2	3.8 ± 0.1	24 ± 1.8	6.7 ± 0.3
Maong	27 ± 1.3	1.8 ± 1	58 ± 2.3	4.3 ± 0.3	23 ± 2.7	8.3 ± 1.9
Nanas	29 ± 1.7	1.8 ± 0.2	62 ± 2.1	3.8 ± 0.5	24 ± 1.1	7.1 ± 1.2
Gondok	28 ± 1.1	1.4 ± 0.3	62 ± 0.9	4.2 ± 0.3	23 ± 0.8	8.1 ± 1.3
Muani	28 ± 2.1	1.9 ± 0.7	**67 ± 3.3**	4.7 ± 0.4	25 ± 1.4	8.2 ± 1.7
Nyuh	27 ± 0.9	1.4 ± 0.6	57 ± 1.1	4.3 ± 0.1	25 ± 0.4	7.7 ± 0.1
Putih	28 ± 0.9	1.2 ± 0.3	61 ± 1.1	3.7 ± 0.7	24 ± 1.5	7.6 ± 0.1

Figure 3.1 Variation in spine density on petioles of Bali salak cultivars (*Salacca zalacca* var. *amboinensis*). A, Nyuh and B Nanas showing sparse and dense spines respectively. C(1) Nyuh petioles showing sparse spines. C(2) Nanas petioles showing more dense spines on their petioles.

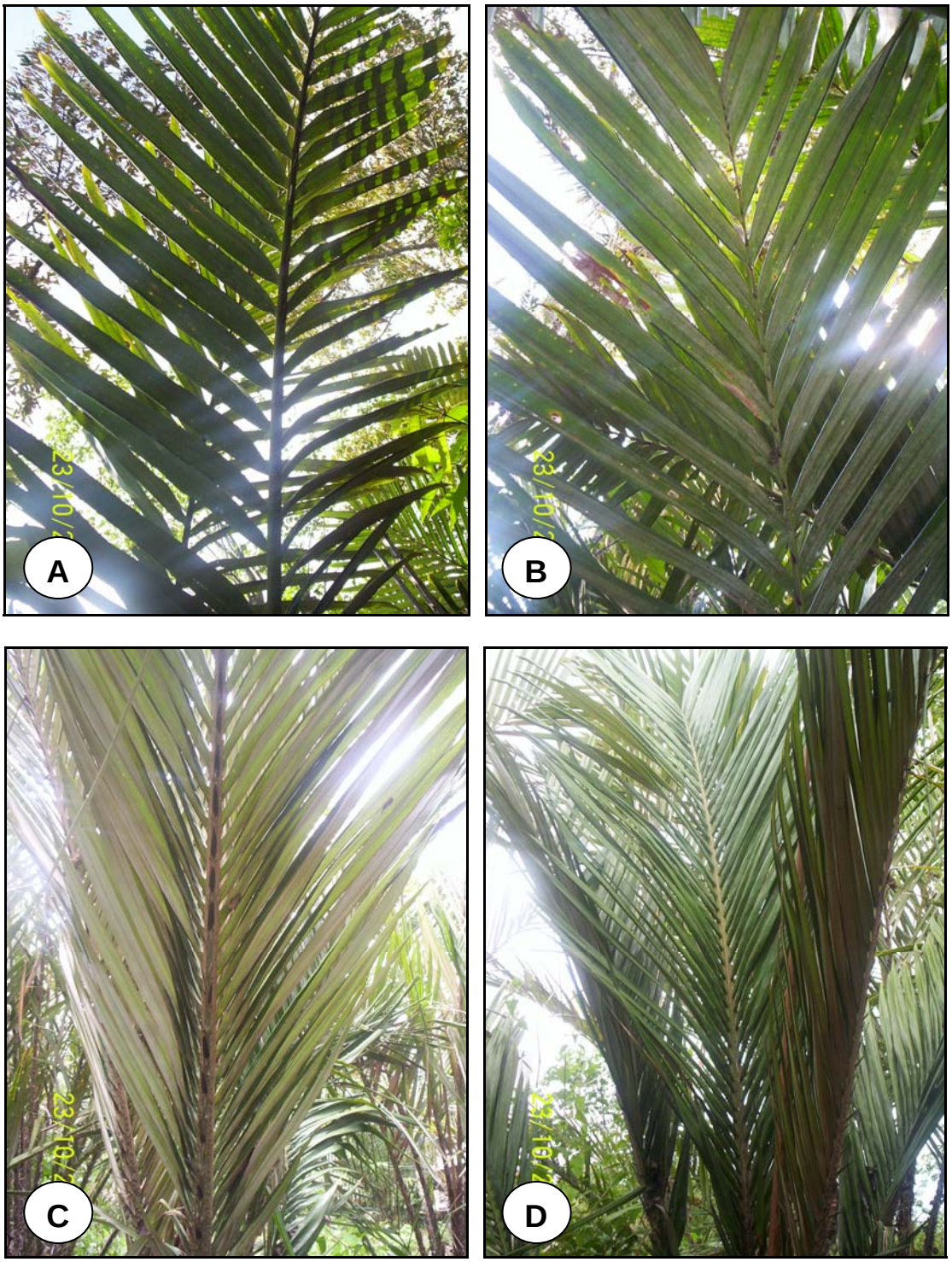


Figure 3.2 Leaves of Bali salak cultivars (*Salacca zalacca* var. *amboinensis*) showing pinnate leaves: A and B; leaves of Nanas cultivar; and C and D, leaves of Bingin cultivar showing partially fused leaflets.

3.4.2 Reproductive morphology

3.4.2.1 Flowers

Table 3.4 showed the mean values and standard errors of the 15 floral characters studied. There was significance differences ($p \leq 0.05$) among the 13 cultivars examined (Table 3.4) in three characters: calyx length of male flowers, length of female flower and corolla length of female flower. The other 12 characters did not show significance differences among cultivars investigated. The inflorescences and flowers of the cultivars are presented in Figure 3.3.

3.4.2.2 Pollen analysis under SEM and LM

The pollen grains of the cultivars are described using the terminology of Moore *et al*. (1991). Photographic documentation of electron microscopy (SEM) is shown in Figures 3.4 and 3.5, and light microscopy (LM) images are given in Figure 3.6.

The pollen morphology of 13 Bali salak cultivars investigated under SEM showed homogenous pollen types among the cultivars with regard to shape, aperture, and exine pattern. The pollen diameter of the cultivars ranged from 2 – 5 µm. The shape of the pollen grains was inaperturate (without aperture), the grains obtusely angular or occasionally rectangular with depression and ridge in their surface. The sculptural elements of the exine were scabrate/granulate (Figure 3.5).

The number of pollen grains observed under LM each of the 13 cultivars varied from 137 to 265 (Table 3.5). The potential viability of the pollen grains of Bali salak cultivars was low which ranged between 19.8 % and 31.6 % (Table 3.5). The low nonaborted of the pollen indicated by the low number of pink grains compared to aborted pollen grains which were indicated by yellow colour (Figure 3.6).

Table 3.4 The reproductive features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on length (L) and width (W) of flower measurements. The table shows mean values and standard errors representing 20 measurements from ten inflorescences of each of 13 cultivars. Character names that are significantly different ($p \leq 0.05$) based on the ANOVA shown in bold.

Character / Cultivar	Male flower (mm)						Female flower (mm)	
	Flower length	Flower width	**Calyx L**	Calyx W	Corolla L	Corolla W	**Flower length**	Flower width
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Gula	8.7 ± 0.4	3.7 ± 0.3	9.3 ± 0.2	2.7 ± 0.1	13.4 ± 0.1	2.6 ± 0.1	**8.9 ± 0.3**	5.5 ± 0.2
Boni	8.4 ± 0.2	3.2 ± 0.2	7.9 ± 0.2	2.4 ± 0.1	13.6 ± 0.2	2.9 ± 0.1	**8.9 ± 0.5**	4.9 ± 0.3
Bingin	8.4 ± 0.3	3.8 ± 0.1	9.5 ± 0.3	2.5 ± 0.1	13.3 ± 0.2	3.1 ± 0.2	9.4 ± 0.4	5.7 ± 0.2
Selem	7.9 ± 0.2	3.4 ± 0.2	9.7 ± 0.2	2.6 ± 0.1	13.1 ± 0.2	2.9 ± 0.1	9.3 ± 0.2	4.9 ± 0.3
Embadan	7.2 ± 0.2	4.2 ± 0.1	10.3 ± 0.1	2.3 ± 0.1	15.1 ± 0.2	3.1 ± 0.1	10.2 ± 0.1	5.1 ± 0.1
Nangka	9.1 ± 0.2	3.3 ± 0.4	9.4 ± 0.2	2.5 ± 0.3	14.3 ± 0.2	3.1 ± 0.1	9.2 ± 0.4	4.6 ± 0.2
Penyalin	9.7 ± 0.3	3.9 ± 0.2	10.1 ± 0.2	2.7 ± 0.3	14.3 ± 0.2	3.2 ± 0.1	9.9 ± 0.2	5.3 ± 0.1
Maong	7.8 ± 0.2	3.4 ± 0.3	9.4 ± 0.2	2.7 ± 0.1	14.0 ± 0.1	2.8 ± 0.2	9.4 ± 0.2	4.7 ± 0.2
Nanas	8.4 ± 0.1	4.1 ± 0.2	**9.1 ± 0.4**	2.5 ± 0.1	14.9 ± 0.2	2.9 ± 0.1	10.4 ± 0.5	5.2 ± 0.3
Gondok	8.4 ± 0.3	3.4 ± 0.1	10.0 ± 0.1	2.9 ± 0.1	13.9 ± 0.3	3.1 ± 0.1	9.4 ± 0.3	4.9 ± 0.3
Muani	9.1 ± 0.1	4.2 ± 0.2	**10.4 ± 0.3**	2.9 ± 0.2	15.4 ± 0.2	3.2 ± 0.1	**11.1 ± 0.6**	5.2 ± 0.3
Nyuh	7.9 ± 0.1	3.5 ± 0.3	9.2 ± 0.4	2.9 ± 0.2	12.3 ± 0.3	2.7 ± 0.1	9.6 ± 0.2	4.9 ± 0.2
Putih	8.7 ± 0.3	3.6 ± 0.2	**9.1 ± 0.4**	2.9 ± 0.2	14.7 ± 0.2	2.9 ± 0.1	9.7 ± 0.5	5.1 ± 0.1

Table 3.4 (Continued) The reproductive features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on length (L) and width (W) of flower measurements. The table shows mean values and standard errors representing 20 measurements from ten inflorescences of each of 13 cultivars. Characters names that are significantly different ($p \leq 0.05$) based on the ANOVA indicated in bold.

Character / Cultivar	Female flower (mm)													
	Calyx L Mean ± SE		Calyx W Mean ± SE		**Corolla L** Mean ± SE		Corolla W Mean ± SE		Style plus stigma L Mean ± SE		Ovary L Mean ± SE		Filament L Mean ± SE	
Gula	10.1 ±	0.2	4.0 ±	0.1	14.3 ±	0.2	4.0 ±	0.1	2.8 ±	0.1	2.9 ±	0.2	3.1 ±	0.1
Boni	9.7 ±	0.2	4.2 ±	0.2	14.8 ±	0.2	3.7 ±	0.1	2.7 ±	0.3	2.5 ±	0.1	3.2 ±	0.1
Bingin	10.3 ±	0.3	4.1 ±	0.1	15.0 ±	0.3	4.1 ±	0.2	2.4 ±	0.1	2.7 ±	0.1	3.2 ±	0.2
Selem	10.2 ±	0.2	4.1 ±	0.2	15.1 ±	0.2	4.1 ±	0.1	2.9 ±	0.3	2.8 ±	0.3	3.1 ±	0.1
Embadan	11.2 ±	0.2	4.3 ±	0.1	15.3 ±	0.1	3.9 ±	0.1	2.7 ±	0.1	2.7 ±	0.1	3.1 ±	0.2
Nangka	10.3 ±	0.2	3.8 ±	0.1	15.3 ±	0.2	3.7 ±	0.2	2.6 ±	0.1	2.7 ±	0.2	3.3 ±	0.2
Penyalin	10.3 ±	0.3	3.7 ±	0.3	14.9 ±	0.4	3.9 ±	0.1	2.6 ±	0.1	2.6 ±	0.1	3.1 ±	0.2
Maong	10.3 ±	0.2	3.8 ±	0.4	14.0 ±	0.2	4.2 ±	0.2	2.9 ±	0.2	2.6 ±	0.2	3.2 ±	0.2
Nanas	9.8 ±	0.2	4.1 ±	0.1	14.0 ±	0.2	3.8 ±	0.2	2.5 ±	0.2	2.5 ±	0.2	3.4 ±	0.1
Gondok	10.9 ±	0.2	4.1 ±	0.2	15.1 ±	0.3	4.2 ±	0.1	2.8 ±	0.1	2.9 ±	0.1	3.1 ±	0.2
Muani	11.3 ±	0.4	4.4 ±	0.2	**15.4 ±**	**0.3**	4.3 ±	0.1	2.9 ±	0.1	2.9 ±	0.2	3.4 ±	0.2
Nyuh	10.2 ±	0.2	4.1 ±	0.1	**13.0 ±**	**0.2**	3.8 ±	0.2	2.5 ±	0.2	2.5 ±	0.2	3.4 ±	0.1
Putih	10.1 ±	0.2	4.1 ±	0.1	14.0 ±	0.2	3.9 ±	0.2	2.7 ±	0.2	2.7 ±	0.1	3.3 ±	0.1

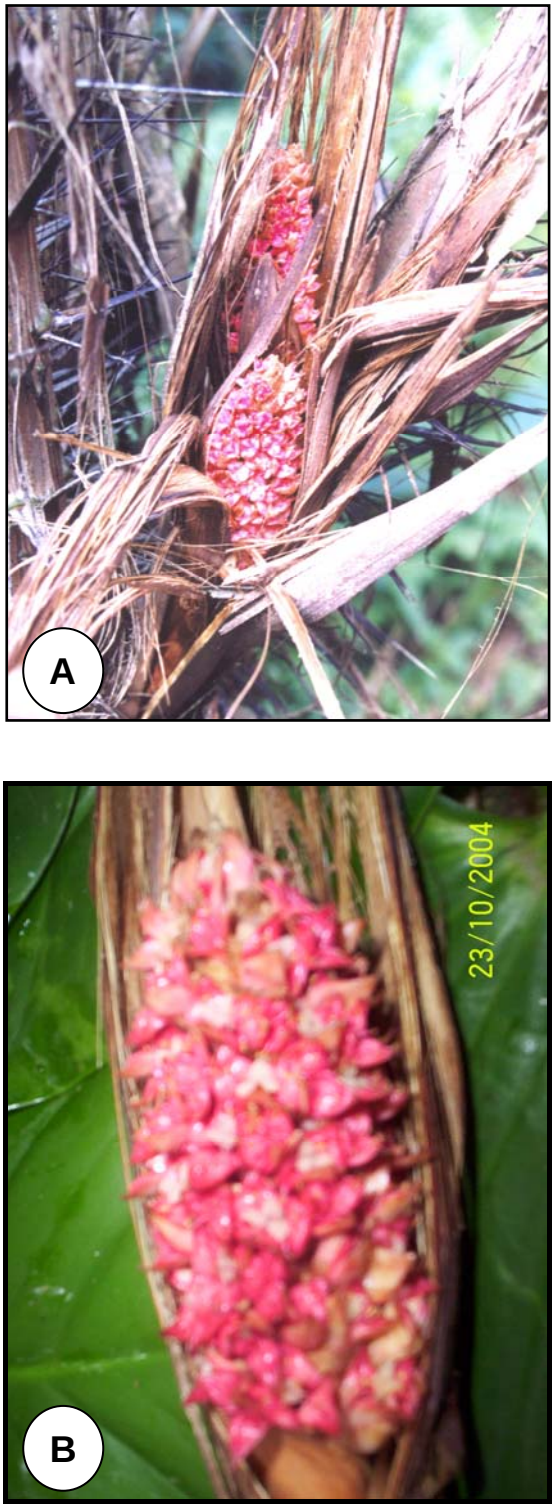


Figure 3.3 Flowers of Bali salak cultivars: A and B inflorescences bearing male and female flowers with white stigma.

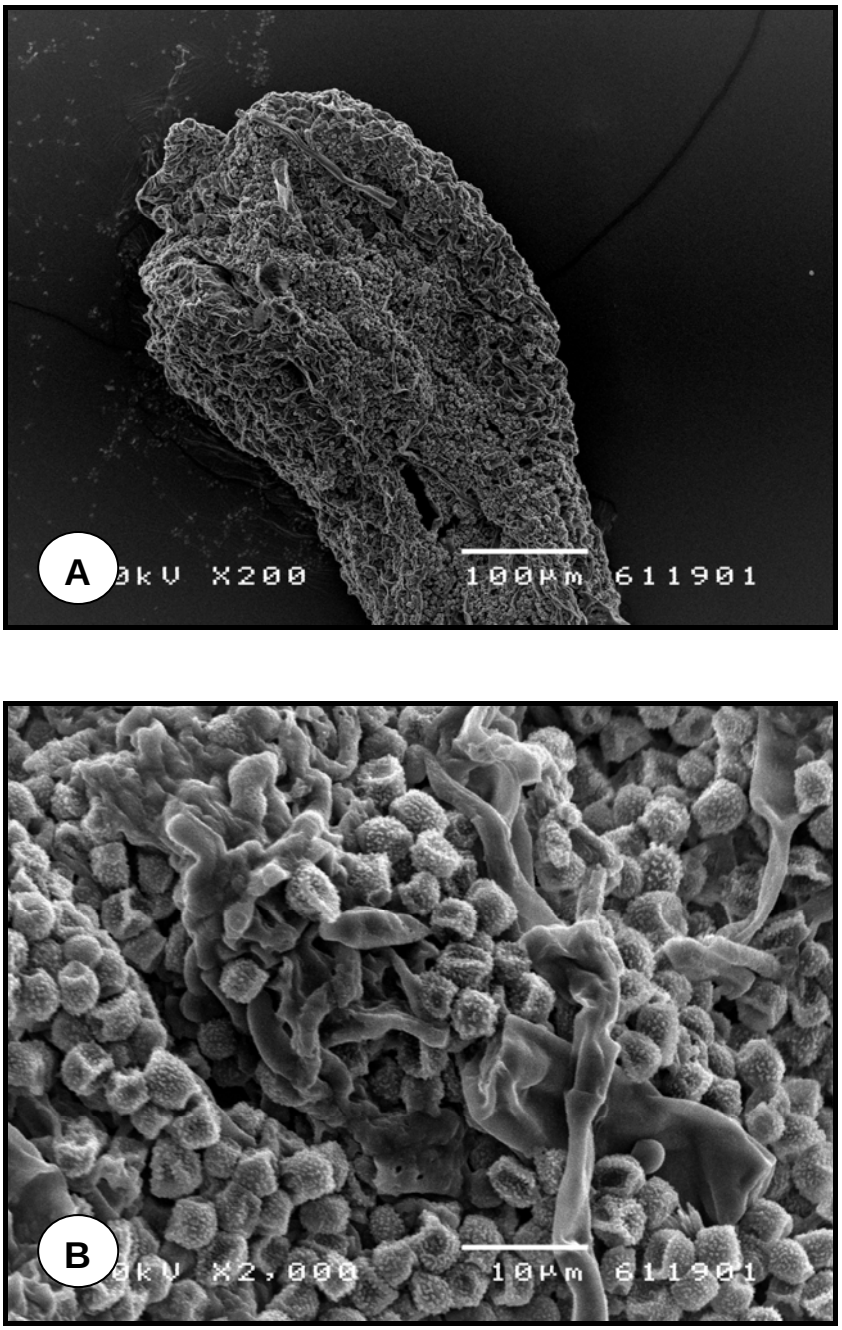


Figure 3.4 Anthers of Bali salak cultivar (*Salacca zalacca* var. *amboinensis*) (A) and numerous of pollen grains within anther (B).

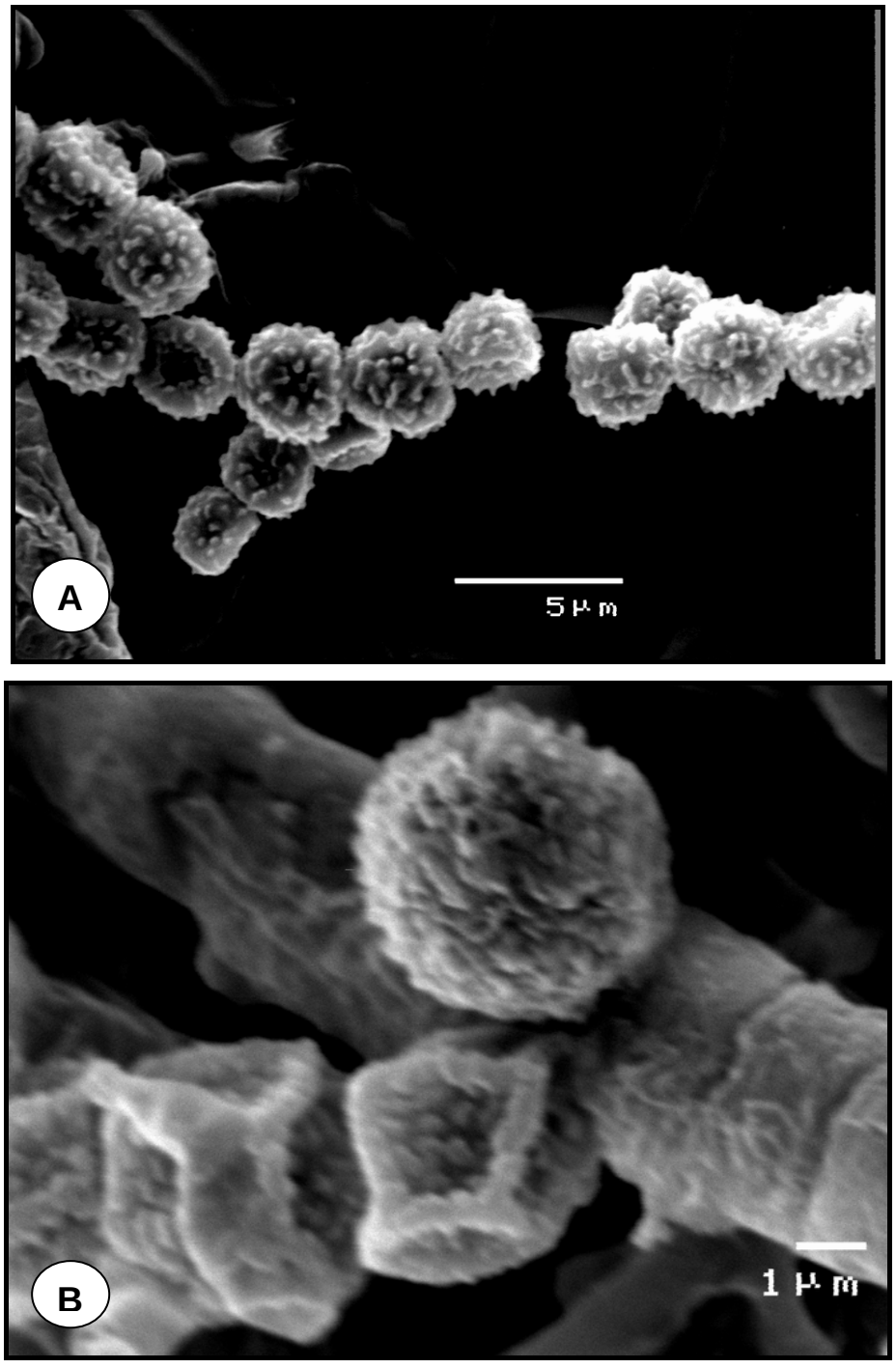


Figure 3.5 Pollen features of Bali salak cultivar (*Salacca zalacca* var. *amboinensis*) observed using scanning electron microscopy. Image A: round pollen grains with supratectal spines, and B, pollen grains with ridges on their surfaces.

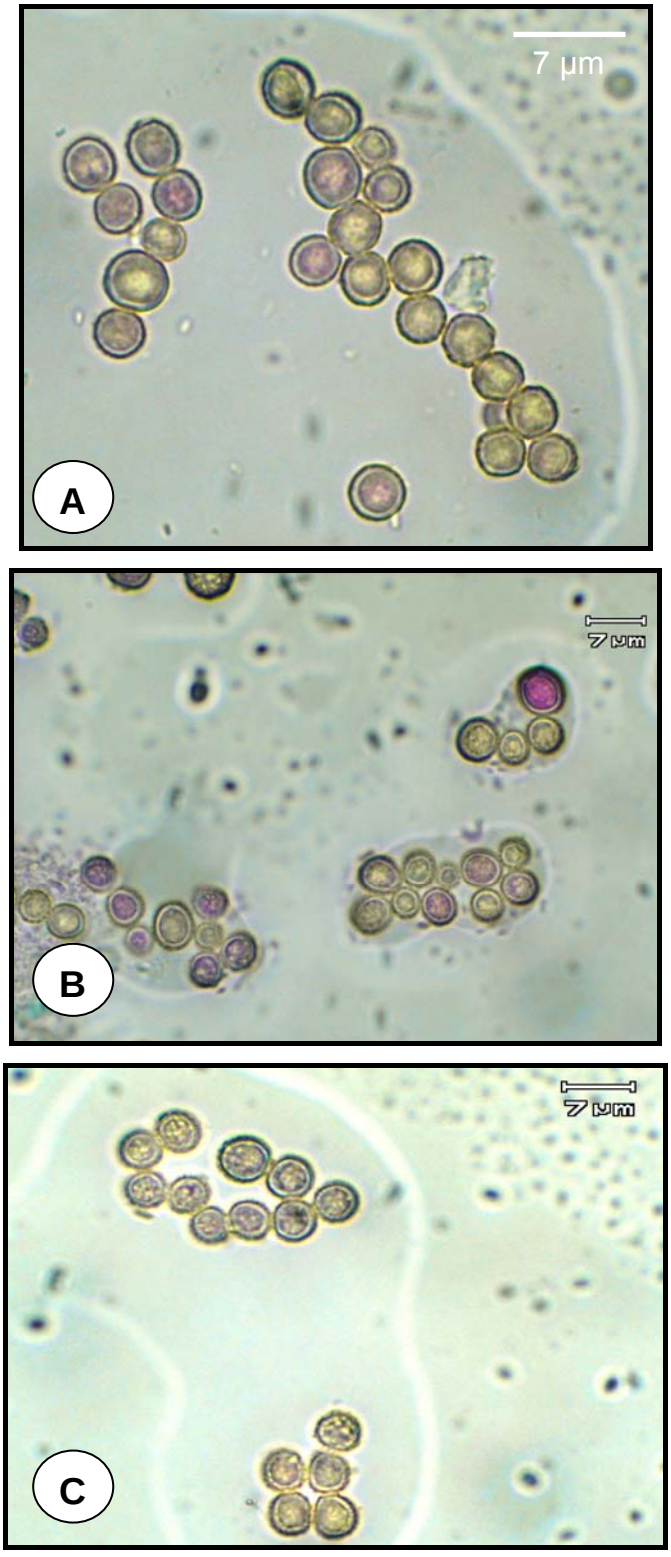


Figure 3.6 Pollen features of Bali salak cultivar (*Salacca zalacca* var. *amboinensis*) observed using light microscopy. The images showing the low number of nonaborted pollen grains as indicated by pink colour compared to aborted pollen grains which are indicated by yellow colour in three cultivars Nyuh (A), Nanas (B), and Gula (C).

Table 3.5 The percentage of nonaborted (dark pink) pollen grains of Bali salak cultivars (*Salacca zalacca* var. *amboinensis*) observed using light microscopy.

Numb.	Cultivar	Number of pollen observed	Nonaborted/ dark pink	Aborted/ yellow	Potential viability (%)
1	Gula	197	39	158	**19.8**
2	Boni	178	53	125	29.8
3	Bingin	231	73	158	**31.6**
4	Selem	109	29	80	26.6
5	Embadan	211	48	163	23.1
6	Nangka	189	40	149	21.2
7	Penyalin	159	37	122	23.3
8	Maong	241	68	173	28.2
9	Nanas	178	56	122	31.5
10	Gondok	137	34	103	24.8
11	Muani	265	58	207	21.9
12	Nyuh	219	60	159	27.4
13	Putih	195	55	140	28.2

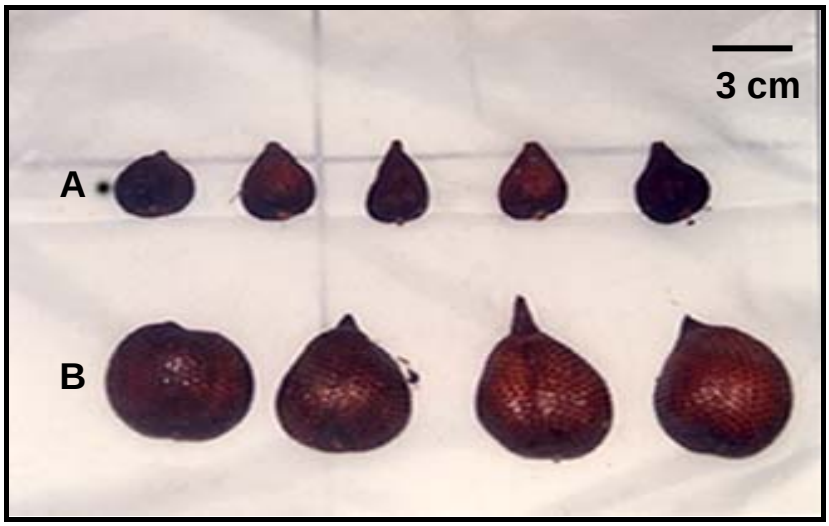
3.4.2.3 Fruits

Variations in fruit taste of Bali salak cultivars based on established criteria (Oka 1995) (Table 3.6). Muani cultivar did not produce fruits, while Bingin cultivar produced fruits without seeds. Bingin also had the smallest fruit weight, it ranged from 12.4 g to 15.6 g compared with the other cultivars, which had fruit weight between 37.8 g and 79.9 g (Table 3.6 and Figure 3.7). Other characteristics which also showed diversity were scales/epicarp colour and flesh/mesocarp colour (Table 3.6 and Figure 3.8).

Table 3.6 The fruit features of 13 Bali salak cultivars (*Salacca zalacca* var. *amboinensis*) based on fruit weight and presence or absence of fruits and seeds. Colour of fruits and flesh, taste of flesh based on established criteria (Oka 1995).

Character / Cultivar	Presence of fruits	Fruit weight (g)	Epicarp colour	Mesocarp taste	Mesocarp colour	Presence of seeds
Gula	present	37.8-79.9	brown	sweet like sugar	white	present
Boni	present	37.8-79.9	dark brown	sweet	red	present
Bingin	present	12.4-15.6	brown	sweet	white yellow	absent
Selem	present	37.8-79.9	brown	sweet	white yellow with black stripes	present
Embadan	present	37.8-79.9	brown	sweet	white yellow	present
Nangka	present	37.8-79.9	brown	sweet	white yellow	present
Penyalin	present	37.8-79.9	brown	sour	white yellow	present
Maong	present	37.8-79.9	brown with white spot	sweet	white yellow	present
Nanas	present	37.8-79.9	brown	sweet	white yellow	present
Gondok	present	37.8-79.9	brown	sweet	white yellow	present
Muani	absent	-	-	-	-	-
Nyuh	present	37.8-79.9	brown	sweet	white yellow	present
Putih	present	37.8-79.9	yellowish brown	sweet	white yellow	present

Figure 3.7 Variations in fruit size of Bali salak cultivars: A, small fruits of Bingin cultivar and B, bigger fruit size of Nanas cultivars.



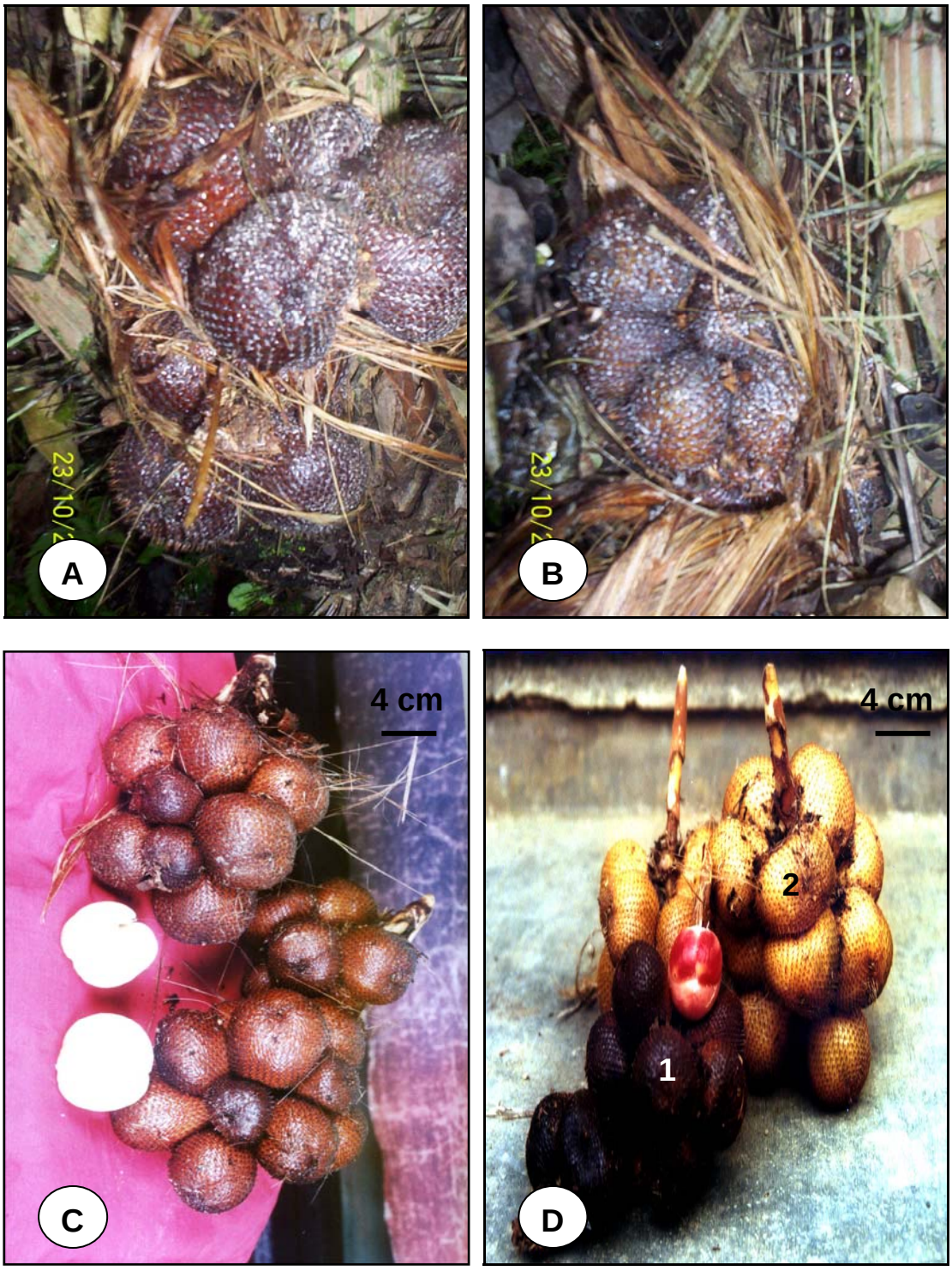


Figure 3.8 Variation in the fruits of Bali salak cultivars (*Salacca zalacca* var. *amboinensis*): A and B, fruits of Maong cultivar with white spots on its scales, C, brown scales and white yellow flesh of Nanas cultivar, D1, red flesh of Boni cultivar, and D2, yellowish brown scales of Putih cultivar.

3.5 Discussion

This investigation has established that there was morphological diversity in the vegetative characteristics of the 13 Bali salak cultivars studied. Seven characters from 12 vegetative variables examined in this analysis were significantly different ($p \leq 0.05$) among the 13 Bali salak cultivars (Table 3.3). However, five other characters: basal leaflet length and width, middle leaflet width, and apical leaflet length and width were not significantly different (Table 3.3). Muani cultivar generally had the highest mean values for 4 of 7 vegetative variables studied that were significantly different, such as plant height, leaf length, middle leaflet length, and spine length.

Morphological variations among the cultivars were also found in leaf form and spine colour of Bingin and Putih cultivar respectively. Of the 13 cultivars studied only Bingin produced partly fused leaves and exhibited delayed fruiting. The latter observation was made by farmers in Sibetan, Karang Asem, Bali. Rare traits such as plicate and late flowering were reported in Tall cultivars of coconut (De Lamothe and Rognon 1977; Ratnambal *et al*. 1995). Plicate palms were observed to begin flowering late compared with other palms (Sugimura *et al*. 1994b). However, plicate leaves have not been reported in *Salacca*.

The yellowish brown spine colour of Putih quite distinct from the other cultivars which had dark brown to almost black spines. Menon and Pandalay (1960) found that morphological variation such as albinism (lack of chlorophyll) and pigment variations occurred in Dwarf cultivars of *Cocos*.

The present study found that spine density was significantly different among cultivars ($p \leq 0.05$) (Table 3.3). The significantly lower spines density in Nyuh than in other cultivars as can be seen in Figure 3.1C. Hodel and Vatcharakorn (1998)

reported that several cultivars were recognised in *Salacca wallichiana*, one of them was characterised by the lack of spines.

Three of 15 flower characters (calyx length of male flower, length of female flower and corolla length of female flower) were significantly different ($p \leq 0.05$) among the cultivars investigated (Table 3.4). However, most of the features (12 characters) did not show any significant differences.

Similarly, the pollen morphology of 13 Bali salak cultivars investigated under SEM was homogenous. All the cultivars examined had similar pollen characters. The diameter of the pollen grains of the cultivars was quite small (3 – 5 µm). This was in contrast with the pollen grains of palms in general which range from 15 - 75 µm in diameter (Mahabale 1966). The shape of the pollen grains was inaperturate, the grains obtusely angular or sometimes rectangular with depressions and ridges in their surfaces. Uhl and Dransfield (1987) reported that the pollen aperture of *Salacca secunda* was disulcate, and meridionosulcate grains were found in *Salacca affinis*. The sculptural elements of the exine of the cultivars were granulate and noticeably varied in size. The tectate exine was finely perforate with supratectal spines in *Salacca affinis*, and in *Salacca secunda* the tectate exine was smooth with sparse perforations (Uhl and Dransfield 1987). This suggested that pollen morphology varies noticeably among the *Salacca* species; however, no variation was noted between the 13 Bali salak cultivars analysed in this study.

The percentage of nonaborted pollen grains in the 13 cultivars investigated in this study was low. From 137 to 265 of pollen grains observed under LM, the potential viability was ranged from 19.8 % to 31.6 %. Hutauruk (1999) found that pollen grains of three Bali salak cultivars (Gula, Boni, and Putih) were sterile or aborted, as none of the grains stained with aceto-carmine. This study, however, found that all of the

13 cultivar flowers examined had nonaborted or potentially viable pollen grains, even though the percentage was quite low (19.8 % to 31.6 %).

Weight, epicarp colour, mesocarp colour, and mesocarp taste were variable in the 13 cultivars studied (Table 3.7). All cultivars produced fruits, except Muani. This cultivar, identified by the farmers in Bali, is known locally as 'male'; flowers abort and no fruits are produced. Bingin had the smallest fruit weight which ranged between 12.4 g and 15.6 g compared with the others cultivars (37.8 – 79.9 g). Arunachalam (1999) reviewed the range of variability of morphological characters in coconut cultivar, in which fruit size ranged from less than 50 g to 3000 g. Environmental factors such as, soil, nutrient, sun light have been shown to significantly influence the weight of Ungurahua palm fruits (*Oenocarpus bataua* subsp. bataua) (Miller 2002). The current investigation where all samples of the 13 cultivars were collected at the same time and places, established that Bingin produced very much lighter fruits. This indicates that fruit size and weight in Bingin may be genetically determined.

These analyses suggest that vegetative morphological characters and reproductive characters are informative and potentially useful in determining the discrimination among 13 Bali Salak cultivars using phenetic analysis. A detailed analysis of the discrimination of the cultivars is presented in Chapter 4.

CHAPTER 4

Discrimination among 13 Bali Salak Cultivars using Phenetic Analysis of Anatomical and Morphological Characters

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4.1 Introduction

Classical taxonomic methods have been used extensively to differentiate taxa, but these methods are descriptive and the outcomes are potentially subjective. Where complex suites of characters occur, such as in Bali salak, unravelling relationships requires a more objective approach than classical taxonomy provides. Phenetic or numerical methods of analysis are techniques which group individuals into taxa based on their character states and are more appropriate to objectively validate any taxonomic structure within a data set (Sneath and Sokal, 1973). These methods were used as the basis for understanding the natural groupings within Bali salak in this chapter.

Distinguishing between Bali salak cultivars is difficult, although Darmadi (2001) recognised eight groups, as outlined in Chapter 1. However, the study was mainly based on morphological features with few replicates of each cultivar examined; the study was not representative of all the cultivars. Additional detailed analysis of relationships between Bali salak cultivars based on a broader range of anatomical and morphological characters was required. Evaluation of anatomical and morphological characters (presented in Chapter 2 and 3) identified 26 variable characters. Of these characters, 17 were quantitative with a significant difference ($p \leq 0.05$) based on the ANOVA, and nine were qualitative. In this chapter these 26

morphological and anatomical features are examined for their combined ability to group cultivars using phenetic analysis.

4.2 Specific aims

The specific aim of this section was to discriminate between 13 Bali salak cultivars on the basis of their anatomical and morphological characters and if possible to derive clear natural groupings from this data using numerical methods.

4.3. Methods

4.3.1 Character selection

A total of 26 characters derived from Chapter 2 and Chapter 3 were used to analyse the discrimination among 13 Bali salak cultivars. The characters included quantitative characters that showed significant differences ($p \leq 0.05$) from the ANOVA and qualitative features that showed distinct variations between the cultivars (Table 4.1).

4.3.2 Multivariate analyses

Multivariate analysis is a statistical method, which is concerned with analyzing multiple measurements of several samples of individuals (Cooly and Lohnes, 1971). The multivariate techniques utilize measures of sample similarity or dissimilarity across all the data provided; for the analyses in this study “samples” generally refers to character means for a large number of replicated measurements. To investigate the discrimination among 13 Bali salak cultivars, two analyses: classification (cluster analysis) and ordination (multidimensional scaling) methods, were performed.

Table 4.1 The characters and their states used in scoring the specimens for multivariate analysis. The table shows the character names, abbreviation and the measurement type used for each character. For the ordinal characters, it illustrates the scoring used for each character.

Numb.	Character	Abbreviation	Measurement
	Nominal		
1	Plant height	PH	(cm)
2	Leaf length	LL	(cm)
3	Petiole length	PL	(cm)
4	Spine length	SL	(cm)
5	Middle leaf length	MLL	(cm)
6	Male calyx length	MCL	(mm)
7	Female flower length	FFL	(mm)
8	Female corolla length	FCL	(mm)
9	Adaxial cell length	AdCL	(µm)
10	Abaxial cell length	AbCL	(µm)
11	Abaxial cell width	AbCW	(µm)
12	Leaflet number	LIN	count
13	Spine density	SpD	number/unit
14	Stomatal density	StD	number/unit
15	Stomatal index	StI	(%)
16	Ratio abaxial cell length to width	RAbClw	cell length/cell width
17	Fruit weight	FrW	12.4-15.6 g/37.8m-79.9 g/absent; 0/1/2
	Ordinal		
18	Spine colour	SpC	brown black/brown yellow; 0/1
19	Leaflet form	LIF	partly fused/straight; 0/1
20	Presence of fruits	PrF	absent/present; 0/1
21	Epicarp colour	EpC	brown/dark brown/brown with white spot/absent; 0/1/2/3/4
22	Mesocarp taste	MsT	sweet/sweet like sugar/sour/absent; 0/1/2/3
23	Mesocarp colour	MsC	white/red/white yellow with black stripe/white yellow/absent; 0/1/2/3/4
24	Presence of seeds	PrS	absent/present/none; 0/1/2
25	Periclinal cell wall pattern	PCwP	flat/convex; 0/1
26	Guard cell position	GcP	raised/level/sunken; 0/1/2

Prior to analyses, the following procedures were conducted: all data sets were log transformed and ranges standardized for comparison of results. Log transformations rescaled the data to values more suitable for association (Belbin, 1995). Range standardisation equalizes both the size and variability of characters so that the smallest state among the variables is coded as zero and the largest state is coded as one (Sneath and Sokal, 1973). However, as the results of analyses using both log transformed data and standardized data were very similar, only the results of log transformed data are presented. A combination of nominal and ordinal data were used in the analyses, as illustrated in Table 4.1. The full data matrix is presented in Table 4.3.

All analyses in these studies were performed using the software Pattern Analysis Package (PATN) version 3.03 of Belbin (2001). Cluster analysis was undertaken using Gower's metric association. This measure is appropriate for continuous biological data. It is also suitable for matrices containing mixtures of quantitative and qualitative characters (Gower, 1971). The unweighted pair-group method using arithmetic means (UPGMA) was employed as fusion strategy in the dendrogram generated. UPGMA is widely used as all the objects are given an equal weight, reflecting real distance between individuals throughout the fusion process (Belbin, 1988). The dendrogram was constructed to reveal the hierarchical discrimination evident in the data.

Ordination analysis provided an n-dimensional co-ordinate plot of the similarity/dissimilarity among samples, such that the distance between any two samples in the ordination space is representative of their dissimilarity (Sneath and Sokal, 1973). The ideal results are a depiction of the majority of the character information presented in the data in as few dimensions as possible. Thus a data set with 80% of the variation depicted in 3 dimensions is a better result than one in

which only 50% of the variation is depicted in 2 dimensions. Multidimensional scaling (MDS) was performed and 1000 random starting points were used to ensure that the global minima were reached. Principal axis correlation (PCC) was used to correlate the characters with the ordination vectors. The values of the correlation coefficient calculated from the PCC can be used to determine the significance of the characters in forming the clusters (Belbin 1995). The values of the PCC range from zero to one, a value of zero indicating no correlation and a value of one indicating complete correlation. Characters with the correlation values of less than 0.7 were not considered to contribute significantly to classification relationships. All analyses were performed in both two and three dimensions, but as the third dimension added quite significant information, the results for three-dimensions are presented.

4.4 Results

4.4.1 Cluster analysis (CA)

The UPGMA dendrogram resulting from the fusion matrix based on Gower's dissimilarity revealed two primary clusters (A and B) separating the cultivars (Figure 4.1). It is evident that Muani (B) was clearly separated from the other 12 cultivars (A) at a dissimilarity value of 61%. Similarly, Bingin also separated out distinctly within group A with approximately 45% dissimilarity where three groups are clearly defined including a large cluster A1 which consisted of 11 cultivars, Bingin (A2) and Muani (B) (Figure 4.1).

Around 23.5 % of variance separated the cultivars into five groups, consisting of Muani (B), Bingin (A2), and groups labelled A1a, A1b and A1c (Figure 4.1). However, if 20% dissimilarity was used to distinguish the 13 cultivars, eight groups were recognized. The groups were Gula (1), Boni (2), Maong (3), Nyuh (5), Putih (6),

Bingin (7), Muani (8) and the largest cluster (4) consisted of six cultivars (Selem, Nangka, Gondok, Embadan, Penyalin, Nanas) (Figure 4.1).

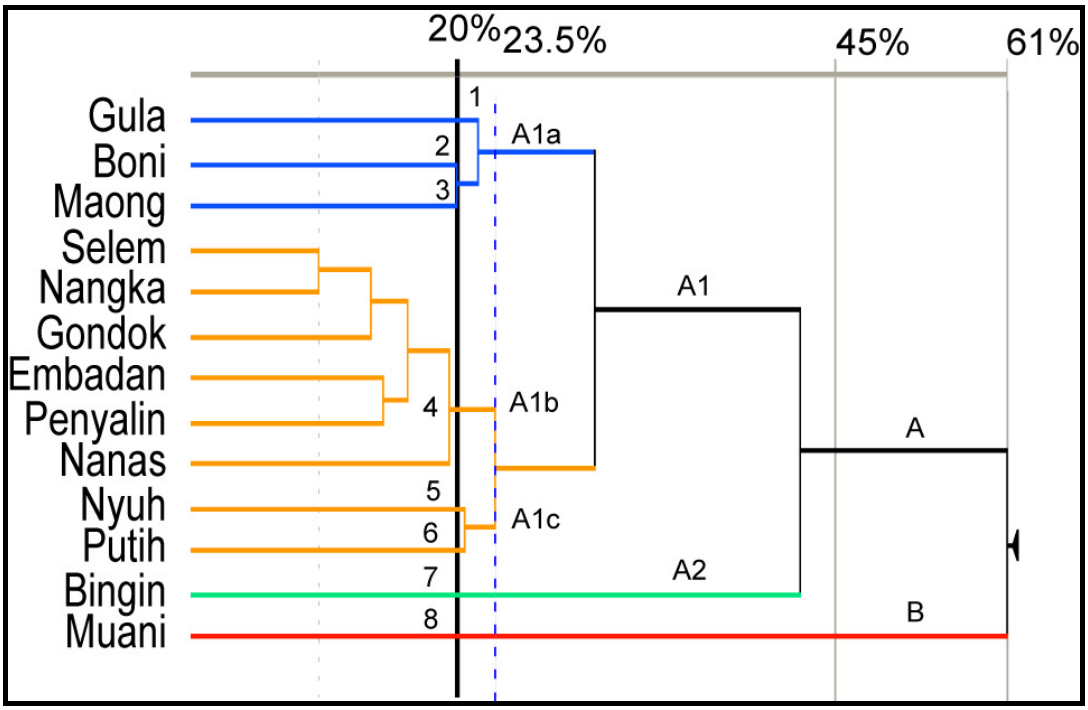


Figure 4.1 The dendrogram of 13 Bali salak cultivars which resulted from the UPGMA fusion strategy based on Gower's dissimilarity. The scale at the top is Gower's matrix association index which ranges from 0 to 1 or in percent dissimilarity. The dendrogram is labelled with groups discussed in the text; A, B, A2, A1a-c and 1-8.

4.4.2 Multidimensional scaling (MDS)

The MDS produced a stress measure of 0.0996 (Figure 4.2), indicating that the three-dimensional ordinations provided a good description of the data set (Figure 4.2). MDS analysis supported the separation of the groups obtained from the cluster analysis, with Muani (11) and Bingin (3) distinctly separated out from the other 11 cultivars. The exception occurred in Gula and Maong which separated in the cluster analysis, whilst in the ordination space they were quite close together. The 11 cultivars clustered into three main groups A, B and C (Figure 4.2).

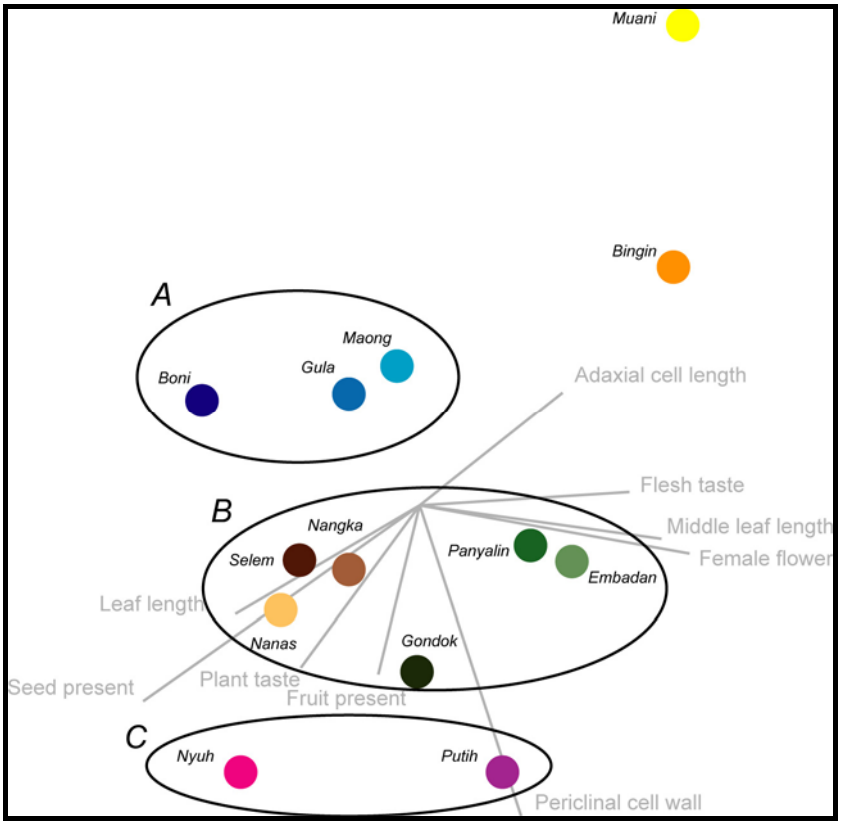


Figure 4.2 The scatter plots of 13 Bali salak cultivars which resulted from the ordination analysis of multidimensional scaling (MDS) in 3 dimensions (Stress: 0.0996). The plot substantiates the data set into five distinct clusters, Muani, Bingin and the groups labelled A, B and C. The characters with a correlation coefficient cut-off greater than 0.7 are presented and the character vectors plotted (in grey).

If a correlation coefficient cut-off value of 0.7 from the principal axis correlation (PCC) was used to determine the character that significantly influenced the formation of the cluster, the group separated primarily on the following characters: plant height, leaf length, middle leaflet length, female flower length, adaxial cell length, presence of fruits, presence of seeds, mesocarp taste and periclinal cell wall pattern. Figure 4.2 shows the characters vectors and their contribution to forming the clusters for the combination of axes. The vectors, dimension spaces and correlation coefficients from the PCC analyses for all 26 characters are shown in Table 4.2. Five quantitative features and four qualitative characters possessed high

correlation coefficients of more than 0.7. Other characters had less than 0.7 correlation coefficients as can be seen in Table 4.2.

Muani was clearly separated from the other cultivars by having the characters that were associated with high scores for adaxial cell length and the absence of fruit and seeds (Table 4.3). Muani therefore can be distinguished from the other cultivars by having high mean values of adaxial cell length and negative correlation with those characters that positively correlated with group A, B and C, such as the presence of fruits and seeds (Table 4.3). While, the separation of Bingin from the other groups A, B, and C was due to this cultivar producing fruits without seeds (Figure 4.2).

Using a correlation coefficient cut-off value of 0.7 from the PCC analysis identified plant height, leaf length, middle leaflet length, female flower length, fruit taste, and presence of fruits and seeds as characters responsible for clustering cultivars of groups B and C. Hence, these cultivars can generally be diagnosed by having fruits and seeds, sweet flesh taste except Penyalin which has sour taste, plant height of 5.30 m – 5.71 m, leaf length of 4.44 m – 4.65 m, and middle leaf length (5.6 m – 6.2 m) (Table 4.3).

Periclinal cell wall pattern significantly influenced the separation of group A from group B and C. Group A: Gula, Boni and Maong can be principally characterised by a flat periclinal cell wall pattern, which the cultivars of group B and C had convex periclinal cell wall patterns.

Table 4.2 The 26 characters and their ordination space in three dimensions (D1, D2, D3) and their correlation values resulting from PCC analysis (r^2). The characters and the correlation values of more than 0.7 are indicated by bold letters.

Numb.	Characters	D1	D2	D3	r^2
1	**Plant height**	**0.86**	**-0.39**	**0.30**	**0.84**
2	**Leaf length**	**0.94**	**-0.26**	**0.18**	**0.77**
3	Petiole length	0.82	-0.19	0.52	0.46
4	Spine length	-0.24	0.69	0.67	0.52
5	**Middle leaf length**	**0.38**	**-0.07**	**0.92**	**0.79**
6	Male calyx length	-0.23	-0.18	0.95	0.59
7	**Female flower length**	**0.32**	**-0.10**	**0.93**	**0.84**
8	Female corolla length	-0.31	0.60	0.73	0.29
9	**Adaxial cell length**	**0.55**	**0.32**	**0.76**	**0.71**
10	Abaxial cell length	0.03	0.95	-0.31	0.43
11	Abaxial cell width	0.40	0.91	-0.05	0.63
12	Leaflet number	0.86	-0.45	-0.20	0.57
13	Spine density	0.05	0.99	-0.09	0.20
14	Stomatal density	-0.05	-0.70	-0.70	0.69
15	Stomatal index	0.23	-0.90	-0.35	0.67
16	Ratio abaxial cell length to width	0.95	0.14	0.25	0.59
17	Fruit weight	0.99	0.02	-0.09	0.60
18	Spine colour	-0.48	-0.72	0.49	0.33
19	Leaflet form	0.87	-0.28	-0.39	0.60
20	**Presence of fruits**	**-0.58**	**-0.60**	**-0.54**	**0.75**
21	Epicarp colour	-0.04	-0.123	0.99	0.48
22	**Mesocarp taste**	**0.47**	**0.06**	**0.88**	**0.73**
23	Mesocarp colour	0.02	-0.40	0.91	0.64
24	**Presence of seeds**	**0.29**	**-0.64**	**-0.70**	**0.72**
25	**Periclinal cell wall pattern**	**0.02**	**-0.94**	**0.33**	**0.84**
26	Guard cell position	0.92	-0.36	-0.09	0.37

Table 4.3 Data matrix used in the multivariate analysis of the 13 Bali salak cultivars (*Salacca zalacca* var. *amboinensis*). All characters and their states are explained in Table 4.1.

Character	PH	LL	PL	SL	MLL	MCL	FFL	FCL	AdCL	AbCL	AbCW	LIN	SpD	StD
Number / **Cultivar**	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gula	470	358	126	2.7	55	9.3	8.9	14.3	39.1	42.8	27.8	67	92	126.1
Boni	496	385	143	2.8	56	7.9	8.9	14.8	37.7	41.5	24.9	82	102	137.1
Bingin	428	342	118	2.9	58	9.5	9.4	15.0	40.6	41.7	26.1	65	89	132.8
Selem	571	456	160	2.6	56	9.7	9.3	15.1	38.5	40.7	24.8	79	93	149.4
Embadan	550	463	199	2.7	57	10.3	10.2	15.3	39.5	41.4	23.5	74	94	138.1
Nangka	543	444	146	2.5	58	9.4	9.2	15.3	37.1	43.1	25.1	78	96	125.8
Penyalin	564	483	204	2.9	62	10.1	9.9	14.9	39.7	39.8	26.4	72	98	135.2
Maong	529	452	167	2.8	58	9.4	9.4	14.0	39.9	44.2	25.6	75	93	124.5
Nanas	563	465	174	2.6	62	9.1	10.4	14.0	44.7	39.3	27.6	80	96	150.8
Gondok	540	445	155	2.9	62	10	9.4	15.1	39.7	38.4	23.2	81	92	153.1
Muani	589	507	178	3.1	67	10.4	11.1	15.4	51.1	43.7	28.7	77	88	113.4
Nyuh	552	449	162	1.9	57	9.2	9.6	13.0	40.7	40.9	22.8	75	54	141.8
Putih	566	454	126	2.7	61	9.1	9.7	14.0	41	40.3	23.1	83	79	139.6

Table 4.3 (Continued) Data matrix used in the multivariate analysis of the 13 Bali salak cultivars (*Salacca zalacca* var. *amboinensis*). All characters and their states are explained in Table 4.1.

Character	StI	RAbClw	FrW	SpC	LIF	PrF	EpC	MsT	MsC	PrS	PCwP	GcP
Cultivar	15	16	17	18	19	20	21	22	23	24	25	26
Gula	14.4	1.7	1	0	1	1	1	0	0	1	0	1
Boni	15.6	1.6	1	0	1	1	0	1	1	1	0	1
Bingin	13.2	1.6	0	0	0	1	1	1	3	0	0	0
Selem	14.5	1.8	1	0	1	1	1	1	2	1	1	1
Embadan	15.1	1.6	1	0	1	1	1	1	3	1	1	2
Nangka	15.5	1.7	1	0	1	1	1	1	3	1	1	2
Penyalin	15.8	1.7	1	0	1	1	1	2	3	1	1	1
Maong	13.8	1.7	1	0	1	1	2	1	3	1	0	1
Nanas	17.4	2	1	0	1	1	1	1	3	1	1	1
Gondok	18.3	1.6	1	0	1	1	1	1	3	1	1	1
Muani	11.9	2.1	2	0	1	0	4	3	4	2	0	2
Nyuh	16.6	1.8	1	0	1	1	1	1	3	1	1	2
Putih	15.4	1.8	1	1	1	1	3	1	3	1	1	1

4.5 Discussion

Cluster analysis and the MDS ordination revealed two cultivars Muani and Bingin, and three groups of cultivars, each showing a distinctive combination of quantitative and qualitative characters. Muani is a very distinct cultivar based on a number of quantitative characters, including higher mean values of plant height, leaf length, middle leaf length, female flower length, and adaxial cell length than in the other cultivars (Table 4.3). This indicated that most of the quantitative characters chosen for analysis were proven to be useful in differentiating the cultivars. In the plantations, for example, Muani cultivar is easily recognised by its height. The cultivar generally has a taller habit than the other 12 cultivars. Results from this study suggested that it is a genetic rather than environmental characteristic. However, the absence of fruits was also significant in contributing to the separation of Muani from the other cultivars. The presence of fruits had a high correlation coefficient (0.75) in separating the other 12 cultivars distinctly from Muani.

The largest cluster which consisted of six cultivars (Figure 4.1 and 4.2) included cultivars which exhibited the following characters: the presence of fruits and seeds, high mean values of fruit weight and stomatal index, and a convex periclinal cell pattern. Cluster analysis indicates that Gula and Maong are quite distinct (Figure 4.1). Based on MDS (Figure 4.2) Gula and Maong appear to overlap, when viewed in one dimension. However, when viewed in three dimensional spaces these two cultivars are in fact well separated. This suggests a distinct relationship between Gula and Maong.

Bingin did not possess seeds and produced small fruits (12.4 - 15.6 g) compared with the other cultivars which had fruit weight between 37.8 g and 79.9 g (Table

4.2). This suggests importance of reproductive characters; presence of fruits and seeds, in defining the discrimination between 13 Bali salak cultivars.

The separation of the cultivars was influenced significantly by the periclinal cell wall pattern. Gula, Boni, Maong, Bingin and Muani are characterised by having a flat periclinal cell wall pattern. The other eight cultivars have convex periclinal cell wall patterns.

The outcomes from these analyses suggested that morphological and anatomical characters can be used in identifying the Bali salak cultivars. Based on the multivariate analysis, the nine useful characters for recognising the eight cultivars (Table 4.3) are plant height, leaf length, middle leaflet length, female flower length, adaxial cell length, presence of fruits, presence of seeds, mesocarp taste and periclinal cell wall pattern. These characters were used in deriving the key to Bali salak cultivars (Chapter 5).

CHAPTER 5

The Taxonomy of Bali Salak Cultivars

5.1 Introduction

Blume (1823) in Mogea (1982) proposed the name *Salacca edulis* for salak palms. This name was apparently suggested by Reinwardt when they collaborated at the Bogor Botanical Garden. In 1828, Reinwardt published an incomplete description of *Salacca* as a new genus based on *Salacca edulis*. This description included observations based on vegetative characters, obtained from plants living in Java. A more detailed description was given by Blume (1830) in Roehmer and Schultes, Systema Vegetabilium Vol. 7 of 1830 (Farr *et. al*. 1979). Blume (1843) gave a comprehensive description of this species under the name *Salacca edulis Reinw*. Therefore, this species may be considered as the type species of the genus (Mogea 1982). Since this taxon is widely cultivated for its fruits in some areas of Java “edulis” was chosen as the specific epithet (Mogea 1978, 1982). In 1982 Mogea proposed that the correct citation for the salak palm was *Salacca zalacca* (Gaertn.) Voss. which is the only species found in Java and is also widely cultivated in Madura and Bali. However, there are differences between the original Javanese forms (*Salacca zalacca* var. *zalacca*) and another variety from Bali (Mogea 1982). The plant from Bali is the same as that from North Sulawesi and Ambon, i.e. *Salacca zalacca* var. *amboinensis* (Mogea 1982).

Govaerts and Dransfield (2005), in the World Checklist of Palms recognised 20 species within the genus *Salacca* without establishing any subspecies. However,

until a revision of the genus has been completed, the research presented in this thesis considers the Bali salak as a cultivar of *Salacca zalacca* (Gaertn.) Voss.

A range of descriptions of the species within the genus has been reported. However, no comprehensive investigations have been carried out on the characteristics found within the Bali salak cultivars or for any species of the genus *Salacca*. Therefore, the research presented in this chapter aims to address this lack of information. Identification keys and descriptions of each cultivar were developed using characteristics of each cultivar based on vegetative anatomical and morphological characters, as well as reproductive features. These keys have potential for wide use in plantations by Balinese farmers in Bali.

The key to species of *Salacca* presented in this thesis (Section 5.4) has been developed based on descriptions from the available literature chiefly: Reinwardt (1828), Martius (1838), Griffith (1845,1850), Beccari (1886, 1909, 1918), Blatter (1926), Burret (1942), Furtado (1949), Whitmore (1973), Mogea (1980, 1981a, 1981b, 1982, 1984, 1986), Dransfield and Mogea (1981), Hodel (1997), Hodel and Vatcharakorn (1998), Darmadi (2001), Martin *et al*. (2002), Govaerts and Dransfield (2005). For some species, complete information was unavailable, and the descriptions were often brief, therefore the descriptions of the species presented here are not always comparable.

5.2 Description of the genus *Salacca*

Salacca Reinw., Syll. Ratisb. 2: 3 (1828).

Salakka Reinw. ex Blume, Catalogus: 112 (1823), orth. var.

Zalacca Rumph. ex Blume in J.J.Roemer & J.A.Schultes, Syst. Veg. 7: 1333 (1830), orth. var.

Lophospatha Burret, Notizbl. Bot. Gart. Berlin-Dahlem 15: 752 (1942).

Palms with a relatively small to robust habit; height ranges from 1 m to 12 m, stems may be creeping or procumbent (acaulescent) or if erect frequently clustered, appearing spiny because of retained petioles. Leaves range from 0.9 to 10 m, pinnate, or flabellate, terminal leaflets often bifid, with pinnate venation, marcescent; leaf-sheath splits opposite the petiole, at the extreme base unarmed, otherwise very sparsely to densely covered with scattered or whorled spines, numerous persistent scales present; petioles are channelled adaxially near the base, rounded distally and abaxially, armed with spines and often a powdery indumentum; rachis also armed but more sparsely; leaflets on pinnate leaves are unbranched, except for the terminal-pair, linear or sigmoid, acuminate or very rarely deeply lobed at the apex, regularly arranged or grouped and fanned within the groups, variously armed with short bristles along the main vein and margin, abaxial blade surfaces often with a dense covering of a powdery indumentum, midribs prominent adaxially and transverse veinlets usually conspicuous. Plants are dioecious or andromonoeceious. Inflorescences are axillary but enclosed within the leaf-sheaths of the subtending leaf, and emerge through a slit along the midline of the abaxial surface of the leaf-sheaths, usually short and sometimes spicate, more often with 1 or 2 orders of crowded or spreading branches, occasionally hidden by detritus, sometimes arching out of the crown. Staminate inflorescences are usually branched to at least one more order than the pistillate; peduncles are usually short; prophylls often inconspicuous, partly enclosed within the leaf-sheaths, the sheath splits and becomes irregularly tattered; peduncular bracts several, tubular at the base, apical portion irregularly tattered, frequently densely scaly; rachis usually longer than the peduncle; rachis bracts similar to the peduncular bracts; rachillae are cylindrical, exposed or hidden by the bracts, bearing a tight spiral of imbricate, triangular or low, rounded bracts sometimes connate laterally to form a continuous

spiral. Staminate flowers are borne in dyads with two small prophylar bracteoles, these are sometimes split but always variously connate to each other, the flower exerted from within at anthesis; calyx tubular, variously splitting into lobes, sometimes divided almost to the base, chaffy, striate; corolla with a short stalk-like base, and long proximal tube, bearing 3 triangular lobes, valvate; stamens 6, borne at the mouth of the corolla tube, filament shorts, broad basally, anthers rounded to elongate, dehiscence introrse; pollen elliptic or obtusely angular, meridionosulcate, disulcate or inaperturate; tectate, finely perforate, with sparse to dense supratectal spines or spinules; pistillode very small or absent. Pistillate flowers are either solitary or borne in a dyad with a similar sterile staminate (or neuter) flower pollen absent; calyx of pistillate flower tubular at the base, distally with 3, triangular lobes, striate; corolla similar with 3 triangular lobes, valvate; staminodes 6, borne at the mouth of the corolla tube; the filaments usually elongate, anthers ± sagittate, empty or with pollen grains; gynoecium tri-carpellate, triovulate, covered in flattened, smooth, or erect spine-tipped scales, styles unbranched, stigma 3-lobed, fleshy, reflexed at anthesis, locules incomplete, ovules basifixed, anatropous. Fruit 1 – 3 seeded, globose to pear-shaped or ellipsoidal, with stigmatic remains; epicarps covered in somewhat irregular vertical rows of reflexed scales, the scale tips smooth or spine-like and upward pointing, mesocarp very thin at maturity, endocarp not differentiated. Seeds basally attached, sarcotesta very thick, sour or sweet, inner seed coat very thin, endosperm homogeneous, the apex with a pit; embryo basal. Germination adjacent ligular; eophyll entire bifid. n = 14 (*Salacca zalacca*). Found through South East Asia, from Burma, Indochina and the Philippines to Sumatra, to Borneo, North Sulawesi, Java, Bali, and Ambon.

5.3 Discussion of the selected characters with diagnostic value.

Characters having diagnostic value were identified for *Salacca* species from literature sources, whilst those for Bali Salak cultivars were identified from analyses associated with this current study. The characters used for distinguishing Bali salak cultivars included 17 quantitative and 9 qualitative characteristics for each cultivar based on vegetative anatomical and morphological characters, and reproductive features (Table 4.1). These quantitative characters showed significantly different mean values ($p \leq 0.05$) based on ANOVA and there were some obvious differences of qualitative characters between cultivars. Multivariate analysis (Chapter 4) indicated nine of these 26 characters that had square correlation values more than 0.7 were the most reliable discriminators of cultivars. Therefore, these 26 characters (Table 4.1) are employed for the descriptions of each cultivar in this section. Although comprehensive discussions of these characters have been given in previous chapters (Chapter 2, 3 and 4) further discussion is required for some of the characters used to distinguish between the species.

Habit

Many descriptions use the term ‘acaulescent’ however, in this study the word is not used because the term is ambiguous. It has been correctly used by some authors to refer to a creeping or procumbent stem, which may be metres long (Beccari 1886). However, other authors have used it to mean that there is no stem above the ground (Griffith 1845). There is further confusion because in many species with procumbent stems, the stem is very often below ground or at least partially below ground or covered in debris or litter. However, the height of the plant can be used to distinguish between several of the species. The height varied distinctly from species to species, and ranged from 1 m to 12 m long (Mogea 1984; Hodel and Vatcharakorn 1998).

Leaves

There is a clear variation in the nature of the leaves as some species have pinnate leaves, while other species have flabellate leaves. However, the leaves of *S. clemensiana* may be pinnate, flabellate, or there may be an intermediate form. The intermediate form is pinnate at the base but from the middle of the rachis it is flabellate and the apex is bifid. The length of the leaves varies between species, the smallest recorded length is 0.9 m in *S. minuta*, whereas in the other species it is up to 7 m long or even reached 10 m long in *S. wallichiana*. Although, leaf-sheaths, petioles and the rachis may vary between species, because the information available for this study was often incomplete, details of these organs could not be used to distinguish between the species. For most species, spine length and arrangement could be used to distinguish between species except for *S. lophospatha* and *S. dolicholepis* where the information was unavailable. In some species such as *S. minuta, S. sarawakensis* and *S. flabellata spines* do not exceed 2 cm long. Whereas, in other species such as *S. secunda and S. wallichiana* spines were found to be up to 10 and 12 cm long respectively. As Mogea (1982) found, size and shape of the leaflets and lamina can be used to distinguish between species of *Salacca*, similarly the appearance of the leaflets or lamina surfaces was variable and there were differences between some species. The surfaces were concolorous and glossy green in *S. magnifica*, *S. sarawakensis*, *S. secunda*, *S. affinis* and *S. glabrescens*, while in other species the adaxial surface was usually glossy or dark green, and whitish on abaxial surface, except in *S. rupicola* where the adaxial surface was dark bluish-green and the abaxial surface was pinky creamy brown.

Inflorescences

Inflorescences, particularly the staminate inflorescences provide many useful characters for distinguishing between species of *Salacca*. Hodel and Vatcharakom (1998) found that the length of the staminate inflorescence easily distinguished particular species. Staminate inflorescences in some species such as *S. zalacca, S. flabellata and S. wallichiana* ranged from 1 m to 4 m long. In *S. minuta* it reached 1.5 m long and was lying on the ground because the plant was just 1 m tall. In *S. stolonifera* the inflorescence sometimes reached 4 m long with the stoloniferous inflorescences rooting and sprouting at their tips. Whereas, in some other species staminate inflorescence lengths were generally between 10 and 80 cm long or were very short from 3 to 6 cm long in *S. affinis*. Staminate rachilla length was quite variable in some species. It was up to 12 cm in *S. magifica*, in contrast to just up to 2.5 cm in *S. multifora*. Pistillate inflorescences, pistillate rachillae, and the flowers could not be used diagnostically because of a lack of comparable information.

Fruits

In those species for which information was obtainable, fruit epicarp colour was variable between species but constant within species. For *S. vermicularis and S. dolicholepis* the epicarp was black: it was reddish brown in *S. wallichiana and S. glabrescens* and pink in *S. magnifica*. However, the fruit of several species may have a similar appearance and the epicarp colour varied from brown to dark brown. In general, the features that are most useful taxonomically were characters associated with plant height, leaf length, leaf shape, spine length and inflorescence length particularly of the staminate inflorescence. If other character information is available then several of these would probably be important for distinguishing between species.

5.4 A key to species of *Salacca*

1 Leaves flabellate
2 Leaves on mature plants more than 3.5 m long …… **1**. *S. magnifica*
2. Leaves on mature plants less than 3.2 m long
3 Leaves up to 0.9 m long ..…….……..……..…… **2**. *S. minuta*
3. Leaves more than 0.9 m long
4 Spines 2.5 cm long or more
5 Staminate rachillae up to 7 cm long, rarely less than 4 cm……..…
……..... **3**. *S. dransfieldiana*
5. Staminate rachillae up to 2.5 cm long ……..……...…… **4**. *S. multiflora*
4. Spines up to 1.8 cm long or less
6 Leaf blades glossy green on both surfaces …..…….. **5**. *S. sarawakensis*
6. Leaf blades glossy green on adaxial and whitish on abaxial surfaces
……..…..... ….. **6**. *S. flabellata*
1. Leaves are pinnate or partially pinnate
7 Leaves partially pinnate ……..……..……..…….**7**. S. clemensiana
7. Leaves entirely pinnate
8 Median length of leaflets 45 cm or less
9 Leaves up to 1.25 m long ……..……..……..…..…… **8**. *S. gracilifora*
9. Leaves more than 1.25 m long
10 Maximum length of the spines no more than 2 cm long ..… **9**. *S. rupicola*
10. Maximum length of the spines up to 8 cm long
11 Maximum leaf length up to 7 m ……..……. **10**. *S. zalacca*[1]
11. Maximum leaf length no more than 5.5 m
12 Staminate inflorescences from 3 to 6 cm long, fruit epicarp chestnut brown ….……..……..…… **11**. *S. affinis*
12. Staminate inflorescences from 30 to 80 cm long, fruit epicarp yellowish to reddish brown ..……..……**12**. *S. ramosiana*
8. Median length of leaflets 50 cm or more
13 Leaflets regularly alternate on rachis ……..……..... **13**. *S. sumatrana*
13. Leaflets arranged variously but not in a regular pattern
14 Inflorescences stoloniferous to 4 m long ……..…. **14**. *S. stolonifera*
14. Inflorescences not stoloniferous
15 Fruit epicarp black
16 Mature leaves up to 7 m long ……..... **15** .*S. vermicularis*
16. Mature leaves up to 5 m long …… **16**. *S. dolicholepis*
15. Fruit epicarp not black
17 Fruit epicarp dark brown ……..…..……..….**17**. *S. secunda*
17. Fruit epicarp reddish-brown
18 Mature leaves up to 10 m long, staminate inflorescenses up to 3 m long ...……..……**18**. *S. wallichiana*
18. Mature leaves up to 3.6 m long ……..……..…….....……**19**. *S. glabrescens*

Note 1: Mogea (1982) recognises a subspecies of *S. zalacca*, but other authorities consider that there are no subspecies (Govaerts and Dransfield 2005).

Note 2: *S. lophospatha* is not included in the key because there was insufficient information to enable the species to be completely keyed out. Data in the description is based on the prologue which was very brief and lacked detail (Burret 1942).

5.5 Description of *Salacca* species

1. *SALACCA MAGNIFICA* MOGEA

Salacca magnifica Mogea, Reinwardtia 9: 468 (1980).

Habit: plant up to 6.3 m tall, erect. **Leaves**: flabellate, 6 m long; leaf-sheaths about 130 cm long, broadly triangular near base, gradually channelled above the base, spiny; petiole about 70 cm long, about the middle in cross-section circular 2.5 cm diameter, the lower side tends to be flat with numerous spines; **spines** up to 6.7 cm long, usually upward pointing, dull blackish brown, spines on leaf-sheaths in small combs of 3 – 7, elsewhere in pairs or solitary on rachis; rachis 3.5 m long, the base 1.2 – 1.7 cm diameter, apex of adaxial surface scurfy; **blade** flabellate, obtriangular, deeply bifid, 4 m long, 38 – 70 cm broad, glossy green on both surfaces. **Inflorescences:** staminate inflorescences erect or somewhat curved, branching of first to third orders, 45 cm long, 9.5 cm broad; rachillae 4 – 9 per inflorescence, 11 – 15 cm long, 1.1 – 1.8 cm diameter, flowers many per rachillae, 6 mm long, 3 mm cm broad; pistillate inflorescences erect, more or less cylindrical, 30 cm long, 7 cm broad, rachillae one per inflorescence, 11.5 cm long, 7 cm broad, pistillate flowers about 100 per rachilla, calyx bell-shaped, deeply splitting between 2 of the lobes, corolla pitcher-shaped, 1.6 – 1.8 cm long, 0.8 – 1.2 cm broad. **Fruits:** pink when mature. **Distribution:** Borneo.

2. *SALACCA MINUTA* MOGEA

Salacca minuta Mogea, Fed. Mus. J. (Kuala Lumpur) 29: 11 (1984).

Habit: plant up to 1 m tall; stems absent or very short. **Leaves:** flabellate, about 0.9 long; leaf-sheaths 10 cm long, in cross-section weakly crescent-shaped, 4 cm long, 3 cm diameter; petiole 12 – 20 cm long, on the lower side spiny; **spines** patent, flat, triangular, up to 1 cm long, at the base 2 mm broad, sparse on the leaf-sheath with some single spines, on the petiole and rachis similar as on the leaf-sheath; **blade** flabellate, obtriangular, leaf margins lobes, each lobe corresponding to a main longitudinal veins, deeply bifid, 50 cm long, 14 – 20 cm broad, adaxial surface glossy green, abaxial surface with brown indumentum. **Inflorescences:** axillary, piercing the subtending back of the leaf-sheaths base, staminate inflorescences slender, unbranched, 15 cm long, at the base covered by three empty bracts, staminate inflorescences sometimes elongate, lying on the ground, whip-like, about 150 cm long, new leafy shoot produced at the apex; 3 flowers per rachilla; pistillate inflorescences unavailable. **Fruits:** unavailable. **Distribution:** Malaysia.

3. *SALACCA DRANSFIELDIANA* MOGEA

Salacca dransfieldiana Mogea, Reinwardtia 9: 463 (1980).

Habit: plant about 1.5 m tall; stems erect clustered, about 3 cm diameter. **Leaves:** flabellate, about 0.8 – 1.3 m long; leaf-sheaths about 20 cm long, above the base gradually channelled; basal part broadly triangular, in cross-section weakly crescent-shaped, about 4 cm long, 5 cm diameter; petiole 40 cm long; **spines** patent, flat, triangular, 3.5 cm long, at the base 0.5 mm broad, sparse on the leaf-sheath with 1 single spine or often with 2 other ones together, on rachis usually only single spines, about 7 cm apart; **blade** flabellate, obtriangular, deeply bifid at apex, 68 – 75 cm long, 24 – 28 cm broad, broadest at the top; adaxial surface glossy green, abaxial surface whitish. **Inflorescences:** staminate inflorescences 20 cm long, erect, somewhat curved, rachillae 1 per inflorescence, 7 cm long, about 1 cm diameter; flowers many on each rachilla, calyx bell-shaped, 3.5 mm long, split almost completely to the base; corolla pitcher-shaped, petals more or less spathulate, about 4 mm long, 0.5 mm broad; pistillate inflorescences unavailable. **Fruits:** unavailable. **Distribution:** Borneo.

4. *SALACCA MULTIFLORA* MOGEA

Salacca multiflora Mogea, Fed. Mus. J. (Kuala Lumpur) 29: 13 (1984).

Habit: plant about 3.2 m tall, erect. **Leaves:** flabellate, to 3.1 m long; leaf-sheaths about 40 cm long, gradually channelled above the base, basal cross-section broadly triangular, crescent-shaped, about 10 cm long, 8 cm diameter, with the very base attached all about the axis; petioles 12 cm long with lateral spines; **spines** pointing horizontally, patent, rarely upward, or downward, in small combs of 3, at a distances of 1.5 – 3 cm, the other scattered in pairs or solitary, brown, up to 2.5 cm long; rachis 180 – 200 cm long, covered with fine brown indumentum on abaxial surfaces; **blade** flabellate, obtriangular, deeply bifid, 200 – 250 cm long, 20 – 28 cm broad, adaxial surface glossy green, abaxial surface with pale brownish indumentum. **Inflorescences:** axillary, piercing the subtending back of the leaf-sheaths base; staminate inflorescences somewhat curved, up to 3 branching orders, 25 cm long, rachis and internodes covered by bracts; rachillae with 17 flowers per inflorescence, cylindrical, 1.5 – 2.5 cm long, 0.3 – 0.6 cm diameter; pistillate inflorescences erect, 5 cm long, bearing only one rachilla with flowers, rachillae with flowers cylindrical, 1.5 cm long, 1 cm diameter, outside glabrous, each bearing not more than 10 flowers. **Fruits:** only known from young fruits, globose, 1 cm diameter, covered by upturned brown scales. **Distribution:** Malaysia.

5. *SALACCA SARAWAKENSIS* MOGEA

Salacca sarawakensis Mogea, Reinwardtia 9: 473 (1980).

Habit: plant stemless. **Leaves:** flabellate, 2.4 m long, basal part of leaf-sheaths unknown, the channelled part with length estimated at 120 cm, at about the middle part in cross-section elliptic to crescent-shaped, deep brown; the lower surface very faintly ribbed dull brownish, spiny; petiole about 87 cm long, the lower section circular, spiny; **spines** patent, not flattened, pale yellowish, on the sheaths placed in 3 longitudinal rows, 1 on the lower side and the other two near the upper side, regular but toward the tip distance apart increasing from 2.5 to 4.5 cm, the lower side bearing the bigger ones 0.6 – 1.8 cm long, its base 0.4 – 0.5 cm broad, those on the petiole length unavailable, at the base 0.5 cm broad, toward the top at decreasing distance of 7 – 9.5 cm, not spiny near the top; rachis 72.5 cm long, 1 cm

diameter at the base; **blade** flabellate, obovate, deeply bifid, 120 cm long, 66 cm broad, concolorous, glossy green on both surfaces. **Inflorescences:** staminate inflorescences unknown; pistillate inflorescences curved in first order branching pattern, 17 – 20 cm long, bracts 2.5 – 6.5 cm long; rachillae 1 – 2 per inflorescence about 2.5 cm long, approximately 12 mm diameter; flowers about 40 on each rachilla, calyx split halfway, corolla swollen or pitcher-shaped. **Fruits:** unavailable. **Distribution:** Borneo.

6. *SALACCA FLABELLATA* FURTADO

Salacca flabellata Furtado, Gard. Bull. Singapore 12: 387 (1949).

Habit: plant stems including leaf-sheaths to 5 cm diameter. **Leaves:** flabellate, 1 – 2.3 m long; leaf-sheaths 27.5 – 34 cm long, channelled suddenly above the base, on the abaxial surface smoothly wrinkled and on the median spiny; petiole 20 – 100 cm long, at the base in cross-section broadly ovate; often rather narrow on the upper side, the lower side spiny; **spines** 0.5 – 1. 5 cm long, ascending or patent, flat, narrow triangular, yellowish pale brown, in pairs, 0.4 – 1 cm apart, at the base 0.4 cm broad and 0.1 cm thick, on the channelled part, in three longitudinal rows, one on the lower side and the other two on the upper side, patent, 0.5 cm apart, but towards the top distances increasing from 3.5 to 8.5 cm apart; rachis 40 – 75 cm long, prominent below, may be spiny; **blade** flabellate, obovate, deeply bifid at apex, 80 – 100 cm long, 20 – 45 cm broad, glossy green on adaxial surfaces, whitish on abaxial surfaces. **Inflorescences:** staminate inflorescences lying on the ground whip-like, slender, unbranched, 1 – 2 m long, sometimes producing a new plant leafy shoot at the apex; rachillae 2 – 4 per inflorescence, 1.5 – 3 cm long, 1 mm diameter; pistillate inflorescence similar to staminate one, except a new leafy shoot is not produced, length unknown; rachilla cylindrical 2 cm long, 1 – 1.2 cm diameter outside glabrous; pistillate flowers 15 – 20 on each rachilla. **Fruits:** only known when immature, globose, about 7 mm diameter, covered by brown scales up to 3 mm long. **Distribution:** Malaysia.

7. *SALACCA CLEMENSIANA* BECC.

Salacca clemensiana Becc., Philipp. J. Sci., C 4: 618 (1909).

Salacca conferta Griff., Calcutta J. Nat. Hist. 5: 16 (1845).

Habits: plant usually appears stemless but occasionally reaches 100 cm long. **Leaves:** may be pinnate or flabellate or a combination of pinnate and flabellate, the intermediate form is pinnate at the base and the upper portion is flabellate and bifid, to 5 m long; the leaf-sheath and petiole covered with long spines; **spines** 5.1 – 7.6 cm long; rachis in the middle portion acutely trigonous, with a line of long spines along the centre of abaxial part; **pinnate leaflets** 45 – 60 cm long, 6 – 7 cm broad, adaxial surface shiny green, abaxial surface dull whitish colour, small spines present along margins; flabellate leaf length information unavailable. Intermediate leaflets in alternating groups of 3-4, apex tapers into a long thin tip, **Inflorescences:** staminate inflorescences 61 – 91 cm long, with several short branches, bearing 6 – 8 rachillae per inflorescence, rachillae 5 – 7.6 cm long; staminate flowers small, 4 mm long; calyx split to the base into 3 linear lobes; corolla slightly longer than the calyx, lower half fused to form a tube, upper divided into 3 lobes, oblong; pistillate inflorescence unavailable. **Fruits:** unavailable. **Distribution:** Philippines, Borneo.

8. *SALACCA GRACILIFORA* MOGEA

Salacca graciliflora Mogea, Fed. Mus. J. (Kuala Lumpur) 29: 6 (1984).

Habit: plant about 1.3 m tall; stems very short, 20 cm long, 2.5 cm diameter. **Leaves:** pinnate, 1.25 m long; leaf-sheaths 15 cm long, channelled gradually above the base, in cross-section weakly crescent-shaped, 5 cm long, 3 cm diameter; petiole; 50 – 60 cm long, the lower surface not scurfy; **spines** patent, flat, narrowly triangular, pale brown, spines on the leaf sheath up to 3 cm long, swollen at the base, 6 mm broad, 2 or 3 form a small comb up to 1.5 cm long, at distances of 2 – 6 cm, toward the top gradually smaller and fewer, on the petiole as on the leaf-sheath but smaller and fewer with single spines on the lower side, at a distances of about 8 cm; **leaflets** at the base of the rachis 14 – 24 cm long, 1.5 – 2 cm broad, at the middle 21 – 24 cm long, 2.5 cm broad, apical part of the leaf more or less obtriangular, 18 cm long, 19 cm broad. **Inflorescences:** staminate inflorescences

two or three, lying on the ground whiplike, slender, unbranched, up to 70 cm long; rachillae one per inflorescence, flowers cylindrical 2 cm long, 4 mm diameter; staminate flowers 0.25 cm long, 0.15 cm broad, calyx with 3 lobes, 0.4 mm from the top; corolla triangular, 2.5 mm long, 0.8 mm; pistillate inflorescences unavailable. **Fruits:** unavailable. **Distribution:** Malaysia.

9. *SALACCA RUPICOLA* J.DRANSF.

Salacca rupicola J.Dransf., Bot. J. Linn. Soc. 81: 36 (1980).

Habit: plant with subterranean stems, in old specimens shortly creeping, to 35 mm diameter without leaf-sheaths. **Leaves:** pinnate, up to 2.25 m long, leaf-sheath dull green, epidermis totally obscured by dense purplish-brown scaly indumentum, densely armed with spines; petioles to 100 cm long, armed with an abaxial row of spines; **spines** may be solitary or pairs or in whorls, those on the sheath in oblique whorls to 15 mm long, the partial whorls about 10 mm apart and comprising up to 8 spines united by their bulbous bases, those on the petioles in groups of 2 subequal spines to 2 mm long, 1 large lateral spine to 6 mm long and 2 small lateral spines or groups of up to 5 spines varying from 2 – 20 mm long; **leaflets** up to 15 on each side of rachis, 25 – 30 cm long, 1.5 – 3 cm broad, in groups of 2 – 3, adaxial surface dark bluish-green, abaxial surface pinkish-creamy brown. **Inflorescences:** staminate inflorescences 15 cm long, up to 3 rachillae per inflorescence, 7 cm long; staminate flowers 0.25 mm long, 0.2 mm broad, sepals 3, free almost to the base; pistillate inflorescences 35 – 40 cm long, unbranched; rachillae to 6 x 1 cm, each bract subtending a pair of flowers one pistillate, the other a sterile staminate or neuter flower. **Fruits:** young fruit spherical, about 1 cm diameter, densely covered with dark brown scales, the tips swept upward, spine-like. **Distribution:** Borneo

10. *SALACCA ZALACCA* (GAERTN.) VOSS

Salacca zalacca (Gaertn.) Voss, Vilm. Blumengärtn. ed. 3, 1: 1152 (1895).

Calamus zalacca Gaertn., Fruct. Sem. Pl. 2: 267 (1791).

Salacca rumphii Wall., Pl. Asiat. Rar. 3: t. 223 (1831).

Salacca edulis Reinw., Syll. Ratisb. 2: 3 (1828).

Salacca blumeana Mart., Hist. Nat. Palm. 3: 202 (1838).

Calamus salakka Willd. ex Steud., Nomencl. Bot., ed. 2, 1: 252 (1840

Salacca edulis var. *amboinensis* Becc., Ann. Roy. Bot. Gard. (Calcutta) 12(2): 74 (1918).

Salacca zalacca var. *amboinensis* (Becc.) Mogea, Principes 26: 71 (1982).

Habit: plant 4 – 8 m tall, stems subterranean, creeping and tillering, forming compact clumps by successive branching at the base; roots not extending to any great depth; stolon with only its terminal leaf bearing part being upright, reaching several metres long and 10 – 15 cm diameter excluding the densely spiny leaf-sheaths, often branching; new roots growing out of the stem immediately under the crown of leaves; internodes very congested, leaf traces inserted almost horizontally. **Leaves:** pinnate, 3 – 7 m long; leaf-sheaths up to 90 cm long, deeply split at base, covered by lime-green to yellowish indumentum, scaly; petiole 2 – 3 m long, indumentum as per leaf-sheath, scaly; **petioles** 1.1 – 2.95 m long, indumentum as per leaf-sheath; **spines** 0.5 – 8 cm long, patent, pointing horizontally, rarely upward, or downward, in small combs of 3 or 4, other scattered in pairs or solitary, grey to blackish; **leaflets** 59 - 95 on both sides of rachis, often unequally spaced, 20 – 70 cm long, 2 – 7.5 cm broad, adaxial surface dark green, abaxial surface whitish. **Inflorescences:** an axillary compound spadix, stalked, at first enclosed by spathes; staminate inflorescences 50 – 100 cm long; flowers in pairs in axils of scale-like bract; staminate flowers, calyx 3 lobes, split below the middle, corolla tubular, reddish, 6 stamens borne on the corolla throat and pistillode; pistillate inflorescences 15 – 32 cm long, bearing 1 – 4 rachillae per inflorescence, rachillae 5 – 12 cm long; corolla tubular, yellow-green outside and dark red inside, ovary trilocular with short trifid, red style and 6 staminodes borne on the corolla throat. **Fruit:** globose to ellipsoid drupe, 15 – 40 per spadix, about 5 – 9 cm long, 4 – 6 cm broad, tapering towards base, rounded at top; epicarp (skin) with numerous yellow to brown, united, imbricate scales, each scale ending in a fragile prickle; mesocarp/flesh yellowish white. **Seeds:** 1 - 3 per fruit, 0.2 – 0.8 cm thick, sarcotesta cream-coloured, stony inner part smooth, 2.3 – 2.9 cm long, 1.5 – 2.7 cm broad, blackish-brown and trigonous with 2 flat surfaces and a curved one; endosperm homogeneous and white. **Distribution:** Sumatra, Java, Bali, North Sulawesi and Ambon. In Indonesia at least 20 intraspecific taxa are distinguished according to place of origin and cultivation, e.g. 'Condet', 'Pondoh', 'Bali', 'Suwaru'. 'Bali' is

andromonoecious; the inflorescences bear both hermaphrodite and staminate flowers, still in doubt on whether the later produce functional pollen.

11. *SALACCA AFFINIS* GRIFF.

Salacca affinis Griff., Calcutta J. Nat. Hist. 5: 9 (1845).

Salacca borneensis Becc., Malesia 3: 68 (1886).

Salacca affinis var. *borneensis* (Becc.) Furtado, Gard. Bull. Singapore 12: 399 (1949).

Salacca dubia Becc., Malesia 3: 68 (1886).

Calamus collinus, Griff. Palms Brit. Ind. T. 186. (1850)

Habit: plant 4 – 7 m tall; stems clustered. **Leaves:** pinnate, to 3 m long; **spines** 1.5 – 6 cm long, thin, whitish, **leaflets** to 33 cm long, to 8.8 cm broad, strongly falcate, flat in one plane at least toward leaf tips, evenly spaced within groups with irregular gaps between, concolorous. **Inflorescences:** erect, rachillae hidden in the subtending spathes; staminate inflorescences 3 – 6 cm long, solitary or in group of 2 - 3, spathes 10 – 18 cm long; pistillate inflorescences 5 – 8 cm long, bracts minute, alternate; rachillae short each with about 3 flowers. **Fruits:** 4 – 6.5 cm long, 4.5 cm broad, tapering at both ends, scales smooth, chestnut brown; seeds 3. **Distribution:** Sumatra, Malaysia, Borneo.

12. *SALACCA RAMOSIANA* MOGEA

Salacca ramosiana Mogea, Principes 30: 161 (1986).

Habit: plant 8 m tall, erect, diameter including leaf-sheaths about 60 cm. **Leaves:** pinnate, about 2.7 – 5.5 m long; leaf-sheaths dull brown with pale brown indumentum and numerous brown spines; petiole 100 cm long, channelled near the base, circular in cross-section, 2 cm diameter; **spines** triangular, pointing upward or downward, pale yellow, those on the petiole about 5 – 8 cm long, 0.6 cm broad, arranged in transverse or oblique combs, about 10 spines, on each comb, those on the base of the rachis in small combs of 2 – 3 spines, in middle rachis borne singly,

at the very tip the spines are upcurved, sometimes with two small ones together in small combs, up to about 1.5 – 3.5 cm long, 0.2 cm broad; rachis 2.4 – 4.2 m long, 2.5 cm diameter, elliptic at the base in cross section; **leaflets** about 40 on both sides, at the base of the rachis the leaflets 11 – 46 cm long, 2.5 – 10 cm broad, somewhat sigmoid, armed along the margins with small upcurved spines, leaflets irregularly grouped, the apical leaflet more or less obtriangular, deeply bifid. **Inflorescences:** staminate inflorescences about 30 – 80 cm long, 4 cm broad including the bracts; rachillae about 25 per inflorescence, 3 – 8 cm long, 0.6 – 1 cm broad; staminate flowers many on each rachilla, calyx bell-shaped, 0.1 – 0.3 cm long, split near the apex only; corolla bell-shaped, 0.5 – 0.6 cm long, about 0.1 mm broad; pistillate inflorescences 45 – 70 cm long, 5.5 cm broad including the bracts, erect, branching to 3 orders; bracts boat-shaped, rachillae many per inflorescence, erect, about 2 cm long, 1.4 cm broad, pistillate flowers 0.5 – 0.7 cm long, split to about halfway or completely, corolla bell-shaped. **Fruits:** more or less ellipsoidal, 6 cm long, 3 cm broad, scales in vertical rows adpressed in the middle of the fruit, rhomboid in shape, yellowish reddish brown. **Distribution:** Philippines, Malaysia.

13. *SALACCA SUMATRANA* BECC.

Salacca sumatrana Becc., Ann. Roy. Bot. Gard. (Calcutta) 12(2): 80 (1918).

Salacca wallichiana (non Mart.). Mig. Prodr. Fl. Sum. 255,592

Habit: plant 4 – 7 m tall; stem usually absent or very short. **Leaves:** pinnate, length unavailable, leaves with regularly alternately arranged and equidistant large elongate leaflets; leaf-sheaths details unavailable; petiole densely armed with very unequal spines; **spines** on the petiole large and robust, subulate, flattened, frequently approximate by their base and obliquely inserted, those on the rachis large flattened, 3 – 5 cm long, 5 – 10 mm broad at their bases, sometimes accompanied by smaller ones; rachis stout, trigonous, armed only along the abaxial surface, especially toward the base; **leaflets** 51 – 75 cm long, 6 – 7.5 cm broad, numerous, all equidistant and regularly two rowed, narrowly lanceolate, slightly sigmoid at the base, falcate apex acuminate, adaxial surface glossy green, abaxial surface dull ashy grey. **Inflorescences:** about 12 cm long; staminate inflorescences unavailable; pistillate inflorescences unavailable. **Fruits:** 6 – 7 cm

long, 4 – 4.5 cm broad, turbinate, tapering to base, scales glossy, chestnut brown, points fine 4 – 6 mm long, flesh red; seeds usually 3. **Distribution:** Sumatra.

14. *SALACCA STOLONIFERA* HODEL

Salacca stolonifera Hodel, Palm J. 134: 35 (1997).

Habit: plant up to 3 m tall, shrubby often creeping; stems up to 70 cm long, 5 – 8 cm diameter, usually creeping. **Leaves:** pinnate, up to 3.5 m long, ascending; leaf-sheaths 30 – 60 cm long, deeply split but tubular and bulbous at the base; petiole to 1.2 m long; **spines** to 4 cm long, black, spines becoming paired apically, spines similar to those on both leaf-sheaths and petioles; rachis 2 – 2.5 m long; **leaflets** 17 on each side of rachis, up to 50 cm long, 5 cm broad, clustered and fanned in remote groups of 2 or 3, end pair with truncate, jaggedly toothed tips, adaxial surface green, abaxial surface whitish. **Inflorescences:** staminate inflorescences 1 – 2 interfoliar, whip-like, stoloniferous inflorescences rooting and sprouting at their tips, up to 4 m long, simple, unbranched, spike-like, partial inflorescences to 20 cm long near the base, reddish, nodding; pistillate inflorescences unavailable. **Fruits:** unavailable. **Distribution:** Thailand.

15. *SALACCA VERMICULARIS* BECC.

Salacca vermicularis Becc., Malesia 3: 66 (1886).

Habit: plant about 8 m tall; stemless, clustered. **Leaves:** pinnate, large, up to 7 m long; leaf-sheaths and petioles densely armed with large, broad base, long spines; **spines** 1 – 6.5 cm long, arranged in whorls below, in pairs or scatter higher up, dark brown; rachis unavailable; **leaflets** 27 – 90 cm long, 3.5 – 8 cm broad, interruptedly fasciculate, those on the middle portion usually alternate and formed by 3 or 4 leaflets on each side of the rachis, elongate lanceolate, adaxial surface dark green, abaxial surface whitish. **Inflorescences:** massive; staminate inflorescences 8 – 12 cm long, 0.8 – 1 cm diameter; male flower red, musty smelling; pistillate inflorescences unavailable. **Fruits:** black. **Distribution:** Sarawak.

16. *SALACCA DOLICHOLEPIS* BURRET

Salacca dolicholepis Burret, Notizbl. Bot. Gart. Berlin-Dahlem 15: 731 (1942).

Habit: plant about 4 m tall; stems clustered, short, prostrate, often rooting at the nodes. **Leaves:** pinnate, up to 5 m long; leaf-sheaths and petioles densely armed with spines; **spines** information unavailable; **leaflets** 40 – 60 cm long, 3.4 - 4.5 cm broad, borne in fanned groups of 3 to 5, terminal pair compound, up to 45 pairs on both sides of rachis, adaxial surface green, abaxial surface dull grey. **Inflorescences:** staminate inflorescences with rachillae 11.5 cm long; pistillate inflorescences unavailable. **Fruits:** black when ripe . **Distribution:** Borneo.

17. *SALACCA SECUNDA* GRIFF.

Salacca secunda Griff., Calcutta J. Nat. Hist. 5: 12 (1845).

Habit: details unavailable. **Leaves:** pinnate, 9 m long; leaf-sheaths and petioles details unavailable; spines 5 – 10 cm long; **leaflets** 50 – 100 cm long, 5 – 10 cm broad, concolorous, linear-lanceolate, veins on abaxial surface spinulose. **Inflorescences:** staminate inflorescences with rachillae 6.4 – 7.6 cm long, 1.3 cm diameter; male flowers with round bracts, densely crowded, calyx tripartite to about the middle flower, corolla about the length of the calyx; pistillate inflorescences branched rachillae tomentose. **Fruits:** 5 cm long, 6 – 6.5 cm broad, scales dark brown, spreading, tips recurved. **Distribution:** Assam.

18. *SALACCA WALLICHIANA* MART.

Salacca wallichiana Mart., Hist. Nat. Palm. 3: 201 (1838).

Calamus zalacca Roxb., Fl. Ind. ed. 1832, 3: 773 (1832).

Salacca macrostachya Griff., Calcutta J. Nat. Hist. 5: 13 (1845).

Salacca beccari Hook.f., Fl. Brit. India 6: 474 (1893).

Habit: plant large, up to 12 m tall, shrubby, stems clustered; 2 – 5 m long, diameter including leaf-sheath 20 – 30 cm. **Leaves:** pinnate, 7 – 10 m long, ascending; leaf-sheaths to 100 cm long, deeply split but tubular and bulbous at base, covered with rusty brown indumentum, scaly; petiole 2 – 3 m long, lime-green to yellowish,

indumentum as for the leaf-sheaths; **spines** similar for both sheath and petiole**,** linear to 8 cm long, in partial whorls, black, apex somewhat reflexed; rachis 5 – 7 m long; **leaflets** 40 – 55 on each side of rachis, 70 – 100 cm long, 8 cm broad, irregularly arranged in groups of 2 – 5 forming fans, terminal leaflets with truncate to filiform tips, adaxial surface green, abaxial surface greyish. **Inflorescences:** 2 – 3 per stem, interfoliar, pendulous, usually laying on the ground, 2 – 3 m long; staminate inflorescences with up to 10, long branches, each with up to 10 rachillae, simple, unbranched, spike-like, reddish, up to 10 cm long; pistillate inflorescences with fewer branches, with up to 4 rachillae, simple, unbranched, spike-like, reddish, to 10 cm long, bracts tomentose enclosing one pistillate flower and two sterile staminate or neuter flowers. **Fruit:** 7 – 8 x 4 cm, obovoid, borne in dense clusters, scales reddish brown with bristle-like reflexed tips. **Distribution:** Thailand, Myanmar, Malaysia, and Singapore.

19. *SALACCA GLABRESCENS* GRIFF.

Salacca glabrescens Griff., Calcutta J. Nat. Hist. 5: 14 (1845).

Habit: plant up to 7 m tall; stems form a ring, shrubby, often clustered, creeping to, shortly erect, to 100 cm tall, diameter 5 – 12 cm. **Leaves:** pinnate, 9 – 18 per stem, 2.45 – 3.6 m long, ascending to spreading, emerging leaves sometimes reddish brown; leaf-sheaths 45 – 60 cm long, deeply split but tubular at the base, densely covered by brownish indumentum, armed with spines; petiole 2 – 3 m long, covered by greenish yellow indumentum; **spines** solitary in a single row or in partial whorls, needle-like, black with yellow base, 1 – 5 cm long; rachis 2 m – 4 m long; **leaflets** 23 – 30 on each side of rachis, 45 – 60 cm long, 5 cm broad, equidistant particularly near tip or slightly fanned forming groups of 3 – 4, slightly falcate, concolorous except when young. **Inflorescences:** 2 – 4 per stem, bursting through bulbous leaf base spreading, short; each branch bearing 1 inflorescence; staminate inflorescences emerge at ground level, 30 – 60 cm long with 10 – 2 branches each terminating in a reddish, spike-like, partial inflorescences 8 – 12 cm long; pistillate inflorescences to 25 cm long, partial inflorescence 5 – 12 cm long. **Fruits:** 4.5 x 3 cm, pear-shaped or obovoid, reddish brown, scaly, tip spine-like; seeds 2 or 3. **Distribution:** Thailand and Malaysia.

SALACCA LOPHOSPATHA J.DRANSF. & MOGEA[2]

Salacca lophospatha J.Dransf. & Mogea, Principes 25: 180 (1981).

Lophospatha borneensis Burret, Notizbl. Bot.Gart. Mus. Berlin-Dahlem 15: 753 (1942)

Habit: plant with stems very short, subterranean or partially erect. **Leaves:** pinnate, about 4 m long; leaf-sheaths and petioles densely armed with black spines; **leaflets** strongly discolorous; leaflets dark green on adaxial surfaces, chalky-white on abaxial surfaces; **Inflorescences:** staminate inflorescences pendulous with discrete rachillae bracts, bracteoles with an apical tuft of airs cf. *S. clemensiana* where the bracteoles are densely fluffy hairy; pistillate inflorescences unavailable. **Fruits:** unavailable. **Distribution:** Borneo.

5.6 A Key to the currently recognised cultivars of Bali salak

1 Plants produce flowers but never produce fruits ……..…..... **1**. Muani

1. Plants produce both flowers and fruits ……...……..……. 2

2 Spine colour is yellowish-brown ……...……...…..... **2**. Putih

2. Spine colour is dark brown to almost black ……..…..… 3

3 Leaf length less than 360 cm ……..…… 4

3. Leaf length more than 360 cm ……..…….. 5

4 Number of leaflets on the rachis usually 67 or less ……...……... 6

4. Number of leaflets on the rachis more than 67 ..…….…… **3**. Embad

5 Number of spines per 30 cm length of petioles is less than 60 …..... **4**. Nyuh

5. Number of spines per 30 cm length of petioles is more than 60 7

6 Leaflets are curly, partly fused, and fruits at maturity 15 grams or less ……. ……..……..……..……...… **5**. Bingin

6. Leaflet are straight, fruits at maturity more than 15 grams …….…… **6**. Gula

7 Mesocarp (flesh) of mature fruits red or white yellow with black stripes ... 8

7. Mesocarp (flesh) of mature fruits neither red or with black stripes ...…..... 9

8 Mesocarp is red ……..……..…….. **7**. Boni

8. Mesocarp white yellow with black stripes ……..…..... **8**. Selem

9 Epicarp (skin) of mature fruits has white spots ……..…..……. **9**. Maong

9. Epicarp (skin) of mature fruits lacks of white spots …..…….……..… 10

10 Mature fruits sour to the taste ……..……..……..…..... **10**. Penyalin

10. Mature fruits sweet to taste ……..……..……. 11

11 Flesh of mature fruits taste like jackfruit ...……...…... **11**. Nangka

11. Flesh of mature fruits does not taste like jackfruit ……...……... 12

12 Mature flesh smell and/or taste like pineapple …….....….. **12**. Nanas

12. Fruit smell and/or taste aromatic as in the flowers of *Michelia champaca* ……. ……..……..……..... **13**. Gondok

5.7 A key to eight cultivars proposed for Bali salak based on the results of this study

1 Plants produce flowers but never produce fruits ……..……...…... **1**. Muani

1. Plants produce both flowers and fruits ……...……..……... 2

2 Spine colour is yellowish-brown, not dark brown to almost black ..... **2**. Putih

2. Spine colour is dark brown to almost black ……..…… 3

3 Leaf length less than 360 cm ……...…… 4

3. Leaf length more than 360 cm ……..……. 5

4 Leaflets are curly, partly fused, and fruits at maturity are 15 grams or less ……
……**5**. Bingin

4. Leaflet are straight, fruits at maturity are more than 15 grams ……. **6**. Gula

5 Epicarp (skin) of mature fruits is with white spots ……..…….…. **9**. Maong

5. Epicarp (skin) of mature fruits is without white spots ……..... 6

6 Number of spines per 30 cm length of petioles is less than 60 ……... **4**. Nyuh

6. Number of spines per 30 cm length of petioles is more than 60 …..... 7

7 Mesocarp is red, periclinal cell wall flat ...……..... **7**. Boni

7. Mesocarp white yellow or with black stripes, periclinal cell wall convex … Biasa (**3**. Embadan, **8**. Selem, **10**. Penyalin, **11**.Nangka, **12**. Nanas, **13**. Gondok)

Note: the numbers associated with each cultivar correspond to the number in the descriptions.

5.8 Description of currently recognised Bali salak cultivars

In the description of the cultivars below, measurements of plant height, petiole length, and leaf length are presented in metres. However, in previous chapters (Chapter 2, 3 and 4) the measurement of these characters are presented in centimetres. The mean values of the characters which are used to describe 13 Bali salak cultivars presented in Table 5.1.

Table 5.1 Mean values and ranges of characters which are used to describe the 13 Bali salak cultivars (*Salacca zalacca* var. *amboinensis*). All character abbreviations are explained in Table 4.1.

Character / Cultivar	PH	LL	PL	SL	MLL	MCL	FFL	FCL	AdCL	AbCL	AbCW	LIN	SpD	StD	StI	RAb Clw	FrW
Gula	470	358	126	2.7	55	9.3	8.9	14.3	39.1	42.8	27.8	67	92	126.1	14.4	1.7	37.8-79.9
Boni	496	385	143	2.8	56	7.9	8.9	14.8	37.7	41.5	24.9	82	102	137.1	15.6	1.6	37.8-79.9
Bingin	428	342	118	2.9	58	9.5	9.4	15.0	40.6	41.7	26.1	65	89	132.8	13.2	1.6	12.4-15.6
Selem	571	456	160	2.6	56	9.7	9.3	15.1	38.5	40.7	24.8	79	93	149.4	14.5	1.8	37.8-79.9
Embadan	550	463	199	2.7	57	10.3	10.2	15.3	39.5	41.4	23.5	74	94	138.1	15.1	1.6	37.8-79.9
Nangka	543	444	146	2.5	58	9.4	9.2	15.3	37.1	43.1	25.1	78	96	125.8	15.5	1.7	37.8-79.9
Penyalin	564	483	204	2.9	62	10.1	9.9	14.9	39.7	39.8	26.4	72	98	135.2	15.8	1.7	37.8-79.9
Maong	529	452	167	2.8	58	9.4	9.4	14.0	39.9	44.2	25.6	75	93	124.5	13.8	1.7	37.8-79.9
Nanas	563	465	174	2.6	62	9.1	10.4	14.0	44.7	39.3	27.6	80	96	150.8	17.4	2	37.8-79.9
Gondok	540	445	155	2.9	62	10	9.4	15.1	39.7	38.4	23.2	81	92	153.1	18.3	1.6	37.8-79.9
Muani	589	507	178	3.1	67	10.4	11.1	15.4	51.1	43.7	28.7	77	88	113.4	11.9	2.1	absent
Nyuh	552	449	162	1.9	57	9.2	9.6	13.0	40.7	40.9	22.8	75	54	141.8	16.6	1.8	37.8-79.9
Putih	566	454	126	2.7	61	9.1	9.7	14.0	41	40.3	23.1	83	79	139.6	15.4	1.8	37.8-79.9

1. MUANI

Plants about 5.82 m tall; petiole usually 1.78 m long; spine 3.1 cm long, spine density/number of spines per 30 cm length of petioles 88, spine colour dark brown to almost black. **Leaves** 4.97 m long, middle leaflets 67 cm long, leaflet number 77 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 51.1 µm long, abaxial cells 43.7 µm long and 28.7 µm wide, ratio adaxial cell length to width 2.1; stomatal density 113.4, stomatal index 11.9 %; **periclinal cell walls** flat as the surrounding epidermal surfaces, guard cells sunken as the surrounding epidermal cells. **Calyx** of male flowers 10.4 mm long, female flower 11.1 mm long, corolla of female flowers 15.4 mm long. **Fruits** are not produced. The local farmers refer to them as 'male' plants due to the plant does not produce fruits.

2. PUTIH

Plants about 5.36 m tall; petiole usually 1.26 m long; spines 2.7 cm long, spine density/number of spines per 30 cm length of petioles 79, spine colour yellowish brown. **Leaves** 4.54 m long, middle leaflets 61 cm long, leaflet number 83 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 41 µm long, abaxial cells 40.3 µm long and 23.1 wide' ratio adaxial cell length to width 1.8; stomatal density 139.6, stomatal index 15.4 %; **periclinal cell walls** convex, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 9.1 mm long, female flower 9.7 mm long, corolla of female flowers 14 mm long. **Fruits** epicarp yellowish-brown, mesocarp white, taste sweet, fruit weight 37.8 – 79.9 g; seeds present.

3 EMBADAN

Plants about 5.50 m tall; petiole usually 1.99 m long; spines 2.7 cm long, spine density/number of spines per 30 cm length of petioles 94, spine colour dark brown to almost black. **Leaves** 3.53 m long, middle leaflets 57 cm long, leaflet number 74 on both sides of rachis, leaflets straight. **Leaf epidermis:** adaxial cells 39.5 µm long, abaxial cells 41.4 µm long and 23.5 wide, ratio adaxial cell length to width 1.6; stomatal density 138.1, stomatal index 15.1 %; **periclinal cell walls** convex, guard cells sunken as the surrounding epidermal cells. **Calyx** of male flowers 10.3 mm

long, female flower 10.2 mm long, corolla of female flowers 15.3 mm long. **Fruits** epicarp brown, mesocarp white yellow, taste sweet, fruit weight 37.8 – 79.9 g; seeds present.

4. NYUH

Plants about 5.32 m tall; petiole usually 1.62 m long; spines 1.9 cm long, spine density/number of spines per 30 cm length of petioles 54, spine colour dark brown to almost black. **Leaves** 4.49 m long, middle leaflets 57 cm long, leaflet number 75 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 40.7 µm long, abaxial cells 40.9 µm long and 22.8 wide, ratio adaxial cell length to width 1.8; stomatal density 141.8, stomatal index 16.6 %; **periclinal cell walls** convex, guard cells sunken as the surrounding epidermal cells. **Calyx** of male flowers 9.2 mm long, female flower 9.6 mm long, corolla of female flowers 13 mm long. **Fruits** epicarp brown, mesocarp white yellow, taste sweet, fruit weight 37.8 – 79.9 g; seeds present.

5. BINGIN

Plants about 4.28 m tall; petiole usually 1.18 m long; spines 2.9 cm long, spine density/number of spines per 30 cm length of petioles 89, spine colour dark brown to almost black. **Leaves** 3.42 m long, middle leaflets 58 cm long, leaflet number 65 on both sides of rachis, leaflets curly, partly fused. **Leaf epidermis**: **adaxial cells** 40.6 µm long, abaxial cells 41.7 µm long and 26.1 wide, ratio adaxial cell length to width 1.6; stomatal density 132.8, stomatal index 13.2 %; **periclinal cell walls** flat as the surrounding epidermal surfaces, guard cells raised as the surrounding epidermal cells. **Calyx** of male flowers 9.5 mm long, female flower 9.4 mm long, corolla of female flowers 15 mm long. **Fruits** epicarp brown, mesocarp white yellow, taste sweet, fruit weight 12.4-15.6 g; seeds absent.

6. GULA

Plant about 4.70 m tall; petiole usually 1.26 m long; spines 2.7 cm long, spine density/number of spines per 30 cm length of petioles 92, spine colour dark brown to almost black. **Leaves** 3.58 m long, middle leaflets 55 cm long, leaflet number 67

on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 39.1 µm long, abaxial cells 42.8 µm long and 27.8 wide, ratio adaxial cell length to width 1.7; stomatal density 126.1, stomatal index 14.4 %; **periclinal cell wall** flat as the surrounding epidermal surfaces, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 9.3 mm long, female flower 8.7 mm long, corolla of female flowers 14.3 mm long. **Fruits** epicarp brown, mesocarp white, taste is sweet like sugar, fruit weight 37.8 – 79.9 g; seeds present.

7. BONI

Plants about 4.96 m tall; petiole usually 1.43 m long; spines 2.8 cm long, spine density/number of spines per 30 cm length of petioles 102, spine colour dark brown to almost black. **Leaves** 3.85 m long, middle leaflets 56 cm long, leaflet number 82 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 37.7 µm long, abaxial cells 41.5 µm long and 24.9 wide, ratio adaxial cell length to width 1.6; stomatal density 137.1, stomatal index 15.6 %; **periclinal cell walls** flat as the surrounding epidermal surfaces, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 7.9 mm long, female flower 8.9 mm long, corolla of female flowers 14.8 mm long. **Fruits** epicarp dark brown, mesocarp red, taste sweet, fruit weight 37.8 – 79.9 g; seeds present.

8. SELEM

Plants about 5.71 m tall; petiole usually 1.60 m long; spines 2.6 cm long, spine density/number of spines per 30 cm length of petioles 93, spine colour dark brown to almost black. **Leaves** 4.76 m long, middle leaflets 56 cm long, leaflet number 79 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 38.5 µm long, abaxial cells 40.7 µm long and 24.8 wide, ratio adaxial cell length to width 1.8; stomatal density 149.4, stomatal index 14.5 %; **periclinal cell walls** convex, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 9.7 mm long, female flower 9.3 mm long, corolla of female flowers 15.1 mm long. **Fruits** epicarp brown, mesocarp white yellow with black stripes, taste sweet, fruit weight 37.8 – 79.9 g; seeds present.

9. MAONG

Plants about 5.29 m tall; petiole usually 1.67 m long; spines 2.8 cm long, spine density/number of spines per 30 cm length of petioles 93, spine colour dark brown to almost black. **Leaves** 4.52 m long, middle leaflets 58 cm long, leaflet number 75 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 39.9 µm long, abaxial cells 44.2 µm long and 25.6 wide, ratio adaxial cell length to width 1.7; stomatal density 124.5, stomatal index 13.8 %; **periclinal cell walls** flat as the surrounding epidermal surfaces, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 9.4 mm long, female flower 9.4 mm long, corolla of female flowers 14 mm long. **Fruits** epicarp brown with white spots, mesocarp white yellow, taste sweet, fruit weight 37.8 – 79.9 g; seeds present.

10. PENYALIN

Plants about 5.64 m tall; petiole usually 2.04 m long; spines 2.9 cm long, spine density/number of spines per 30 cm length of petioles 98, spine colour dark brown to almost black. **Leaves** 4.83 m long, middle leaflets 62 cm long, leaflet number 72 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 39.7 µm long, abaxial cells 39.8 µm long and 26.4 wide, ratio adaxial cell length to width 1.7; stomatal density 135.2, stomatal index 15.8 %; **periclinal cell walls** convex, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 10.1 mm long, female flower 9.9 mm long, corolla of female flowers 14.9 mm long. **Fruits** epicarp brown, mesocarp white yellow, taste sower, fruit weight 37.8 – 79.9 g; seeds present.

11. NANGKA

Plants about 5.43 m tall; petiole usually 1.46 m long; spines 2.5 cm long, spine density/number of spines per 30 cm length of petioles 96, spine colour dark brown to almost black. **Leaves** 4.44 m long, middle leaflets 58 cm long, leaflet number 78 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 37.1 µm long, abaxial cells 43.1 µm long and 25.1 wide, ratio adaxial cell length to width 1.7; stomatal density 125.8, stomatal index 15.5 %; **periclinal cell walls** convex, guard cells sunken as the surrounding epidermal cells. **Calyx** of male flowers 9.4 mm

long, female flower 9.1 mm long, corolla of female flowers 15.3 mm long. **Fruits** epicarp brown, mesocarp white yellow, taste sweet like jackfruit, fruit weight 37.8 – 79.9 g; seeds present.

12. NANAS

Plants about 5.73 m tall; petiole usually 1.74 m long; spines 2.6 cm long, spine density/number of spines per 30 cm length of petioles 96, spine colour dark brown to almost black. **Leaves** 4.90 m long, middle leaflets 62 cm long, leaflet number 79.8 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 44.7 µm long, abaxial cells 39.3 µm long and 27.6 wide, ratio adaxial cell length to width 2; stomatal density 150.8, stomatal index 17.4 %; **periclinal cell walls** convex, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 9.1 mm long, female flower 10.4 mm long, corolla of female flowers 14 mm long. **Fruits** epicarp brown, mesocarp white yellow, sweet like pineapple taste, fruit weight 37.8 – 79.9 g; seeds present.

13. GONDOK

Plants about 5.40 m tall; petiole usually 1.55 m long; spines 2.9 cm long, spine density/number of spines per 30 cm length of petioles 92, spine colour dark brown to almost black. **Leaves** 4.45 m long, middle leaflets 62 cm long, leaflet number 81 on both sides of rachis, leaflets straight. **Leaf epidermis**: adaxial cells 39.7 µm long, abaxial cells 38.4 µm long and 23.2 wide, ratio adaxial cell length to width 1.6; stomatal density 153.1, stomatal index 18.3 %; **periclinal cell walls** convex, guard cells at the same level as the surrounding epidermal cells. **Calyx** of male flowers 10 mm long, female flower 9.4 mm long, corolla of female flowers 15.1 mm long. **Fruits** epicarp brown, mesocarp white yellow, taste is sweet and smell is aromatic as in the flowers of *Michelia champaca*, fruit weight 37.8 – 79.9 g; seeds present.

5.9 Discussion

5.9.1 *Salacca* species

Based on the literature it was evident that the differences between some of the *Salacca* species are minimal and that the number of described species could be reduced. For example, S*alacca sumatrana* is differentiated from S*alacca zalacca* based only on the arrangement of the leaflets (Beccari 1918). Additional research would establish whether or not *S. sumatrana* and *S. zalacca* are valid species or they are geographical variants of the same species. A survey of the literature (Burret 1842; Dransfield and Mogea 1981) indicated that there were inadequate or poor diagnoses of the species characteristics in *S. dolicolepis* and *S. lophosphata*. Hence, further detailed work is required to establish the validity of these species.

5.9.2 Bali salak cultivars

Although Govaerts and Dransfield (2005) did not recognize a subspecies within *Salacca zalacca*, there is strong support for differentiating Bali salak as a subspecies from the original javanese material (*S. zalacca* var *zalacca*). Bali salak cultivars are andromonoeceious plants (Darmadi 2001), while the plants from Java are dioecious (Mogea 1978). There is a space between the mesocarps and seeds of mature fruits of Bali salak which can be detected by shaking the fruits. By contrast, in the mature fruits of *Salacca* from Java, the mesocarps tightly cover and adhere to the seeds. However, detailed study is required to establish the differences between plants from Bali and those from Java, as the breeding system of the Bali salak cultivars is not yet clearly understood.

This study supports a reduction in the number of Bali salak cultivars by combining some cultivars, as extremely minor variations were found between several cultivars. Based on the results of the multivariate analysis (Section 4.4), of the 13 cultivars

investigated, eight taxa are recognised. These taxa consist of the 7 distinct cultivars Muani, Bingin, Putih, Nyuh, Maong, Boni, Gula, plus one ‘composite cultivar’ designated as “Biasa”. This contains the six cultivars Selem, Nangka, Gondok, Embadan, Penyalin and Nanas which can only reliably be distinguished based on taste. The name ‘Biasa”, which means ‘common’, has actually often been used by the farmers for the cultivars such as Nanas and Nangka. These are the most common cultivars grown by the farmers

CHAPTER 6

General Conclusions

6.1 Leaf epidermal study conclusion

When leaf epidermal features were analysed using ligh microscope, there was little difference between the cultivars. However, using the higher resolution of scanning electron microscope, it was evident that two qualitative characters: guard cell position and periclinal cell wall pattern varied among cultivars. Furthermore, ANOVA of quantitative measurements indicated that six characters (adaxial cell length, abaxial cell length, abaxial cell width, ratio of adaxial cell length to width, stomatal density, stomatal index) were significantly different ($p \leq 0.05$) among the 13 cultivars investigated. Hence, these characters can be regarded as informative characters for identification of Bali salak cultivars.

6.2 Vegetative morphological and reproductive characters

This study showed that some quantitative, vegetative morphological characters and reproductive characters varied ($p \leq 0.05$) among the 13 cultivars investigated. These characters were plant height, leaf length, petiole length, leaflet number, spine length, spine number, middle leaflet length, male calyx length, female flower length, and female corolla length. Variations in qualitative characteristics both vegetative and reproductive included; presence or absence of fruit, epicarp colour, mesocarp colour, mesocarp taste, presence of seeds and spine colour. Vegetative morphological characters and reproductive characters were informative and useful for identifying the 13 Bali salak cultivars.

6.3 Phenetic Analysis

A phenetic analysis using multivariate techniques indicated that the Bali salak cultivars consisted of eight groups, with Muani and Bingin clearly separated from the main group of 11 cultivars. Also identified as distinct were the five cultivars Gula, Boni, Maong, Nyuh and Putih, as well as the characters that were critical to their separation. The six other cultivars: Selem, Nangka, Gondok, Penyalin, Nanas and Embadan clustered into one main group. The results of the multivariate analysis also revealed strong statistical support for nine characters which have correlation values of more than 0.7 that strongly influenced the separation of the cultivars. These characters were plant height, leaf length, middle leaf length, female flower length, flesh/mesocarp taste, presence of fruits, presence of seeds, adaxial cell length and periclinal cell wall pattern. These results support the recognition of eight taxa with *Salacca zalacca* var. *amboinensis.*

6.4 Taxonomy

Based on the data developed throughout this study selected characteristics for each of the 13 cultivars were determined. However, because minor variations were found in some cultivars, it is proposed that the number of cultivars recognised be reduced from thirteen to eight. The eight cultivars recognised based on results from this study were seven distinct cultivars Muani, Bingin, Putih, Nyuh, Maong, Boni, Gula, plus one ‘composite cultivar’ designated as “Biasa”. This contains the six currently recognised cultivars Selem, Nangka, Gondok, Embadan, Penyalin and Nanas. Additionally, short descriptions and a key to the species of *Salacca* has been provided based on a literature review. This survey indicated that further research is required to determine species delimitations and that several species are inadequately known.

6.5 Future Research in the Bali Salak Cultivars

Although this study has covered many anatomical, morphological and reproductive characters more extensive scanning electron microscopy analysis of reproductive characters should be conducted. This is because results of reproductive characters particularly pollen structures were based on material extracted from dried flowers. It was difficult to extract the anthers from this material and to determine the degree of dehiscence. Investigation of pollen structures should be taken from fresh flowers, preferably unopened buds so as to assess the percentage of viable and non viable pollen. Comprehensive analysis of the breeding systems of Bali salak cultivars also be investigated, since the breeding system of the variety is not clearly understood. As *Salacca zalacca* var. *amboinensis* is the only recognised variety which is andromonoecious and is thus distinct from *Salacca zalacca* var. *zalacca* and other species of the genus which are all represented by dioecious plants. As there is considerable variability among cultivars, there is the potential for new cultivars to be developed through the establishment of a breeding program incorporating commercially desirable characteristics from *Salacca zalacca* var. *amboinensis* and possibly other species.

References

Artschwager, E. (1930) A comparative study of the stem epidermis of certain sugarcane varieties. *Journal of Agricultural Research*, 41: 853-865.

Arunachalam, V. (1999) Prospection and collection of coconut diversity. Pp 12-19 in Improvement of Plantation Crops (M.J.Ratnambal, P.M. Kumaran, K. Muralidharan, V. Niral, and V. Arunachalam, Eds.). CPCRI, Kasaragod.

Baas, P. (1975) Vegetative anatomy and the affinities of Aquifoliaceae, *Sphenotemon, Phelline*, and *Onchotheca. Blumea*, 22: 311-407.

Banson, J.A. and Velasco, J.R. (1982) Coconut: production and utilisation. *Philippine Coconut Research and Development Foundation* (PCRDF), Metro Manila, Philippines, pp. 351.

Bavappa, K.V.A. (1966) Morphological and anatomical studies in *Areca catechu* Linn. and *A. triandra* Roxb. *Phytomorphology*, 16: 346-443.

Beccari, O. (1886) Nuovi studi sulle palmae Asiatiche. *Malesia*, 3: 54-149.

Beccari, O. (1909) Notes on Philippine Palms. II. Philipp. *Journal of Science Bot.*, 4 (5): 601-637.

Beccari, O. (1918) Asiatic Palms - Lepidocaryaeae. *Annals of the Royal Botanic Garden* (Calcutta) 12 (2): 1-231.

Belbin, L. (1988) PATN, pattern analysis package. CSIRO - Canberra, Australia.

Belbin, L. (1995) PATN, pattern analysis package. CSIRO - Canberra, Australia.

Belbin, L. (2001) PATN, pattern analysis package. Blatant Fabrications Pty Ltd.

Blatter, S.J.E. (1926) The palms of British India and Ceylon. Oxford University press.

Blume, C.L. (1843) *Salacca*. *Rumphia*, 2: 161.

Bragg, L.H. (1969) Pollen size variation in selected grass taxa, *Ecology*, 50 (1): 124-127.

Burret (1942) Neue Palmen aus der Gruppe der Lepidocaryoideae. *Notizbl. Bot. Gart. Berlin-Dahlem*, 15: 728-755.

Cavazoz, M.L. and Moya, E.G. (2002) Morphological and pollen differentiation in *Solanum cardiophyllum* ssp. *cardiophyllum* and *S. cardiophyllum* ssp. *ehrenbergii*. *Botanical Journal of the Linnean Society*, 140 (4): 415-426.

Chaturvedi, S. (1998) Leaf morpho-anatomy of six species of *Pinu*s L. (Abietaceae). *Philippine Journal of Science*, 127: 49-64.

Chay-Prove, P. and Goebel, R. (2000) Salak (Snake fruit) (*Salacca* sp.). Department of Primary Industry (DPI) Notes. Queensland Horticulture Institute, Centre for Wet Tropics Agriculture, South Johnstone, Queensland - Australia.

Christophel, D.C. and Rowett, A.I. (1996) Leaf and cuticle atlas of Australian leafy Lauraceae. Australian Biological Resources Study, Canberra.

Christodoulakis, N.S. and Fasseas, C. (1991) Seasonal dimorphism of *Phlomis fructicosa* under controled environmental condition. *Acta Oecologica*, 12 (3): 323-330.

Clifford, H.T. and Watson, L. (1977) Identifying grasses: data, methods and illustrations. University of Quensland Press.

Cronquist, A. (1981) An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.

Cooly, W.W. and Lohnes, P.R. (1971) Multivariate data analysis. John Wiley and Sons, INC. New York.

Cutler, D.F. (1982) Applied Plant Anatomy. Longman, London and New York.

Dafni, A. and Firmage, D. (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant Systematics and Evolution,* 222: 113-132.

Darmadi, A.A. (2001) Taxonomical study of Bali salak cultivars (*Salacca zalacca,* var*. amboinensis* (Becc.) Mogea). Postgraduate thesis, Bogor Agricultural Institute, Bogor, Indonesia.

Dasti, A.A., Bhokhari, T.Z., Malik, S.A., Malik, S.A. and Akhtar, R. (2003) Epidermal morphology in some members of family Boraginaceae in Baluchista, *Asian Journal of Plant Sciences*, 2 (1): 42-47.

De Lamothe, N. De M. and Rognon, F. (1977) The Dwarf coconuts at Port Bouet. *Oleageneux,* 32 (8-9): 373-375.

Dilcher, D.L. and Zeck, C.A. (1968) A study of the factors controlling variation of cuticular characters. *Indiana academic Science*, 78, 1115 (Abstract).

Dilcher, D.L. (1974) Approaches to the identification of angiosperm leaf remains. *Botanical Review*, 40 (1): 1-157.

Dransfield, J. and Mogea, G.P. (1981) A Reassesment of the Genus Lophospatha. *Pricipes*, 25 (4): 178-180.

Farr, E.R., Leussink, J.A. and Stafleu, F.A. (1979) Index Nominum Genericorum 3. Dr. Junk, Den Hag.

Furtado, C.X. (1949) Palmae Malasicae X. The Malayan species of *Salacca*. *Garden Bulletin Singapore*, 12: 378-403.

Gari, N.M. (2003) Epidermal studies of Bali salak cultivars (*Salacca zalacca* var. *amboinensis* (Becc.) Mogea). Research report for the fulfillment of the Graduate Diploma of Research Methods, James Cook University, Australia.

Ghimiray, T. and Das, P.K. (1996) Stomatal characters and diurnal variation in two cultivar groups of cowpea *Vigna unguiculata* (L.). *Environment and Ecology*, 14: 86-88.

Ghose, M. and Bhattaacharya (1996) Leaf epidermal characteristics of some tall and dwarf cultivars of coconut, *Cocos nucifera* L. in the Sundarbans. *Mooreana*, 6: 79-83.

Ghose, M. and Davis, T.A. (1973) Stomata and trichomes in leaves of young and adult palms. *Phytomorphology*, 23: 216-229.

Gogoi, R., Bokolial, D., and Das, D.S. (2002) Leaf epidermal morphology of some species of Zingiberaceae. *Plant Archives*, 2: 257-262.

Govaerts, R. and Dransfield, J. (2005) World Checklist of Palm. Royal Botanical Garden Kew, UK.

Gower, J.C. (1971) A general coefficient of similarity and some of its properties. *Biometrics*, 27: 29-76.

Griffith, W. (1845) The Palms of British East India. *Calcutta Journal of Natural History*, 5: 1-103.

Griffith, W. (1850) Palms of British India, Charles A. Searao, Calcutta.

Hallam, N.D. and Chambers, T.C. (1970) The leaf waxes of the genus *Eucalyptus* L. Heritier. *Australian Journal of Botany*, 18: 335-386.

Harley M.M. and Baker W.J. (2001) Pollen aperture morphology in Arecaceae: application within phylogenetic analyses, and a summary of record of palm-like pollen the fossil.

Haron, N.W. and Moore, D.M. (1996) The taxonomic significance of leaf micromorphology in the genus *Eugenia* L. (Myrtaceae). *Botanical Journal of the Linnean Society*, 120: 265-277.

Heenan, P.B. (1994) The origin and identification of *Hebe* x *franciscana* and its cultivars (Scrophulariaceae). *Horticulture in New Zealand*, 5: 15-20.

Hilu, K.W. and Randall, J.L. (1984) Convenient method for studying grass leaf epidermis. *Taxon*, 33: 413-415.

Hodel, D.R., (1997) New species of Palms from Thailand. *Palm Journal*, 134: 28-37.

Hodel, D.R. and Vatcharakorn, P. (1998) The Palms of Thailand. Nong Nooch Tropical Garden, Thailand.

Hussin, K.H. and Sani, Z.M. (1998) Comparative leaf anatomical studies of some *Sterculia* L. species (Sterculiaceae). *Botanical Journal of the Linnean Society*, 127: 159-174.

Hutauruk, D. (1999) Seed formation of Bali salak (*Salacca zalacca* var. *amboinensis*). Postgraduate thesis, Bogor Agricultural Institute, Bogor, Indonesia.

Ilic, J. (1985) The family key for hardwood identification. CSIRO, Division of Chemical and wood technology, Autralia, Technical paper, No. 8.

Juniper, B.E. (1959) The effect of pre-emergent treatment of peas with trichlorocetic acid on the sub-microscopic structure of the leaf surface. *New Phytologist*, 58: 1-4.

Karang Asem Tourism Office (2000) Introducing agrotourism Sibetan salak fruit farming. Karang Asem Tourism Office, Amlapura, Bali-Indonesia.

Kong, H.Z. (2001) Comparative morphology of leaf epidermis in the Chlorantaceae. *Botanical Journal of the Linnean Society*, 136: 279-294.

Kotresa, K. and Seetharam, Y.N. (2000) Epidermal micromorphology of some species of *Cassia* L. (Caesalpiniaceae). *Phytomorphology*, 50: 229-237.

Krishnamurthy, K.H. and Kannabiran, B. (1973) Histomorphology of foliar epidermis and pharmacognosy in Asclepiadaceae. *Journal of the Indian Botanical Society*., 40: 105-113.

Lestari, R. and Ebert, G. (2002) Salak [*Salacca zalacca* (Gaertner.) Voss.] The snake fruit from Indonesia. Conference on International Research on Food Security, Natural Resource Management and Rural Development. Deutscher Tropentag- Witzenhausen.

Mahabale, T.S. (1966) Pollen grains in palmae. *Review of Palaeobotany and Palynology*, 4: 299-304.

Martin, G.J., Beaman, J.H., Agama, A.L. and Nais, J. (2002) Project Ethnobotany Kinabalu, The United Nations Educational, Scientific and Cultural Organization, France.

Martius, C.F.P. (1838) *Historia Naturalis Palmarum*, 3: 201.

Menon, K.P.V. and Pandalay, K.M. (1960) Coconut palm – a monograph. Indian Coconut Central Committee, Ernakulam.

Metcalfe, C.R. and Chalk, L. (1979) Anatomy of the Dicotyledons, 2nd edn. Crown Agents, Oxford, U.K.

Miller, C. (2002) Fruit Production of the Ungurahua Palm *(Oenocarpus bataua subsp. bataua, Arecaceae)* in an Indigenous Managed Reserve. *Economic Botany*, 58: 165-176.

Mogea, J.P. (1978) Pollination in *Salacca edulis*. *Principes*, 22 (2): 56-63.

Mogea, J.P. (1980) The flabellate-leaved species of *Salacca* (Palmae) *Reinwardtia*, 9 (4): 56-63.

Mogea, J.P. (1981a) Three new species of *Salacca* (Palmae) from the Malay Peninsula, *Federation Museums Journal*: Museums Department, Peninsular Malaysia Kuala Lumpur, Vol.29.

Mogea, J.P. (1981b) Notes on *Salacca wallichiana*. *Principes*, 25 (3): 120-123.

Mogea G.P. (1982) *Salacca zalacca*. The correct name for the salak Palm. *Principes*, 26 (2): 70-72.

Mogea, J.P. (1984) Three new species of *Salacca* (Palmae) from the Malay Peninsula, *Federation Museums Journal*: Museums Department, Peninsular Malaysia Kuala Lumpur, Vol.29.

Mogea, J.P. (1986) A new species in the Genus Salacca. *Principes*, 30 (4): 161-164.

Moon, H.K. and Hong, S.P. (2003) Pollen morphology of the genus *Lycopus* (Lamiaceae), *Annales Botanici Fennici*, 40: 197-198.

Moore, P.D. and Webb, J.A. (1978) An illustrated guide to pollen analysis. Hodder and Stoughton, London.

Moore, P.D., Webb, J.A. and Collinson, M.E. (1991) Pollen Analysis. Blackwell Scientific Publications, London.

Ogundipe, O.T. and Akinrinlade, O.O. (1998) Epidermal micromorphology of some species of *Albizia durazz* (Mimosaceae). *Phytomorphology*, 48: 325-333.

Oka, I.B. (1995) Study on some cultivars of Bali salak. Faculty of Agriculture, Udayana University, Bali.

Olowekudejo, J.D. and Sheteolu, O.P. (1988) The taxonomic value of epidermal characters in the genus *Ocimum* (Lamiaceae). *Phytomorphology*, 38: 147-158.

Olowekudejo, J.D. (1993) Comparative epidermal morphology of West African species of *Jatropha* L. (Euphorbiaceae). *Botanical Journal of the Linnean Society*, 139: 139-154.

Patil, S.G. and Patil, V.P. (1987) Stomatal studies in the genus *Chlorophytum* and their taxonomic sigificance. *Phytomorphology*, 37: 155-158.

Parveen, S.N., Murthy, K.S.R., and Pullaiah (2000) Leaf epidermal characters in *Crotalaria* species (Papilionoideae) from eastern Ghats. *Phytomorphology*, 50: 205-212.

Rajagopal, T. (1979) Distributional patterns and taxonomy of foliar stomata. *Indian Journal of Botany*, 2: 63-69.

Ram, H.Y.M. and Nayyar, V.L. (1974) A rapid method of obtaining epidermal peels in plants by treatment with cupric sulphate and hydrochloric acid. *Stain Technology*, 49: 114-116.

Ratnambal, M.J., Muralidharan, K., Nair, N.K., Kumaran, P.M., Bhaskara Rao, E.V.V. and Pillai, R.V. (1995) Coconut descriptors – part I. CPCRI, Kasaragod.

Reinwardt, C.G.C. (1828). *Zalacca*. *Sylloge Plantarum Nova,* 2: 3.

Salisbury, E.J. (1927) On the causes and ecological significance of stomatal frequency with special reference to the woodland flora. *Philosophical Transactions of the Royal Society of London. Series B,* 216, 1-65.

Schuiling, D.L. and Mogea, J.P. (1992) Plant resources of South-East Asia. Edible fruits and Nuts. *Prosea*, 2: 278-284.

Shaheen, A.M. (2002) Morphological variation within *Ricinus communis* L. in Egypt: Fruit, Leaf, Seed and Pollen, *Pakistan Journal of Biological Sciences*, 5 (11): 1202-1206.

Sharma, R.C.; Sharma, S., and Gupta, A.K. (2001) Stomatal characters of *Populus ciliata* in relation to leaf rust and growth parameters. *Phytomorphology*, 51: 199-205.

Sneath, P.H. and Sokal, R.R. (1973) Numerical taxonomy. W.H. Freeman and Company, San Fransisco.

Sowunmi, M.A. (1968) Pollen Morphology in the Palmae, with special Reference to Trends in Aperture Development. *Review of Palaeobotany and Palynology*, 7: 45-53.

Stace, C.A. (1965a) Cuticular studies as an aid to plant taxonomy. *Bull. Br. Mus. (Nat. Hist.) Bot.*, 4 (1): 1-78.

Stace, C. A. (1984) The taxonomic importance of the leaf surface. In: Current concepts in plant taxonomy, Eds. (H. Heywood and D. M. Moore) Academic Press, London, pp. 67-94.

Steiner, J.J. (1999) Birdsfoot trefoil origins and germplasm diversity. In the trefoil: the science and technology of *Lotus*), Special report, Eds. (PR. Beucelinck). American Society of Agronomy and Crop Science Society of America; Madison , WI), No. 48.

Sugimura, Y., Rocat, D.A., Salud, C.D. and Kamata, N. (1994b) Characteristics of coconut palms with plicata leaves. *Japan Journal of Tropical Agriculture*, 38 (2): 119-123.

Tomlinson, P.B. (1961) Palmae. In "Anatomy of the Monocotyledons," Eds. (C.R.Metcalfe). Oxford, The Clarendon Press.

Tomlinson, P.B. (1990) The structural biology of palms. Oxford University Press, pp. 477.

Uhl, N.W. and Dransfield, J. (1987) Genera Palmarum. A classification of palms based on the work of Harold E. Moore, Jr. Allen Press, Inc., Lawrence, Kansas, pp. 610.

Utami, N. (1989) Seedling leaf anatomy of *Salacca zalacca* var. *zalacca* and *Salacca zalacca* var. *amboinensis*. Bogor: Herbarium Bogoriensis, Balitbang Botani, Puslitbang Biologi, LIPI.

Van Wyk, A.E.; Robbertse, P.J., and Kok, P.D.F. (1982) The genus *Eugenia* L. (Myrtaceae) in Southen Africa: the structure and taxonomic value of stomata. *Botanical Journal of the Linnean Society*, 41-56.

Whang, S.S. and Pak, J.H. (2001) Cuticle micromorphology of leaves of *Pinus* (Pinaceae) from Mexico and Central America. *Botanical Journal of the Linnean Society*, 135: 349-373.

Whitmore, T.C. (1973) Palms of Malaya. 132, Oxford University Press, Singapore, Kuala Lumpur, Singapore, London.

Wijana, G. (1997) Cultivar diversity and selection process of salak plants in Indonesia.Yayasan Wisata Agro Dewata, Denpasar, Bali.

Wilkinson, H.P. (1979) The plant surface (mainly leaf). In "Anatomy of the Dicotyledons," Eds. (C.R. Metcalfe and L. Chalk), Vol. 1, pp 97-165. Oxford, U.K: Clarendon Press.

Winsor, L. (1994) Tissue processing. In: Laboratory Histopathology, a complete reference. Eds. (A.E. Wood and R.C. Ellis). Churchill Livingstone, Tokyo, 1994.

Yukawa, T., Ando, T., Karasawa, K., and Hashimoto, K. (1992) Existence of two stomatal shapes in the genus *Dendnrobium* (Orchidaceae) and its systematic significance. *American Journal of Botany*, 79: 946-952.

Zar, J.H. (1999) Biostatistical analysis. Prentice Hall International, Inc. New Jersey.

Appendix 2.1 The leaf epidermal features of 13 Bali salak cultivars (*Salacca zalacca* var*. amboinensis*) based on qualitative observations under light microscopy (LM).

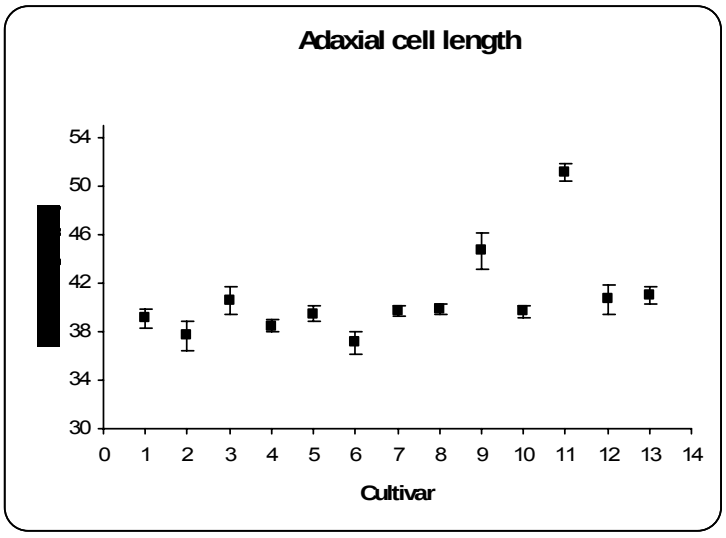
Cultivar / Character	Stomatal distribution	Stomatal shape	Adaxial cell shape	Adaxial cell shape	Thickening on anticlinal cell walls	Pattern of anticlinal cell walls	Thickening on guard cells
Gula	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Boni	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Bingin	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Selem	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Embad	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Nangka	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Penyalin	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Maong	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Nanas	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Gondok	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Muani	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Nyuh	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present
Putih	amphistomatic	paraciytic	rectangular to rhomboid	rectangular	present	ridged	present

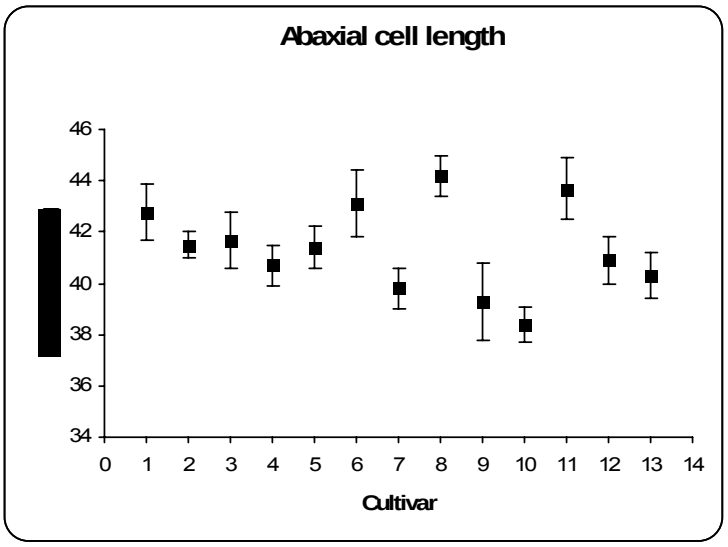
Appendix 2.2 Summary of statistical results from analyses of variance (ANOVA) of 10 leaf epidermal characters of 13 Bali salak cultivars. ***** indicates that there were significant differences ($P \leq 0.05$) among the means of the cultivars.

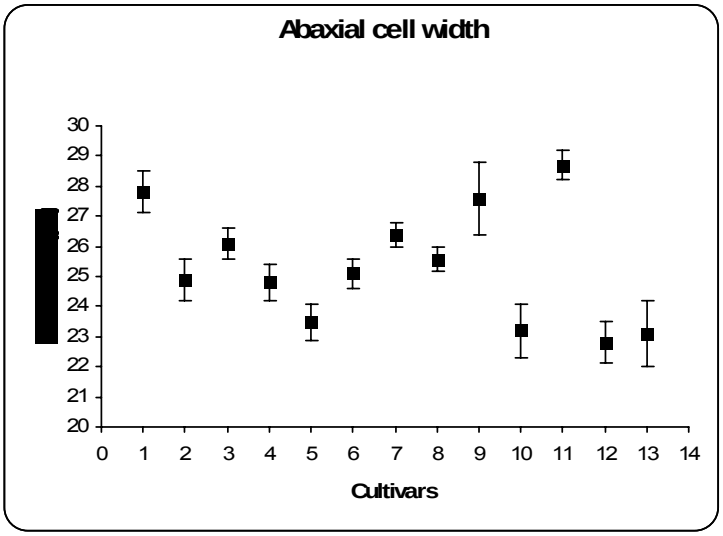
Character		Sum of Squares	df	Mean Square	F	Sig. (P)
Adaxial cell length	Between Groups	938.467	12	78.206	4.843	0.000*
	Within Groups	839.643	377	16.147		
	Total	1778.109	389			
Adaxial cell width	Between Groups	50.192	12	4.183	.833	0.617
	Within Groups	261.185	377	5.023		
	Total	311.377	389			
Ratio adaxial cell length to width	Between Groups	6.720	12	.560	2.158	0.018*
	Within Groups	30.366	377	.260		
	Total	37.086	389			
Abaxial cell length	Between Groups	1552.185	12	129.349	5.401	0.000*
	Within Groups	1245.241	377	23.947		
	Total	2797.426	389			
Abaxial cell width	Between Groups	505.866	12	42.155	3.828	0.000*
	Within Groups	572.590	377	11.011		
	Total	1078.456	389			
Ratio abaxial cell length to width	Between Groups	2.752	12	.229	1.102	0.365
	Within Groups	24.351	377	.208		
	Total	27.103	389			
Guard cell length	Between Groups	184.278	12	15.357	1.259	0.271
	Within Groups	634.243	377	12.197		
	Total	818.521	389			
Guard cell width	Between Groups	5.611	12	.468	1.134	0.354
	Within Groups	21.437	377	.412		
	Total	27.048	389			
Stomatal density	Between Groups	293.492	12	24.458	6.003	0.000*
	Within Groups	476.700	377	4.074		
	Total	770.192	389			
Stomatal index	Between Groups	286.320	12	23.860	9.932	0.000*
	Within Groups	281.072	377	2.402		
	Total	567.391	389			

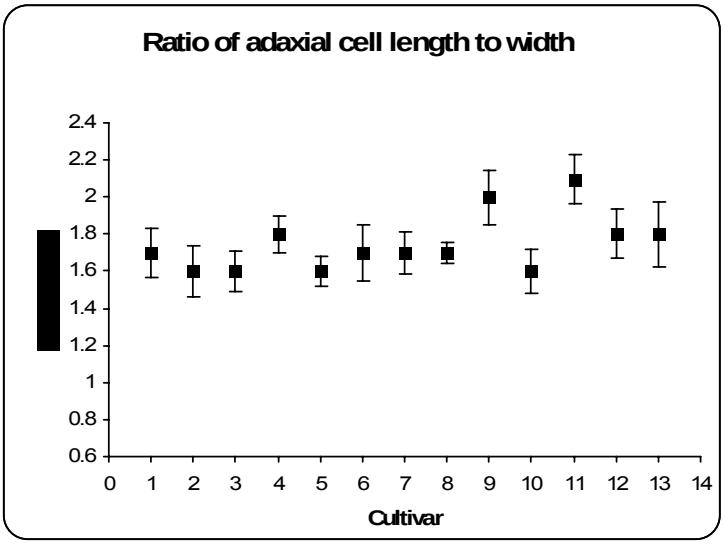
Appendix 2.3 Scatter plots of six leaf epidermal characters that showed significant differences ($p \leq 0.05$) among 13 Bali salak cultivars based on ANOVA.

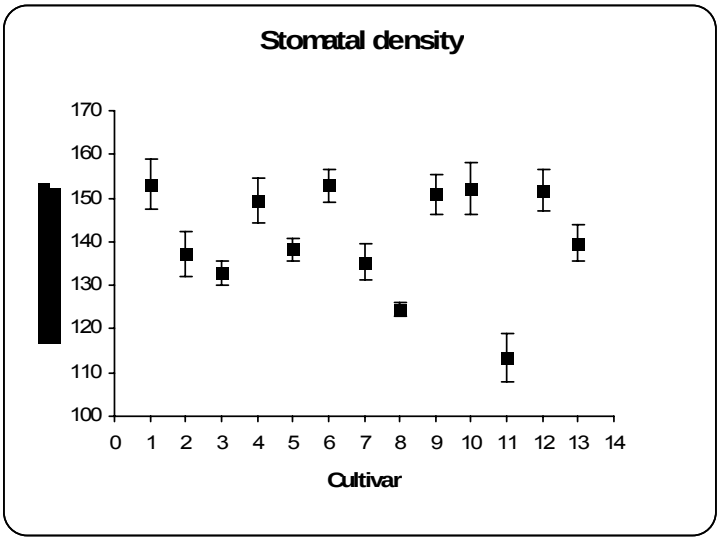
The figures show mean values and standard errors of adaxial (Ad) cell length, abaxial (Ab) cell length, abaxial (Ab) cell width, ratio adaxial cell length to width (R.l:w), stomatal density (SD); (number:mm^2), and stomatal index (%). Measurements were made from 90 observations of 30 leaves from each of 12 cultivars plus 9 leaves of Bingin. Cultivars are designated as numbers 1 to 13, where cultivar 1 = Gula, 2 = Boni, 3 = Bingin, 4 = Selem, 5 = Embad, 6 = Nangka, 7 = Penyalin, 8 = Maong, 9 = Nanas, 10 = Gondok, 11 = Muani, 12 = Nyuh, and 13 = Putih.

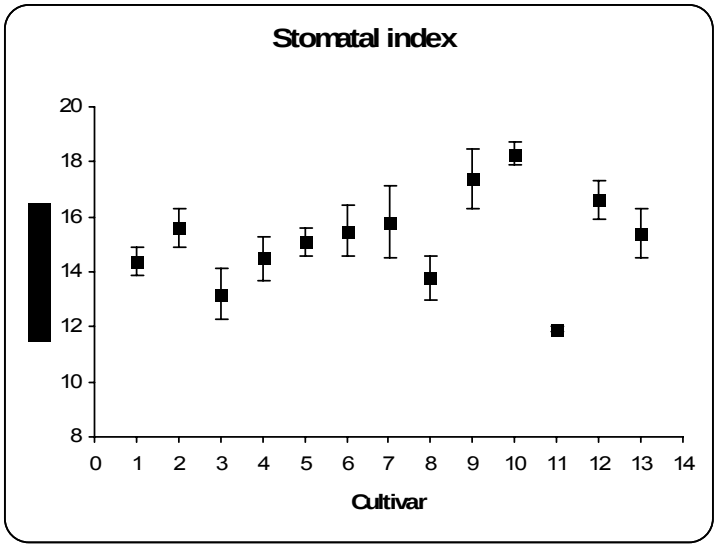





Abaxial cell width
30
29
28
27
26
25
24
23
22
21
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivars


Ratio of adaxial cell length to width
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Stomatal density
170
160
150
140
130
120
110
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Stomatal index
20
18
16
14
12
10
8
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar

Appendix 3.1 Summary of statistical results from analyses of variance (ANOVA) of 12 vegetative morphological characters of 13 Bali salak cultivars. ***** indicates that there were significant differences ($P \leq 0.05$) among the means of the cultivars.

Character		Sum of Squares	df	Mean Square	F	Sig. (p≤0.05)
Plant height	Between Groups	5054315.958	12	421192.996	88.868	0.001*
	Within Groups	1786805.417	377	4739.537		
	Total	6841121.374	389			
Leaf length	Between Groups	1059188.049	12	88265.671	19.316	0.001*
	Within Groups	1722768.387	377	4569.677		
	Total	2781956.436	389			
Petiole length	Between Groups	222280.369	12	18523.364	11.730	0.001*
	Within Groups	595331.662	377	1579.129		
	Total	817612.031	389			
Leaflet number	Between Groups	10740.026	12	6542.987	29.776	0.001*
	Within Groups	21466.333	377	743.940		
	Total	32206.359	389			
Spine length	Between Groups	332780.171	12	43765.765	13.450	0.001*
	Within Groups	483112.523	377	1879.134		
	Total	209846.234	389			
Spine density	Between Groups	23121.432	12	895.002	15.718	0.001*
	Within Groups	18236.333	377	56.940		
	Total	25343.843	389			
Basal leaf length	Between Groups	184.278	12	15.357	1.259	0.271
	Within Groups	634.243	377	12.197		
	Total	818.521	389			
Basal leaflet width	Between Groups	5.611	12	.468	1.134	0.354
	Within Groups	21.437	377	.412		
	Total	27.048	389			
Middle leaflet length	Between Groups	293.492	12	24.458	6.003	0.001*
	Within Groups	476.700	377	4.074		
	Total	770.192	389			
Middle leaflet width	Between Groups	6.320	12	13.860	9.932	0.121
	Within Groups	21.072	377	2.402		
	Total	67.391	389			
Apical leaflet length	Between Groups	114.232	12	28.357	1.359	0.234
	Within Groups	234.234	377	12.534		
	Total	418.598	389			
Apical leaflet width	Between Groups	9.654	12	2.468	1.134	0.123
	Within Groups	85.812	377	7.243		
	Total	123.034	389			

Appendix 3.1 (continued) Summary of statistical results from analysis of variance (ANOVA) of 15 reproductive characters of 13 Bali salak cultivars. ***** indicates that there were significant differences ($P \leq 0.05$) among the means of the cultivars.

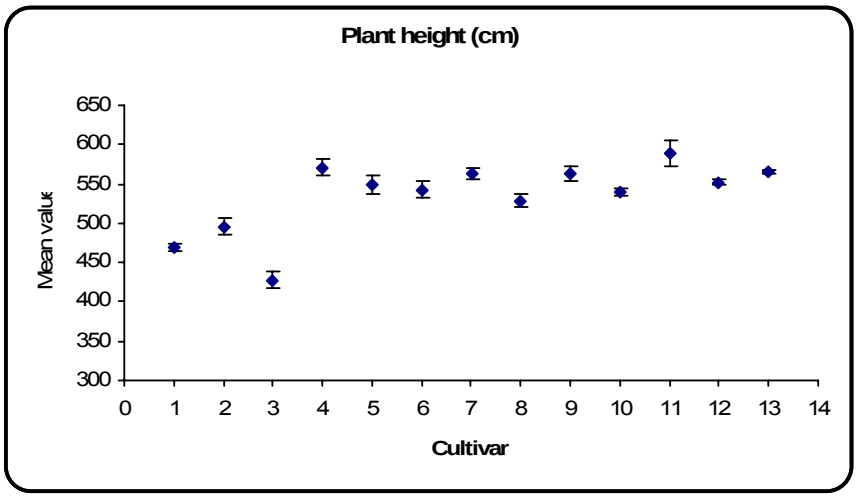
Character		Sum of Squares	df	Mean Square	F	Sig. (p≤0.05)
Male flower length	Flower width	6.123	12	.546	2.154	0.143
	Within Groups	31.445	247	.984		
	Total	134.063	259			
Male Flower width	Between Groups	7.392	12	1.468	1.134	0.154
	Within Groups	22.765	247	.812		
	Total	131.654	259			
Male calyx length	Between Groups	286.320	12	23.860	9.932	0.001*
	Within Groups	281.072	247	2.402		
	Total	567.391	259			
Male calyx width	Between Groups	8.864	12	2.454	1.156	0.145
	Within Groups	81.814	379	7.253		
	Total	127.036	332			
Male corolla length	Between Groups	54.176	12	3.185	0.737	0.564
	Within Groups	275.098	379	6.097		
	Total	351.876	332			
Male corolla width	Between Groups	7.345	12	3.456	1.134	0.154
	Within Groups	31.345	247	6.459		
	Total	147.456	259			
Female flower length	Flower width	293.492	12	24.458	6.003	0.001*
	Within Groups	476.700	247	4.074		
	Total	770.192	259			
Female flower width	Between Groups	5.611	12	4.468	1.165	0.251
	Within Groups	21.437	247	6.412		
	Total	27.048	259			
Female calyx length	Between Groups	8.686	12	.468	1.134	0.213
	Within Groups	31.4567	247	.412		
	Total	187.876	259			
Female calyx width	Between Groups	4.410	12	3.468	3.145	0.129
	Within Groups	23.109	247	1.412		
	Total	145.067	259			
Female corolla length	Between Groups	184.278	12	25.357	4.259	0.002*
	Within Groups	634.243	247	7.197		
	Total	818.521	259			

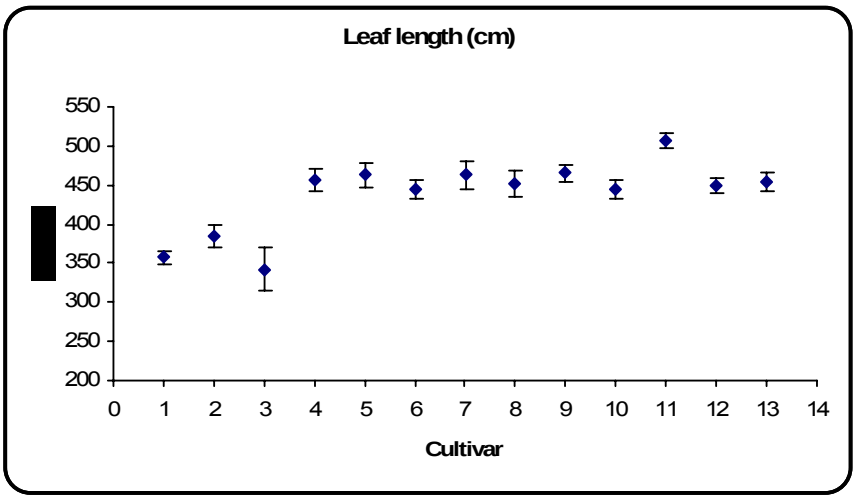
Appendix 3.1 (continued)

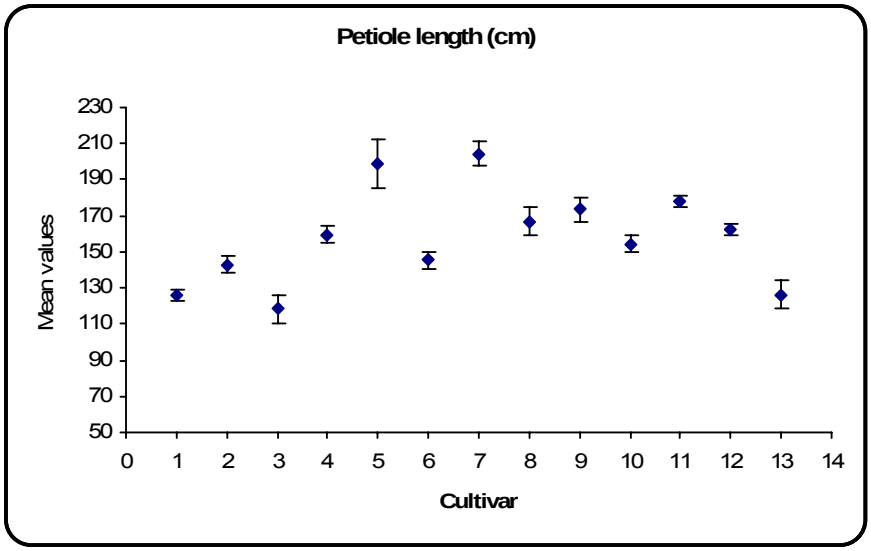
Character		Sum of Squares	df	Mean Square	F	Sig. (p≤0.05)
Female corolla width	Between Groups	5.901	12	3.467	1.345	0.197
	Within Groups	76.941	379	8.298		
	Total	128.876	332			
Stigma L	Between group	5.611	12	7.567	1.165	0.491
	Within Groups	27.486	247	9.412		
	Total	39.087	259			
Ovary L	Between Groups	9.092	12	3.441	3.345	0.345
	Within Groups	37.4871	247	5.476		
	Total	165.8723	259			
Filament L	Between Groups	5.64	12	4.468	1.134	0.153
	Within Groups	27.445	247	6.412		
	Total	37.056	259			

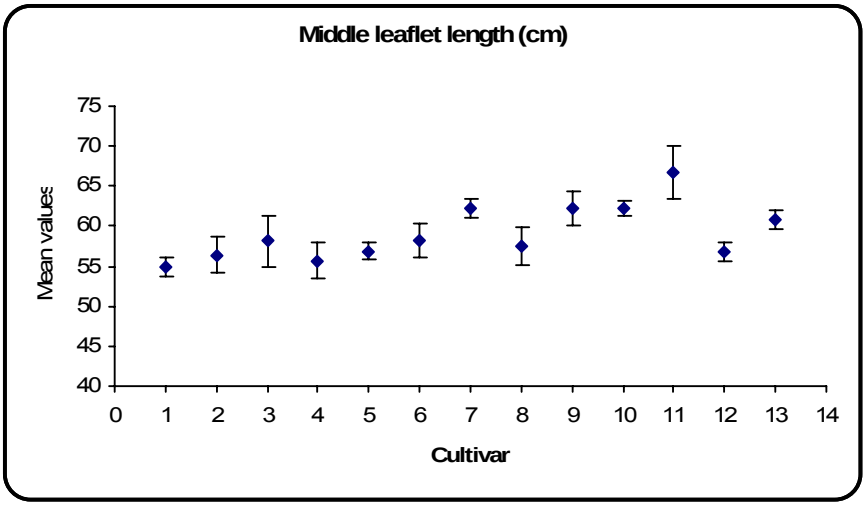
Appendix 3.2 Scatter plots of seven vegetative morphological characters and three reproductive characters that showed significant differences ($p \leq 0.05$) among 13 Bali salak cultivars based on ANOVA.

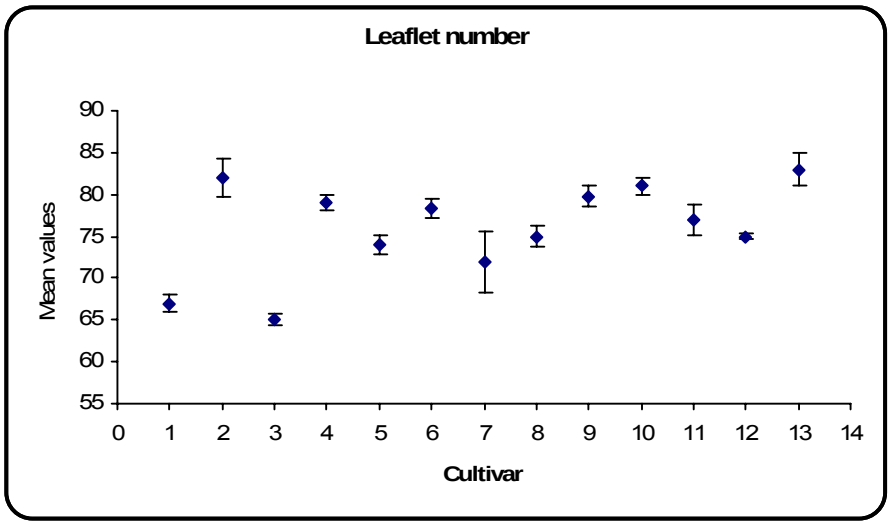
The figures show mean values and standard errors of plant height, leaf length, petiole length, middle leaflet length, leaflet number, spine length, spine density, male calyx length, female flower length, and female corolla length. Measurements were made from 30 observations of vegetative morphological characters each of 12 cultivars plus 9 plants of Bingin and 20 observations of reproductive characters (flowers). Cultivars are designated as numbers 1 to 13, where cultivar 1 = Gula, 2 = Boni, 3 = Bingin, 4 = Selem, 5 = Embad, 6 = Nangka, 7 = Penyalin, 8 = Maong, 9 = Nanas, 10 = Gondok, 11 = Muani, 12 = Nyuh, and 13 = Putih.

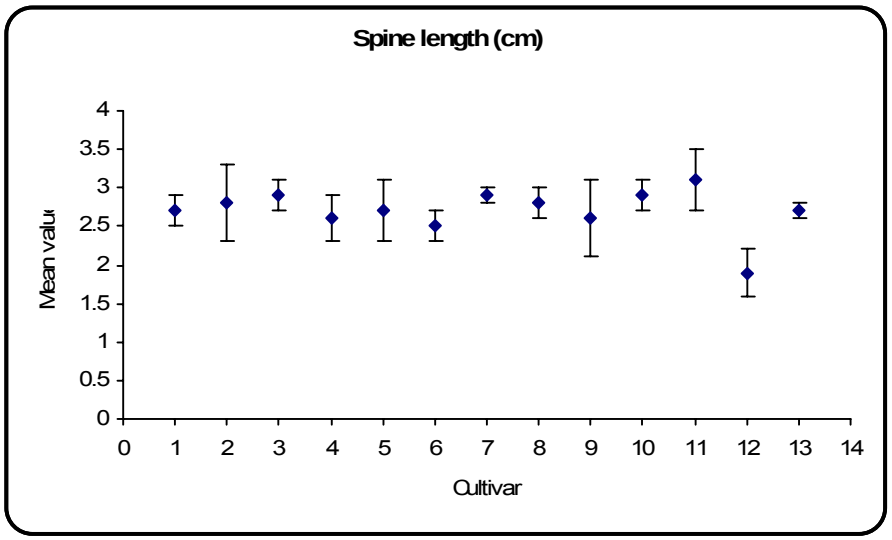


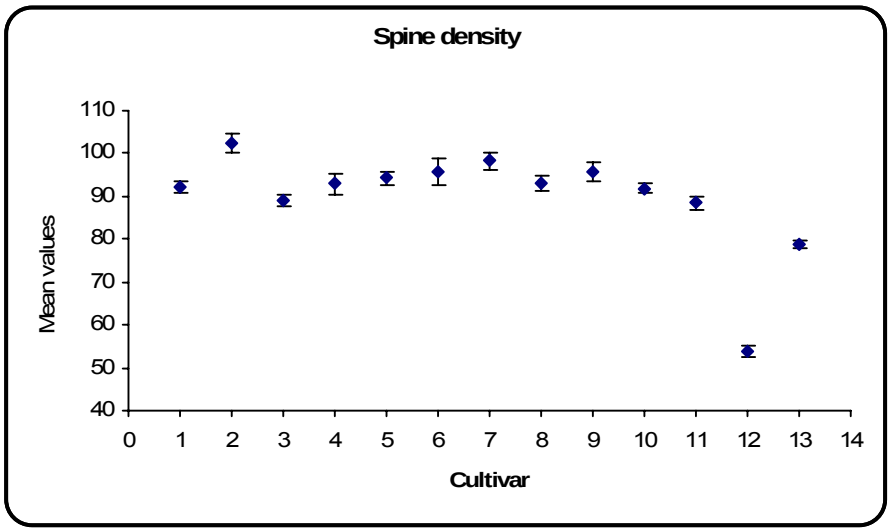


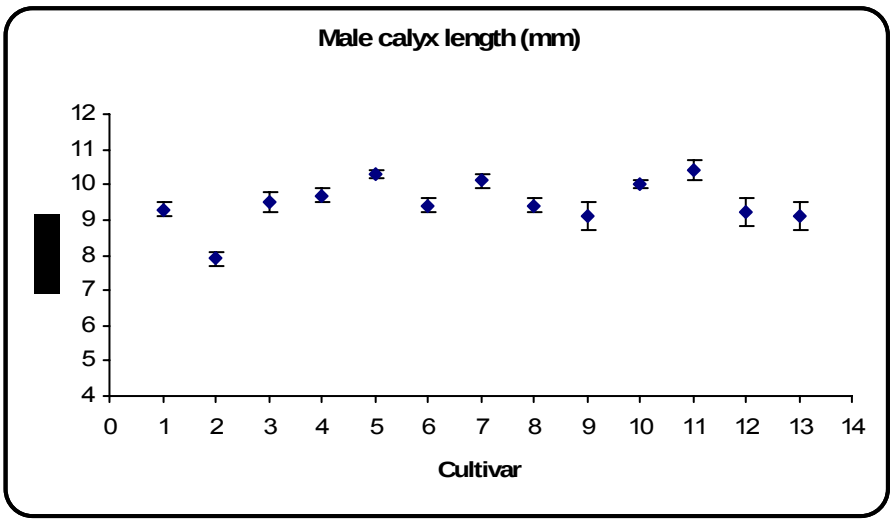

Petiole length (cm)
Mean values
230
210
190
170
150
130
110
90
70
50
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar

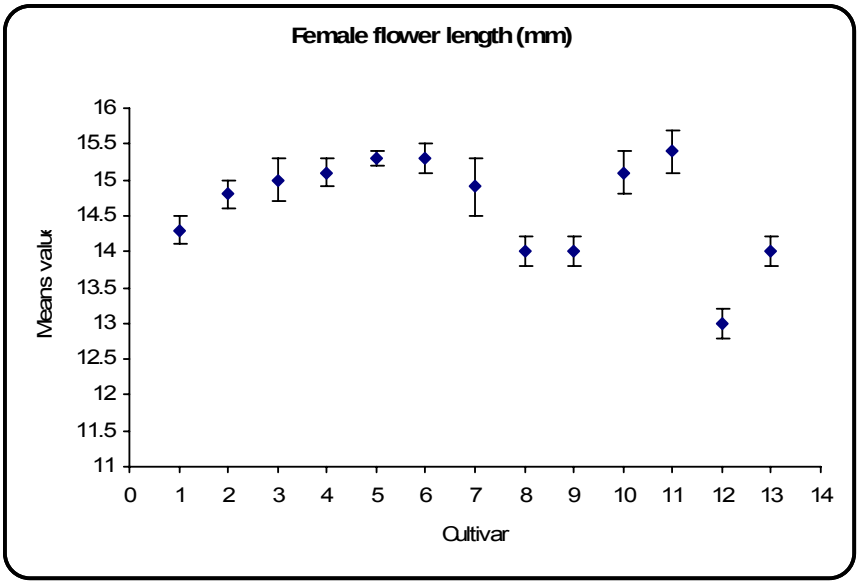

Middle leaflet length (cm)
Mean values
75
70
65
60
55
50
45
40
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar

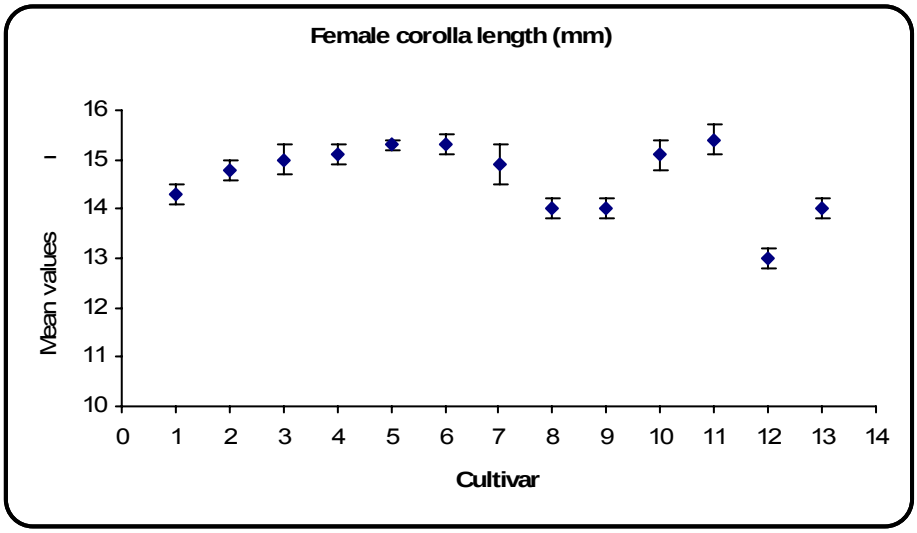

Leaflet number
Mean values
90
85
80
75
70
65
60
55
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Spine length (cm)
Mean valu
4
3.5
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Spine density
Mean values
110
100
90
80
70
60
50
40
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Male calyx length (mm)
12
11
10
9
8
7
6
5
4
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Female flower length (mm)
Means valu
16
15.5
15
14.5
14
13.5
13
12.5
12
11.5
11
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar


Female corolla length (mm)
Mean values
16
15
14
13
12
11
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cultivar