INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.1 The genus *Citrus*

The genus *Citrus* belongs to the family Rutaceae, subfamily Aurantioideae, tribe Citreae and subtribe Citrinae (Swingle, 1967). It is believed that *Citrus* originated in the New Guinea-Melanosia region but the evolution into cultivated species took place in southeastern Asia (Swingle, 1967; Simpson and Corner-Ogorzaly, 1986). *Citrus* was then introduced to the other part of the world by immigrants, traders and explorers (Soost and Cameron, 1975; Reitz, 1984). Today it is widely planted throughout the world in tropical and subtropical climates. The producing regions located approximately between 40° north and south of the equator (Soost and Cameron, 1975; Reitz, 1984; Spiegel-Roy and Vardi, 1984; Rouse, 1988). The world major producing countries are Brazil, United States, Spain, China, Japan, Mexico and Italy. Today the most widely grown *Citrus* fruits in the world is sweet orange (*Citrus sinensis*) followed by, lemon (*Citrus limon*), lime (*Citrus aurantifolia*), grapefruit (*Citrus paradisi*) and pomelo (*Citrus maxima*) (Simpson and Corner-Ogorzaly, 1986). Among all the commercial *Citrus* varieties, the most important is Valencia sweet orange. Other famous *Citrus* varieties being the Washington navel, the Satsuma mandarins of Japan, the Pera orange of Brazil, the Doblefina and other blood oranges of Spain and the Shamouti orange of the eastern Mediterranean (Reitz, 1984).

Citrus plants are evergreen shrubs or bushy small trees. However one of the wild species, *Citrus halimii* reaches 75 ft. in height with a girth of over 1 m (Stone, 1972). Most species are thorny. The leaves are winged petiole in some species and are characterised with the presence of oil glands. The flowers are axillary, solitary or clustered in small terminal cymes. They are usually white, with purplish pink on the outer surface of the petals, or sometimes yellow or cream (Hume, 1957; Swingle, 1967). *Citrus* fruits are special kind of berries called hesperidium with leathery rinds (exocarp and endocarp) dotted with numerous oil glands on the exocarp (Opeke, 1982; Simpson and Corner-Ogorzaly, 1986). The exocarp and mesocarp are also called flavedo and albedo respectively. The centre of the fruits are filled with stalked fusiform pulp-vesicles sacs containing juice surrounding the seeds. The seeds are obovoid or flattened obovoid, they contained one embryo (monoembryonic) or many embryos (polyembryonic). *Citrus* fruits range in size from small such as *Citrus madurensis* up to the football sized, *Citrus maxima*. Another feature that shows variability, is the colour of the peels, they range from yellow green to orange. Moreover, the oil filled-cavities found in *Citrus* leaves and fruit peels have a sweet smelling aroma which is a special characteristic of the *Citrus* family (Hume, 1957; Swingle, 1967; Simpson and Corner-Ogorzaly, 1986).

The classification of species within the genus *Citrus* is complicated because of the occurences of polyploidy, polyembryony and mutants. Several classifications systems have been proposed by various taxonomists to define the species in the genus *Citrus*. Engler (1986) recognised only 4 species in this genus, Swingler (1943) established 16 species and later Tanaka (1954) proposed 159 species. The systems which are commonly used are the Swingle (Fig.1.1) and Tanaka Systems. According to Swingle, *Citrus* can be divided into two subgenus namely subgenus *Citrus* and subgenus *Papeda*. The subgenus *Citrus* consists of all the commonly cultivated species of sweet juicy fruits, whereas species of *Citrus* belonging to the subgenus *Papeda* have non edible fruits (Swingle, 1967). All the classification systems have been based on morphological characters. The classification based on morphological criteria is valid until now due to the stability of morphological criteria which is less affected by environmental conditions (Galun, 1988).

Figure 1.1: Classification of the genus *Citrus* (Swingle, 1967)

Subgenus *Citrus*	**Subgenus *Papeda***
1. *Citrus medica*	12. *Citrus latipes*
var. *sarcodactylis*	13. *Citrus micrantha*
var. *ethrog*	var. *microcarpa*
2. *Citrus limon*	14. *Citrus celebica*
3. *Citrus aurantifolia*	var. *south wickii*
4. *Citrus aurantium*	15. *Citrus macroptera*
var. *myrtifolia*	var. *kerrii*
5. *Citrus sinensis*	var. *annamensis*
6. *Citrus reticulata*	16. *Citrus hystrix*
var. *austera*	
7. *Citrus maxima*	
var. *racemosa*	
8. *Citrus indica*	
9. *Citrus tachibana*	
10. *Citrus halimii*	
11. *Citrus madurensis*	

1.2 *Citrus* in Malaysia

Some *Citrus* (limau) species are commonly cultivated, whilst the others are either rarely cultivated or grow only in the wild. The genus *Citrus* in Malaysia comprises 12 species (Table 1.1). Malaysia is known as a home of wild species of *Citrus* such as *Citrus halimii* and *Citrus macroptera* (Stone *et al.*, 1973; Jones, 1983; Jones, 1984; Jones and Ghani, 1987).

The commonly cultivated species which are grown in Malaysia are *Citrus reticulata, C. maxima, C. limon, C. hystrix* and *C. madurensis*. Besides these species, a number of varieties and hybrids of *Citrus* species exist locally (Jones, 1984).

In Malaysia, *Citrus* is grown mainly in the highland region, as in the Cameron Highlands district in the state of Pahang (Ko, 1992). The species which are planted here predominantly are sweet and mandarin oranges. Lime and pomelo are planted in the states of Perak and Johor, which are the lowland areas (Jones and Zainudin, 1984). The highland areas are more suitable for *Citrus* cultivation because of environmental condition which favour growth.

Although *Citrus* is produced locally, it is not enough for local consumption. In 1988, Malaysian *Citrus* production was estimated at 47.3 tonnes. Nevertheless, for local consumption Malaysia need to produced 170,000 tonnes (Saamin *et al.*, 1991).

Since the early 1950's, *Citrus* is grown widely in plantations. The planting sites are located in the states of Kelantan, Terengganu, Perak and Johor. But in 1970's the production has declined due to the problem of diseases. The diseases which affect *Citrus* production are root rot which is caused by *Phytophthora* fungi, tristeza virus, bacterial canker and greening disease. The greening disease which is caused by virus is probably the main cause of decline in *Citrus* production (da Graca, 1991; Saamin, *et al.* 1991; Saamin and Bakar, 1991).

To overcome this problem, Malaysia Agricultural Research and Development Institute (MARDI) had set up research programmes to study and improve *Citrus* production. Programmes that have been carried out include *Citrus* planting improvement, disease control, weed control and monitoring the import of *Citrus* planting materials (Jones and Ghani, 1987).

For *Citrus* cultivation improvement, MARDI and some local universities (UKM, UM and UPM) have established a germplasm collection programme. In this programme, wild *Citrus* species are collected and conserved as a living plant in a nursery.

Table 1.1: *Citrus* in Malaysia (Jones, 1984)

Subgenus *Citrus*:		
Scientific name	English name / local name	Status
Citrus aurantifolia (Christm.) Swing	Lime / Limau asam	cultivated, wild ?
Citrus aurantium Linn.	Sour orange / Limau samar	sparingly cultivated
Citrus maxima Linn. (Burm.) Merrill	Pomelo, Shaddock / Limau besar, Limau bali	cultivated
Citrus halimii Stone	? / Limau kedut raja, Limau kedangsa	wild
Citrus limon (Linn.) Burm.f.	Lemon / Limau mata	cultivated
Citrus madurensis Loureiro	Calamondin orange / Limau kesturi	cultivated
Citrus medica Linn.	Citron / Limau susu	cultivated
Citrus paradisi Macf.	Grapefruit / Limau gedang	sparingly cultivated
Citrus reticulata Blanco	Tangerine, Mandarin / Limau manis, Limau langkat	cultivated
Citrus sinensis (Linn.) Osbeck	Sweet orange / Limau potong	sparingly cultivated

Subgenus *Papeda* :		
Scientific name	English name / Local name	Status
Citrus hystrix D.C.	Papeda /Limau purut	cultivated, wild
Citrus macroptera Montrouzier	False shaddock / Limau hantu	cultivated ?, wild

1.3 Economic importance of *Citrus*

Citrus fruits are rich in nutrient content especially vitamin C (Ghosh, 1990; Barlass and Skene, 1986). Moreover they have an ability to grow in different climates. This make them one of the most popular and important product in the world of fruit trade.

Citrus fruits such as sweet orange, mandarin, grapefruit and lemon are usually consumed fresh or as fruit juices. Besides the fresh fruits, *Citrus* by-products are consumed and important in *Citrus* industry. The fruit segments are canned, the pulps are processed into jam and marmalade, the juice is extracted and concentrated or diluted into soft drinks. Molasses and *Citrus* dried pulps are used as dairy cattle feed (Reitz, 1984). In citric acid industry, lemon and lime are processed to produce citric acid (Samson, 1980; Ghosh, 1990).

The most commercially significant by-products in *Citrus* industry are essential oils. Essential oils are extracted from fruits, flowers and leaves of *Citrus*. The chemical constituents in essential oils are important as fragrances in perfumery and cosmetic products, and as flavouring agents in the food and beverage industry as well as in drugs (del Rio *et al.*, 1991). The finest and most expensive essential oils have been reported extracted from flowers of nerol and fruit of bergamot sour orange. Essential oils from bitter orange, bergamot, lemon and mandarin are used in soap manufacturing industry (Baaliouamer *et al.*, 1985).

1.4 *Citrus* essential oil

Essential oil is defined as the volatile oil obtained by steam distillation of plant parts (Haagen-Smit, 1972). The essential oil components can be divided into four major groups of compounds based on the following functional characteristics:

1) Hydrocarbons

2) Hydroxy compounds

3) Carbonyl compounds

4) Acids and Esters

In plants, essential oils are commonly found in families of Compositae, Labiatae, Graminae, Geraniaceae, Rosaceae, Lauraceae, Umbelliferae and Rutaceae (Guenther, 1965; Guenther, 1967). They are commercially important in many industries for the scenting and flavouring of all kinds of consumer finished products (Usai *et al.*, 1992). Chemical components of essential oils have also been used for taxonomic purposes (Scora, 1988).

Essential oils can be obtained by cold-pressing or steam distillation of plant parts such as flowers, fruits and leaves. The complex mixture of components of essential oils are separated and analyzed using gas chromatography and mass spectometry. In the family Rutaceae, especially in *Citrus*, review or quantitative analyses of *Citrus* essential oils have been reported (Shaw, 1979).

Table 1.2 represents compounds identified in *Citrus* species. Hydrocarbon compound is found to be the major constituents of *Citrus* essential oils. The hydrocarbon compounds are myrcene, α-terpinene, γ-terpinene, p-cymene, terpinolene, α and β-phellandrene, d-limonene, α and β-thujene, sabinene, 3-carene, α and β-pinene, camphene, β-bisabolene, γ and δ-cadinene, selinene, β-caryophyllene, longifolene, humulene and copaene. Aromadendrene is absent from all species.

Myrcene, d-limonene and α-pinene is present in all species. α-phellandrene is found in peel oils of *Citrus limon, C. aurantium* and *C. aurantifolia*. This compound has been identified in *C. limon* leaf oils. β-phellandrene is identified in petal oils of *C. sinensis, C. paradisi C. reticulata* and juice oil of *C. paradisi*. β-thujene which is absent from all species is only present in *C. limon* (Eureka) leaf oils. β-pinene is identified in most of the oils except in peel oils of *C. aurantium* and juice oils of *C. sinensis* and *Citrus* cultivar *jyabon*. γ-cadinene is found present only in peel oils of *Citrus junos* and leaf oil of *C. paradisi* var. *Duncan*. γ-selinene and longifolene are found only in peel oil of *C. aurantium*.

Hydroxy compounds which are found to be present in most of the oils are linalool, α-terpineol, nerol and geraniol. However linalool is absent only in fruit oils of *Citrus* cultivar *jyabon* while α-terpineol is missing in leaf oils of *C. paradisi* var. *Duncan* and *C. halimii*. Furthermore fenchol is absent from all species. Only in flavedo oil of *C. grandis* Osbeck forma Tosa-buntan, β-terpineol is identified.

Carbonyl compounds identified in most of the oils are geraniol, neral and citronellal and the esters compounds are neryl acetate and geranyl acetate.

Key:

Compounds

a) Hydrocarbon

Monoterpenes

Acyclic terpenes

1. Myrcene

Monocyclic terpenes

2. α-Terpinene

3. γ-Terpinene

4. p-Cymene

5. Terpinolene

6. α-Phellandrene

7. β-Phellandrene

8. d-Limonene

Bicyclic terpenes

9. α-Thujene

10. β-Thujene

11. Sabinene

12. 3-Carene

13. α-Pinene

14. β-Pinene

15. Camphene

Sesquiterpenes

Monocyclic

16. β-Bisabolene

Bicyclic

17. γ-Cadinene

18. δ-Cadinene

19. γ-Selinene

20. β-Caryophyllene

21. Longifolene

22. Humulene

23. Copaene

24. Aromadendrene

b) Hydroxy

Terpenoids

25. Geraniol
26. Nerol
27. Linalool
28. Citronellol
29. Thymol
30. α-Terpineol
31. β-Terpineol
32. Isopulegol
33. Carveol
34. Borneol
35. Fenchol
36. Nerolidol
37. Elemol

c) Carbonyl

Terpenoid

38. Geranial
39. Neral
40. Citronellal
41. Carvone
42. Camphor
43. Nootkatone

Esters

44. Methyl geranoate
45. Citronellyl acetate
46. Neryl acetate
47. Geranyl acetate
48. Neryl propionate
49. Geranyl propionate
50. Undecyl acetate

Table 1.2: Compounds identified in *Citrus* oils

Species	Refference	Plant part	Compound 1	2	3	4	5	6	7	8	9	10
C. aurantifolia	Clark *et al.* (1987)	Peel	+	+	+	+	+	+	-	+	+	-
C. aurantifolia (Mexican lime)	Azzouz and Reineccius (1976)	Fruit	+	-	+	+	+	+	-	+	+	-
C. aurantium	Attaway *et al.* (1966a)	Leaf	+	-	-	+	+		-	+	-	-
C. aurantium	Mac Leod *et al.* (1988)	Peel	+	-	+	+	+	+	-	+	-	-
C. grandis Osbeck forma Tosa-buntan	Sawamura and Kuriyama (1988)	Flavedo	+	-	+	+	+	-	-	+	-	-
C. halimii	Scora *et al.* (1976)	Leaf	+	-	-	+	-	-	-	+	-	-
C. jambhiri Lush	Lund *et al.* (1981)	Leaf	+	+	+	+	+	-	-	+	+	-
C. junos	Shinoda *et al.* (1970)	Peel	+	-	+	+	+	-	-	+	-	-
C. limon (Eureka)	Baaliouamer *et al.* (1985)	Leaf	+	+	+	+	+	+	-	+	-	+
C. limon " Rough lemon"	Attaway *et al.* (1966a)	Leaf	+	-	-	+	+	-	-	+	+	-
C. limon (Sicilian lemon)	Chamblee *et al.* (1991)	Peel	+	+	+	+	+	+	-	+	+	-
C. limon (California lemon)	Chamblee *et al.* (1991)	Peel	+	+	+	+	+	+	-	+	-	-
C. paradisi var. *Duncan*	Attaway *et al.* (1966a)	Leaf	+	+	-	+	+	-	-	+	-	-
C. paradisi var. *Marsh*	Attaway *et al.* (1966a)	Leaf	+	+	-	+	-	-	-	+	-	-

Table 1.2: continued

Species	Refference	Plant part	Compound									
			1	2	3	4	5	6	7	8	9	10
C. paradisi	Attaway *et al*. (1966b)	Petal	+	+	+	+	+	-	+	+	-	-
C. paradisi	Wilson and Shaw (1980)	Fruit	+	-	+	-	-	-		+	-	-
C. paradisi	Cadwallader and Yu (1994)	Juice	+	-	-	-	-	-	+	+	-	-
C. reticulata var. *Murcot*	Attaway *et al*. (1966a)	Leaf	+	+	-	+	+	-	+	+	-	-
C. reticulata var. *Dancy*	Attaway *et al*. (1966a)	Leaf	+	+	-	+	+	-		+	+	-
C. reiculata	Attaway *et al*. (1966b)	Petal	+	+	+	+	+	-	+	+	-	-
C. sinensis var. *Pineapple*	Attaway *et al*. (1966a)	Leaf	+	+	+	+	+	-	-	+	-	-
C. sinensis var. *Pineapple*	Moshonas and Shaw (1994)	Juice	+	-	+	-	-	+	-	+	-	-
C. sinensis var. *Valencia*	Attaway *et al*. (1966a)	Leaf	+	-	-	+	-	-	-	+	-	-
C. sinensis var. *Valencia*	Moshonas and Shaw (1994)	Juice	+	-	+	-	-	+	-	+	-	-
C. sinensis var. *Hamlin*	Attaway *et al*. (1966a)	Leaf	+	-	-	+	+	-	-	+	-	-
C. sinensis var. *Hamlin*	Moshonas and Shaw (1994)	Juice	+	-	+	-	-	+	-	+	-	-
C. sinensis	Attaway *et al*. (1966b)	Petal	+	+	+	+	+	-	+	+	-	-
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Peel	+	-	+	-	-	-	-	+	-	-
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Juice	+	-	+	-	+	-	-	+	-	-

Table 1.2: continued

Species	Refference	Plant part	Compound									
			11	12	13	14	15	16	17	18	19	20
C. aurantifolia	Clark *et al.* (1987)	Peel	+	-	+	+	+	+	-	-	-	+
C. aurantifolia (Mexican lime)	Azzouz and Reineccius (1976)	Fruit	-	-	+	+	+	+	-	-	-	+
C. aurantium	Attaway *et al.* (1966a)	Leaf	+	-	+	+	-	-	-	-	-	-
C. aurantium	Mac Leod *et al.* (1988)	Peel	+	+	+	-	-	+	-	+	+	+
C. grandis Osbeck forma Tosa-buntan	Sawamura and Kuriyama (1988)	Flavedo	-	-	+	+	-	-	-	+	-	+
C. halimii	Scora *et al.* (1976)	Leaf	-	+	+	+	+	-	-	-	-	+
C. jambhiri Lush	Lund *et al.* (1981)	Leaf	+	-	+	+	+	-	-	-	-	+
C. junos	Shinoda *et al.* (1970)	Peel	-	-	+	+	-	+	+	-	-	+
C. limon (Eureka)	Baaliouamer *et al.* (1985)	Leaf	+	+	+	+	+	+	-	+	-	+
C. limon " Rough lemon"	Attaway *et al.* (1966a)	Leaf	+	+	+	+	+	-	-	-	-	+
C. limon (Sicilian lemon)	Chamblee *et al.* (1991)	Peel	+	-	+	+	+	-	-	-	-	+
C. limon (California lemon)	Chamblee *et al.* (1991)	Peel	+	-	+	+	+	-	-	-	-	+
C. paradisi var. *Duncan*	Attaway *et al.* (1966a)	Leaf	+	+	+	+	+	-	+	+	-	+
C. paradisi var. *Marsh*	Attaway *et al.* (1966a)	Leaf	+	+	+	+	+	-	-	-	-	+

Table 1.2: continued

Species	Refference	Plant part	Compound									
			11	12	13	14	15	16	17	18	19	20
C. paradisi	Attaway *et al*. (1966b)	Petal	+	+	+	+	-	-	-	-	-	+
C. paradisi	Wilson and Shaw (1980)	Fruit	+	-	+	+	-	-	-	+	-	+
C. paradisi	Cadwallader and Yu (1994)	Juice	+	-	+	+	-	-	-	-	-	-
C. reticulata var. *Murcot*	Attaway *et al*. (1966a)	Leaf	+	-	+	+	+	-	-	-	-	+
C. reticulata var. *Dancy*	Attaway *et al*. (1966a)	Leaf	+	-	+	+	+	-	-	-	-	-
C. reiculata	Attaway *et al*. (1966b)	Petal	+	+	+	+	-	-	-	-	-	+
C. sinensis var. *Pineapple*	Attaway *et al*. (1966a)	Leaf	+	+	+	+	+	-	-	-	-	+
C. sinensis var. *Pineapple*	Moshonas and Shaw (1994)	Juice	+	+	+	-	-	-	-	-	-	-
C. sinensis var. *Valencia*	Attaway *et al*. (1966a)	Leaf	+	+	+	+	+	-	-	-	-	+
C. sinensis var. *Valencia*	Moshonas and Shaw (1994)	Juice	+	+	+	-	-	-	-	-	-	-
C. sinensis var. *Hamlin*	Attaway *et al*. (1966a)	Leaf	+	+	+	+	+	-	-	-	-	-
C. sinensis var. *Hamlin*	Moshonas and Shaw (1994)	Juice	+	-	+	-	-	-	-	-	-	-
C. sinensis	Attaway *et al*. (1966b)	Petal	+	+	+	+	-	-	-	-	-	+
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Peel	+	-	-	+	+	+	-	-	-	-
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Juice	-	-	+	-	-	-	-	-	-	-

Table 1.2: continued

Species	Refference	Plant part	Compound									
			21	22	23	24	25	26	27	28	29	30
C. aurantifolia	Clark *et al*. (1987)	Peel	-	+	+	-	+	+	+	-	-	+
C. aurantifolia (Mexican lime)	Azzouz and Reineccius (1976)	Fruit	-	+	-	-	+	+	+	-	+	+
C. aurantium	Attaway *et al*. (1966a)	Leaf	-	-	-	-	+	+	+	-	-	+
C. aurantium	Mac Leod *et al*. (1988)	Peel	+	-	+	-	-	-	+	-	-	+
C. grandis Osbeck forma Tosa-buntan	Sawamura and Kuriyama (1988)	Flavedo	-	-	-	-	-	-	+	-	-	+
C. halimii	Scora *et al*. (1976)	Leaf	-	+	+	-	-	-	+	-	-	+
C. jambhiri Lush	Lund *et al*. (1981)	Leaf	-	-	-	-	+	+	+	+	-	+
C. junos	Shinoda *et al*. (1970)	Peel	-	+	+	-	+	+	+	+	-	+
C. limon (Eureka)	Baaliouamer *et al*. (1985)	Leaf	-	-	-	-	+	+	+	+	-	+
C. limon " Rough lemon"	Attaway *et al*. (1966a)	Leaf	-	-	-	-	+	+	+	-	-	+
C. limon (Sicilian lemon)	Chamblee *et al*. (1991)	Peel	-	+	-	-	+	+	+	+	-	+
C. limon (California lemon)	Chamblee *et al*. (1991)	Peel	-	+	-	-	+	+	+	+	-	+
C. paradisi var. *Duncan*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	+	+	+	-	-	-
C. paradisi var. *Marsh*	Attaway *et al*. (1966a)	Leaf	-	+	-	-	+	+	+	-	-	+

Table 1.2: continued

Species	Refference	Plant part	Compound									
			21	22	23	24	25	26	27	28	29	30
C. paradisi	Attaway *et al*. (1966b)	Petal	-	-	-	-	-	-	+	+	-	+
C. paradisi	Wilson and Shaw (1980)	Fruit	-	+	+	-	-	-	+	-	-	+
C. paradisi	Cadwallader and Yu (1994)	Juice	-	-	-	-	-	-	-	-	-	-
C. reticulata var. *Murcot*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	-	+	-	-	-
C. reticulata var. *Dancy*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	-	+	-	+	+
C. reiculata	Attaway *et al*. (1966b)	Petal	-	-	-	-	-	-	+	-	-	+
C. sinensis var. *Pineapple*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	+	+	+	-	-	+
C. sinensis var. *Pineapple*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	+	-	-	+
C. sinensis var. *Valencia*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	+	+	+	-	-	+
C. sinensis var. *Valencia*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	+	-	-	+
C. sinensis var. *Hamlin*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	+	+	+	-	-	+
C. sinensis var. *Hamlin*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	+	-	-	+
C. sinensis	Attaway *et al*. (1966b)	Petal	-	-	-	-	-	-	+	+	-	+
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Peel	-	+	-	-	-	+	-	-	-	+
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Juice	-	-	-	-	+	+	-	-	-	+

Table 1.2: continued

Species	Refference	Plant part	Compound									
			31	32	33	34	35	36	37	38	39	40
C. aurantifolia	Clark *et al.* (1987)	Peel	-	-	-	+	-	-	-	+	+	+
C. aurantifolia (Mexican lime)	Azzouz and Reineccius (1976)	Fruit	-	-	-	-	-	-	-	+	+	-
C. aurantium	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	+	-	-
C. aurantium	Mac Leod *et al.* (1988)	Peel	-	+	+	-	-	-	-	+	+	-
C. grandis Osbeck forma Tosa-buntan	Sawamura and Kuriyama (1988)	Flavedo	+	-	+	-	-	-	-	+	-	+
C. halimii	Scora *et al.* (1976)	Leaf	-	-	-	-	-	-	-	-	-	+
C. jambhiri Lush	Lund *et al.* (1981)	Leaf	-	+	-	-	-	-	-	+	+	+
C. junos	Shinoda *et al.* (1970)	Peel	-	-	+	-	-	-	+	-	-	+
C. limon (Eureka)	Baaliouamer *et al.* (1985)	Leaf	-	+	-	-	-	+	-	+	+	+
C. limon " Rough lemon"	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	+	+	+
C. limon (Sicilian lemon)	Chamblee *et al.* (1991)	Peel	-	-	-	+	-	+	-	+	+	+
C. limon (California lemon)	Chamblee *et al.* (1991)	Peel	-	-	-	+	-	+	-	+	+	+
C. paradisi var. *Duncan*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	-	+	+
C. paradisi var. *Marsh*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	+	+	+

Table 1.2: continued

Species	Refference	Plant part	Compound									
			31	32	33	34	35	36	37	38	39	40
C. paradisi	Attaway *et al.* (1966b)	Petal	-	-	-	-	-	+	-	+	+	-
C. paradisi	Wilson and Shaw (1980)	Fruit	-	-	-	-	-	-	-	+	+	+
C. paradisi	Cadwallader and Yu (1994)	Juice	-	-	-	-	-	-	-	-	-	-
C. reticulata var. *Murcot*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. reticulata var. *Dancy*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. reiculata	Attaway *et al.* (1966b)	Petal	-	-	-	-	-	+	-	-	-	-
C. sinensis var. *Pineapple*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	+	-	+
C. sinensis var. *Pineapple*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	-	-	+	+
C. sinensis var. *Valencia*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	+	+	+
C. sinensis var. *Valencia*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	-	-	+	+
C. sinensis var. *Hamlin*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	-	-	+
C. sinensis var. *Hamlin*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	-	-	+	+
C. sinensis	Attaway *et al.* (1966b)	Petal	-	-	-	-	-	+	-	+	+	-
Citrus cultivar *jyabon*	Ogihara *et al.* (1989)	Peel	-	-	+	-	-	-	-	-	+	+
Citrus cultivar *jyabon*	Ogihara *et al.* (1989)	Juice	-	-	+	-	-	-	-	+	+	+

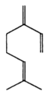
Table 1.2: continued

Species	Refference	Plant part	Compound									
			41	42	43	44	45	46	47	48	49	50
C. aurantifolia	Clark *et al.* (1987)	Peel	-	-	-	-	-	-	+	-	-	-
C. aurantifolia (Mexican lime)	Azzouz and Reineccius (1976)	Fruit	-	-	-	-	-	+	+	-	-	-
C. aurantium	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	+	-	-	-	-
C. aurantium	Mac Leod *et al.* (1988)	Peel	+	-	-	-	-	-	-	-	-	-
C. grandis Osbeck forma Tosa-buntan	Sawamura and Kuriyama (1988)	Flavedo	+	-	+	-	-	+	-	-	-	-
C. halimii	Scora *et al.* (1976)	Leaf	-	-	-	-	-	-	-	-	-	-
C. jambhiri Lush	Lund *et al.* (1981)	Leaf	-	-	-	-	-	+	+	-	-	-
C. junos	Shinoda *et al.* (1970)	Peel	+	-	-	-	+	-	+	-	-	-
C. limon (Eureka)	Baaliouamer *et al.* (1985)	Leaf	-	-	-	-	+	+	+	-	-	-
C. limon " Rough lemon"	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. limon (Sicilian lemon)	Chamblee *et al.* (1991)	Peel	-	+	-	+	+	+	+	+	+	+
C. limon (California lemon)	Chamblee *et al.* (1991)	Peel	-	+	+	+	+	+	+	+	+	+
C. paradisi var. *Duncan*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. paradisi var. *Marsh*	Attaway *et al.* (1966a)	Leaf	-	-	-	-	-	+	-	-	-	-

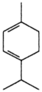
Table 1.2: continued

Species	Refference	Plant part	Compound									
			41	42	43	44	45	46	47	48	49	50
C. paradisi	Attaway *et al*. (1966b)	Petal	-	-	-	-	-	-	-	-	-	-
C. paradisi	Wilson and Shaw (1980)	Fruit	+	-	+	-	+	+	+	-	-	-
C. paradisi	Cadwallader and Yu (1994)	Juice	-	-	-	-	-	-	-	-	-	-
C. reticulata var. *Murcot*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. reticulata var. *Dancy*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. reiculata	Attaway *et al*. (1966b)	Petal	-	-	-	-	-	-	-	-	-	-
C. sinensis var. *Pineapple*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	+	-	-	-	-
C. sinensis var. *Pineapple*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	-	-	-	-
C. sinensis var. *Valencia*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	+	-	-	-	-
C. sinensis var. *Valencia*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	-	-	-	-
C. sinensis var. *Hamlin*	Attaway *et al*. (1966a)	Leaf	-	-	-	-	-	-	-	-	-	-
C. sinensis var. *Hamlin*	Moshonas and Shaw (1994)	Juice	-	-	-	-	-	-	-	-	-	-
C. sinensis	Attaway *et al*. (1966b)	Petal	-	-	-	-	-	-	-	-	-	-
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Peel	-	-	-	-	-	-	+	-	-	-
Citrus cultivar *jyabon*	Ogihara *et al*. (1989)	Juice	-	-	-	-	-	+	+	-	-	-

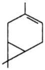
Figure 1.2: Some of *Citrus* essential oil compounds



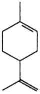
Myrcene



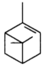
α-terpinene



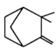
3-carene



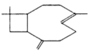
d-limonene



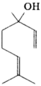
α-pinene



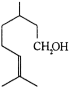
Camphene



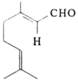
Caryophyllene



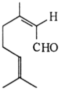
Linalool



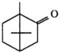
Citronellol



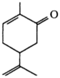
Geranial



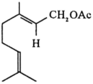
Neral



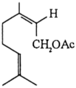
Camphor



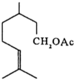
Carvone



Geranyl acetate



Neryl acetate



Citronellyl acetate

1.5 Isoenzymes of *Citrus*

Isoenzymes are multiple molecular forms of enzymes sharing catalytic activity (Brewer, 1970). They provide a useful application as a genetic marker in genetic analysis and taxonomic studies for plants, especially for cultivar improvement (Soost and Torres, 1981). Compared to other biochemical markers, isoenzymes have the advantage of not being a product of a series of biosynthetic reactions (Torres *et al.*, 1978). Moreover, the techniques for their separation are comparatively rapid and simple (Soost and Torres, 1981).

Genetic analysis of several isoenzyme systems to determine relationships among species of *Citrus* have been reported (Hirai and Kajiura, 1987; Ashari *et al.*, 1989). Esen and Soost (1976) studied the peroxidase polymorphisms in *Citrus* leaves. They found that enzyme peroxidase exist in multiple molecular forms in the 30 taxa of *Citrus* and related genera. In 1977, Esen and Scora investigated amylase polymorphisms in *Citrus* and some related genera. From their investigation, they suggested that amylase isoenzyme profiles might have potential uses as markers in genetic studies in *Citrus.* Three other enzyme systems analyzed were malate dehydrogenase (MDH), hexokinase (HK) and isocitrate dehydrogenase (Soost and Torres, 1981).

Hirai *et al.* (1986) analyzed isoenzymes of glutamate oxaloacetate transaminase (GOT) and malate dehydrogenase (MDH) in leaves of *Citrus* to elucidate the relationships between pomelo and sour orange. From the analysis, sour orange is suggested to be a hybrid of pomelo and mandarin.

Isoenzyme is also important in providing genetic marker that could be used to distinguish nucellar from zygotic seedlings. The methodology of isoenzymes determination of nucellar from zygotic seedlings depends on the codominance of alleles that specify isoenzymes. Iglesias *et al.* (1974) analyzed the isoenzymes of peroxidase and esterase in 20 days old seedlings progenies of *Citrus* grown *in vitro*. The origin of the seedlings were confirmed by analysing the bands detected in the zymogram exhibited by seedlings at earlier stages of development of the progenies.

The potential of combining restriction fragment length polymorphisms (RFLPs) and isoenzymes analysis to create a linkage map for *Citrus* has been investigated (Durham *et al.*, 1992). Combination of these techniques provide reliable methods to determine whether *Citrus* seedlings and rootstocks originate from selfing, outcrossing or apomixis and to distinguish any cultivars which originated by hybridization (Roose, 1988).

1.6 Browning of young shoot homogenates of *Citrus*

Browning usually occurs when plant tissues are injured or extracted. This is a result of oxidation of phenolic compounds (substrates) by the copper containing enzyme, polyphenol oxidase (PPO) (o-diphenol oxidase or catechol oxidase or phenolase) (Mayer and Harel, 1979). In plant cell most phenolics, which are generally present as esters or glycosides, are held within the vacuole (Rhodes, 1977; Vaughn and Duke, 1984). They are separated from the enzyme (PPO) which is found in the plastid (Vaughn and Duke, 1984). When the cell is ruptured, the substrates (phenolic compound) would mix with the PPO, leading to the hydrolisis of the phenolic glycosides and oxidation of the released phenolic compounds by PPO to form o-quinones (Rhodes, 1977; Valero *et al.* 1988). The o-quinones which are very reactive oxidizing agents undergo polymerization and also react with amines, α-amino, imino and thiol groups in proteins by forming covalent linkages (with the NH_2 and -SH groups of proteins); yielding brown pigments with high molecular weights. This process is known as enzymic browning (Van Sumere *et al.*, 1975).

The presence of enzymatic browning of young shoot extracts is useable as a chemical characteristic in taxonomic studies (Scora, 1988). Surveys of *Citrus* and related taxa have been done (Esen and Soost, 1974a; Esen and Soost, 1974b; Esen and Scora, 1975). Observations reveal that aqueous extracts from certain taxa turned brown when left standing, while those of others retained their original green-yellow colour. *Citrus* taxa can be grouped into two phenotypic classes: browning and nonbrowning, based on the occurence or absence of browning in young shoot homogenates (Esen and Soost, 1974a). The difference between the two phenotypic groups is that the browning taxa has a phenolic which serves as a substrate for polyphenol oxidase activity, whereas the nonbrowning taxa were devoid of both. From the survey, browning appears to be a dominant trait and is suggested to be useful as a genetic marker and a taxonomic criterion. This is supported by the fact that crosses between the browning and nonbrowning parents produce hybrids of the browning phenotype (Esen and Soost, 1974b).

1.7 Tissue culture in *Citrus*

Citrus is one of the horticultural crops with major economic importance in world fruit production and is being cultivated extensively. However, *Citrus* is susceptible to disease problems which consequently affects production. Disease control in conventional breeding is found to be less effective, thus an alternative method to this problem is to establish plants through propagation via tissue culture techniques.

Plant tissue culture is the culture of isolated cells, tissues and organs explanted from plants and grown in aseptic conditions under controlled environment to allow differentiation and preservation. Using this method propagules require less space compared to conventional breeding. And since this technique is carried out in a sterile environment, the propagules susceptibility to diseases are controlled. The kind of explant chosen, will depend on type of culture and species to be used (George and Sherrington, 1984).

Citrus propagation through tissue culture can be accomplished in three ways:

1) Multiplication of shoots from axillary buds (eg. formation and outgrowth of shoots from single nodes) (Sim *et al.*, 1989).

2) Shoot morphogenesis (formation of adventitious shoot) (Moore, 1986; Duran-Vila *et al.*, 1989).

3) Somatic embryogenesis (formation of adventitious somatic embryos or embryoids). Embryoids can be induced from three sources of cultured diploid cells (Dodds and Roberts, 1982):

i) Vegetative cells of mature plants.

ii) Reproductive tissues other than zygote (eg. ovule and anther) (Hidaka, 1984; Marin and Duran-Vila, 1988; Ling *et al.*, 1990).

iii) Hypocotyl of embryo. (Hidaka and Kajiura, 1988).

Production of somatic embryos and adventitious shoots may occur either directly or indirectly. Direct mode shoot morphogenesis and somatic embryogenesis involve the induction of new shoots without prior formation of callus and formation of an asexual embryo from explant tissue respectively. In the second method, shoots and somatic embryo formation are initiated on unorganised callus tissues. Callus tissue arises from disorganised proliferation of cells from segments (explants) of plant organs which consists of an amorphous mass of loosely arranged parenchyma cells. (George and Sherrington, 1984; Dodds and Roberts, 1982; Tisserat, 1985; Merkle *et al.*, 1990).

Successful experiments on *Citrus* tissue culture have been employed extensively and the summary of results obtained are shown in Table 1.3.

Nutrient media composition and plant growth regulator types are important factors influencing the growth of tissue culture. From Table 1.3, it is observed that the most common medium employed in *Citrus* tissue culture is the formulations developed by Murashige and Skoog (MS) in 1962 and Murashige and Tucker (MT) 1969. The Murashige and Tucker medium differs from the Murashige and Skoog in organic substance concentrations: the B-vitamins thiamin HCl, nicotinic acid and pyridoxin HCl were increased to 10, 5 and 10 mg/l respectively whereas sucrose was increased to 5%

(Murashige and Tucker, 1969). Plant growth regulators which are most important in Citrus tissue culture are auxins and cytokinins. Auxins growth regulators that usually incorporated into media are 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-Napthalene acetic acid (NAA). 2,4-D is frequently used to initiate callus growth and enhance embryogenic callus production (Merkle *et al.*, 1990; Bonga and Von Aderkas, 1992) whereas NAA is used to promote rooting. Cytokinins which are usually employed in this work are kinetin and 6-benzylaminopurine (6-BAP). 6-BAP is very effective in promoting adventitious shoots initiation. Cytokinins are often used in conjunction with auxins. A high level of 6-BAP to NAA ratio is required for axillary shoot proliferation. While treatment with a mixture of high auxins and low cytokinins levels induced root formation (George and Sherrington, 1984; Tisserat, 1985). Complex organic supplements, malt extract, yeast extract, casein hydrolysate, citric acid and coumarin have been used for Citrus culture (Grosser and Chandler, 1986; Teo *et al.*, 1988; Tusa and Geraci, 1988; Song *et al.*, 1991b). It has been observed that malt extract enhanced embryogenesis and shoot regenaration (Raj Bhansali and Arya, 1979; Kunitake *et al.*, 1991; Song *et al.*, 1991b; Carimi *et al.*, 1994; Carimi *et al.*, 1995). The addition of casein hydrolysate to the culture medium will induce callus initiation (Erner *et al.*, 1975; Beloualy, 1991). Citric acid, natural component of citrus juice promote growth activity in callus (Erner *et al.*, 1975; Einset, 1978; Duran-Vila *et al.*, 1989; Amo-Marco and Picazo, 1994). Growth promoting activity involves cell division and cell enlargement (Erner *et al.*,1975).

Table 1.3: Summary of results obtained from tissue culture of *Citrus* species

Species	Explants	Results	Basical Medium	Plant Growth Regulators (mg/l)	Authors
Citrus aurantifolia	Stem internode	Shoot and bud proliferation	Duran-Vila *et al.* (1989)	6-BAP (1)	Duran-Vila *et al.* (1989)
C. aurantium	Stem internode	Adventitious shoots	Murashige and Tucker (1969) (MT)	6-BAP (5) NAA (1)	Moore (1986)
C. aurantium	Shoots	Shoots rooted	MT	NAA (1)	Moore (1986)
C. aurantium	Callus induced from embryo	Embryos induction	MT	(1000 / 1500) malt extract	Beloualy (1991)
C. aurantium	Callus induced from embryo	Embryoids and shoots induction	MT	NAA (1) 6-BAP (10)	Beloualy (1991)
C. grandis	Immmature ovules	Embryogenic callus	MT	2,4-D (0.1)	Song *et al.* (1991a)

Table 1.3: continued

Species	Explants	Results	Basical Medium	Plant Growth Regulators (mg/l)	Authors
C. junos	Callus	Shoots initiation	Murashige and Skoog (1962) (MS)	IBA (0.5) 6-BAP (0.5)	Oh *et al.* (1991)
C. junos	Callus	Somatic embryo induction	MT	IAA (0.1 / 1.0) BAP (1.0)	Song *et al.* (1991b)
C.limon	Ovule callus	Highest number of embryoids	MS	-	Kochba *et al.* (1982)
C. limon	Seedling root apices	Shoots initiation on root swellings	Dale (1975)	-	Sauton *et al.* (1982)
C. limon	Mature or seedling node	Shoot growth	MS	6-BAP (1.25)	Barlass and Skene (1982)

Table 1.3: continued

Species	Explants	Results	Basical Medium	Plant Growth Regulators (mg/l)	Authors
C. madurensis	Seedling stem section	Plantlets via callus	Grinblat (1972)	NAA (0.1)	Grinblat (1972)
C. microcarpa	Seed cotyledon	Adventitious shoots and roots	MS	NAA (5) Kinetin (2.5) 15 % CM	Rao *et al*. (1982)
C. mitis	Seedling epicotyl	Shoot regeneration	MS	6-BAP (0.5-1.0)	Sim *et al*. (1989)
C. mitis	Seedling leaf segments	Shoot regeneration	MS	6-BAP (2.0)	Sim *et al*. (1989)
C. mitis	Seedling shoot tip	Multiple shoot buds	MS	6-BAP (1.0)	Sim *et al*. (1989)
C. mitis	Seedling nodes	Multiple buds	MS	6-BAP (0.5)	Sim *et al*. (1989)

Table 1.3: continued

Species	Explants	Results	Basical Medium	Plant Growth Regulators (mg/l)	Authors
C. nobilis Lour. X *C. deliciosa* Tenora (Kinnow mandarin)	Epicotyl segments	Embryogenic callus	Modified MT	NAA (6.0) Kinetin (0.2) Malt extract (500)	Gill *et al.* (1994)
C. paradisi X *Poncirus trifoliata* (Citrumello)	Stem node	Several rooted plantlets	MT	Coumarin (13.2-21.9)	Grosser and Chandler (1986)
Citrumello	*In vitro* stem internode	Several adventitious plantlet	MT	Coumarin (13.2-21.9)	Grosser and Chandler (1986)
Citrumello	Shoot	Shoot multiplication	MS	6-BAP (1) IBA (0.5) Adenine (4.0)	Starrantino and Caruso (1988)

Table 1.3: continued

Species	Explants	Results	Basical Medium	Plant Growth Regulators (mg/l)	Authors
Citrumello	Shoot	Shoot rooted	MS	NAA (1)	Starrantino and Caruso (1988)
C.reticulata	Shoot tips	Multiple shoots	MS	6-BAP (1) Kinetin (0.5) NAA (0.5)	Singh *et al.* (1994)
C. sinensis	Mature or seedling node	Shoot growth	MS	6-BAP (2.25)	Barlass and Skene (1982)
C. sinensis	*In vitro* hypocotyl sections	Adventitious shoots	MT	NAA (0.02) 6-BAP (2)	Burger and Hackett (1986)
C. sinensis X *Poncirus sp.*	Mature or seedling node	Shoot growth	MS	6-BAP (2.25)	Barlass and Skene (1982)

Table 1.3: continued

Species	Explants	Results	Basical Medium	Plant Growth Regulators (mg/l)	Authors
C. sinensis X *Poncirus sp.*	Epicotyl segments	Shoots from 30 % explant	Navarro *et al.* (1975)	NAA (0.1) 6-BAP (0.5)	Edriss and Burger (1984)
C.sinensis X *Poncirus sp.*	Stem internode	Shoots via callus	MT	6-BAP (5)	Moore (1986)
C. sinensis X *Poncirus sp.*	Seedling root apices	Shoots initiation on root swellings	Dale (1975)	-	Sauton *et al.* (1982)

1.8 Species description

Citrus halimii **B.C. Stone**

(Plate 1)

Habit: Tree, about 22-23 m.

Leaves: Elliptic to narrowly elliptic or slightly ovate or obovate-elliptic. Upper surface dark subglossy green, lower surface paler green.

Flowers: Solitary in leaf axils, petals 5, white.

Fruits: Subglobose, slightly pyriform.

Seeds: Monoembryonic.

(Stone *et al.*, 1973)

Citrus hystrix **D.C.**

(Plate 2)

Habit: Shrub, about 2-12m.

Leaves: Alternate or biseriate, stalked unifoliate, orbicular-ovate or ovate oblong, lanceolate.

Flowers: Solitary or terminal. Petals yellowish white, fragrant.

Fruits: Medium. Pendulous, globose, ovoid or elliptic.

Seeds: Monoembryonic.

(Swingle, 1967)

Plate 1: *Citrus halimii*

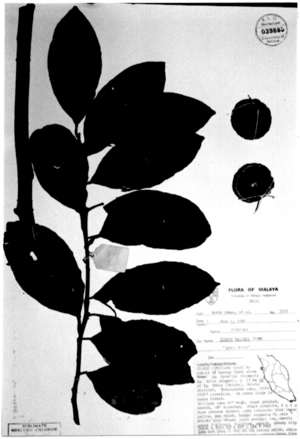
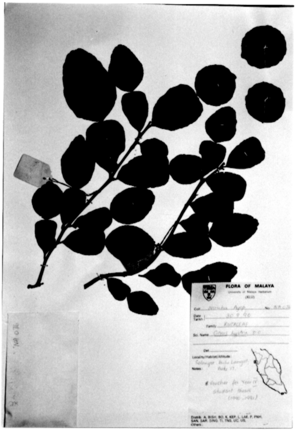


Plate 2: *Citrus hystrix*



***Citrus madurensis* Loureiro.**

(Plate 3)

Habit: Shrub, about 10-12 m.

Leaves: Spiral, small, green, elliptic-ovate.

Flowers: Axillary or terminal. Petal white with lower surface slightly pinkish.

Fruits: Very small, globose to oblate.

Seeds: Monoembryonic.

(Ko, 1992)

Citrus *micrantha* var. *microcarpa* Wester.

(Plate 4)

Habit: Shrubs, about 4.5 m.

Leaves: Ovate to ovate-oblong or elliptical.

Flowers: Compact axillary, small. Petal white with trace of purple on the outside.

Fruits: Small, roundish in outline.

Seeds: Monoembryonic.

(Swingle, 1967).

Plate 3: *Citrus madurensis*

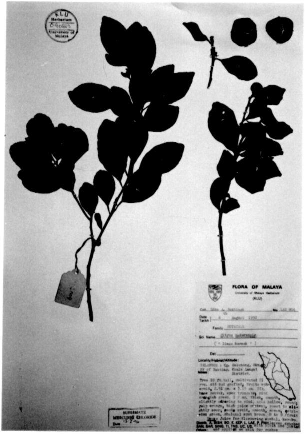
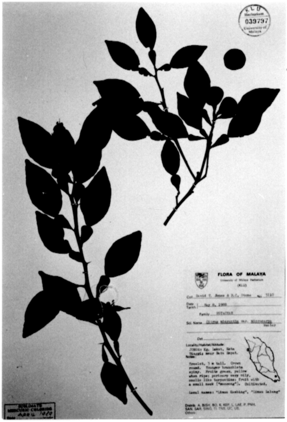


Plate 4: ***Citrus micrantha*** **var.** ***microcarpa***



Citrumello (*Citrus paradisi* X *Poncirus trifoliata*)

(Plate 5)

Habit: Moderately vigorous.

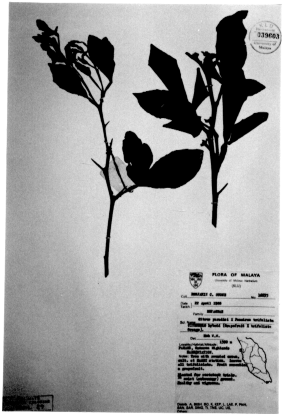
Leaves: Large, trifoliate.

Fruits: Medium, globose.

Seeds: Polyembryonic.

(Ko, 1992)

Plate 5: Citrumello (*C. paradisi* X *P. trifoliata*)



1.9 Objectives of present study

The main objectives of this study were:

1) To determine the relationships between species studied using characteristics of essential oil components, isoenzymes analysis and presence of enzymatic browning in young shoot extracts.

2) To determine the best explant and regeneration media for propagation via tissue culture technique.