CHAPTER 2

LITERATURE REVIEW

Local vegetables or Pak-Puen-Baan (Thai) are defined as the plant species locally grown for food by native Thais. The plants include those indigenous to the country as well as the species that had long been introduced from other parts of the world. As for indigenous species, it was reported that quite a number of forest plants had been domesticated and utilized. These plants, having gone through the long process of natural selection in flora evolution are tolerant and capable of adjusting themselves to local environments, to a certain extent. They are of trees, shrubs or annuals and the parts that are used as vegetable are stem, shoot, root, bulb, leaf or flower, depending upon the species. Those of the introduced ones, usually called by local Thai names and having been grown for generations, are practically accepted as the native varieties (Poungmanee, 2007; Sapcharoen, 2005).

1. Survey, collection and utilization of local vegetable

Surveys of local vegetables have been conducted in several parts of Thailand with more or less similar objectives. Some scientists include collection of the plants in germplasm conservation while others preserve them as herbarium specimens for botanical study. Information concerning utilization of those plants are mostly gathered, accordingly. Trisonthi and Trisonthi (1999) conducted surveys of local vegetables in the Upper-North of Thailand in search for relevant information serving the purpose of conservation of the becoming-rare specimens. Local vegetables were collected from local markets, home gardens and forests. From this collection, 163 plant species were identified via morphological characterization. Methods of food preparation from the plant parts were also recorded. In this study some favourable vegetables of the North were indicated, e.g. *Brousonnetia kurzii*, *Dolichos lablab*,

Ficus lacor, *Gymnema inodorum*, *Houttuynia cordata*, *Polygonum odoratum* and *Raphanus sativus* var. *caudatus*. Among these, two species, *Erythropalum sandens*, found only in Nan Province, and *Brassasiopsis palmata* were considered rare and worth conserving.

Legkerm (1991) made a survey on most demanded varieties of local vegetables in some districts of Chiang Mai reported 6 kinds of vegetables as the most favourites, i.e. *Broussonetia kurzii*, *Bauhinia* spp., *Dregea volubilis*, *Gymnema inodorum* and *Melientha sauvis*. These vegetables were seasonal and were not seriously grown in commercial scale.

Apart from the North, surveys and collection of local vegetables were carried out in particular parts of Thailand. In the Central, research were done in the Provinces of Lop Buri and Kanchanaburi by Cheancharoen (1999) and Thongpairoj (1999), respectively. The former author collected 152 species of plants, most of flowering ones with only 4 species of ferns while the latter did 155 species of angiosperms with 3 species of fern and 1 of algae. Plants collected by both authors were preserved either in spirit or as dry herbarium specimens.

A study carried out in the southern part of Thailand were more of ethnobotanic. Upho (2005) reported her work on utilization of local plants by Buddhist and Muslim Thais in the Lower-South. Indigenous knowledge concerning utilization of the plants was also observed. Records made from the study showed that the plants used for food and medicine were of 236 and 327 species, respectively.

Although there are various kinds of local vegetables being grown and consumed in rural and urban areas, particular kinds of them have been recognized to have certain commercial potential. They were judged from their nutritional and medicinal values as well as that of consumption demands, according to Deewiset (1999), Pookajorn (2000), Poungmanee (2007) and Sapcharoen (2005). These local vegetables include Chiang Da, Nang Laeo, Phak Plang and some others.

2. Botanical aspects of *Peliosanthes teta* Andr., *Basella alba* L. and *Gymnema inodorum* Decne.

2.1 *Peliosanthes teta* Andr.

Peliosanthes teta Andr., having a local Thai name of Nang Laeo (Smitinand, 2001), falls into the monocotyledonous type of Angiosperm. Its ecology is primarily in wet evergreen forest, from 0-3,000 m above sea-level (Chen and Tamura, 2000; Jessop, 1976; 1979), distributed in Northeast and Southeast India; tropical Southeast Asia, i.e. Thailand, Laos, Myanmar, Vietnam, through Indo-China into Malaysia, Sumatra, Java, Lesser Sunda Island (Sumbawa) and Borneo; and Southern China including Taiwan (Chen and Tamura, 2000; Conran and Tamura, 1998; Jessop, 1979).

Peliosanthes Andr. was earlier a member of the family Liliaceae. It was later placed into the family Convallariaceae instead, after the revision of *Peliosanthes* (Liliaceae) as reported by Jessop in 1976. During that time, the previously recognized species of *Peliosanthes* were united into a single species, *P. teta*, comprising two subspecies: subsp. *teta* and subsp. *humilis* (Andr.) Jessop ex Gandhi (Chen and Tamura, 2000). Nevertheless, it was stated otherwise by Conran and Tamura (1998) that approximately ten species of *Peliosanthes* were indentified.

Convallariaceae were subdivided with 3 tribes: Tribe Polygonateae, Tribe Ophiopogoneae and Tribe Convallarieae. *Peliosanthes teta* Andr. is in the Tribe Ophiopogoneae which comprises three genera; *Liriope* Lour., *Ophiopogon* Ker Gawler and *Peliosanthes* Andr. Seventeen genera and about 130 species of Convallariaceae distributed in North and Central America, Europe, India and Asia, and Bali and Lombok (Conran and Tamura, 1998).

The genus *Peliosanthes* is a perennial herb. **Rhizome** is usually very short, most frequently less than 5 cm (Jessop, 1976; 1979) or rarely up to 40 cm in length (Conran and Tamura, 1998; Jessop, 1976). **Root** is thick (Chen and Tamura, 2000; Conran and Tamura, 1998; Jessop, 1976; 1979). **Leaf** is often crowded at the

base (Jessop, 1979), rarely at cauline (Chen and Tamura, 2000). It is arranged in alternate form (Conran and Tamura, 1998) and separated from one another by several nodes bearing scale-leaves (Jessop, 1976). Leaf blade is linear (Chen and Tamura, 2000) or sublinear (Conran and Tamura, 1998; Jessop, 1976; 1979) to oblong (Jessop, 1976), elliptic (Chen and Tamura, 2000), ovate-oblong (Conran and Tamura, 1998), ovate (Jessop, 1976; 1979). Petiole is usually distinct (Jessop, 1976, 1979; Chen and Tamura, 2000; Conran and Tamura, 1998), sometimes stipulated (Conran and Tamura, 1998). **Inflorescence** is of simple raceme (Chen and Tamura, 2000; Conran and Tamura, 1998; Jessop, 1976; 1979) or reduced panicle (Chen and Tamura, 2000). **Flower** is in clusters of 2-5 (Chen and Tamura, 2000; Jessop, 1979). Perianth is companulate or subglobose, varying in colour, i.e. white, green, blue, violet or purple (Jessop, 1976; 1979). It is constructed with 6 stamens (Chen and Tamura, 2000; Conran and Tamura, 1998) of very short filaments. Ovary is superior to inferior (Chen and Tamura, 2000; Jessop, 1976; 1979), bearing 2-5 ovules (Chen and Tamura, 2000; Jessop, 1979) at the base (Conran and Tamura, 1998; Jessop, 1976; 1979). Ovary wall ruptures in early development, exposing a young seed. **Seed** is ellipsoid to pyriform (Jessop, 1976; 1979) or globose (Chen and Tamura, 2000) with blue colour when mature (Chen and Tamura, 2000; Conran and Tamura, 1998; Jessop, 1976; 1979).

Botanical aspects of *Peliosanthes teta* Andr. can be described as follows:

2.1.1 Root and Stem

Root is thick (Chen and Tamura, 2000; Jessop, 1976; 1979) and fibrous (Conran and Tamura, 1998). **Stem** is rhizomous, usually short (Chen and Tamura, 2000; Jessop, 1976).

2.1.2 Leaf

The plant obtains 4-8 (Chen and Tamura, 2000), sometimes 2-12, leaves (Jessop, 1976; 1979). **Leaf blade** is almost linear (Jessop, 1976) or lanceolate to elliptic (Chen and Tamura, 2000; Jessop, 1976), ovate or obovate (Jessop, 1976), 7.5-47.5 × 1.5-11.5 cm in size (Chen and Tamura, 2000; Jessop, 1976; 1979) with acute to acuminate (Chen and Tamura, 2000; Jessop, 1976), or rarely slightly arcuate apex

(Jessop, 1976). Petioles, 5-30 cm (Chen and Tamura, 2000) or 4-50 cm in length (Jessop, 1979), are well-defined and slightly compressed (Chen and Tamura, 2000; Jessop, 1976; 1979).

2.1.3 Inflorescence

Inflorescence is of raceme (Jessop, 1976; 1979) or reduced panicle (Chen and Tamura, 2000), 10-15 cm (Chen and Tamura, 2000) or 35-75 cm in length (Jessop, 1976; 1979). **Bract** is sublinear to ovate (Jessop, 1976) or lanceolate (Chen and Tamura, 2000), smaller towards the apex of the raceme (Jessop, 1976; 1979), usually spreading at the base and ascending distally (Jessop, 1976). **Pedicel,** 3-8 mm (Chen and Tamura, 2000) or 1-10 mm in length (Jessop, 1976; 1979), is articulated, usually below (Jessop, 1976) or close to the flower (Jessop, 1979). It enlarges after flowering (Jessop, 1979).

2.1.4 Flower

Perianth, adnating to the ovary, is purple in colour (Chen and Tamura, 2000). **Anther,** usually adpressed closely to the style (Jessop, 1976; 1979), is 0.5-2 mm in length (Chen and Tamura, 2000; Jessop, 1976; 1979). **Style,** with 3-6 ridges, is short (Chen and Tamura, 2000), 0.75-2 mm in length (Jessop, 1976; 1979). **Stigma** is capitated (Chen and Tamura, 2000). **Ovary,** conical to ovoid, is half-inferior (Jessop, 1976; 1979). It bears 3 locules with 1-5 basal ovules in each (Conran and Tamura, 1998). Ovary lobes, 2.5-4 × 1.5-2 mm in size (Chen and Tamura, 2000), are suborbicular, broadly ovate, elliptic, obovate (Jessop, 1976; 1979) or oblong to linear (Chen and Tamura, 2000; Jessop, 1976; 1979).

2.1.5 Seed

Seed is suboblong, 5-7 mm (Chen and Tamura, 2000) or up to 10-12 mm in length (Jessop, 1976; 1979).

As earlier mentioned, the species *Peliosanthes teta* was subdivided into two subspecies: subsp. *teta* and subsp. *humilis* (Andrews) Jessop ex Gandhi. According to

Jessop (1976; 1979), identification of these two subspecies was regarded on a distinct constancy character of the flower number in each bract. The description of these 2 subspecies is as follows:

P. teta* supsp. *teta (Jessop, 1976; 1979)

Leaf lamina 2-34 times as long as broad. **Pedicels** 2-6 in the axial of each fertile bract. **Flowers** usually green, rarely blue. **Anthers** 0.5-0.6 mm in length.

P. teta* supsp. *humilis (Jessop, 1976; 1979)

Leaf lamina 2-10 times as long as broad. **Pedicels** solitary in the axial of each fertile bract. **Flowers** sometimes green, often white, blue, violet or purple. **Anthers** 0.5-2.0 mm in length.

2.2 *Basella alba* L.

Basella L. is in the family Basellaceae, a small pantropical and subtropical family, composing of 5 genera and about 20 species (Bailey, 1969; Bhattacharyya and Johri, 1998; Eriksson, 2007; Lawrence, 1951). The plants of this family are the climbing perennial herbs. **Stem** is succulent (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Eriksson, 2007; Larsen, 1992; Lawrence, 1951), sometimes suffrutescent, scandent or trailing vines (Eriksson, 2007; Lawrence, 1951), often mucilaginous but without milky latex (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Eriksson, 2007; Lawrence, 1951). **Leaf,** petiolated and estipulated (Bhattacharyya and Johri, 1998; Eriksson, 2007; Lawrence, 1951), is of simple type, also fleshy (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Eriksson, 2007; Larsen, 1992; Lawrence, 1951) and arranged alternately (Bailey, 1969; Bhattacharyya and Johri, 1998; Lawrence, 1951) or spirally (Backer and Bakhuizen van den Brink, 1963; Eriksson, 2007; Larsen, 1992). Leaf blade varies in shape among species. Most species have entire blades, sometimes with a reddish margin. Venation is pinnate, rarely actinodromous (Eriksson, 2007). **Inflorescence** is of spike (Eriksson, 2007), raceme (Larsen, 1992) or panicle (Backer and Bakhuizen van den Brink, 1963; Bhattacharyya and Johri, 1998; Lawrence, 1951). **Flower** is bisexual (Bailey, 1969;

Larsen, 1992; Lawrence, 1951) and actinomorphic (Bhattacharyya and Johri, 1998; Lawrence, 1951). It is constructed with 2 sepals (Bailey, 1969), 5 petals (Bailey, 1969; Bhattacharyya and Johri, 1998; Lawrence, 1951), 5 stamens (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Eriksson, 2007; Larsen, 1992; Lawrence, 1951) and 1 style with 3-parted stigma (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Larsen, 1992; Lawrence, 1951). Its superior ovary comprises 1 locule with 3 carpels (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Larsen, 1992; Lawrence, 1951). **Fruit** is of drupe type, indehiscent (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Larsen, 1992), enveloped by persistent fleshy-accrescent perianth (pseudo-berry) (Backer and Bakhuizen van den Brink, 1963; Bhattacharyya and Johri, 1998; Lawrence, 1951). **Seed** is globular (Backer and Bakhuizen van den Brink, 1963).

A species of *Basella*: *B. alba* L., having many vernacular names of Phak Plang or Phak Pang (Thai) (Smitinand, 2001), Malayan Nightshade, Malabar Spinach, Ceylon Spinach, Indian Spinach (Eriksson, 2007; Larsen, 1992; Smitinand, 2001) distributes in tropics and warm countries, mainly in Southeast Asia, Northwards to China and Japan, and also Africa (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Eriksson, 2007; Larsen, 1992; Lawrence, 1951). It can be found in local naturalized areas, grassy wilds, brush woods, open forest, waste places, also often cultivated especially in natural garden (Backer and Bakhuizen van den Brink, 1963). Its native area is unknown, but somewhere in Africa or probably Asia (Eriksson, 2007).

Botanical aspects of *Basella alba* L. can be described as follows:

2.2.1 Stem

Stem is of climbing type (Backer and Bakhuizen van den Brink, 1963), scandent, or sometimes suffrutescent (Lawrence, 1951), and can be up to 10 m in length (Larsen, 1992). It is glabrous (Bailey, 1969; Eriksson, 2007), varying in

colour from red to green, often fleshy and mucilaginous (Bhattacharyya and Johri, 1998; Eriksson, 2007).

2.2.2 Leaf

Leaf is of simple type and alternate (Bhattacharyya and Johri, 1998; Lawrence, 1951). Leaf blade is ovate to suborbicular or ovate-lanceolate (Bailey, 1969) or oblong (Backer and Bakhuizen van den Brink, 1963), 2-15 × 2-13.5 cm in size (Backer and Bakhuizen van den Brink, 1963; Eriksson, 2007; Larsen, 1992). Leaf margin is entire (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Bhattacharyya and Johri, 1998; Larsen, 1992) or undulate (Bailey, 1969). Leaf apex is obtuse or acute (Backer and Bakhuizen van den Brink, 1963; Larsen, 1992), sometimes acuminate (Eriksson, 2007) or emarginated (Bailey, 1969). Leaf base is often cordate or subcordate (Bailey, 1969; Larsen, 1992) to cuneate or attenuate (Eriksson, 2007). It is petiolated, glabrous (Larsen, 1992), estipulated (Bhattacharyya and Johri, 1998; Lawrence, 1951), 1-3 cm in length (Larsen, 1992).

2.2.3 Inflorescence

Inflorescence, 3-25 cm in length, is of simple spike (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Eriksson, 2007; Larsen, 1992) to raceme or panicle (Bhattacharyya and Johri, 1998), with thick rachis (Backer and Bakhuizen van den Brink, 1963) or stout and fleshy axis (Eriksson, 2007). It is floriferous only in the upper part (Larsen, 1992). **Flowers** cluster on elongated thickened peduncles in branching inflorescence (Bailey, 1969). Very young flowers are crowded while older ones are wide apart (Backer and Bakhuizen van den Brink, 1963). **Bract** is minute and caducous (Larsen, 1992), acute and much shorter than perianth (Backer and Bakhuizen van den Brink, 1963). **Bracteole** is well developed (Eriksson, 2007) and enclosed, oval or obtuse (Backer and Bakhuizen van den Brink, 1963) or ovate to triangular (Eriksson, 2007).

2.2.4 Flower

Flower is sessile in axillary (Backer and Bakhuizen van den Brink, 1963), small, 3-4 mm in length (Backer and Bakhuizen van den Brink, 1963; Larsen,

1992). **Perianth** is white to dark purple (Larsen, 1992), white with purple tip (Backer and Bakhuizen van den Brink, 1963) or reddish (Backer and Bakhuizen van den Brink, 1963; Eriksson, 2007). The petal is 3.5-5.5 × 1-2.5 mm in size, erect and ovate to elliptic in shape. It is connated about 1/3-2/3 of their length (Eriksson, 2007) and does not or very little expand at anthesis stage (Backer and Bakhuizen van den Brink, 1963). The flower is usually cleistogamous (Eriksson, 2007). **Sepal,** 2-2.5 × 3.5-5.5 mm in size (Eriksson, 2007), may be carinat but not winged in flower (Bailey, 1969). **Stamen** is 5, opposite sepals and adnate to sepal base. Filament is free and erect (Bhattacharyya and Johri, 1998). Anther is longitudinally dehiscent (Backer and Bakhuizen van den Brink, 1963), bicelled and basifixed (Bhattacharyya and Johri, 1998) with pale colour (Eriksson, 2007). **Pistil** is composed of 1 style with 3-parted stigmas almost to the base (Bhattacharyya and Johri, 1998; Eriksson, 2007) or of 3 styles (Backer and Bakhuizen van den Brink, 1963; Eriksson, 2007). Its superior ovary is unilocular with a single basal ovule (Bhattacharyya and Johri, 1998).

2.2.5 Fruit and Seed

Fruit is of drupe type (Bhattacharyya and Johri, 1998; Eriksson, 2007; Lawrence, 1951) surrounded by persistant and fleshy perianth (Eriksson, 2007; Lawrence, 1951). Its shape is ovoid to globose or almost sphere (Bailey, 1969; Larsen, 1992), or forming as pseudo-berry, depressed-globous, slightly lobed (Backer and Bakhuizen van den Brink, 1963), 4-7 × 7-10 mm in size (Backer and Bakhuizen van den Brink, 1963; Bailey, 1969; Eriksson, 2007). The fruit is violet or purple to shining black with very fleshy, violet juice (Backer and Bakhuizen van den Brink, 1963; Eriksson, 2007; Larsen, 1992). **Seed** is of sphere shape with a large annualar or spirally twisted embryo (Bhattacharyya and Johri, 1998; Lawrence, 1951).

Bailey (1969) described 2 species of *Basella*: *Basella alba* and *Basella rubra*, as follows:

***B. alba* L.**

A very similar but with narrower leaves. Leaves are ovate to ovate-lanceolate, prevailingly longer than broad, rounded or tapering at base, acute or slightly

obtuse, somewhat undulate, and whitish flowers in longer-peduncled spikes arranged in very loose clusters.

***B. rubra* L.**

A rampant-growing vein with green or purplish stems. Leaves are 2-6 or more inches wide, about as broad as long, and are ovate to suborbicular, and cordate at base, obtuse or somewhat emarginated. Flowers are on short spikes, in small clusters, their color is reddish.

2.3 *Gymnema inodorum* Decne.

Gymnema Decne. is a member of Asclepiadaceae, milkweed family (Lawrence, 1951). This family comprises 250 genera and over 2,000 species (Li *et al*., 1995) or about 3,000 species (Bhattacharyya and Johri, 1998). Generally, the plant of this family are perennial (Lawrence, 1951) or annual herbs (Bhattacharyya and Johri, 1998). They can be climber, shrubs, but rarely treelike (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995). **Stem** is usually lianous and generally with milky sap or less often clear latex (Lawrence, 1951; Li *et al*., 1995). Some genera are fleshy or cactuslike (Lawrence, 1951), sometimes covered with dense tomentum or with wax. **Leaf** is of simple type, arranged in opposite (Bhattacharyya and Johri, 1998) or occasionally whorled, and very rarely alternate (Lawrence, 1951; Li *et al*., 1995). Leaf is petiolated and estipulated or stipulated (Lawrence, 1951; Li *et al*., 1995). Leaf margin is entire (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995). **Inflorescence** is of dichasial or monochasial cyme (Bhattacharyya and Johri, 1998; Lawrence, 1951), or condense umbelliform, occasionally raceme (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995). **Flower** is bisexual (Lawrence, 1951; Li *et al*., 1995) and actinomorphic, typically pentamerous (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995). It is constructed with 5 sepals joined at base only (Lawrence, 1951; Li *et al*., 1995), 5 gamopetalous petals, or only basally fused (Bhattacharyya and Johri, 1998). Five stamens are usually adnate to the gynoecium to form a gynostegium (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995). Pistil comprises 2 styles with a common single 5-lobed often much-enlarged stigma (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995). Ovary is unilocular

with 1 carpel (Lawrence, 1951) of numerous ovules (Bhattacharyya and Johri, 1998; Li *et al*., 1995). **Fruit** is of follicle type. **Seed** is usually flattened and crowned with a tufted micropylar coma of long silky hairs (Lawrence, 1951).

The species of *Gymnema* that distributes in tropical countries of Asia, i.e. Thailand, China, Vietnam, Philippines, India and Nepal is that of *G. inodorum* Decne., having a local Thai name of Chiang Da (Smitinand, 2001). This plant can be found in brushland or open woods at 200-1,000 m above sea-level (Li *et al.*, 1995).

Botanical aspects of *Gymnema inodorum* Decne. can be described as follows:

2.3.1 Stem

Stem is lianous and glabrous up to 10 m in length (Li *et al.*, 1995) and 0.5-5.0 cm in diameter (Klangsab, 2006). Young branchlets are pale grey, become grayish green when older. It is lenticellate and puberulent, with milky sap (Bhattacharyya and Johri, 1998; Klangsab, 2006; Lawrence, 1951; Li *et al.*, 1995).

2.3.2 Leaf

Leaf is of simple type and opposite (Bhattacharyya and Johri, 1998; Klangsab, 2006; Lawrence, 1951) with petiole 2-6 cm long (Klangsab, 2006; Li *et al.*, 1995). Leaf blade is ovate-oblong to ovate or broadly ovate, 4-13 × 2-9 cm in size. It is glabrous or thin puberulent along veins, with acuminate to caudate apex. Leaf base is rounded to shallowly cordate. Lateral veins are of 4-6 pairs (Li *et al.*, 1995). The leaf margin is entire (Bhattacharyya and Johri, 1998; Lawrence, 1951).

2.3.3 Inflorescence

Inflorescence, up to 4 cm in length, is raceme-like with umbel-like cymes (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995), arranged in spiral. Peduncle and pedicel are 1-2 cm and 1-1.5 cm long, respectively (Li *et al.*, 1995).

2.3.4 Flower

Flower is bisexual and pentamerous. **Calyx** is of 5 basally connate sepals, imbricate or open (Bhattacharyya and Johri, 1998; Lawrence, 1951). Sepals are oblong, 2-3 × 1-4 mm in size, shorter than corolla tube with puberulent and ciliate. **Corolla** is yellow, 6-7 mm long with minutely puberulent outside and corolla tube is cylindrical (Li *et al.*, 1995). It is composed of 5 lobes, contorted or less commonly valvate (Bhattacharyya and Johri, 1998; Lawrence, 1951). The lobes are oblong, 3-4 × 1.6-1.8 mm in size, with rounded apex and ciliate margin (Li *et al.*, 1995). Five **stamens** are usually adnate to the gynoecium to form a gynostegium (Bhattacharyya and Johri, 1998; Lawrence, 1951). **Anthers** are bilocular, and united in pairs bearing a translator arm joined with a rough sagittate body called the gland (Li *et al.*, 1995) by a pendages (caudicles) (Bhattacharyya and Johri, 1998) called pollinia. It is oblong (Li *et al.*, 1995). **Gynoecium** consists of apocarpous bicarpellate pistil (Bhattacharyya and Johri, 1998; Lawrence, 1951) and 2 distinct styles (Bhattacharyya and Johri, 1998). Stigma head is dome-shaped, exserting from corolla tube (Li *et al.*, 1995). It is commonly 5-lobed, often much-enlarged stigma (Lawrence, 1951). Its superior ovary is unilocular (Bhattacharyya and Johri, 1998; Lawrence, 1951), comprising numerous anatropous ovules (Lawrence, 1951).

2.3.5 Fruit and Seed

Fruit is of follicle type (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al*., 1995) with lanceolate in outline, up to 16 × 3 cm in size. Its wall is thick with slightly fibrous and glabrous peel (Li *et al*., 1995). **Seeds** are numerous (Bhattacharyya and Johri, 1998). Each is 1.5 × 1 cm in size (Li *et al*., 1995), usually flattened and crowned with a tufted micropylar coma of long silky hairs (Bhattacharyya and Johri, 1998; Lawrence, 1951).

3. Plant characterization

It has been recognized among botanists that environment can play important roles on plant diversity distributing in different ecosystems. Wild plants as well as

domesticated ones being cultivated in particular environment for a long period of time could develop some differential botanical characters of which, to a certain extent, could be referred to as varietal identities. Phootyanan (2003) and Thatsaneeyakorn (2000) mentioned that utilization of wild plants as well as cultivated plants have been vast and for extensive purposes. Quite a number of them have been used in the fields of breeding and improvement. Recognition was made from the workers involved in such fields that varietal identity of the parent plants and the hybrids is essential, since characters of the plants can reveal genetic relationship among them at certain levels. Junsongduang *et al.* (2003) and Stuessy (1990) stated that taxonomial identification of plants, though conventional, could only accurately classify the plants to species or subspecies while Phootyanan (2003) and Thatsaneeyakorn (2000) indicated that other means of characterization could supplement adequate identity at varietal level. These aspects involve anatomical (Junsongduang *et al.*, 2003; Stuessy, 1990), cytological, chemical, biochemical and molecular biological characterizations (Chengkun *et al.*, 1995).

Recent works supporting the above mentioned statement are those done with some terrestrial orchid species located in different natural habitats. The works revealed that different orchid ecotypes of the same species portrayed different anatomical characters of the plant parts as well as that of chromosome numbers, relevant to their genetic relationship analysed via isozyme patterning. Investigated orchid species were *Calanthe cardioglossa* Schltr., *Geodorum attenuatum* Griff., *G. citrinum* Jacks, *G. recurvum* (Roxb.) Alston, *G. siamensis* Rolfe. ex Downie., *Liparis paradoxa* (Lindl.) Rchb. f, *L. regnieri* Finet., *L. siamensis* Rolfe. ex Downie, *Malaxis acuminata* D. Don, *M. calophylla* (Rchb. f.) Kze., *M. latifolia* J. E. Sm., *Nervilia aragoana* Gaud., *N. crociformis* (Zoll. & Mor.) Seidenf., *N. plicata* (Andr.) Schltr., *Spathoglottis eburnea* Gagnep. and *S. pubescens* Lindl. (Fupanya, 2008; Klongklaw, 2008; Saisuwan, 2008; Thainurak, 2008; Thongsan, 2008; Uncharisanggard, 2008).

As for local vegetables, Suwanthada *et al*. (2004) characterized a group of *Basella alba* L. varieties collected from some areas of the Provinces of Chiang Mai, Chiang Rai, Khon Kaen and Lampang via acrylamide gel electrophoresis. The isozyme systems used were acid phosphatase, aspartate amino transferase, glutamate

dehydrogenase, malate dehydrogenase, shikimate dehydrogenase and superoxidase dismutase. Genetic relationship analyses among the varieties were able to divide the plants into 4 groups relevant to their morphological characterization.

Although plant characterization can be conducted in many ways, most scientists prefer to rely on those which are effective, accurate, convenient, applicable and sufficient to serve their objectives of study, as had been often reviewed.

3.1 Morphology of plant parts

Plant morphology is the study of the form and structure of plants (Hartmann *et al*., 1988; Kenneth, 1963; Raven *et al*., 1992). This study, sometimes alongwith that of development of the plants, have been widely used in taxonomial work for identification and classification of flora (Bhattacharyya and Johri, 1998; Chase, 2005; Hartmann *et al.*, 1988; Soltis *et al.*, 2005; Stuessy, 1990).

Morphological work, serving the purpose of identification of plant species, is usually done with the plant parts of root, stem, leaf, inflorescence, flower, fruit and seed. Particular plant parts are occassionally characterized in detail to classify specific groups of plants, especially for varietal, and/or clonal identification (Stuessy, 1990)

Tanming and Chantaranothai (2009) enumurating *Ficus* found in different parts of Thailand including some of introduced varieties indicated that morphological characters capable of classifying the plants were those of inflorescence and leaf while Kumnuan (2001) identifying strawberry hybrids depended on more distinct morphological characters of canopy density, colour of leaf and petiole, stipule shape, fruit shape, fruit skin colour, achene colour and achene position, to differentiate the hybrids.

Yapwattanaphun (1998) working on lychee (*Litchi chinensis* Sonn.) varieties grown in germplasm field at Chiang Rai Horticultural Research Centre, reported that specific morphological character that could distinctively identify 19 varieties of those lychee was that of the leaf.

Wichaipanich and Ramingwong (2004) carrying out morphological characterization of 20 clones of a longan variety, Daw, indicated that the colour of mature leaf and its petiole, the shape of fruit, the colour of rind and aril and the seed shape could successfully use in identify those clones.

Specimens of *Litsea cubeba* Pers., Ta-krai-ton (Thai), were collected from 3 different locations, i.e. Doi Angkhang and Doi Inthanon in Chiang Mai province and Phuka National Park in Nan province by Phupan (2003). She succeeded in classifying them via morphological characterization into 2 varieties. The distinct evaluation was based on the absence or presence of sericeous-pubescent on the leaf surface.

Chokthaweepanich (2002) morphologically classified 16 different types of *Curcuma* and 1 type of *Smithatris* (Zingberaceae) reported that these plants could be separated in groups based on the presence of their spur and anther crest. *Curcuma* was classified into 2 groups according to the spur morphology and within each group the plants could be divided into sub-group by fusion of the bracts and from the bract colour.

3.2 Pollen morphology

Palynology, according to Kenneth (1963) and Simpson (2006), is the study of spores and pollen grains of which having a number of morphological and ultrastructural features substantiable to refer phylogenetic relationships of plants. These features can often be used to identify particular plant taxon.

3.2.1 Terminology

The terminology applied to pollen morphology and ultrastructure varies from author to author. Simpson (2006) or stated otherwise, referring to those of Punt *et al.* (1994), Reitsma (1970) and Walker and Doyle (1975), described some of the compulsory terminologies as follows:

3.2.1.1 Pollen unit

Pollen unit refers to the number of pollen grains united together at the time of release. Single, unfused pollen grains are called **monads**, found

in the great majority of angiosperms. Pollen grains rarely fused in pairs, each of the pair is known as a **dyad**. More commonly, the four haploid products of meiosis remain fused together, comprising a **tetrad**. Pollen grains that are connate in precise unit of more than four are called **polyads**, usually consist of a multiple of eight fused grains. Fusion of pollen grains in large, often irregular numbers, but less than an entire theca, are called **massulae.** The fusion of all pollen grains of an entire theca is called a **pollinium.**

3.2.1.2 Pollen polarity

Pollen polarity refers to the position of one or more apertures relative to a spatial reference, a polar axis. This polar axis is the extended pollen grain diameter that passes through the centre of the original pollen tetrad (Figure 1). The intersection of the polar axis with the grain surface near the centre of the tetrad is the **proximal pole**. This pole is the surrounding area being the proximal face or proximal hemisphere. The pole which is away from the tetrad centre is the **distal pole.** This pole is the surrounding area being the distal face or distal hemisphere. The intersection with the pollen surface of a plane at a right angle to the polar and passing through the centre of the grain defines the pollen **equator**, the surrounding area being the equatorial region. Observing a pollen grain from the direction of either pole is a polar view while that from the equatorial direction is an equatorial view.

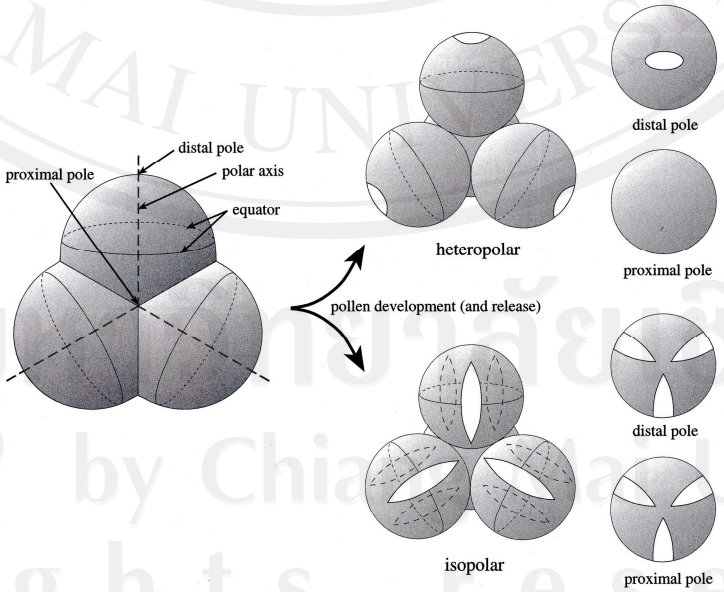

Figure 1 Pollen polarity (Simpson, 2006)

The general types of pollen polarity are **isopolar**, **heteropolar** and **apolar.** The isopolar is the type in which the two polar hemispheres are the same but can be distinguished from the equatorial region. The heteropolar is the type that the two polar hemispheres are different, because of differential displacement of one or more apertures while the apolar refers to the type that polar and equatorial regions cannot be distinguished, after pollen grain separation from the tetrad, as also seen in Figure 1.

3.2.1.3 Pollen aperture

A **pollen aperture** is a specially delimited region of the pollen grain wall. Its function primarily is to serve as the formation site of a pollen tube exiting from the pollen grain body. Pollen aperture type refers to the shape, number, position and arrangement of the aperture(s) of a pollen grain. Two general types of apertures, **colpus** and **porus**, correspond to shape. A colpus is an elongate aperture with a length/width ratio of greater than 2:1. Colpi can be elliptic, oblong or fusiform in outline shape. A porus is a circular to slightly elliptic aperture with a length/width ratio of less than 2:1. An elongate aperture similar in shape to a colpus occurring at the pole is called a **sulcus** while that similar in shape to a porus, also occurring at the pole, is called an **ulcus**.

3.2.1.4 Pollen symmetry

Pollen symmetry is generally either **radially symmetric**, i.e. with two or more planes of symmetry, or **bilaterally symmetric**, with a single plane of symmetry.

3.2.1.5 Pollen size

Pollen size can vary tremendously across taxa. Size is typically measured in terms of both the polar and equatorial diameters. Typical pollen grains are ca. 25-50 μm in diameter, but pollen diameter can range from < 5 μm to > 200 μm. Erdtman (1972) allocated the size of the pollen into 6 groups as follows:

very small pollens	**(perminutae : PI)**	**< 10**	**µm**
small pollens	**(minutae : MI)**	**10-25**	**µm**
medium size pollens	**(mediae : ME)**	**25-50**	**µm**
large pollens	**(magne : MA)**	**50-100**	**µm**
very large pollens	**(permagnae : PA)**	**100-200**	**µm**
gigantic pollens	**(giganteae : GI)**	**> 200**	**µm**

3.2.1.6 Pollen shape

Pollen shape refers to the three-dimensional shape of a pollen grain, e.g., boat-shaped, ellipsoid, fusiform or globose/spheroidal. Shape may be assessed by the two-dimensional outline shape either in polar view or equatorial view. A series of pollen shape classes based on the relation between the length of the polar axis (P) and of the equatorial diameter (E), termed P/E ratio (Erdtman, 1972) is shown in Table 1.

Table 1 Pollen shape classes and the ratio of polar diameter (P) and equatorial diameter (E) (Erdtman, 1972)

Shape classes	P/E ratio
Peroblate	< 0.500
Oblate	0.500-0.750
Subspheroidal	0.750-1.333
suboblate	0.750-0.875
oblate spheroidal	0.875-1.000
prolate spheroidal	1.000-1.143
subprolate	1.143-1.333
Prolate	1.333-2.000
Perprolate	> 2.000

3.2.1.7 Pollen sculpturing

Pollen sculpturing refers to the external features of the pollen wall. Specialized pollen sculpturing terms include: **baculate**, having rod-shaped elements; **clavate**, having club-shaped elements; **echinate**, having spinelike elements; **fossulate**, having longitudinal grooves; **foveolate**, having pitted surface caused by pores in the surface; **gemmate**, having globose or ellipsoid elements; **psilate**, having smooth sculpturing; **reticulate**, having netlike sculpturing; **rugulate**, having irregular to sinuous, tangentially oriented elements; **spinulose** or **scabrate**, having spinelike elements < 1 μm long; **striate**, having thin, cylindrical, tangentially oriented elements and **verrucate**, having short, wart-like elements.

3.2.1.8 Pollen wall structure

Pollen wall structure refers to the internal form of the pollen wall. Mature pollen walls almost always consist of two major layers: **intine** and **exine**. The intine is the innermost layer, which is composed primarily of cellulose and pectines. The exine is the outermost, desiccation-resistant wall layer. The exine of many taxa may be divided into two layers, an inner **endexine** and an outer **ektexine**. The endexine typically forms a more or less homogenous inner layer while the ektexine may exhibit a variety of structural forms of **tectum**, **columellae** and **foot layer.** The layers of tectum and columellae are sometimes called **sexine** and the rest of the wall is called **nexine**. The intine is composed of 2 layers, the one attached to the endexine is called ektitine and the other is called enditine, as shown in Figure 2.

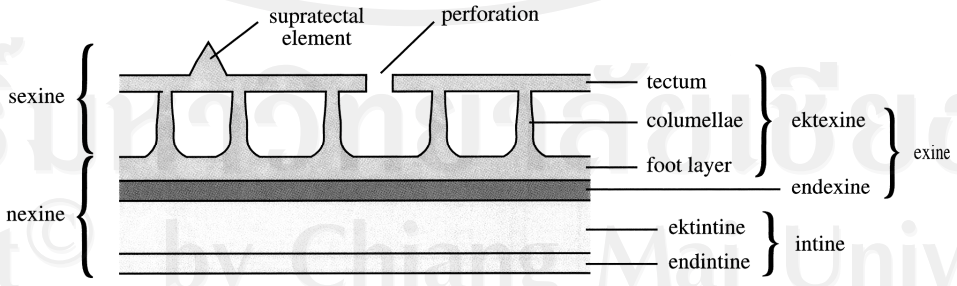

Figure 2 Pollen wall structure (Simpson, 2006)

3.2.2 Morphological studies of pollen

Agashe and Caulton (2009) acknowledged pollen morphology as a tool for precise identification of plant species. Morphological characteristics of the pollen has been widely used by systemic botanists in plant identification at high taxonomic levels, applicable for phylogenetic research (Kantachot, 2008). Such characteristics can specify varietal characters of plants and often readily identify to the genus level, sometimes to the species level, if adequate reference material is available (Cutler *et al.*, 2008). Similar to other plant parts, pollen characters are so varied that the classification system of plants can be built up entirely on the basic morphology and ultrastructure of the pollen (Agashe and Caulton, 2009; Fægri and Iversen, 1989; Moore *et al.*, 1991; Ruksat, 1991). Raven *et al.* (1992) specifically indicated that sculpturing of the pollen walls is very exact and distinctively different from one species to another.

Pollen morphology can be studied through proper techniques (Agashe and Caulton, 2009; Fægri and Iversen, 1989). The pollen grains can be obtained from different sources such as from living plants, from herbarium specimens or even from fossils. The efficient method of pollen preparation for morphological observation suggested by several authors (Agashe and Caulton, 2009; Erdtman, 1972; Fægri and Iversen, 1989; Kermanee, 2008; Moore *et al.*, 1991; Ruksat, 1991; Shivanna, 2003), was that of acetolysis. Pollen samples collected from the source are placed in vials at low temperature or stored in the vials containing the chemicals such as 95% ethanol, glacial acetic acid, 4% formaldehyde or Karnovsky's fixative if kept at room temperature. Consequently these pollen grains are subjected to serial steps of Erdtman's acetolysis. Via this technique, the protoplast, the intine and the pollen coat substances are thus removed, leaving only the exine of the pollen grains. The acetolyzed pollens then show their surface features, such as ornamentation, apertures and exine stratification (Agashe and Caulton, 2009; Shivanna, 2003). The reagent used in acetolysis, i.e. concentrated sulphuric acid and acid anhydride are not only corrosive but also react vigorously with water, thus precautions must be seriously considered (Moore *et al.*, 1991).

Morphological criterias required in pollen identification of plant taxa are very subtle and very often difficult to perceive under light microscope (Fægri and Iversen, 1989; Moore *et al.*, 1991), thus very fine details of the objects could only be obtained under powerful microscopes. Moore *et al.* (1991) stated that electron microscope utilizes a beam of electrons rather than light illumination. The beam has a much shorter wavelength thus greatly increasing the resolution of the microspore.

In recent years, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have provided powerful techniques for studying the finer details of the pollen exine (Shivanna, 2003). The exine stratification is best studied by making ultra thin sections and examining them under TEM while the exine surface is three-dimentional viewed with precise details from SEM (Agasha and Caulton, 2009).

Several authors reported various techniques suitable for pollen preparation for TEM and SEM studies. Some recent examples are seen from the plant specimens belonging to Amaryllidaceae (Arayakitcharoenchai and Suwanthada, 2010, 2011; Dönmez and Işik, 2008), Asclipiadaceae (Bhattacharyya and Johri, 1998; Lawrence, 1951; Li *et al.*, 1995; Wongsawad *et al.*, 1996), Basellaceae (Bhattacharyya and Johri, 1998; Erdtman, 1972; Eriksson, 2007), Callitrichaceae (Cooper *et al.*, 2000), Celastraceae (Perveen and Qaiser, 2008), Compositae (Bunwong and Chantaranothai, 2008), Euphorbiaceae (Kunwasee *et al.*, 2009), Iridaceae, Ixialiriaceae (Dönmez and Işik, 2008), Liliaceae (Pehlivan and Özler, 2003; Wongsawad *et al.*, 1996), Nelumbonaceae (Kreunen and Osborn, 1999), Polygalaceae (Krachai *et al.*, 2009) and Zingiberaceae (Paisooksantivatana and Thepsen, 2001; Sotthikul and Apavatjrut, 2003). The techniques of preparing the specimen pollen suggested most was that via acetolysis. The pollen was acetolyzed, dehydrated and air-dried before placing them on the stub with adhesive tape. The pollen grains were coated with heavy metal, e.g. gold, palladium or a mixture of the two, before investigating under SEM. Some authors chose the modified method of placing fresh pollen grains directly on the stub without acetolytic treatment (Celik *et al.*, 2005; Wongsawad *et al.*, 1996).

As for Amaryllidaceae, palynological studies are rather scarce (Alves-Araújo *et al*., 2007). Erdtman (1972) illustrated pollen morphology of several taxa distributing among several families of angiosperms, including approximately 90 species in 60 genera of classified Amaryllidaceae. Among these were *Crinum* (Erdtman, 1972; Huang, 1972), *Galanthus* (Dönmez and Işik, 2008; Sahin, 2000), *Hippeastrum* (Alves-Araújo *et al*., 2007), *Hymenocallis* (Huang, 1972; Meerow and Dehgan, 1985), *Leucojum* (Dönmez and Işik, 2008), *Lycoris* (Ren *et al*., 1995), *Narcissus* (Chen and Ueda, 1977; Dönmez and Işik, 2008), *Pancratium, Sternbergia* (Dönmez and Işik, 2008) and *Zephyranthes* (Alves-Araújo *et al*., 2007).

Arayakitcharoenchai and Suwanthada (2010; 2011) investigating pollen morphology of 2 diploid and 6 tetraploid *Hippeastrum* via SEM reported that the pollen of all varieties were of ellipsoidal monads with different sizes ranging from 81.20 to 121.24 μm and from 30.25 to 38.36 μm in length on polar axis and equatorial axis, respectively. Significant variation was found in sculpturing of the pollen grains of different specimens.

Bhattacharyya and Johri (1998), Lawrence (1951), Li *et al.* (1995) and Wongsawad *et al.* (1996) studying pollen morphology of *Gymnema inodorum* Decne., Asclepiadaceae, revealed that the pollen grains of this plant aggregated into a waxy pollinium of which united in pairs of oblong shape. Wongsawad *et al.* (1996) indicated further that the pollen portrayed the shape of circular-lobate from the polar view and the suboblate from equatorial view. The aperture was dicolpate and the surface was fine-knobby.

Bhattacharyya and Johri (1998) and Eriksson (2007) observing morphological characters of the pollen of *Basella alba*, Basellaceae, found that the pollen grain of this plant was a perfectly cuboidal, hexacolpate with reticulate, and non-spinulose tectum. Erdtman (1972) investigated another species of *Basella, B. rubra*, described the pollen having a shape of a dice (length of sides 30 μm). The sexine consists of low elements forming a fine reticulum.

Cooper *et al*. (2000) working on an aquatic family of dicots, Callitrichaeceae, using SEM and TEM, described the comparative pollen morphology and ultrastructure of 13 *Callitriche* species that the pollen from all taxa obtained similar features but the pollen sculpture and their exine thickness varied. The palynological data produced from this study revealed correlation between the growth habits and pollination biology of the species.

Kunwasee *et al.* (2009) examined pollens of 68 genera and 216 euphorbiaceaous species found in Thailand. The pollens were obtained from fresh and herbarium specimens. Using the TEM and SEM, they concluded from a conspicuous morphological diversity of the pollens that 61 pollen types were classified from apertural system and ornamentation of the exine.

Pehlivan and Özler (2003) studied the pollen grains of 14 species of *Muscari* (Liliaceae), with 5 of them endemic to Turkey under LM, SEM and TEM. They stated that the pollens from all species were of monad. They were monosulcate, isopolar and of bilaterally symmetry. The species *M. coeleste* sometimes shed the pollen as dyads. Characteristic structures of pollen ornamentation were generally reticulate and suprareticulate with different details among the species.

Boonlert (1994) making an observation in Chiang Mai University on pollen morphology of some trees identified the plants into 74 species in 25 families and 56 genera. The results showed that most of the pollens were monad, with the size ranging from 12.25 to 131 μm. The shape of the pollens varied, i.e. spherical, globose or ovoid. With the respects to shape, number and position of aperture, the pollens were classified into 9 forms, inaperturate, monocolpate, 3-coplate, 3-colporate, 4-colporate, 3-porate, 4-porate, periporate and polyads. Almost all of the pollens obtained reticulated surface.

Paisooksantivatana and Thepsen (2001) working on phenetic relationships of some Thai *Curcuma* species (Zingiberaceae) based on morphological, palynological and cytological evidences described some of distinguished pollen characters of the plants. The pollens of all species were inaperturated except one species, *Curcuma alismatifolia* Gagnep., being monoporate. The shape of the pollens could be spherical, oblate-spheroidal or sub-oblate. The exine was either granulated or psilated.

3.3 Internal structure of plant parts

Plant anatomy, a study dealing with internal structures of plant organs (Esau, 1977; Evert, 2006), plays important role in the understanding of plant biology (Yeung, 1998). This study reveals development of plant structure and physiological processes involved (Fahn, 1977; Little and Jones, 1980; Santanachote, 2002). Working on plant systematics, Cutler *et al.* (2008) indicated that external morphology is sufficient to botanically identify plant species provided that the complete plant specimens are available. When morphological features of the plants are indistinct anatomical characters can take part to help identify them with a certain accuracy since anatomy of the plants, according to Esau (1965), is a result of evolutionary specialization of a long duration.

According to Cutler *et al*. (2008), Kantachot (2008) and Stuessy (1990), studies concerning internal structure provide not only significant information for identification and classification of plant species but also contribute anatomical data useful in solving problems concerning genetic relationship among them by giving evidences on homologies of anatomical character status capable of suggesting evolutionary interpretation.

3.3.1 Histological techniques

Internal structures of plants are generally investigated under microscopy. Sections of plant parts made for histological investigation are required to be very thin to allow precise inspections. Such sections can be prepared by hand or with the aid of the machine known as microtomes, depending upon the nature of the specimens and the objectives of the practitioners (Yeung, 1998).

Free hand sectioning is acknowledged as the simplest method of preparing specimens for microscopic viewing. It allows researchers to examine the specimens in a few minutes since the preparation is only temporarily and fixation of the materials is not generally required. This method is usually reliable not only for soft tissues but also for a variety of plant materials such as soft herbaceous stems and small woody twigs (Berlyn and Miksche, 1976; Yeung, 1998). However, successful sectioning yielding good sections of even thickness is limited to skillful technicians who can handle the razor blades effectively (Johansen, 1940).

Although the term “free-hand sections” was originally applied to sections which were cut by means of a razor from material held in the hand or placed against a length of elder pith, it also includes the sections cut from unembedded live or preserved materials via sliding microtome. As a mater of fact, all sections handled loosely and unattached to glass slides are also considered as free hand sections. In addition, with this sliding microtome, it has been made possible for technicians to produce the sections of hardwood specimens (Johansen, 1940).

Sections prepared by the techniques mentioned above are usually too thick, no thinner than 30 μm, for perfect histological studies. Thus, researchers prefer to rely on paraffin embedding technique and cut their embedded samples with rotary microtome to obtain very thin sections. Modifications of such microtechnique to obtain good sections of fresh plant tissues, e.g. fixing, dehydrating, embedding and staining, have widely been made by individual researchers to suit their objectives (Johansen, 1940; Keating, 1996; Kermanee, 2008; Sass, 1966).

Staining is considered a very important step of the section preparation. Modifications of staining procedure have been tremendously taken part by technicians to improve and enhance the visibility of their sectioned tissues through wide range of stains available for specific purposes (Hodson and Acuff, 2005; Kermanee, 2008). Stains used for plant tissues are of two types, according to their sources, i.e. natural dye and synthetic dye. Natural dyes are made from natural materials, e.g. plants, animals and minerals. Among these, only three natural dyes are used by botanical technicians, i.e. brazilin, hematoxylin and cochineal and its derivatives (Johansen, 1940). **Brazilin** is obtained from the plants belonging to Caesalpiniaceae, but principally from *Caesalpinia crista* or *C. echinata*. It has come into extensive use as a stain for smears. **Hematoxylin** is a chromogen derived from logwood (*Hematoxylin campechianum* L.) and is one of the most important of all stains. It is a homologue of brazilin, possessing one or more hydroxyl group in its chemical constitution. The dye solution itself has little or no affinity for tissues, unless iron or aluminum is present, consequently mordanting in some form is necessary. **Cochineal** is a yellow-red powder obtaining from the female cochineal insects. The dye itself is not used by botanists to any great extent but its derivative, **carmine**, is better known and more ulitilized. Another type of dye is abundant in member. These synthetic dyes are used both as single stain or in combinations. The widely used ones are, for example, acid fuchsin, anilin blue, basic fuchsin, crystal violet, eosin, erythrosine, fast green, methylene blue, neutral red, orange G, safranin O and sudan (Johansen, 1940; Kermanee, 2008; Ruzin, 1999).

Attempts had been made in Thailand to develop natural dyes for plant tissue staining as seen from the work of Suwattanacoupt *et al*. (2009). The authors extracted dyes from jack-fruit (*Artocarpus heterophyllus* Lamk.), lipstick tree (*Bixa orellana* L.), sappan tree (*Caesalpinia sappan* L.), coconut (*Cocos nucifera* L.) and turmeric (*Curcuma longa* L.), using 5 mordants, i.e. alum, cupper sulfate, ferrous sulfate, sodium chloride and cobalt nitrate. Staining of each dye were tested with stem transverse sections of *Vernonia cinerea*. Good results were obtained from all dyes with different degrees of satisfaction.

3.3.2 Anatomical studies

Anatomical studies, especially those of comparative, provide characters which are taxonomical useful in identification and classification of plant species (Cutler *et al*., 2008; Kantachot, 2008). Such studies are commonly carried out in several plant parts, i.e. root, stem, leaf, flower, fruit, seed or pod in a large number of plant species.

Padmini and Shanmukharao (1995) investigated structure, distribution and taxonomic importance of foliar stomata in 45 taxa belonging to 19 genera of Indian Amaranthaceae. They reported that, in all, six stomatal types could be recognized, i.e. anomocytic, anisocytic, diacytic, paracytic, hemiparacytic and brachyparacytic. The majority of the taxa were amphistomatic while hypostomatic leaves were confined to only three taxa. Stomatal diversity was common but most of the taxa showed either dominance or codorminance. Stomatal distribution was helpful in distinguishing the three tribes of the family. The tribe Celosieae revealed exclusive presence of anomocytic and anisocytic stomata while Amarantheae and Gymphreneae showed other stomatal types, i.e. paracytic and diacytic in addition to anomocytic and anisocytic stomata. Moreover, the latter two tribes were each distinguishable into two subtribes on the basis of stomata.

Systematic root anatomy of Asparagales and some taxa formerly included in this order was studied and described by Kauff *et al*. in 2000. The authors mentioned that the presence of a dimorphic outer layer with long and short cells was known as widespread in monocotyledons indicating that it originated early in the monocot lineage whereas in Asparagales and Araceae this layer was hypodermal. They also suggested possible correlation between the presence of a velamen or a persistent rhizodermis in many Asparagales and Araceae and that of the dimorphic hypodermal layer. Many other root anatomical characters, such as the presence of vascular bundles in the central pith and a multi-layered schlerenchymatous cylinder, were as well considered being ermorphic and developed convergently.

Some histo-anatomical aspects concerning the leaf structure of *Basella alba* L. and *B. rubra* L. (Basellaceae) were studied by Busuioc and Ifrim (2004). Results analysed from the structure showed a few differences between the two taxa, i.e. the outline of transverse section of the petiole, the petiole vascular bundle and the lamina mesophyll.

Cutler (1992) studied vegetative anatomy of some Ophiopogoneae (Convollariaceae), i.e. *Ophiopogon*, *Liriope* and *Peliosanthes*, focussing on that of the leaf. Observations under LM and SEM provided a syndrome of leaf characters including epidermal features, hypodermal fibre-like cells; raphides and unusual short, square-ended prismatic crystals; phloem with abundant sclerenchyma and frequent individual strands each composed of a sieve tube element and its associated companion cell, and vascular bundles with unusual orientation which indicated the very close inter-relationship between *Ophiopogon* and *Liriope*. *Peliosanthes* also showed the phloem type, hypodermal fibre-like cells and raphides, but was less similar in epidermal characters and vascular bundle orientation. The significance of the unusual phloem type was thus considered in relation to similar types in other members of the Liliiflorae.

Comparative anatomy of six genera and 11 species in Cucurbitaceae: *Cucumis melo* L., *C. sativus* L., *Cucurbita moschata* Duchesne, *C. pepo* L., *Diplocyclos palmatus* (L.) C. Jeffrey, *Luffa acutangula* (L.) Roxb., *L. aegyptiacea* Mill., *Momordica charantia* L., *M. cochinchinensis* (Lour.) Spreng., *M. subangulata* Blume and *Trichosanthes cucumerina* L. were studied by Khamphio and Thammathaworn (2008). The plant parts investigated were petioles, stems and tendrils. Transverse sections of these organs were prepared via paraffin embedding method to construct the key to species based on anatomical characters of the organs. It revealed that bicollateral bundles, glandular and non-glandular trichomes, anomocytic stomata, presence of perivascular fibre in the stems and calcified crystals accumulated in the organs were the common characters of the studied species. The anatomical characters that could be used for species identification were those of the trichome shape, epidermal cell shape on adaxial epidermis, presence or absence of

stomata on adaxial epidermis, presence or absence of lithtocysts and cystolith shape appeared in the leaf blades.

Srinual *et al*. (2008) reported the wood anatomical characters of the genus *Vatica* L. (Dipterocarpaceae) in Thailand gathered from the sections produced via sliding microtome of which could be used as a tool for species identification of the plant. The result showed that the following features were commonly found in all species of the genus; 1) indistinct growth ring boundaries, 2) solitary vessel, 3) diffused porous-wood, 4) intervessel pits with opposite, alternate and scalariform; 5) ray parenchyma with procumbent and upright cells, 6) the presence of resin canals and 7) the presence of starch grains in ray parenchyma cells. The wood anatomical characteristics capable of distinguishing the species were 1) perforation plates types, 2) axial parenchyma arrangement, 3) ray parenchyma arrangement, 4) resin canal arrangement, 5) presence or absence of septate fibre and 6) prismatic crystals in parenchyma cell. In addition, these characters could be used to classify the genus into groups.

Study on comparative anatomy of *Litsea cubeba* Pers., Ta-krai-ton (Thai), Lauraceae, collected from 3 different locations, i.e. Doi Angkhang and Doi Inthanon in Chiang Mai province and Phuka National Park in Nan province was carried out. It can be concluded from morphological study that the plant could be classified into 2 varieties, *cubeba* and *formosana* by the presence or absence of sericeous-pubescent on the leaf surface, respectively. From anatomical study, the stems in transverse sections showed the similarity of the arrangement of tissue system. The leaves of all specimens possessed paracytic type of stomata on the abaxial surface and oil cells in palisade layer. The number of stomata per square millimetre and the stomatal index of the specimens in each location were different. The fruit wall of all specimens were distinctly separated into exocarp, mesocarp of several parenchymatous layers and endocarp of many oil cells (Phupan, 2003).

Jaturat (2000) collected 26 local legume seeds from the provinces of Chiang Mai, Nan and Mae Hong Son. The plants grown from these seeds were then

morphologically and anatomically studied. It was shown that there were differences in external structures of each sample, in growth habit, shape, size, number and colours of leaves, flowers, fruits and seeds. Anatomical differences were expressed in the number of layer and of collenchyma and fibre in cortex and the substances accumulated in the cells as well as the arrangement of the vessel in vascular bundles. The differences in layer and arrangement of sclerenchyma and type of vascular bundle in pod were also formed. Characteristic of cuticle, shape and length of macrosclereid, size and number of layers of lagenosclereid were unliked in anatomy of the seed coat. Upper and lower epidermis of the leaves were also studied and it revealed that stomata were of paracytic type and differences in number of stomata per square millimetre and stomatal index were found.

Dai and Liang (1991) reported the epidermal features of leaves in subfamily Ophiopogonoideae (Liliaceae). Thirty-nine species and one variety were examined under SEM. Transactions of stomatal apparatuses of 6 species were also made and examined under LM. It appeared that epidermal features of leaves were recognized as cuticular processes type, non-cuticular processes type and non-stomatal band type. The cuticular processes type could be further divided into three patterns, i.e. fibrillose, massive and wrinkled-massive. The taxonomic value of the epidermal features of leaves in Ophiopogonoideae was rather evident, i.e. 1) epidermal features were distinguishable among the species of *Ophiopogon*, *Liriope* and *Peliosanthes*, even in their vegetative state, 2) different patterns of cuticular processes could classify some species in *Ophiopogon*, and 3) epidermal features of leaves provided evidences for further discussion on relationships among *Ophiopogon*, *Liriope* and *Peliosanthes*. As for stomatal apparatus, it occurred that *Liriope*, *Ophiopogon* and *Peliosanthes* were of the anomocytic type.

Talingtaisong (2008) concluded from his study on comparative anatomy of the leaves of 9 species of *Lagerstroemia* L. (Lythraceae) that from epidermal peels and transverse sections all species have bifacial and hypostomatic leaves with anomocytic stomata. Features regarded as particularly distinctive included 1) absence of trichomes, and presence of branched/unbranced trichomes, 2) presence

of secretory cells in adaxial cuticle layer only or both adaxial and abaxial, 3) shapes of abaxial epidermal cells in rectangle to hexagonal with smooth surfaces or in amorphousness with rough surfaces, 4) presence of idioblasts in the mesophyll, and 5) bicollateral midveins of heart-shaped, U-shaped and C-shaped. These epidermal features and anatomy of the leaf therefore provided taxonomically characters capable of species classification.

Phattalamanon (2000) worked on systematic leaf anatomy of 48 species of Orchidaceae via peeling and paraffin embedding. The result revealed that the plants could be classified into 4 groups based on presence of trichome, fibre in mesophyll, palisade layer, cuticular sculpturing, stegmata and bundle sheath.

Stern and Judd (1999) investigated comparative vegetative anatomy and systematics of 17 species of *Vanilla* (Orchidaceae) and described the results in detailed. Foliage leaves of *Vanilla* appeared glabrous, having abaxial, tetracytic stomatal apparatuses, and a homogeneous mesophyll. Species might or might not have a uniseriate hypodermis. Crystals occurred in the foliar epidermis of some species, but all species have crystalliferous idioblasts with raphides in the mesophyll. Vascular bundles in leaves were collateral and occurred in a single series alternating large and small. Sclerenchyma might or might not be associated with the vascular bundles. Scale leaves might be crescent or C-shaped and usually obtained abaxial stomatal apparatuses. A hypodermis might or might not be present; the mesophyll contained raphide bundles in idioblasts. Vascular bundles were collateral and occurred in a single row, sometimes aligned close to the adaxial surface. They could or could not be associated with sclerenchyma. Stems of leafy vanillas showed a sclerenchyma band separating cortex from ground tissue while none appeared in those of the leafless. Ground tissue of the stem could consist solely of assimilatory cells or mixed assimilatory and water-storage cells. In some species centrally located assimilatory cells were surrounded by layers of water-storage cells. A uniseriate hypodermis was present in all stems. Sclerenchyma could completely surround the scattered collateral vascular bundles, occurred only on the phloem side, or be absent. Both aerial and terrestrial roots were notable for their uniseriate velamen the cell walls of which may be

unmarked or ornamented with anticlinal strips. Exodermis was uniseriate with cells varied from barely thickened to strongly thickened but only the outer and radial walls were thickened. Cortical cells of aerial roots generally obtained chloroplasts that were lacking from the same tissue of terrestrial roots. Raphide bundles occurred in thin-walled cortical idioblasts. Endodermis and pericycle were uniseriate with the cells of O-thickened type located opposite the phloem. Cells of the endodermis were either O- or U-thickened, as well opposite the phloem. Vascular tissue might be embedded in thin- or thick-walled sclerenchyma or in parenchyma. Metaxylem cells were always wider in terrestrial than in aerial roots of the same species. Pith cells were generally parenchymatous but sclerotic in a few species.

Comparative anatomy of leaf of species representing the entire seven genera of Penaeaceae using LM and SEM was studied by Dickie and Gasson in 1999. It was declared that, due to variability and inconsistency, leaf anatomical characters were not regarded as particularly useful for systematics within or among genera in this family. Across the family, a number of taxa exhibited a trend towards amphistomatous, isobilateral leaves, generally associated with increased leaf thickness and amount of palisade mesophyll. This trend was not apparent in closely related families, e.g. Alzateaceae, Crypteroniaceae, Oliniaceae and Rhynchocalycaceae.

Lakoet (2004) studied comparative anatomy of leaf surface of 29 species from 23 genera of Thai grass (Poaceae). The authors reported the results of the investigation of epidermal leaf peels inspected from the light microscope that there were variations in presence or absence of cutin sculpturing on epidermis, the shape and wall of long cells, the shape of short cells, the number of basal cells on macro hairs, the type of micro hairs, the shape of subsidiary cells, the presence or absence of papillae and of silica bodies in long cells.

Gonzalez *et al*. (2004) described the leaf architecture (venation and cuticle) of the eight extant species of Argentinean Proteaceae and discussed the diagnostic value of the characters. All species have brachyparacytic stomata restricted to the abaxial surface of the leaves and multicellular trichomes. Cuticle features, that

differed among the species, were the presence or absence of glands and papillae, and stoma size in relation to epidermal cell size. Architectural and cuticular features were also useful for differentiating tribes, subtribes, genera and species within the subfamily Grevilleoideae. The authors basically concluded a key for the identification of Argentinean Proteaceae gathered from leaf and cuticle characters.

3.4 Cytogenetical and cytotaxonomical studies

According to Kenneth (1972) cytogenetics refers to the cytological aspect of genetics while cytotaxonomy concerns classification of organisms according to relationship discovered by cytological research. Jones and Luchsinger (1979), Soontornchainaksaeng (2005) and Stebbins (1971) stated that cytogenetical investigations are used to determine the taxonomic botany as well as breeding research. For taxonomic botany, chromosome numbers are generally applied in classification of plant species. The DNA information of each species is organized in a characteristic number of chromosomes which is a reliable indicator of the relatedness of similar species. Observation of chromosomes under microscopy can reveal much about their roles in inheritance, adaptation and evolution and also a small role in differentiation and development (Chulalaksananukul *et al.*, 2001). Chromosome information plays an important role in various usefulness especially in identification and classification using the numerical and structural differences of chromosomes and karyotype diversity in different taxonomic unit (Sharma and Sharma, 1999).

3.4.1 Chromosome techniques

Determination of number and type of plant chromosomes, known as karyotypic study, can be conducted from various meristematic tissues at metaphase stage of mitosis and meiosis (Chaiyasut, 1989; Singh, 1993; Stebbins, 1971). Appropriate preparation of meristematic tissues can yield accurate chromosome counts and other karyotypic recording. Normally, the tissues taken for somatic chromosome investigation are those from the root tip, the calyx base, young bud, the tip of young leaf or endosperm inside the seeds (Apisitwanich and Masuthon, 2000; Chaiyasut, 1989; Jones and

Luchsinger, 1979; Pignone *et al*., 1994; Roy *et al*., 2010; Withner, 1974) where mitotic dividing cells occur while reproductive tissues bearing microspore mother cells and megaspore mother cells are for meiotic studies (Apisitwanich and Masuthon, 2000; Baimai, 1993; Bootrat, 1985; Campiranon, 1997).

Cytologists devise cytological techniques in producing precise information on chromosome numbers and chromosome structures to examine and analyse genome in plant species (Soontornchainaksaeng, 2005). Squash and smear techniques were found to be the basic methods of handling mitotic chromosomes of plant species along with karyotypic analysis and meiotic configuration (Campiranon, 2003; Soontornchainaksaeng, 2005). Among chromosomal techniques, Feulgen's squash is the most popular one for karyotypic studies since it gives flattened chromosomes aligning at the same level easier for inspection (Campiranon, 2003; Dyer, 1979; Krasaechai, 1996).

Dyer (1979), Sharma and Sharma (1999) and Singh (1993) similarly described the basic principles for handling mitotic and meiotic chromosomes of plant species consisting collection, fixation, maceration and staining of specimens. Modification of the practice can be made depending upon crop species, objectives of the experiments and personal preference of technicians.

3.4.2 Karyotype analysis

Campiranon (2003), Chaiyasut (1989), Singh (1993) and Stebbins (1971) described karyotype as the exact haploid chromosome set of an organism and that karyotype is generally constant within a species, though modifications can occur along its evolution. According to these authors, karyotypic study is an investigation of the chromosome complement yielding the number and morphology of the chromosomes. Scientists generally produce mitotic chromosomes of organisms via squash methods of vegetative meristematic tissues as stated earlier. The karyogram, a physical measurement of the chromosomes in haploid set from photomicrograph where chromosomes are arranged in descending order from longest to shortest, and the idiogram which represents a diagrammatic sketch of the karyogram, are consequently constructed

(Stebbins, 1971). Such karyogram/idiogram provide the scientists with karyotype formulae, useful for indicating relatedness of similar species. Moreover, this karyotype formula of a plant species can also be used as its specific identity applicable for cytological characterization in the respect of cytotaxonomical study (Chaiyasut, 1989; Singh, 1993; Soontornchainaksaeng, 2005; Stebbins, 1971).

3.4.2.1 Chromosome morphology

Chromosome morphology is usually studied at the metaphase stage of mitosis, when chromosomes have become contracted to the maximum amount or nearly the maximum in their cycle, and when they are most easily stained. The principal landmarks which may be seen at this stage are the centromeres or kinetochores, to which the spindle fibres attached. Some chromosome pairs in the somatic complement of a species bear at one end a satellite which appears as a single small spherical body, or a pair of such bodies, attached to the remainder of the chromosome by a slender thread as illustrated in Figure 3. In most chromosomes, the centromere is localized in one particular region and chromosomes are designated as telocentric, acrocentric, sub-metacentric and metacentric chromosome in accordance with its position (Stebbins, 1971).

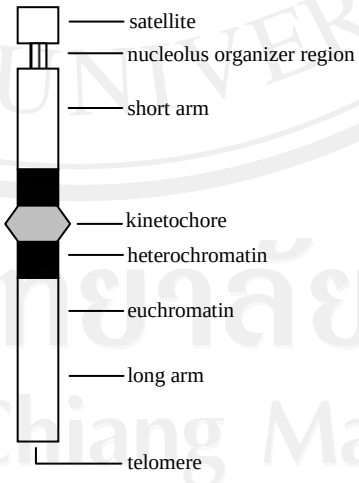

Figure 3 An idiogram of a metaphase chromosome (Singh, 1993)

3.4.2.2 Chromosome nomenclature

Singh (1993) discussed the statement of Levan *et al*. (1964) in his review that the centromere position is a very useful landmark for morphological identification and nomenclature of chromosomes. From such position an individual chromosome is divided by centromere into two opposite arms. These two arms can be equal or unequal in length. The longer arm is called the long arm while the other is the short arm.

Several scientists proposed the chromosome nomenclature based on the centromere position. Dyer (1979) and Levan *et al*. (1964) grouped chromosomes in categories as shown in Table 2 from their arm ratios of which calculated from the relative length (r) of the long arm (l) and short arm (s), i.e. r = l/s. But, other scientists included the centromeric index (CI) in the designation of the nomenclature (Chaiyasut, 1989). This CI referred to the ratio of the length of the long-arm (Ll) and the total length of individual chromosomes (LT). From these relative length of the chromosomes the idiogram of the chromosome complement are then produced.

Table 2 Nomenclature of chromosomes based on Dyer (1979) and Levan *et al.* (1964)

centromere position	arm ratio (l/s)		chromosome designations
	Dyer (1979)	Levan *et al*. (1964)	
median	1.0	1.0-1.7	metacentric (m)
submedian	1.0-3.0	1.7-3.0	submetacentric (sm)
subterminal	> 3.0	3.0-7.0	acrocentric (a)
terminal	∞	> 7.0 to ∞	telocentric (t)

Karyotypes of most plant species consist of the chromosomes which are comparable to each other in size, designated as large (L), medium (M) and small (S). The L, M and S chromosomes in each chromosome complement can be derived from the size arrangement of the chromosomes. In a karyogram of which the chromosomes are arranged in descending order from longest to shortest, the longest chromosome is allocated as the chromosome number 1 which is the largest (L) while

the shortest chromosome is that of the last number. In such arrangement the medium (M) chromosome refers to those obtaining the length less than that half of the average of the longest chromosome and the shortest chromosome while the small (S) chromosome are those having the length less than half of the average large chromosomes (Chaiyasut, 1989).

3.4.2.3 Karyotype formula

Karyotype formula describes a combination of various chromosome size in a chromosome complement of individual organism. Comparative analysis of karyotypic formulae compiling from the plant species can reliably indicate similarities and relatedness among them (Chaiyasut, 1989), as has been widely practiced by a large number of scientists.

3.4.3 Karyotypic studies in plant species

Karyotype investigation has been widely carried out in various plant species by scientists with more or less similar goals.

In Amaryllidaceae, Buathong and Suwanthada (2008) produced an appropriate protocol of root-tip preparation for chromosome investigation of Amazon lily (*Eucharis grandiflora* Planch. & Lind.) and reported somatic chromosome number of the plant being 2n = 68. Similar experiments were conducted with *Hippeastrum* and it was reported by Arayakitcharoenchai and Suwanthada (2001) that the successful procedure of root-tip squash done with a local variety and 2 commercial varieties of the plant could yield the chromosome numbers as 2n = 2x =22 and 2n = 4x = 44, respectively. When the same technique was applied to the hybrids of the two ploidies it revealed different tripoid numbers of 33, 34 and 36. Chawalid and Suwanthada (2003) studied the karyotype of 3 local varieties of *Hippeastrum* reported the chromosome number of the plants 2n = 2x = 22 and their karyotypic formulae as $2n = L_{10}^{a} + L_{2}^{sm} + M_{2}^{a} + M_{2}^{sm} + S_{4}^{m} + S_{2}^{sm}$; $2n = L_{2}^{sm} + L_{8}^{a} + M_{4}^{a} + S_{6}^{sm} + S_{2}^{m}$ and $2n = L_{4}^{sm} + L_{10}^{a} + M_{2}^{m} + S_{6}^{m}$.

Li *et al*. (1995) working on the basic chromosome numbers of plant species belonging to the family Asclepiadaceae reported the basic number of x = 8-12. Their finding was supported by that of Bhattacharyya and Johri (1998) claiming the x = 11. Soontornchainaksaeng (2005) reviewing the chromosome numbers of plants in Thailand included in her review the research result of 2n = 22 in *Gymnema inodorum* Decne. found by Suwanthada *et al*. (1998).

Gunjan and Roy (2010) studying the karyotype of dominant species of *Aloe* (Asphodelaceae) from eastern India reported that the karyotype analysis of *Aloe* showed that all species contained equal diploid chromosome number of 2n = 14. The morphological features of the chromosomes were mostly 8L and 6S, predominant with submedian, median and subterminal centromeres. *A. vera* and *A. indica* showed a relationship on the basis of karyotype formula ($S_6^m + S_8^t$) and other parameters with homogenous karyotypes, whereas *A. ferox* was considered to have heterogeneous karyotypes with the karyotype formula $S_4^m + S_{10}^t$ of more subtelocentric and telocentric chromosomes, distinguishable from the other 2 species.

Roy *et al*. (2010) investigated the chromosome number of 2 species of *Basella* (Basellaceae), i.e. *B. alba* and *B. rubra* through meiotic smears. They found that the haploid chromosome number of both species of *Basella* were 24. This result was supported by that of Bhattacharyya and Johri (1998) of which the basic chromosome number of the species belonging to the Basellaceae family was x = (11-) 12. Peter reported in the same year that the chromosome number of *B. alba* was 2n = 48 while that of *B. rubra* was 2n = 44. Another karyotypic study concerning *Basella alba* were done by Grasso *et al*. (1997). Apart from karyotypic studies, DNA analysis in three populations of *B. alba*, i.e. Congo Native, Congo Domesticated, and an introduced cultivar from Sri Lanka was carried out revealing the chromosome numbers ranging from 2n = 36 to 2n = 48.

Chromosome number and karyotype analysis of *Kalidiopsis wagenitzii* Aellen (Chenopodiaceae), an endemic halophytic plant of Turky, were studied by Gömürgen and Altinözlü (2005). It was found that the chromosome number of the plant

was 2n = 18. From karyotype analysis, it showed that the average length of metaphase chromosomes was 3.202 μm, varying from 2.653 to 3.860 μm and the total chromosome length was 31.786 μm. There were no satellite chromosomes. The arm ratio ranged from 0.609 to 0.825 μm. Eight of nine chromosome pairs had median centromeres while only one (the third) had submedian centromere.

As for Convallariaceae Conran and Tamura (1998) mentioned karyological aspects of the genus *Peliosanthes* in the respect of the chromosome number that the plants belonging to this genus obtained the basic number of chromosomes of x = 18. This information coincided with those stated by Chang and Hsu (1974), Chen and Tamura (2000), Jones and Smith (1967), Larsen (1966) and Sato (1942) that *P. teta* (Liliaceae) contained the chromosome number of 2n = 36.

Cytogenetical study of Euphorbiaceae in Thailand was conducted by Soontornchainaksaeng *et al*. (2004) from natural populations in several parts of the country. The number and morphology of the chromosomes of 45 genera 161 species, 14 cultivars and 33 unidentified specimens were investigated using modified Feulgen's squash or propiono-carmine squash techniques. It was reported that the members of this family showed a great diversity of chromosome numbers between and within genera ranging from 2n = 16 (*Croton hirtus*) to 124 (*Codiaeum variegatum*). Most species obtained very small chromosomes. Polyploids were found in both natural groups and cultivated plants. Chromosome numbers of more than 140 species were recorded for the first time. The authors claimed that the fundamental data obtained from their research was valuable, giving substantial support to systematics and phylogenetics of the plants.

Sunarin (1999) investigating the chromosome of some varieties of Thai rice (*Oryza sativa*) reported that two varieties of white rice, i.e. Neaw San Pa Tong and Khao Dok Mali 105 and three of the purple rice, i.e. Kham Doi Saket, Kham 88468 and Kham 87046 obtained the same number of chromosomes, 2n = 24. The karyotype analysis showed that, in general, metacentric and submetacentric

chromosomes were found in all varieties and that subtelocentric chromosomes were found only in Khao Dok Mali 105.

Seijo and Fernández (2003) studied the karyotypes of 10 species and 1 variety of South American *Lathyrus* (Leguminosae) comparing to those obtained from 5 entities from the Northern Hemisphere. The authors claimed that although all the species contained a chromosome number of 2n =14, but they could be differentiated by their karyotype formula and quantitative parameters of karyotypes. Arzani, later in 2006, studied the karyotype of 20 accessions of cultivated grass pea (*L. sativus* L.) and some wild species of *Lathyrus* L. of Iran. It turned out that all populations studied were diploid with 2n = 2x = 14. There were significant variations among populations in the number of metacentric, submetacentric and subtelocentric chromosomes, the longest and the shortest length, total haploid complement, arm ratios and CI.

Silayoi and Sompen (1991) carrying out the karyotype analysis of 30 accessions of Thai bananas (*Musa* spp.), both of wild and cultivated, indicated that among the accessions tested 11 were diploid, 18 were triploid and only one was tetraploid. The number of metacentric, submetacentric, subtelocentric and telocentric chromosomes in the complement varied with accessions. The length of the chromosome ranged from 1.22 to 3.93 μm.

Chromosome investigation of some terrestrial orchids was widely carried out as seen in the examples of *Eulophia*, *Liparis* and *Malaxis*. Prarasri and Suwanthada (2006) using modified root-tip squash technique to perform the chromosome count of *Eulophia graminea* Lindl. reported that the plant obtained the chromosome number of 2n = 56. Fupunya and Suwanthada conducted the chromosome studies of *Liparis siamensis* Rolfe ex Downie and *Malaxis latifolia* J. E. Sm., later in 2008. The chromosome counts of the 2 species were stated to be 2n = 42 and 2n = 40, respectively.

Root-tip squash of two species of Sapindaceae, *Dimocarpus longana* Lour. (longan) and *Litchi chinensis* Sonn. (lychee), were conducted to investigate their chromosomes. Ramingwong *et al*. (1998) came up with the suitable procedure in obtaining

cells with mitotic metaphase and reported the chromosome number of longan being 2n = 30. Yapwattanaphun and Ramingwong (1999) studying karyotype of 19 lychee varieties claimed that all of them had the same chromosome number of 2n = 30. They also reported that varietal identification of those lychees could be made based on the size and feature of the chromosome complement of each variety.

Paisooksantivatana and Thepsan (2001) investigated chromosomes of 14 different types of Thai *Curcuma* (Zingiberaceae). They devided the plants into 2 categories based on their chromosome number. The first category consisted of the species with the basic chromosome number of x = 21, having the somatic number of 2n of 42 or 63. These species included *C. aeruginosa* (2n = 63), *C. aromatica* (2n = 63), *C.* cf. *comosa* (2n = 63), *C. longa* (2n = 63), *C. parviflora* (2n = 42), *C. petiolata* (2n = 42), *C. roscoeana* (2n = 42) and *C. zedoaria* (2n = 42). The second category consisted of an assemblage species with the chromosome number varied from 2n = 20 (*C. harmandii)* to 2n = 32 (*C. alismatifolia*), 2n = 36 (*C. thorelii*), 2n = 40 (*C. gracillima*) and 2n = 40 (*Curcuma* 'Bualailao').

3.5 Chemotaxonomy

Chemotaxonomy, also known as chemosystematics, biochemical systematics and comparative phytochemistry, refers to a classification of plants by means of chemistry and biochemistry. The compounds of chemotaxonomic interests, both of primary and secondary metabolic, are, for example, phenolic compounds, nitrogen containing compounds, terpenes, sugars and their derivatives. Each of the compound groups requires specific detection technique. Particular information derived from detected compounds is valuable for identification and classification of plants (Sriprasertsak *et al.*, 1987).

3.5.1 Isozyme analysis

Paisooksantivatana *et al*. (2001) reviewed applications of isozymes in evaluation of genetic variation in plants, relevant to their work on isozyme analyses revealing genetic diversity among natural and cultivated populations of *Curcuma alismatifolia* Gagnep. (Zingiberaceae) in Thailand. The authors referred to those reviews that isozymes were discovered by Hunter and Markert way back in 1957 but received little attention among plant geneticists until after the discovery done by Lewontin and Hubby in 1966 on variations within the same population of *Drosophila* via isozyme analyses. Since then isozyme had become a powerful tool for evaluation of genetic variation in plants of both intra- and inter-population levels. They were used successfully for identification of closely related crop cultivars (Wendel and Parks, 1983) as well as for detecting genetic diversity and phylogenetic relationships of both cultivated and wild species (Crawford, 1990).

Schlegel *et al*. (1989) working on European orchids described the problems arising in some groups of the orchid species having high potential of hybridization that evolutionary changes among their populations occurred so rapidly that the reconstruction of phylogenetic relationship in each group of well-known species was extremely difficult. It was thus necessary to search for additional species-specific characters for the analysis of taxonomic, systematic and evolutionary problems. With the development of biochemical methods of protein and nucleic acid analyses these methods were applied to the studies of systematics and evolutionary problems in a wide range of organisms. Among them, enzyme analysis by starch gel electrophoresis proved to be an effective tool allowing comparison of many samples in a relatively short time. Moreover, the substrate specific of the enzyme reactions make it possible to compare homologous gene products, which are essential for genetic and phylogenetic analyses. From the above mentioned principles, Steinbrück *et al*. (1986) attempted on characterization of interspecific hybrids between *Orchis mascula* and *O. pallens* by enzyme electrophoresis and found that such method could provide a suitable tool for species identification and for the analysis of the hybrids of two closely related species.

Lawrence (2008) stated that gel elctrophoresis is a widely used electrophoretic technique, efficient for separation of proteins or nucleic acid fragments. The gels mostly applied are those of agarose, starch and acrylamide. Of all these, acrylamide gel is more favourable among the technicians for its suitable properties. It is a synthetic polymer, acrylamide monomer, thus uncontaminatable. In term of size, the gel pores are homogenous and adjustable. Other reliable qualifications of this gel include stabilities towards pH, temperature and ionic strength. Moreover, the gel is non-electroosmosis, thus no reaction with sample molecules can occur. Since the gel is translucent clear perception of the sample molecules is therefore affordable (Chokthaweepanich, 2002; Soontaros, 2006).

3.5.2 Isozyme analysis research

Isozyme analyses have been used to generate valuable information in various related fields of genetical studies, e.g. population genetics, plant systematics, determination of phylogenetic relationships, biodiversity and germplasm management (Belay and Furuta, 2001; Paisooksantivatana *et al*., 2001). Extraction of the plant proteins for the analyses are usually prepared from several plant parts, i.e. root, stem, leaf, shoot, seed as well as aseptic callus of the plant parts. The techniques mostly applied for such analyses are those of starch gel and polyacrylamide gel electrophoresis (Paisooksantivatana *et al*, 2001).

Suwanthada *et al*. (2004) identified 5 accessions of *Basella* (Basellaceae) collected from the provinces of Chiang Mai, Chiang Rai, Khon Kaen and Lampang, via isozyme analysis. Trials of extraction technique were carried out. It revealed that the extract solutions of 0.1 M sodium-phosphate buffer pH 7.5, 5 mM β-mercaptoethanol, 2 mM DTT, 2 mM EDTA, 1% PVP and 2% PVPP produced clear and distinct isozyme patterns while 2 out of 7 enzymes, i.e. malate dehydrogenase (MDH) and shikimate dehydrogenase (SKD) failed to yield the bands. Another group of enzymes, comprising esterase (EST), acid phosphatase (ACP), aspartate amino transferase (AAT), superoxide dismutase (SOD) and glutamate dehydrogenase (GLDH) gave rise to different isozyme patterns, of which 4 groups of *Basella* samples could be divided accordingly. Among these, 2 accessions showed 96% similarity.

Geraci *et al*. (2004) working on some endanger-threatened populations of 5 species of *Brassica* (Brassicaceae) in Sicily performed isozyme analyses to assess the genetic diversity degree at population and species levels in order to assist the design of conservation management programmes. From the analysis of allelic presence in the populations, 37 alleles were detected. *Brassica rupestris* and *B. villosa* showed a more similar composition whereas *B. incana* and *B. macrocarpa* resulted more distinct and more differential. The presence of 6 exclusive alleles was a very important remark because it characterized the populations. The same structure of diversity was observed for each species with intra-populational diversity representing the higher part of total diversity. The authors concluded that isozyme analysis was very helpful to characterize *Brassica* sect. Brassica populations as regarded allelic structure and variability with population.

Isozymes of eight enzymes, i.e. GLDH, glutamate oxaloacetate transminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), MDH, 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucose isomerase (PGI), SKD, in *Cucumis* species (*C. hystrix* Chakr., *C. sativas* L. and *C. melo* L.), Cucurbitaceae were analysed electrophoretically to investigate the biosystematics of these three species. Cluster analysis using data from six enzymes indicated that considerable genetic distance existed between *C. hystrix* and melon and between *C. hystrix* and cucumber. It was also considered that *C. hystrix* might be a key species for studying the evolution and taxonomy of genus *Cucumis* (Chen *et al*., 1997).

Examination of specific isozyme patterns from the seeds of 2 cultivars of rice (Gramineae), i.e. Kaodawkmali 105 (KDML 105) and Chainat 1 (CN 1) via polyacrylamide gel electrophoresis, using 5 enzymes, EST, GOT, LAP, malic enzyme (ME) and MDH was conducted. It turned out that esterase was the only enzyme that could distinguish the isozyme pattern of the rice seeds cv. KDML 105 from that of the cv. CN 1. As for other enzymes, the isozyme patterns could be developed but unable to differentiate the differences between the two cultivars of rice (Kaewmala, 2005).

Characterization of isozyme variation in walnut (*Juglans regia* L.) was carried out by Solar *et al*. in 1994. Four different enzymes from the leaves of 17 walnut cultivars, i.e. MDH, 6-PGD, peroxidase (POX) and AAT and two enzymes from pollens of 15 walnut cultivars, i.e. MDH and 6-PGD were analysed via horizontal starch gel electrophoresis. Walnut cultivars of different origin exhibited different numbers of electrophoretic band and also different relative mobility. Different activity levels and phenotypic groups were detected in studied enzyme systems. Pollen enzymes revealed higher variability than enzymes extracted from the leaves. Fifteen walnut cultivars were classified into 10 MDH phenotypic and 14 6-PGD phenotypic groups based on pollen analyses. Seventeen cultivars were classified into 9 POX phenotypic groups and 7 6-PGD phenotypic groups based on analyses of the leaves. All of the 15 walnut cultivars could be identified and distinguished with electrophoretic analyses of MDH and 6-PGD from the pollen while only 10 cultivars were distinguishable with analyses of 6-PGD and POX from the leaves. No variability useful for cultivar identification was observed in MDH and AAT from the leaves.

Dendrobium scabrilingue Lindl. (Orchidaceae) collected from four locations, i.e. two locations of Mae Sa Riang and Pang Ma Pa in Mae Hong Son province and of Chiang Dao in Chiang Mai province and Doi Khun Tan in Lampang province. Most of morphological characters and some quantitative characters of the plants were similar, making it very difficult to distinguish the plants in term of origins. Isozyme pattern analyses of 32 samples of *D. scabrilingue* Lindl. were compared with those of *D. cariniferum* Reichb.f. and *D. bellatulum* Rolfe, using 6 enzyme systems, i.e. EST, GOT, MDH, SKD, glucose phosphate isomerase (GPI) and LAP. It revealed that the polymorphism of 4 enzymes, i.e. EST, GOT, MDH and SKD could separate *D. scabrilingue* Lindl. populations from *D. cariniferum* Reichb.f. and *D. bellatulum* Rolfe. Moreover, they could separate 32 samples of *D. scabrilingue* Lindl. into 4 distinct groups with their putative location, but the samples of *D. scabrilingue* Lindl. could not be grouped in accordance with their origins (Thunla, 2000).

Skunareewattana (2003) studying 11 species of *Paphiopedilum* (Thai Lady's Slipper), Orchidaceae, indicated that these species showed very high variability in morphological characteristics, both within and between species. Attempts had been made later to establish isozyme patterns among those plants via polyacrylamide gel electrophoresis. Twenty enzyme systems were used but only 6 stood out and produced different polymorphic isozyme bands. Those enzymes were EST, GOT, LAP, MDH, SKD and SOD. It was then concluded from the results that EST was the most efficient enzyme capable of identifying 11 species of *Paphiopedilum*.

Prasatsuwan (2005) investigated isozyme patterns produced from immature leaves of 18 species in 7 genera of plicated-leaf terrestrial orchids (Orchidaceae) via polyacrylamide gel electrophoresis. Twenty enzyme systems were used but only 9 of them, i.e. ACP, diaphorase (DIA), EST, GOT, LAP, MDH, POX, SKD and SOD, yielded unique polymorphic isozyme bands from the 18 species. Analyses of all isozyme bands from all of the 9 enzymes were able to classify the plants into 18 species at only 10% genetic difference.

Chokthaweepanich (2002) developed isozyme patterns of 16 different types of *Curcuma* and 1 type of *Smithatris* (Zingiberaceae) from 8 enzyme systems via polyacrylamide gel electrophoresis. All enzyme systems, i.e. alcohol dehydrogenase (ADH), phosphoglucomutase (PGM), LAP, GLDH, GOT, glucose-6-phosphate dehydrogenase (G_6PDH), MDH and EST, showed polymorphism. Isozyme patterns of G_6PDH, MDH and EST could distinguish all species. Classification of the plants based on isozyme patterns using NEI method for distance and UPGMA method for cluster analysis revealed three clusters at genetic distance (D) = 1.989 with *Smithatris* in a separated unit. Classification of *Curcuma* species based on isozyme polymorphisms was concluded to be incongruent with morphological characters.

Pankong (2003) made an attempt to use isozyme patterns via polyacrylamide gel electrophoresis in classifying the *Globba* plants (Zingiberaceae) of her collection of which showing high diversity in their morphological characteristics among 12 species. Eighteen enzyme systems were used but only half of them produced

polymorphic isozyme bands. Those enzymes were ACP, DIA, EST, GOT, LAP, MDH, POX, SKD and SOD. When the isozyme pattern from SOD or those from the 9 enzymes were analysed using UPGMA cluster analysis by SPSS, it was possible to group together all the five clones within each species. Banding patterns from the zymograms showed that the 12 studied groups could well be classified into 12 species by only 4 and 13% genetic difference. The author concluded that the results obtained from her study could be used to support taxonomical identification, separating similar taxa into different species.