CHAPTER 5

CONCLUSION AND DISCUSSION

Studies on characteristics and varietal phylogenetics of three plant species, *Peliosanthes teta* Andr., *Basella alba* L. and *Gymnema inodorum* Decne. collected from some areas in the Upper-North of Thailand comprised several aspects of investigations. Surveys of the plants and collection of the plant specimens were done in some locations in the provinces of Chiang Mai, Chiang Rai, Lampang, Lamphun, Mae Hong Son, Nan, Phayao and Phrae. Planting materials of collected plant accessions, 349 in number, 72 of *Peliosanthes teta* Andr., 172 of *Basella alba* L. and 105 of *Gymnema inodorum* Decne. were grown in cultivation plots at Huai Hong Khrai Royal Development Study Centre. Observations were made on growth and development of the plants while they were growing to their maturity. They were then sampled for characterization via various methods. Varietal phylogenetics of individual species was evaluated from some characters of the plant specimens. Results obtained from those investigations were concluded and discussed below.

1. Surveys and collections

Surveys to cultivation locations and natural habitats of the plant species were planned to cover as much of the areas as time permitted to collect as much specimens as possible. Due to unfamiliarity of the places in most provinces and the lack of local connections, visits were thus mostly made around communities with only a few trips to the natural habitats in the forests.

However, satisfactorily amount of specimens were collected and adequate information concerning cultivation practices, consumption cultures and market demands were gathered via interviews made to the locals and through local market

inspections. Indigenous knowledge towards utilization of the plants were scarcely learned since elderly people who usually are valuable sources of this kind of knowledge were seldom found, reflecting disturbed status of sustainability of folklore wisdoms of indigenous and local plant species around the rural areas. This particular situations occurred in every province that the visits were made to, concealing unstability of pass-on knowledge system of which undoubtedly affecting the path of conservation and sustainable utilization of indigenous plant species.

Anyhow, considerable diversity of the plant accessions belonging to individual targeted plants had now been grown and conserved (*ex situ*), accessible for further studies. They were grown under the environment closest to those of original places.

2. Growth pattern

Growth pattern of the 3 species were made from available plant samples, 72 of *Peliosanthes teta* Andr., 172 of *Basella alba* L. and 105 of *Gymnema inodorum* Decne. Records were made on annual circle of growth, particularly on reproductive phase, since the edible part of 2 plant species are the inflorescence. On the other hand, reproductive organs are usually important to the plants themselves by means of propagating materials.

In fact, for conservation point of view, it is necessary to observe, study and make records on both vegetative and reproductive growth of those plants to be filed as basic information of growth and development of the plants grown under different environment from their original places. Environments, different from those of natural habitats or domestic cultivation, can play the part in modifying the growth of the plants. For specimens collected from those provinces, at this time of report, it is still too early to conclude about such influence. It could only be stated here that collected plants grow well in the environment of collection plots and flower normally.

3. Morphological characterization

Morphological characterization of individual plant species were conducted from available samples. Measurement and records including botanical drawings of the plant parts were done with root, stem, leaf, inflorescence, flower, fruit and seed. Descriptions of botanical characters were commenced and numerical taxonomy was analysed from leaf characters. Phylogenetics relationship of the plant samples within a species were exhibited.

For *Peliosanthes teta* Andr., the samples collected from various locations in different provinces expressed similar characteristics of the plant parts as observed externally. Analysis of genetic relationship of 72 samples revealed that the plants could be allocated into 4 groups at the coefficient indices of 63%-76%.

Basella alba L. was different, although classified as *alba* species, the samples collected from 8 provinces appeared different and could be differentiated into 2 groups from naked eyes by prominent features of stem including the colour of stem and tepal tips. For *Basella* the total samples of 80 were classified into 3 groups at the coefficient indices of 55%-70%.

Characterization of *Gymnema inodorum* Decne. was done, also from 80 samples. These plants were similar in characters but the dendrogram produced from leaf character analysis at the coefficient indices of 47%-76% was able to allocate the samples into 3 groups.

4. Pollen morphology

Inspecting under LM, pollen of different species revealed different shape and feature but under SEM, more detail such as aperture and exine sculpture showed up. Photomicrography of the pollens provided perfect record of references of specific identity at species level.

From this study, seen from SEM, pollen of *Peliosanthes teta* Andr. was monad with regulate exine sculpture. Pollen of *Basell alba* L. appeared as cuboidal monad of which coincides with the work reported by Bhattachryya and Johri (1998), and Eriksson (2007) and the work of Erdtman (1972) on *Basella rubra*. As for *Gymnema inodorum* Decne., its pollen was different appearing in the form of pollinia. This result agrees to that stated by Bhattacharyya and Johri (1998), Lawrence (1951), Li *et al*. (1995) and Wongsawad *et al*. (1996).

The pollens obtained from the plants of the same species are similar, both in shape, feature and exine sculturing. This finding indicates that pollen morphology could classify the plants at species level but not able to differentiate the plants at variety level as supported by the statement gave by Agashe and Caulton (2009).

5. Anatomical characterization

Longitudinal and transverse sections of various plant parts taken from different samples of individual plant species as shown in the results are valuable for comparative anatomical studies providing characters applicable for identification and classification of the plants (Cutler *et al*., 2008; Kantachot, 2008).

Further studies concerning surface anatomy could supplement above mentioned investigation of comparative anatomy of experimented plants.

Anatomical examination carried out in this research is not only useful for the plants identification and classification but also important in evaluating physiological phenomenon concerning growth and development of the plants, vegetatively and reproductively.

For example, biological structure seen through floral anatomy from *Basella* flower sections at mature stage could explain self-fertilization phenomenon occurring in the plant's flower as seen in Figure 194.

From this Figure it can be inspected that both orientation of male and female organs and timing of anther dehiscence conveniently allow self-pollination to occur inside the closed floral buds.

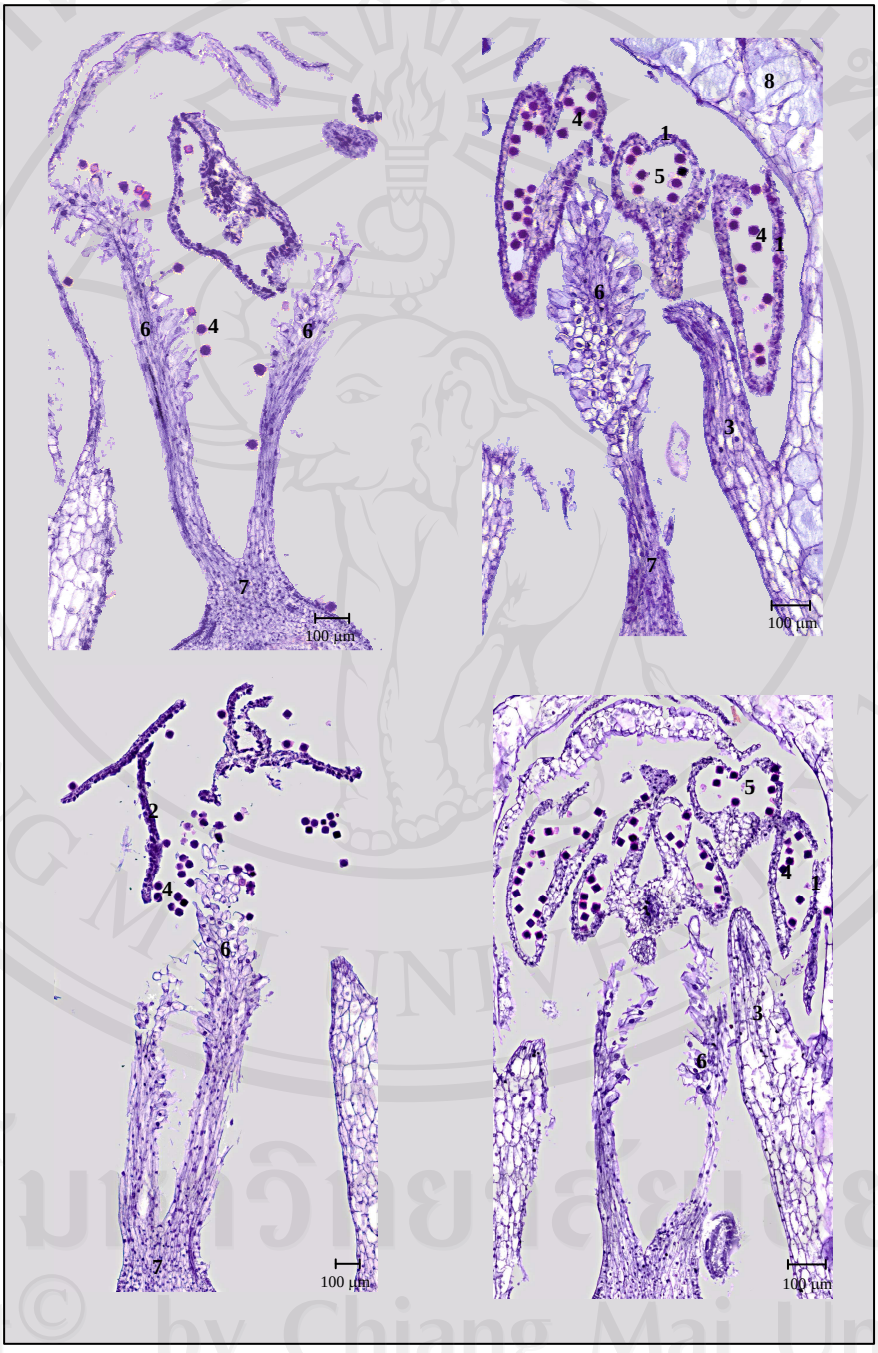

Figure 194 Longitudinal sections of *Basella alba* L. flower showing orientation of floral parts at the time of anther dehiscence

1 = anther; 2 = dehisced anther; 3 = filament; 4 = pollen;

5 = pollen sac; 6 = stigma; 7 = style; 8 = tepal

Indigenous vegetables and local vegetations are known of accumulating inorganic substances in the cells appearing in particular areas. These substances are usually in forms of crystals in various shape. It was found here too that such accumulation appeared in the cells of ground tissue of roots/stems and/or leaves of the three species. They occurred in different forms of crystals. Nevertheless, these crystals were not found in the flower tissue.

6. Karyotypic characterization

In this experiment, prior to the trials of chromosome investigation, tests were made to compose the most appropriate protocol of root-tip squash procedure based on the classic Feulgen's squash.

The results of these preliminary tests yielded the suitable protocol of sampling the root tips at 10.00 a.m., pre-treating the root tips with PDB solution for 6, 8 and 2 hours for *Peliosanthes teta* Andr., *Basella alba* L. and *Gymnema inodorum* Decne., respectively, staining in carbol fuchsin for 6, 8 and 4 hours, also respectively. This procedure has been very successful, with high accuracy, in preparing the root tip tissue of experimental plants for karyotypic studies.

For chromosome counting, results showed here that the somatic number of *Peliosanthes teta* Andr. was 2n = 54 while Conran and Tamura (1998) found his counts as 2x = 18 (2x = 36 by Chang and Hsu (1974), Chen and Tamura (2000), Jones and Smith (1967), Larsen (1966) and Sato (1942). Somatic number of *Gymnema inodorum* Decne. gathered from this research was 2n = 22. For *Basella alba* L., the chromosome counts presented in 2 numbers, i.e. 2n = 38 in Phak Plang Khao and 2n = 44 in Phak Plang Daeng. Different results of chromosome number studied by other authors was 2n = 48 in *B. alba* and 2n = 44 in *B. rubra* (Peter, 1998) while Grasso *et al*. (1997) reported his work of *B. alba*, Sri Lankan cultivar as having 2n = 36 to 2n = 48.

As for *Basella* case, it is interesting that Phak Plang Khao and Phak Plang Daeng have been classified as the same species of *alba*, according to systematic classification stated by Baily (1969). Since it was found here that the chromosome number of Phak Plang Daeng is different from that of Phak Plang Khao and its morphological structure of stem, leaf and flower as well as anatomical characters of those organs also differ, thus, it is suggested here that revision of *Basella* classification should be made.

Another aspect of karyotypic studies, conducted here was the karyotype formula. Such formulae of various samples of three plant species proposed the conclusion that the plant samples representing the provinces of cultivation sites expressed different karyotypic formulae reflecting different configurations of the chromosome complements in the diploid set.

7. Isozyme pattern

Isozyme patterns of individual plant species were investigated via polyacrylamide gel electrophoresis using 8 enzyme systems of acid phosphatase, esterase, glucose dehydrogenase, glutamate oxaloacetate transminase, leucine aminopeptidase, malate dehydrogenase, peroxidase and shikimate dehydrogenase. It was found that 6 enzyme systems were able to reproduce the bands and 2 of them, i.e. glucose dehydrogenase and shikimate dehydrogenase failed. Genetic relationships among the samples of individual species were then exhibited and it turned out that 72 samples of *Peliosanthes teta* Andr. were allocated into 5 groups at the coefficient index of 64%-72%, 80 samples of *Basella alba* L. were divided into 9 groups at the coefficient index of 45%-59% and 80 samples of *Gymnema inodorum* Decne. were separated into 9 groups at the coefficient index of 70%-79%.

For the subject of plant characterization, various methods of characterization were conducted here. The results of evaluation varied with the techniques being used. Accuracy or justifications of those practices cannot be conclude here since they were

not done for comparison but were carried out to supplement taxonomial classification of the plant species, of which relies only on morphological characters. Thus, the results obtained from characterization of various methods are not comparatively discussed here. Instead, they were reported in order to suggest capacities in differentiating plant species or varieties regarding their genetical relatedness.