

CHAPTER 5

SUMMARY AND FUTURE STUDIES

5.1 Reproductive biology of the highland and lowland varieties of *P. chinensis* var. *chinensis*

The highland variety of *Persicaria chinensis* var. *chinensis* is a native species found in the tropics growing at lower montane forest at the altitude 1200 m and above. This scrambling herb is monoecious, non-seasonal and bears bisexual flowers. *P. chinensis* var. *chinensis* is heterostylous with short-styled (thrum) and long-styled (pin) flowers. The flowers are borne in compound corymb, usually terminal. The lowland variety of *P. chinensis* var. *chinensis* is an erect herb commonly grown in household compound and easily cultivated by cuttings. *P. chinensis* var. *chinensis* is a highly varied species, i.e. two main differences: the growth habit and leaf shape between the highland and lowland varieties. The lowland variety has yet to set fruit during three years observation in more than three different populations. Usually, the lowland variety is reproduced vegetatively by cuttings as this is frequently used as a traditional medicinal herb in Chinese medicine.

The time taken from the appearance of very young flower buds to the first anthesised flower was 12–14 days for the highland variety and 9–12 days for the lowland variety. The fertilized open flowers developed into mature fruit in about 14–18 days for the highland variety. The average fruit set of the highland variety in Gunung Ulu Kali at the altitude range from 1300 m–1400 m (namely population H2, H3 and H5) was 48% and only 2% in population H4 located at 1600 m. In the lowland variety, there was a high percentage of flower abortion and very high percentage of fruit degeneration; viable fruits had not been collected.

The anthesis time of the wild highland population was at 0600–0730 hours whereas that of the lowland variety was at 0745–0930 hours. The anther dehiscence and stigma receptivity are synchronous.

P. chinensis var. *chinensis* has eight stamens with five epipetalous stamens and three distinct and free stamens. The gynoecium of *P. chinensis* var. *chinensis* is superior, tricarpellary, syncarpous, and each carpel is uniovulate with a single orthotropous, bitegmatic and crassinucellate ovule with basal placentation. The anther development and female gametophyte formation are similar in the highland and lowland varieties.

The anther is tetrasporangiate and the wall development conforms to the Monocotyledonous type. The endothecium has fibrous thickenings and this layer together with the epidermis remains until the time of dehiscence. The single middle layer is ephemeral and the cells of the glandular tapetum are initially uninucleate but become multinucleate with 3- to 4- nucleate cells which begin to degenerate after the formation of the microspore tetrads. Cytokinesis in the microspore mother cells is simultaneous forming both tetrahedral and decussate tetrads in the highland variety and mostly tetrahedral tetrads in the lowland variety. The mature pollen grains are shed at the three-cell stage.

The development of the embryo sac conforms to the monosporic *Polygonum* type. The embryo sac elongates more than three times of its original size (from 150 μm to 500 μm) before fertilization. An occurrence of twin eggs within an ovule was observed in the lowland variety. The ovule together with the embryo sac and ovary degenerate in the lowland variety and further development of the endosperm has not been observed. Nucellar beak is well-developed by the time the embryo sac is formed.

In the highland variety, the development of the endosperm follows the *ab initio* Nuclear type and free endosperm nuclei gather around the micropylar, chalazal and also at the periphery of the embryo sac. The endosperm is completely cellular only at the late

heart-shaped embryo stage. The zygote undergoes a short resting period. It initially develops into a dyad, proembryo, globular and later a heart-shaped and finally a slightly curved dicotyledonous embryo. The seed coat is formed from both the outer and inner integuments.

Heterostyly has been observed in the highland variety. The pin flowers have taller style, shorter filaments, less thick and less wide stigma than the thrum flower. The number of pollen grains in a pin flower was higher than that of in a thrum flower. The pollen grains of the thrum flower were bigger than those of the pin flower but they have similar pollen aperture, pollen tectal surface and pollen ornamentation. The pin and thrum pollen grains are 3-colpate, medium-sized, suboblate to oblate spheroidal (oblate according to Hesse *et al.* classification) and have reticulate exine with thick, narrow muri and free clavae in the deep lumina.

Comparing the morphology of the thrum flowers and their pollen in the highland and lowland varieties, the lowland thrum flowers have shorter style, longer filaments, thicker and wider stigma than that of the highland thrum flowers. The size of pollen grain of the lowland variety is bigger than that of the highland thrum pollen; they have similar pollen aperture and tectal surface. However, the exine ornamentation of the lowland thrum pollen is not similar in terms of the lumina and the muri; the Their lumina were less wide and the muri were thicker, less narrow and shorter than those of the highland thrum pollen with free clavae.

The pollen grains of the lowland variety do not germinate in the 'complex medium' and the 'modified medium'. In the highland variety, the optimum sucrose and PEG concentration for germination was 15% and 30% respectively. No germination was found in the medium without PEG and bursting of pollen grains occurred in the medium without PEG. Pollen grains reached maximum germination (50%) in the modified medium with an

average pollen tube length of 140 μm . In the complex medium, the pollen tube produced double tubes and branching, reaching a maximum length of 525 μm while the average length of the pollen tube was 350 μm showing 21% germination.

The highland variety of *P. chinensis* var. *chinensis* was self-compatible. Open pollination produced three times more fruits than self pollination and shows a xenogamous breeding system indicating cross-compatibility and outbreeding. The pollen-ovule ratio suggested the breeding system as facultative xenogamy for the highland and lowland varieties.

The possible pollinating agents of the highland variety of *P. chinensis* are diverse. The most common insect visitors are from the Order of Hymenoptera, followed by Diptera along with a lesser number of Lepidoptera and Coleoptera.

Seed germination is epigeal showing 77% viability. The average percentage of germination for the fresh seeds was 47% under the natural highland environment and 34% under the lowland laboratory environment. The first seedling appears approximately one week after sowing in the highland environment and about 12 days in the lowland laboratory environment.

To improve our understanding of the reproduction of this variety, careful observation on the pollinator behavior and pollen load, seed predation and dispersal need to be further monitored.

5.2 Cytotoxic activity of the highland and lowland varieties of *P. chinensis* var. *chinensis*

The *in vitro* neutral red cytotoxicity assay was successfully applied to screen the cytotoxic activity against 18 crude methanol, dichloromethane and water extracts from the highland and lowland varieties of *P. chinensis* var. *chinensis* derived from the leaf, stem

and whole plant (leaf and stem). Although the results from the *in vitro* assay are not sufficient to predict the final effectiveness of potential natural products in cancer chemoprevention, this practical screening tool is able to identify the active and non-active extracts from the test samples.

Overall results showed that the methanol crude extracts from the leaf samples of the highland and lowland varieties, whole plant methanol crude extract of the highland variety, whole plant dichloromethane crude extract of the highland variety and also the stem dichloromethane crude extract of the highland variety possessed greater cytotoxicity effect against CaSki, SKOV-3, MCF-7 and HT-29 cancer cell lines. Two active extracts obtained from the methanol leaf extracts of the highland and lowland varieties were more effective in suppressing the expression of HPV-16 in cervical cancer-derived, CaSki cells and ovary cancer-derived, SKOV-3 cells. Results indicated that the methanol leaf and whole plant extracts of these highland and lowland varieties maybe a more promising agent for the therapy of HPV-16 associated cervical cancer and ovary cancer as compared to their dichloromethane and water counterparts. In addition, the dichloromethane extract from the stem and whole plant sample of the highland variety showed significant cytotoxic activities against colon cancer-derived, HT-29 cells.

Since the *P. chinensis* var. *chinensis* of the highland and lowland populations showed signs of the selectivity for CaSki, SKOV-3, MCF-7 and HT-29 cells, it is therefore necessary to also evaluate the cytotoxic effect of these extracts on other types of human cancer cells. Further studies should be initiated to isolate and identify the specific active compound(s) from the active extracts which demonstrated significant cytotoxic activities. *In vitro* pharmacological studies on the active metabolites isolated should be carried out to elucidate and understand the toxicity level and mechanism of action of the extracts.