CHAPTER 3

MATERIALS AND METHODS

Plant materials: Survey and Plant Collection.

The survey and plant collection were done at Khun Wang Development Center, Royal Project Foundation located in Khun Wang village, Maewin sub district, Mae Wang district, Chiang Mai Province, 1,200-1,400 meters above mean sea level (MSL) at latitude N 18° and longitude E 98° (Boonprakob and Byrne, 2004). The survey area was about 10 kilometers in diameter (Figure 1). The survey was done twice, in November 2002 and then in February 2003. The samples were photographed, wrapped in newspapers and kept in polyethylene bags. After collection, the plant samples were taken back to the nursery of the Department of Horticulture, Faculty of Agriculture, Chiang Mai University, 330 meters MSL.

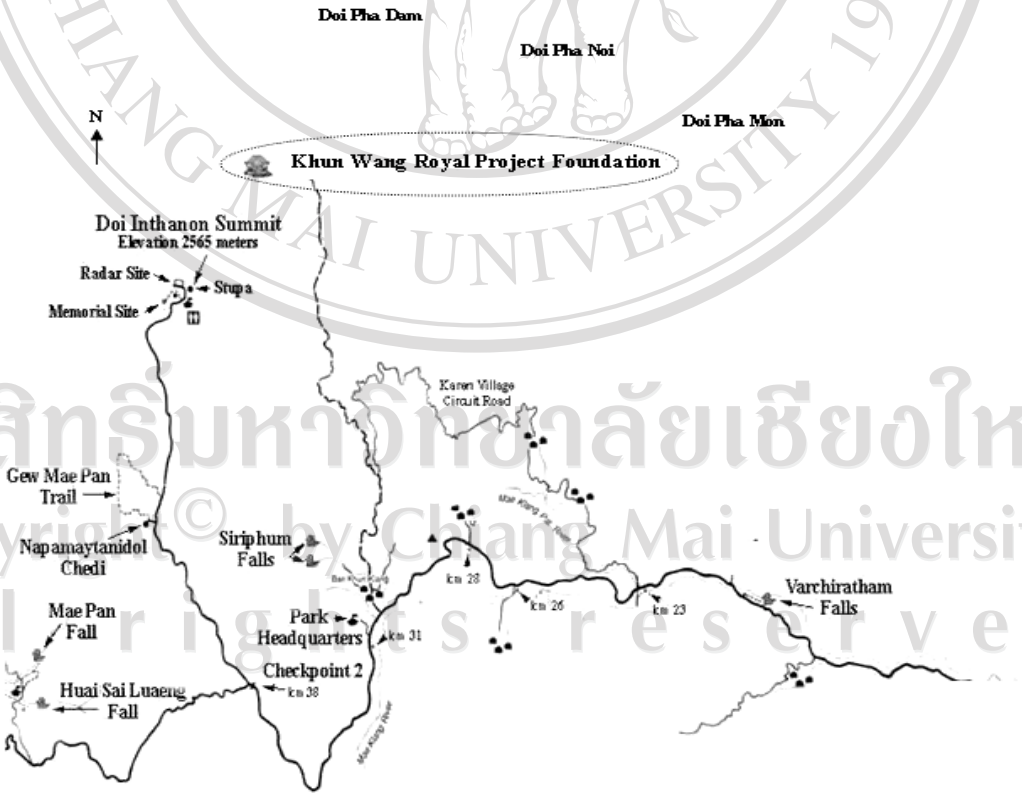

Figure 1 The surveying and plant collection area at Khun Wang Development Center, Royal Project Foundation, Chiang Mai Province.

Experiment 1: Plant taxonomy study.

Plant samples were taken for taxonomy, identification and classification at the Department of Biology, Faculty of Science, Chiang Mai University by comparing them with the samples in the herbarium.

Experiment 2: Study on plant adaptation and survival.

2.1 Growth under greenhouse condition.

Collected samples were cut into 1–3 pieces. The sections were dipped in Seradix (0.8%) solution for 30 seconds and then transferred to rooting media consisting of 1 part of rice husk charcoal and 1 part of coarse sand in plastic baskets and covered with polyethylene bags. Water was given to the samples once a week and the samples were sprayed with 15–15–15 (N–P–K) liquid fertilizer every 2 weeks. After rooting, they were transplanted in 8-inch diameter pots. Foliar spray with 15–15–15 (N–P–K) fertilizer was also applied once a month. Captan, carbaryl, and metaldehyde were used to control plant diseases, insects and snails respectively.

2.2 Effects of temperature and light intensity.

Five samples of *Aeschynanthus jarrettii*, *Parabaena sagittata*, *Selaginella siamensis*, *Gynostemma pentaphyllum*, *Trachelospermum asiaticum* and *Hoya thomsoni* were grown under two conditions (Figure 2): (A) greenhouse condition at 30±2° C, 50,000 cd/m^2 light intensity and 78 % relative humidity, and (B) growth room condition at 25±2°C, 4,200 cd/m^2 light intensity and 52 % relative humidity. The studies lasted for 12 weeks.

Figure 2 Experimental site : (A) greenhouse and (B) growth room.

Experiment 3: Cytology study.

Root-tips for cytological preparations were collected from potted plants and pretreated in an aqueous solution of 8-hydroxyquinoline for 4 hour at 18° C and fixed in 1:3 acetic alcohol for 18-24 hour. Root-tips were transferred to 70 % alcohol, stored at 4°C and hydrolyzed in 1 N HCl for 8 min at 60°C. The samples were then stained by the Feulgen's squash method. Squashes were made on slides in 1 % aceto-carmine solution and the slides were mounted by Canada balsam. At least 10 metaphases of each species were examined for chromosome counts. Each sample was counted and recorded under a microscope.

Experiment 4: DNA Fingerprint study.

Four collected samples and two commercial plant species were used. The DNA fingerprints were done by the Randomly Amplified Polymorphic DNA method (appendix A). DNA banding and width were counted and measured together with the distances of band movements.

Experiment 5: Effects of different concentrations of IBA on rooting.

Eleven creepers of those known genus and 5 IBA concentrations, IBA 0.0 (control), Seradix 0.8 %, 4000, 8,000 and 12,000 mg/l were studied. The cuttings consisted of 3 nodes. There were 4 replications, 10 cuttings per replication, and a total of 200 cuttings per genus. Cuttings were dipped in IBA solutions for one minute and rooted in the baskets, 50 cuttings in each basket. Each basket was put in a polyethylene bag to control moisture. Root numbers and root length were recorded every week for 4 weeks.

Experiment 6: Anatomical study of selected 5 species.

The study on the anatomy of root, leaf and shoot of the well adapted plant species was conducted using the paraffin embedding technique (appendix C).

Experiment 7: Study on vase life of cut green of *Raphidophora glauca* and *R. peepla.*

Five holding solutions were tested: distilled water (control), 8–HQS 250 mg/l, 8–HQS 250 mg/l with $AgNO_3$ 50 mg/l, citric acid 100 mg/l and citric acid 100 mg/l with $AgNO_3$ 50 mg/l. Leaf colour and freshness were recorded every week for two months (appendix D). The experimental design used in this study was completely randomized design (CRD). There were ten leaves (replications) in each treatment. Records were done as follows: vase life (days), petiole decurve, lamina wilt, chroma and hue.

Experiment 8: Tissue culture propagation of *Raphidophora glauca* and *R. peepla*.

Two best well adapted samples were used. Treatments of the media were as follows: MS with 0.0 mg/l BAP and 0.0 mg/l NAA (control), MS with 2.0 mg/l BAP and 0.0 mg/l NAA, MS with 4.0 mg/l BAP and 0.0 mg/l NAA, MS with 8.0 mg/l BAP and 0.0 mg/l NAA, MS with 10.0 mg/l BAP and 0.0 mg/l NAA, MS with 0.0 mg/l BAP and 2.0 mg/l NAA, MS with 2.0 mg/l BAP and 2.0 mg/l NAA, MS with 4.0 mg/l BAP and 2.0 mg/l NAA, MS with 8.0 mg/l BAP and 2.0 mg/l NAA, MS with 10.0 mg/l BAP and 2.0 mg/l NAA. This experiment was conducted by using the completely randomized design (CRD) with ten replications per treatment. The explants were apical shoot meristem and internodes sections. The samples were thoroughly washed by distilled water 3 times, cut under aseptic condition, placed on the media and kept in the air condition room at 25° C under fluorescence lamp for 17 hours per day for two months.

Experimental Site

Greenhouse and the laboratories of the Department of Horticulture, Faculty of Agriculture and the Department of Biology, Faculty of Science, Chiang Mai University were used.

Experimental Duration

November 2002 – September 2007