CHAPTER 3

MATERAILS AND METHODS

I. Plant collecting

Materials

1. plant collecting materials

- spade
- plastic bags
- label tags
- field notebook
- hand lens
- altimeter
- wooden presses and rope
- pressing paper (newspaper)
- corrugated cardboard
- digital camera

2. plant investigation materials

- stereo microscope
- laboratory needle
- Petri dishes
- razor blade
- forceps
- related taxonomic literatures

3. herbarium specimens preparing materials

- hot air oven
- mounting paper
- latex glue
- white paper cover
- needle and thread
- label pad
- deep freezer
- 70 % ethanol

Methods

1. The herbarium specimens of the genus *Argostemma* available in the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University (PSU) and the Forest Herbarium (BKF), Department of National Park Wildlife and Plant Conservation has been studied.

2. Exploration and collection

- Field collections were made in the localities, following Sridith (1999b).
- Plant collections were made with field notes. The morphological characters of each specimen, such as color, habit etc. were noted and photographed.
- Specimen processing following the directions specified in "The Herbarium Handbook" (Foreman and Bridson, 1992).
- The collected specimens were identified and described by using both keys and descriptions from taxonomic literatures.
- The duplicates of plant specimens were deposited at the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University (PSU); The Forest herbarium, Department of National Park Wildlife and Plant Conservation

(BKF), Royal Botanic Gardens, Kew, Surrey, UK (K), Singapore Botanic Gardens (SING), Royal Botanic Gardens Edinburgh (E), Leiden Botanic Garden (L) and The herbarium of the Forest Research Institute of Malaysia (KEP).

II. Chromosomal study

Materials

- the 22 specimens of *Argostemma* spp. from all over Thailand
- 0.1 % colchicine
- glacial acetic acid
- 95% ethanol
- 70% ethanol
- carbol fuchsin
- immersion oil
- absolute ethanol
- acetone
- nail enamel
- vials
- beaker 50 ml.
- hot plate
- needle
- scalpel
- forceps
- microscopic slides and cover-glasses
- label stickers

- light microscope: Nikon Optiphot-2, Olympus BX51
- Exposure control unit: Nikon UFX-DX II, Olympus PM-30, Olympus DP11
- black-white negative films

Methods

1. Preparation of meiotic study : Smear technique (modified from Chaiyasut, 1989; Sharma & Sharma, 1980)

1. Fixation : Fix the young flower buds in 95% ethanol-glacial acetic acid (3:1) (Carnoy's solution) for 24 hours at 10 ^{0}C.

2. Washing : Wash in 95% ethanol 3 times.

3. Storage : Store in 70 % ethanol at 10 ^{0}C.

4. Washing : Wash in water 3 times.

5. Staining : Transfer the young flower buds to carbol fuchsin for 5 minutes at room temperature.

6. Smearing : Dissect out anthers from the flower bud. Put the anthers in a drop of carbol fuchsin on a clean dry slide, cut off the edges of the anthers with a scalpel, squeeze out the inner fluid and reject the empty anther lobes, then smear the fluid with a clean scalpel and cover with a cover-glass on the fluid.

7. Observation : Observe under the microscope. Count 10-30 of first metaphase cells and take photographs 10 well spread first metaphase cells at 100x, using an oil immersion objective.

2. Preparation of mitotic study : Squash technique (modified from Chaiyasut, 1989; Sharma & Sharma,1980)

1. Pretreatment: Pretreat fresh young flower buds in 0.1% colchicine for 5 hours at 10 ^{0}C.

2. Fixation : Transfer to 95% ethanol-glacial acetic acid (3:1) (Carnoy's solution) for 24 hours at 10 ^{0}C.

3. Washing : Wash in 95% ethanol 3 times.

4. Storage : Store in 70 % ethanol at 10 ^{0}C.

5. Washing : Wash in water 3 times.

6. Staining : Transfer the young flower buds to carbol fuchsin for 5 minutes to 5 hours at room temperature.

7. Squashing : Cut off the corolla part of the young flower buds and put it in a drop of carbol fuchsin on a slide. Place a cover-glass on the material and squash by applying uniform pressure on the cover-glass with the tip of pencil through a piece of blotting paper.

8. Observation : Observe under the microscope. Count 10-30 metaphase cells and take photographs 10 well spread metaphase cells.