CHAPTER 5

DISCUSSIONS

Taxa in the present study

Twenty-two taxa of *Argostemma* in Thailand have been collected to achieve the karyological studies, which are 66.66 % of Thai taxa reported by Sridith (1999[b]).

The morphological characters of *Argostemma* exhibit high variation. In the present study, both vegetative and reproductive organs play the greatly different appearances.

The growth forms vary in leafy stems (Sridith, inpress[a]) e.g. *A. condensum* Craib, *A. dispar* Craib and pseudo-verticilate e.g. *A. ebracteolatum* Geddes, *A. verticillatum* Wall.. The leaves arrangement vary in two types: slightly anisophyllous leaves e.g. *A. neurocalyx* Miq., *A. plumbeum* Craib and strongly anisophyllous leaves e.g. *A. unifolioides* var. *glabra* King, *A. elatostemma* Hook.f.

The reproductive organs show two types of distinguished corolla shapes: the star-shaped corolla and bell-shaped corolla. According to Sridith and Puff (2001), who have proposed four major groups in *Argostemma*, i.e. star-shaped and 5-merous flower, star-shaped and 4-merous flower, bell-shaped and 5-merous flower, bell-shaped and 4-merous flower. In this study, almost of star-shaped corolla are found anthers fused and forming an anther cone like structure with the anthers opening by longitudinal slits (Sridith & Puff, 2001). These characters are found in ten taxa: *A. condensum* Craib, *A. dispar* Craib, *A. elatostemma* Hook.f., *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith, *A. ophirense* Maingay ex Hook.f., *A. propinquum*

Ridl., *A. rotundicalyx* K. Sridith, *A. subcrassum* King, *A. unifolioides* var. *glabra* King and *A. khasianum* C.B. Clark (the only one star-shaped species which have 4-merous). But some star-shaped species the stamens are free with anthers opening by apical pores (Sridith, inpress[a]). These characters are found in six taxa: *A. argostemon* K. Sridith, *A. diversifolium* Ridl., *A. kurzii* C.B. Clark, *A. laxum* Geddes, *A. pictum* Wall. and *A. verticilatum* Wall., except *A. argostemon* K. Sridith and *A. pictum* Wall. which the anthers opening by longitudinal slits and *A. verticilatum* Wall. the anthers opening by oblique elongate pores. All of the bell-shaped flower are found with free stamens and anthers opening by apical pores (*sensu* Sridith, inpress). These characters are found in six taxa from both 4-and 5-merous flower: *A. ebracteolatum* Geddes, *A. lobulatum* var. *variabile* Geddes, *A. puffii* K. Sridith, *A. neurocalyx* Miq., *A. neurosepalum* Bahk.f. and *A. plumbeum* Craib.

Moreover, there are two addition records to the previous list by Sridith (1999b). The newly described species are star-shaped corolla. *A. argostemon* K. Sridith, free stamens and opening by longitudinal slits, distinguish from the others by white anthers. The other one is a new record to Thailand, *A. kurzii* C.B. Clarke. The corolla shape of this species is different from the others by the semi star-shaped as corolla tube and shorter than corolla lobes, free stamens with opening by apical pores.

According to the tremendous diversity, the infrageneric division was proposed by many taxonomists, in the last five decades, based on vegetative characters and floral features. The taxonomic status of this genus is discussed in cytological aspect in order to support the relationships between various taxa in the genus.

Karyological study in *Argostemma* Wall.

Chromosome numbers

All twenty-two taxa from four morphological groups (star-shaped and 5-merous flower, star-shaped and 4-merous flower, bell-shaped and 5-merous flower, bell-shaped and 4-merous flower) have the same somatic chromosome numbers as 2n = 22. These chromosome numbers agree with six taxa which were reported (Mangenot & Mangenot, 1962; Hellmayr *et al.*, 1994; Kiehn, 1995; Puangsomlee & Puff, 2001). The chromosome numbers of three species revealed that *A. diversifolium* Ridl., *A. pictum* Wall. and *A. neurocalyx* Miq., are coincident with Puangsomlee and Puff (2001). The microsporocytes of *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith and *A. kurzii* C.B. Clark showed 11 bivalents at first metaphase cells. This number agrees with previous reports of *Argostemma*'s basic chromosome numbers x = 11 (Kiehn, 1996; Puangsomlee & Puff, 2001). From the data suggested that *Argostemma* in Thailand are diploid with basic chromosome numbers x = 11 (2n = 2x = 11).

A. verticillatum Wall. from Thailand showed chromosome numbers of 2n = 22, which differed from the report of Khoshoo & Bhatia (1963). This chromosome number n = 14 of *A. verticillatum* Wall. was achieved from Himalayan materials. The contradiction with the previous study is probably due to the misidentifying or the difficulties in obtaining proper methods to determine chromosomal identities remained the imprecise counting in Rubiaceae. So commented by Kiehn (1995). In any case, there were reports of different chromosome numbers in a given species from different geographic ranges of variations (Soejima & Peng, 1998). Moreover, the nondisjunction in asexual reproduction could be one cause of the different chromosome numbers in plants. Concerning the herbaceous plants with

rhizomes like *Argostemma*, Sharma & Bhattacharya (1969) suggested that the origin of different basic chromosome numbers in various genera of Zingiberaceae, a herbaceous taxon which propagate with rhizomes are connected with asexual reproduction.

The meiotic chromosomes of all twenty-two taxa were investigated, however, only two taxa were achieved. This might due to the time of plant collecting is not coincident with the proper flower-development period (too old developed flowers). In addition, most *Argostemma* have flowers once a year (annual herbs) and the downfall in cultivation together with the limiting time of this research, the numbers of taxa with success in meiotic chromosome study in this work are also limited.

New chromosome counts of *Argostemma* are reported for eighteen taxa, i.e. star-shaped and 5-merous flower group**:** *A. argostemon* K. Sridith, *A. condensum* Craib*, A. dispar* Craib*, A. elatostemma* Hook.f.*, A. kurzii* C.B. Clark, *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith*, A. laxum* Geddes*, A. ophirense* Maingay ex Hook.f., *A. propinquum* Ridl.*, A. rotundicalyx* K. Sridith*, A. subcrassum* King and *A. unifolioides* var *glabra* King**;** star-shaped and 4-merous flower group: *A. khasianum* C.B. Clark**;** bell-shaped and 5-merous flower group**:** *A. ebracteolatum* Geddes*, A. lobulatum* Craib var. *variabile* K. Sridith and *A. puffii* K. Sridith**;** bell-shaped and 4-merous flower group**:** *A. neurosepalum* Bahk.f. and *A. plumbeum* Craib.

Chromosome size and shape

Most of four morphological groups seem to have chromosomal similarity in size and shape. The chromosomes are relatively small and clump together in metaphase as in many taxa of Rubiaceae (Kiehn, 1995). The difficulty in observing the centromeres is an obstruction to study the karyotype of *Argostemma*.

The satellites were usually found in one or two pairs of chromosomes in Rubiaceae (Kiehn, 1995). Naiki and Nagamasu (2004) reported that one pairs of satellite chromosomes were found in six taxa and two pairs of satellite chromosomes were found in one taxa of *Damnacanthus* C. F. Gaertn.. Four taxa of *Argostemma*, i.e. *A. condensum* Craib, *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith and *A. diversifolium* Ridl. and *A. lobulatum* Craib var. *variabile* K. also possess one pair of satellite chromosomes.

The satellites, however, are difficult to be observed in metaphase, whereas in prophase or pro-metaphase are obvious to be distinguished in those taxa as in *Sipanea hispida* Benth. Ex Wernham (Rubiaceae) (Kiehn, 1995). Additionally, more details information on satellites of other *Argostemma* 's chromosomes may be needed for supporting the classification in the genus level as it was achieved in *Citrus* L. (Guerra *et al.*, 1997) and *Hordeum* L. (Linde-Laursen *et al.*, 1995).

Recently, the banding techniques are used to distinguish the differences between chromosomes and allow more details comparisons between complements of different taxa (Stuessy, 1989). Although, Kiehn (1995) reported the failure in Giemsa technique to various taxa of Rubiaceae with small chromosomes size and/or the interfering of the presence of tannin, the chromosomal differentiate of small size and uniform chromosomes in *Coffea* L. (Rubiaceae) could be distinguished

by the C and NOR banding with the DAPI and CMA_3 (Pinto-Maglio, 2006). Thereafter, the chromosomes study of *Argostemma*, which have small chromosomes by banding techniques might be achieved.

Infrageneric relationship of *Argostemma* Wall. according to the karylogical point of view

The same chromosome numbers in a genus is an evidence of the natural groups in most cases, i.e. *Zingiber*: the 27 species are 2n = 22 (Beltran and Kiew, 1984; Chen, 1989; Saensouk, 2000; Eksomtramage *et al.,* 2001; Eksomtramage *et al.,* 2002; Augsonkitt *et al.*, 2004) and *Lathylus* 2n=14 (Seijo & Fernández, 2003). In this study, chromosome data has supported that *Argostemma* could be a natural group. The regular cell division in meiosis could be assumed that the genus is normal fertile in their natural habitat. The variation within the genus is rather high which could be easily seen from the morphological variations, in spite of the chromosomal similarities according to the karyological view points. The morphological variations are possibly due to the physical environments (Fosket, 1994) or changing in the gene or molecular level as in *Jatropha* L. (Soontornchainaksaeng & Chaiyasut, 1999). Currently, the molecular techniques have been considered useful to support the relationship among taxa. Vanijajiva *et al*. (2003) have studied the molecular phylogenetic in Zingiberaceae by using isozyme analysis and RAPD technique. The isozyme patterns and RAPD fingerprintings have indicated that *Bosenbergia* is closer to *Scaphochlamys* than to *Kaempferia*. Therefore, the morphological differences among the populations or between the taxa in the genus might be investigated more with isozyme analysis technique.

The chromosome data in the present work suggested that the genus *Argostemma* might remain a good taxon. And the infrageneric division of the genus might not be necessary at the moment.

Note on chromosomal studying technique

The sources of mitotic data commonly are from meristem of shoots, young leaves and roots (Stuessy, 1989). But in this study, the somatic chromosomes could not be achieved from root-tips due to their fibrilliform roots that always attached to substrate, especially in the species with tubers which are often found specificly to limestone areas. The cultivation of the collected plants to get root-tips were impossible due to the specific environments needed, such as high humidity; low temperature etc. Nevertheless, the new finding technique of chromosomal study for this genus was proposed here. All of the somatic chromosomes were investigated from *corolla part of the young flowering buds*. The suitable corolla length was 1-3 mm. And it is to be affirmed that the metaphase cells would be detected in the flowering buds at the age of the length 1-3 mm only. From the study, the division of the nucleus were rarely found in more than 3 mm corolla length. Additionally, the region of cell division is at the base of the corolla whereas the other corolla regions are rarely found especially at corolla tips.

The tannins are found in some species of *Argostemma*, i.e. *A. argostemon* K. Sridith, *A. ebracteolatum* Geddes, *A. kurzii* C. B. Clarke, *A. laxum* Geddes, *A. ophirense* Maingay ex Hook.f., *A. pictum* Wall., *A. plumbeum* Craib, *A. propinquum* Ridl., *A. puffii* K. Sridith, *A. rotundicalyx* K. Sridith, *A. subcrassum* King and *A. unifolioides* var. *glabra* King as in many Rubiaceae (Kiehn, 1995). The self-

tanning effects are often encountered in chromosome fixations by using Carnoy's solution, resulting in changing the tissues to tan color (Figures 23-26 in appendix) and reducing the stainability. These problems could be avoided by using formaldehyde as a fixative (Greilhuber, 1988). Anyhow, the hydrolysis process for Feulgen staining may be necessary in somatic chromosomes study from root-tips or other tissues (Sharma & Sharma, 1980). But in this study, the staining without hydrolysis process could be obtained even though it was interfered by self-tanning effects. These outcomes were due to the thin and soft tissues of corolla parts. The tissues could be stained at least 5 minutes to 5 hours, depending on the tissues of a given species. The over staining resulted that the dye could be imbued thoroughly to the cytoplasm.