Chromosome Numbers of Thai Herbs in the *Plumbago*

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Abstract

In Thailand, there are only two known types of herbs in the *Plumbago* genus: Ceylon leadwort (*P. zeylanica*) and rose-coloured leadwort (*P. indica*). They are at risk of becoming extinct due to the difficulty in propagation. This leads to a need for the study of their chromosome numbers for database storage. In this study, the root tips and inflorescences were investigated by using the aceto-carmine smear method. It was found that *P. zeylanica* and *P. indica* have different numbers of chromosomes, which are 2*n* = 22 and 2*n* = 14, respectively. Because the size of metaphase chromosomes is extremely small, the study of the chromosome numbers needs to be done from the microsporocyte in the anther by collecting inflorescences between November and January. For *P. zeylanica,* 11 bivalent chromosomes were found in the first meiotic metaphase. *P. indica,* 7 chromosomes were found in the second meiotic metaphase.

INTRODUCTION

The genus *Plumbago* belongs to the family Plumbaginaceae. This family comprises 10 genus, approximately 500 species. So far, there have been three species found in Thailand: *P. zeylanica*, *P. indica* and *P. capensis* (Damrongsiri, 1996). However, only *P. zeylanica* and *P. indica* have been used as medicinal plants. Chettamuun Phloeng Khao and Chettamuun Phloeng Daeng are Thai names of *P. zeylanica* and *P. indica* respectively. They are small shrubs, 0.8-1.5 m high. Roots of *P. indica* have been used as a carminative, an emmenagogue, and for the treatment of hemorrhoids. They contain plumbagin, which stimulates uterine and intestinal contractions, increases digestive enzyme secretion and stimulates the appetite (Saralamp, 1996). The roots of *P. zeylanica* have been used for the treatment of hemorrhoids, dyspepsia and rheumatism. (Dhamasaroch, 1975).

The availability of genus *Plumbago* in Thailand is limited. In addition, it is likely that *Plumbago* becomes extinct due to the difficulty in its propagation. There is, however, only one reported study on chromosome numbers of *P. capensis*. According to the literature, it was found that *P. capensis* has numbers of chromosome 2*n* = 14. This study responds to a need for the study of the chromosome numbers of *P. zeylanica* and *P. indica* for the database storage. The knowledge gained from this study is essential for the development of germplasm conservation and will help plant breeding strategies.

MATERIALS AND METHODS

Plant materials were collected from the Center for Developmental Education at Huaysai Amphur Cha-am Phetchaburi Province and the Center for Developmental Education at Khaohinson Amphur Panonsarakam Chachoengsao Province (both projects undertaken through the Initiative of His Majesty the King Bhumibol Adulyadej of Thailand). They were maintained in the greenhouse of the Biology Department, Faculty of Science, Burapha University, Chonburi, Thailand.

Mitotic chromosomes were studied in the meristematic cells of healthy root tips which were obtained from cuttings, pretreated for 4-8 hr in 0.2% colchicine solution at room temperature, washed and fixed in fresh Carnoy's solution (3:1 absolute ethanol:

glacial acetic acid) for one hr. The root tips were stored under refrigeration in 70% alcohol, hypotonic for 15 min in 0.01 M KCl, then rinsed in distilled water, hydrolyzed with 1 N HCl at 60°C for 5 min. Slides were prepared with the aceto-carmine smear method. Immature inflorescences were collected during November-January for the study on meiotic chromosomes. Slides were fixed in Carnoy's solution and stored under refrigeration in 70% alcohol.

Approximately 20 cells with good spread of mitotic and meiotic chromosomes were photographed using a microscope (Olympus BX-50 System and Olympus PM-30 Automatic Photomicrographic System) at 100x magnification.

RESULTS AND DISCUSSIONS

This study found that *P. zeylanica* and *P. indica* have different numbers of chromosome. As show in Fig. 1A, the mitotic chromosomes of *P. zeylanica* have chromosome numbers $2n = 22$. Measurements made in metaphase chromosome showed average length range 0.4-1.6 μm. The mitotic chromosome number of $2n = 14$ was found in *P. indica* (Fig. 1B). This numbers corresponds to the chromosome numbers of *P. capensis*. In addition, uniformly stain, except for the secondary constriction regions of the satellite chromosome pair, was found in short arms. The size of metaphase chromosomes ranges from 1.6-2.4 μm. The size of chromosomes of Thai herbs in this genus range from 0.4-2.4 μm. This data indicates that it is relatively small when compared with that of the other cultivars such as *Aster* (2.0-5.5 μm)or Zingiberaceae (64.80-98.12 μm) (Huziwara, 1962; Das and Das, 1998).

The meiotic chromosome of *P. zeylanica* was quite regular showing 11 bivalent in the first meiotic metaphase (Fig. 2A). For *P. indica*, 7 chromosomes were found in second meiotic metaphase (Fig. 2B). These results indicated that their patterns of meiotic configuration are regular and thus reconfirm the results of chromosome numbers from root tips.

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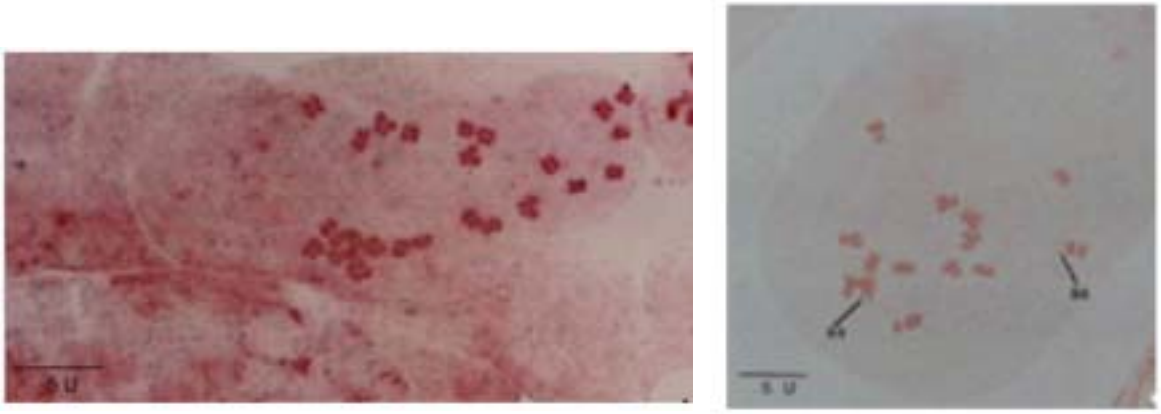
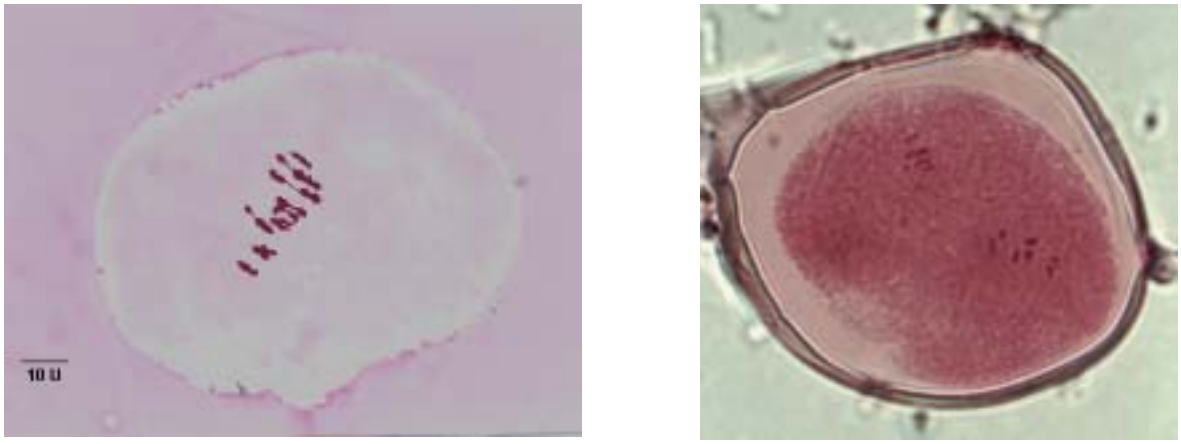
Figures



A B

Fig. 1. Somatic metaphase chromosomes A) *P. zeylanica* (2n = 22) and B) *P. indica* (2n = 14). Bar = 5 μm.



A B

Fig. 2. Meiotic chromosomes of *P. zeylanica* in first meiotic metaphase, n = 11 (A) and *P. indica* in second meiotic metaphase, n = 7 (B). Bar = 10 μm.