筑波実験植物園研報 17 : 51-54, 1998.
Ann. Tsukuba Bot. Gard.

Comparison of Chromosome Number and Karyotype between *Strongylodon lucidus* and *S. macrobotrys* (Leguminosae)

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The genus *Strongylodon,* subtribe Erythrininae, tribe Phaseoleae, family Leguminosae, consisted of twelve species (Huang 1991). *Strongylodon lucidus* (G. Forster) Seem. is widely distributed from Hawaii to Madagascar. On the other hand, *S. macrobotrys* A. Gray is endemic to the Philippines (Huang 1991), and is very threatened in the natural habitat (Andrew and Lewis 1984). Konishi *et al.* (1993), thus, performed a study of the conservation and the reproduction in *S. macrobotrys.*

With respect to basic chromosome number, Goldblatt (1981) advocated a basic chromosome number of X=14 for the genus *Strongylodon* on the basis of a count of 2n=28 in *S. macrobotrys* (Goldblatt and Davidse 1977). However, Huang (1991) and Goldblatt (1981) pointed that it was necessary to perform cytotaxonomical investigation of more *Strongylodon* species, because the basic chromosome number of X=14 in *S. macrobotrys* had no connection to that of X=11 in most members of the tribe Phaseoleae which reported by Goldblatt and Davidse (1977), Lackey (1980) and Goldblatt (1981).

Materials and Methods

Taxonomical treatment in the present study followed Huang (1991) for two species of *Strongylodon* examined.

Strongylodon lucidus (G. Forster) Seem. investigated here was collected in Vanuatu by T. Konishi on October 28, 1997 (Konishi *et al.* 1998), and cultivated in the greenhouse of the Tsukuba Botanical Garden (Table 1). Voucher specimen of *S. lucidus* investigated was deposited in the herbarium of National Science Museum (TNS). *Strongylodon macrobotrys* A. Gray was cultivated in the Tsukuba Botanical Garden (Table 1) after it has been shared from the Singapore Botanic Garden via the Botanic Garden, Faculty of Science, University of Tokyo (Koishikawa Botanic Garden). Polhill (1972) reported that *S. macrobotrys* which was cultivated in the Singapore Botanic Garden was collected from Mt. Makiling, Luzon Island, Philippines by H. M. Curran in 1937. Thus, *S. macrobotrys* investigated in the present study is supposed to be originated from Mt. Makiling.

Table 1. *Strongylodon lucidus* and *S. macrobotrys* used for chromosomal comparisonin the present study

Species	Location	Accession No.
S. lucidus	Vanuatu: Mt. Bernier, Efate Is. 150m alt.	TBG122777
S. macrobotrys	Philippines: Mt. Makiling, Luzon Is.	TBG33040

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For chromosome observation, root tips were harvested, and pretreated in 2mM 8-hydroxyquinoline at 20℃ for two hours. They were fixed in 45% acetic acid at 4℃ for ten minutes, and then macerated in a mixture of 1N hydrochloric acid and 45% acetic acid (2:1) at 60℃ for ten seconds. They were placed on the glass slides, and stained with 2% acetic orcein at 20℃ for two hours. The squash method was used for the conventional karyotype analysis. For counting chromosome number, three chromosome complements at mitotic metaphase were observed per species at least. Chromosomes at mitotic metaphase were classified according to centromeric position defined with arm ratios (Levan *et al.* 1964). Karyotype asymmetry value of arm ratio [$=1-\Sigma(b_i/B_i)/n$; b_i: length of short arm; B_i: length of long arm; n: chromosome number], and that of chromosome length ($=s/x$; s: standard deviation of chromosome length; x: mean chromosome length) followed Zarco (1986) with some modifications.

Results and Discussion

Strongylodon lucidus showed the chromosome number of 2n=22 at mitotic metaphase (Fig. 1A), which was reported for the first time in the present study. On the other hand, *S. macrobotrys* showed the chromosome number of 2n=28 at mitotic metaphase (Fig. 1B), that verified the previous document (Goldblatt and Davidse 1977).

S. lucidus studied showed the karyotype consisted of 14 median-, 7 submedian- and 1 subtermianl-centromeric chromosomes, and the chromosome length of *S. lucidus* varied from 2.0 to 5.9 μm (Fig. 1C). The asymmetry value of arm ratio and chromosome length in the karyotype of *S. lucidus* was 0.35 and 0.23, respectively. On the other hand, the karyotype of *S. macrobotrys* consisted of 13

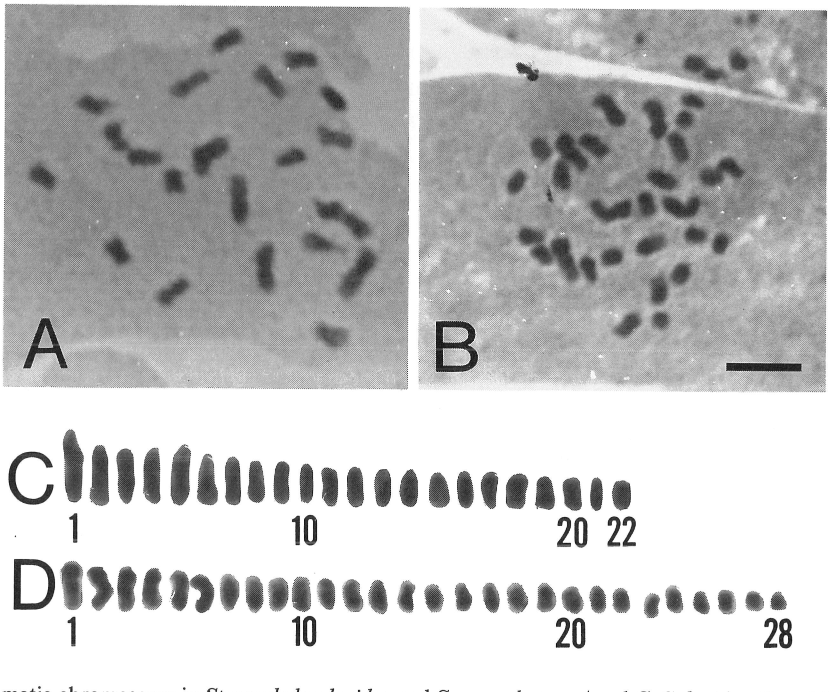

Fig. 1. Somatic chromosome in *Strongylodon lucidus* and *S. macrobotrys*. **A**and **C**: *S. lucidus*. **B** and **D**: *S. macrobotrys*. **A** and **B**: Chromosome complement at mitotic metaphase. **C** and **D**: Chromosomes aligned from the longest to the shortest. Bar shows 10 μm.

median-, 13 submedian- and 2 subterminal-centromeric chromosomes, and the chromosome length in the karyotype of *S. macrobotrys* varied from 3.1 to 7.8 μm (Fig. 1D). The asymmetry value of arm ratio and chromosome length in the karyotype of from *S. macrobotrys* was 0.34 and 0.30, respectively.

Some instances of heteroploidization by the Robertsonian chromosomal rearrangement (centromeric fission and fusion; Robertson 1916) were previously reported in some other plants, *e.g.*, the centromeric fusion in the genus *Haplopappus*, Asteraceae (Jackson 1962). Generally, the asymmetry value of arm ratio is different between two taxa which must show simple Robertsonian relationship. Concerning two *Strongylodon* species studied here, the asymmetry value of arm ratio was very similar to each other. Moreover, the number of the chromosome arm of 44 in *S. lucidus* was different from that of 56 in *S. macrobotrys*. These results intimate that the chromosome number might increase from *S. lucidus* to *S. macrobotrys*, or decrease from *S. macrobotrys* to *S. lucidus* without simple Robertsonian chromosomal rearrangement.

Golodblatt (1981) suggested the basic chromosome number of X=14 for the genus *Strongylodon* on the basis of a count of 2n=28 in *S. macrobotrys* reported by Goldblatt and Davidse (1977). On the other hand, the basic chromosome number of X=11 in most members of tribe Phaseoleae was suggested by Goldblatt and Davidse (1977), Lackey (1980) and Goldblatt (1981). Goldblatt (1981) stated that it was equally difficult to reconcile the basic chromosome number of X=14 in *S. macrobotrys* with that of X=11 in most genus of the tribe Phaseoleae including the genus *Mucuna* which was morphologically close to *Strongylodon* within the subtribe Erythriniae (Huang 1991). In the present karyotype analysis, the basic chromosome number of X=11 in *S. lucidus* cytotaxonomically connected. that of X=11 in most members of tribe Phaseoleae reported by Goldblatt and Davidse (1977), Lackey (1977) and Goldblatt (1981). The present investigation, thus, intimated that *S. lucidus* may be a taxon, or one of taxa which reconciles the cytological relationship between *S. macrobotrys* exhibiting X=14 and most other members of Phaseoleae exhibiting X=11.

Acknowledgements

This study was partially supported by International Scientific Research Program, No. 08041165, Ministry of Education, Science, Sports and Culture, Japan.

Summary

Chromosome number and karyotype of *Strongylodon lucidus* and *S. macrobotrys* were investigated with the standard acetic orcein staining method. *S. lucidus* showed the chromosome number of 2n=22 and the karyotype consisted of 14 median-, 7 submedian- and 1 subterminal-centromeric chromosomes. On the other hand, *S. macrobotrys* showed the chromosome number of 2n=28 and the karyotype consisted of 13 median-, 13 submedian- and 2 subterminal-centromeric chromosomes.

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