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CYTOLOGICAL STUDY OF THE FERN GENUS *DIPLAZIUM* IN GUNUNG GEDE-PANGRANGO NATIONAL PARK, JAVA

Ngatinem Praptosuwiryo & Dedy Darnaedi
(Herbarium Bogoriense, Puslitbang Biologi—LIPI, Bogor)

NGATINEM PRAPTOSUWIRYO & DEDY DARNAEDI. 1994. Cytological study of the fern genus *Diplazium* in Gunung Gede-Pangrango National Park, Java. *Floribunda* 1 (15) : 57—60. — A cytological study of the fern genus *Diplazium* (*Diplazium accedens*, *D. bantamense*, *D. dilatatum*, *D. opacum*, *D. pallidum* and *D. polypodioides*) growing along a narrow strip of forest in Gunung Gede-Pangrango National Park, West Java was carried out. Somatic and gametic chromosome number counts of 76 plants were made by using standard squash methods to determine their ploidy level. Mode of reproduction was examined by a spore counting method. Except for that of *Diplazium accedens* and *D. pallidum*, all species examined were found to have various ploidy levels, but there were significant differences in their spore size and stomatal index. The agamosporous race consistently has bigger spore size than the sexual one. The high percentage of the occurrence of polyploidy, and high degree of the ploidy level, in *Diplazium* is expected to have been enhanced by cold.

The importance of cytological information and mode of reproduction in taxonomic studies of fern species was first demonstrated by Manton (1950), who described polyploidy, hybridization and apomixis. For the Malesian flora a cytological survey was reported from Malaya Peninsula by Manton (1955), but reports are still lacking for Java. A preliminary cytological survey of fern species of Gunung Gede-Pangrango National Park, West Java was reported by Darnaedi (1981, 1992). The results demonstrated a high degree of polyploidy and apomixis.

Fern genus *Diplazium* is the biggest fern genera of the Athyriaceae, and comprises about 400 species world-wide, 25 species distributed in Malesian region and 17 growing on Java (Backer & Posthumus 1939). The cytological study of genus *Diplazium* represented only 4% of the genera (Walker 1979). The percentage of species examined in Java island will be much lower

than 4%. *Diplazium* has basic chromosome numbers of 40 and 41, and tetraploid races are dominant in the genus (Kato & Darnaedi 1988). Taxonomy and morphology of the fern species at Gunung Gede-Pangrango National Park will be described separately by Darnaedi in the Fern Flora of Gunung Gede-Pangrango National Park.

In this study, we examine the cytology on the base of chromosome number and chromosome size as well as reproductive mode of the fern genus *Diplazium* growing along narrow strip in the Gunung Gede-Pangrango National Park.

Materials and Methods

Plants used in this study were collected in the lower part of Gunung Gede-Pangrango National Park at

1,000 to 1,500 m alt. in 1992-1993. Living plants were brought into cultivation at Herbarium Bogoriense experimental gardens. For quick examination living plants kept in wet newspaper and plastic bags for a few days to stimulate the development of new roots. Cytological investigations were made on mitosis and meiosis in spore mother cells. For somatic chromosome observation, actively growing root tips (ca 1 cm long) were pretreated with 0.002 M 8 hydroxyquinolin and placed in darkness at 18-20°C for 3-5 hrs. Selected root tips without root caps were then washed, fixed in 45% acetic acid for 10 min, transferred 1 N HCl solution at 60°C for 3-4 min, and then squashed gently in 2% aceto-orcein solution. Good material was immediately examined under a light microscope, and photographs were taken by using Nikon Camera. Chromosome numbers were counted during observation.

To determine reproductive means (sexual or agamosporous reproduction) spore numbers in each sporangium were counted. Plants with 64 spores in each sporangium were considered to be sexual form, while those with 32 spores is to be agamosporous one (Manton 1950). All voucher specimens and cytotypes are deposited at Herbarium Bogoriense.

Results and Discussions

Chromosome Numbers and Mode of Reproduction

The results of cytological observation of mitotic and meiotic chromosomes of *Diplazium* confirms that the basic chromosome numbers of the genus is $x = 41$, as reported for the first time by Manton (1950). Individual plants with $n = 41$ and or $2n = 82$ is considered to be diploid.

Cytological data provide information of reproductive mode. In a sexual plant, chromosome number of the sporophyte is double the number of the gametophyte. In an agamosporous plant, chromosome numbers of its sporophyte and gametophyte are the same (Manton 1950, Walker 1979).

The results of cytological examination of 76 plants are shown in Table 1, and Fig. 1. Five cyto-reproductive types of *Diplazium* are recognized: diploid sexual, triploid agamosporous, tetraploid sexual, hexaploid sexual and octoploid sexual. Triploid agamosporous types of *Diplazium dilatatum*, *D. polypodioides*; tetraploid sexual types of *D. dilatatum*, *D. opacum*, *D. pallidum*; hexaploid sexual types of *D. opacum* and octoploid sexual types of *D. bantamense* are new cytological records for the species mentioned (Table 1).

Sixteen plants of *Diplazium accedens* examined had $2n = 82$ chromosomes (Fig. 1a). *D. bantamense* has two cyto-reproductive types, $2n = 164$ as tetraploid sexual (Fig. 1b) and $2n = 328$ as octoploid sexual (Fig. 1c). Morphological features distinguishing the tetraploid and octoploid type are unclear. It is presumed

that they have had autopolyploid relationship. Thirteen plants of *D. dilatatum* had $n = 123$ chromosomes (Fig. 1e), and one had $2n = 164$ (Fig. 1d). *D. opacum* has two cytotypes, one of $n = 82$ and $2n = 164$, presumed to be tetraploid sexual (Fig. 1f). The other type has $2n = 246$ with 64 spores. Fifteen plants of *D. pallidum* consistently had $2n = 164$ chromosomes (Fig. 1i). One individual of *Tin* 2-6 had $n = 82$ and $2n = 164$ chromosomes, indicating a tetraploid sexual. *D. polypodioides* has two cytotypes, one with $2n = 82$ (Fig. 1g) the other $2n = 123$ (Fig. 1h). Their reproduction modes are presumed to be sexual and agamosporous respectively.

Table 1. Species, chromosome number, ploidy level & reproductive mode

Species (number of specimens examined)	Chromosome number (n/2n)	Ploidy level & reproductive mode**
<i>Diplazium accedens</i> (16)	-/82	2X sexual
<i>D. bantamense</i> (17)	-/164	4X sexual
(2)	-/328*	8X sexual
<i>D. dilatatum</i> (13)	-/123*	3X agamosporous
(1)	-/164*	4X sexual
<i>D. opacum</i> (3)	82/164*	4X sexual
(1)	-/246*	6X sexual
<i>D. pallidum</i> (1)	82/164*	4X sexual
(14)	-/164	4X sexual
<i>D. polypodioides</i> (1)	41/82	2X sexual
(5)	-/82	2X sexual
(2)	-/123*	3X agamosporous

* New cytological record for the species examined

** 64 spores in each sporangium is expected as sexual reproductive mode, 32 spores as agamosporous one

If we examine the series of ploidy levels in each species, there is no specific pattern of polyploidization mechanism. *D. accedens* is diploid sexual, while *D. pallidum* is tetraploid sexual. The other species varied irregularly. Tetraploid sexual races are the commonest among the species examined, that is 47% of all plants. The same situation was demonstrated in an intensive study of *Dryopteris sparsa* complex with a wide distribution range (East and SE Asia), where about 70% of plants examined were found to be tetraploid and reproductively sexual (Darnaedi et al. 1984). *D. rheophylla*, an endemic species in Thailand, also has a monocytotype 4x sexual. Kato (1992) pointed out that 83% of 372 Japanese fern species examined are sexual in reproduction, and 30% of the sexual species are tetraploid. The tetraploid sexual races are common both in tropical and subtropical areas. As far as the origin of tetraploid is concerned it is expected as duplication of basic chromosome number by both autopolyploid or allopolyploid. Further experimental information is needed.



Fig. 1. Photographs of somatic chromosomes. Scale bar = 3 μ m for all figures, except for c = 6 μ m. a. *Diplazium accedens*, $2n = 82$. b.c. *D. bantamense*, $2n = 164$; $2n = 328$. d.e. *D. dilatatum*, $2n = 164$; $2n = 123$. f. *D. opacum*, $2n = 164$. g.h. *D. polypodioides*, $2n = 82$; $2n = 123$. i. *D. palidum*, $2n = 164$.

Spore Size

Spores size of agamosporous plants are larger and more diverse than those of the sexual plants. Triploid agamosporous *D. dilatatum* has spores with a mean size of 53.45 μm long and 33.25 μm wide, while tetraploid sexuals spores are 42.06 μm long and 28.69 μm wide.

The hexaploid and octoploid races are only in that of *D. opacum* and *D. bantamense*. Within one species, with the same reproduction mode, spores of plant with higher ploidy level were larger in size. Hexaploid races of *D. opacum* have spores of 38.25 μm long and 24.00 μm wide, while the tetraploids have a mean size 32.9 μm long and 22.5 μm wide. A relationship between spore size and ploidy level is thus indicated.

Polyploidy

Chromosome numbers of plants examined in this study demonstrated that *Diplazium* has 80% polyploids with up to 8-ploidy levels (Table 1). The high percentages of polyploidy and high ploidy levels found in this study may have a correlation with low temperature is the mountain range of Gede-Pangrango National Park. Love et al. (1977) hypothesized that polyploidy was enhanced by cold. The same principle can be applied to the mountain ranges of tropical regions.

In Tropical West Africa and Jamaica, a similarity to our data is seen with 60% of species polyploid, and with a number of species attaining levels of 12-ploidy or more. In each of these regions there are moderately high mountains, very varied climatic conditions and a history of great environmental disturbance.

Implication of the Finding

The implication of this study for plant biodiversity at the population level is significant. Where any of the species we studied found to be rare and in need of a conservation strategy, it would be possible only through such genetic analysis to determine what should be conserved. Without such an analysis, researcher would be at risk of selecting only certain genotypes which may not be suitable for replanting in other areas.

It is tragic that at a time when the world is focussing on species conservation, the bases of the species are still ignored. Without an understanding of a species structure in plants, future conservation strategies are likely to be poorly throughout.

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