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SUMMARY OF DOCTORAL THESIS

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Title:
CYTOLOGICAL AND GENETICAL STUDIES IN PEARL MILLET, *PENNISETUM GLAUCUM*, AND ITS WILD RELATIVES

トウジンビエ、*Pennisetum glaucum*、および その近縁種の細胞学および遺伝学的研究

In this study, I investigated the chromosome variation of ten *Pennisetum* species including pearl millet (*P. glaucum*) by fluorescence *in situ* hybridization (FISH) probed with a highly repetitive centromeric satellite and 45S rDNA sequences. I also analyzed the genetic relationship using EST microsatellite markers derived from pearl millet. FISH with the centromeric satellite sequence showed strong to medium hybridization signals at the centromeric regions of all the chromosomes in six species, weak signals on two chromosome pairs in one species and no signal in three species. 45S rDNA sites were varying both in number and strength among the species. Two species showed the signal on one chromosome pair, five species on two chromosome pairs, one species on three chromosome pairs and two species on four chromosome pairs. In addition, DAPI staining exhibited heterochromatin blocks at centromeric, telomeric or both regions in the tested species. Prominent signals were observed at the telomeric region in two species. Results of the EST-microsatellite markers and phylogenetic tree among these species fitted well with the chromosome variation of these species. The discrepancy between the morphological classification and the phylogenetical grouping suggested that the classification of *Pennisetum* species should also consider genomic level rather than only morphological features.

I also analysed karyotypes of *P. schweinfurthii* and *P. glaucum* using acetocarmine condensation pattern, centromeric satellite DNA and 45S rDNA sequences. The chromosome complement of *P. schweinfurthii* consisted of four metacentric chromosomes (I, III, IV and V) and three sub-metacentric (II, VI and VII) chromosomes, whereas chromosome complement of *P. glaucum* consisted of four sub-metacentric (I, III, IV, V and VI), one metacentric (II) and one sub-telocentric (VII) chromosomes. The chromosome length was ranged between 2.20 and 4.90 μm in *P.*

schweinfurthii and between 2.95 and 4.70 µm in *P*. *glaucum*. Large heterochromatin regions, mainly located at centromeric and telomeric regions in most chromosomes of the two species were observed after staining with acetocarmine. Marker bands were clearer in *P*. *schweinfurthii* than in the *P*. *glaucum*. Sites of 45S rDNA sequence were found at two metacentric chromosome pairs in *P*. *schweinfurthii* and one sub-metacentric and one sub-telocentric chromosome pair in *P*. *glaucum*. The intensity of the 45S rDNA signal was higher in *P*. *schweinfurthii* than in *P*. *glaucum*. Results indicated that combining both centromeric satellite and 45S rDNA sequences was useful in differentiating some chromosomes of the two species.

The occurrence of endopolyploidy chromosomes in root tip cells of two different *Pennisetum* species (*P*. *glaucum* and *P*. *mollissimum*, 2n=2x=14 for both of them) was also reported in this study. The two species showed one cycle of DNA endoreduplication and attained a maximum DNA level of 4C and a maximum number of four copies of each chromosome (28 chromosomes). The frequency of occurrence of endopolyploidy chromosome in this study was low (0.9% and 0.6% in *P*. *glaucum* and *P*. *mollissimum*) respectively, from the total observed cells (1041 for *P*. *glaucum* and 798 for *P*. *mollissimum*). More lines and more detailed investigations are needed for better understanding of the physiological and the cytogenetical significance of the occurrence of this phenomenon in pearl millet, *P. glaucum*, and its relative species.

Generally, *Pennisetum* species I used in this study were very good representatives in revealing the chromosome evolution and variation occurred among *Pennisetum* species.