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Induction of multi-karyotypes and genome stability in *Zebrina pendula* (2n=23) after irradiation of X-rays

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Abstract. Karyotypes of 8M+5A+7T+2f, 8M+4A+8T+2f and 8M+3A+9T+3f for *Zebrina pendula* were induced and established from the parental karyotype of 7M+5A+9T+2f (2n=23) by irradiation of X-rays during the course of study. Those new karyotypes seemed to be basically produced by adding M (metacentric chromosome). Centric fissions of M- and f- (fragment) chromosomes were observed here for the first time. 2A- (acrocentric) chromosomes were established by reciplocal translocations between two arms of M-chromosomes and two little arms of f-chromosome and disappearance of a M-chromosome. A centric fission firstly occurred synthesized decrease of M-chromosomes, production of A- and T-chromosomes and then, karyotype display of 5M+8A+10T at the appearance frequency of 7.5%, originally seen in the wild type of *Z. pendula* 23-plant. However, below 5M-chromosomes were not shown in any individual plant and thus, the volume of 5M-chromosomes seemed to be the downward limit of the genome security-stabilization. Some individual plants of *Z. pendula* showed some variants such as thin leaves, glossy leaves and albino leaves.

Keywords : Centric fission, Centric fusion, Deletion, Genome, Karyotype, Reciprocal translocation, Specific differentiation, *Zebrina pendula* Scnizl.

Introduction

Structural changes, aneuploidy and so on in chromosome combined together with sexual isolation may be factors of species differentiation and speciation (*e.g.*, Stebbins, 1971).

Zebrina pendula Schnizl. is a Mexican member of the Commelinaceae is well-known to have the chromosome number of 2n=24 (called "24-plant")(Simmonds, 1954 ; Mattson, 1971 ; Bhattacharya, 1975 ; Jones, 1978 ; Lalithambika and Kuriachan, 1981, 1983). However, a plant with 2n=23 (called "23-plant") was found in the 24-plant population (Saruwatari and Shigenobu, 1989). The karyotype of the common, normal 24-plant of the species was 2n=24=4M+8A+12T, while that of the 23-plant was 2n=23=5M+8A+10T followed White (1945). The symbols "M," "A" and "T" designated metacentric, acrocentric and telocentric chromosomes, respectively. Compaired the karyotypes of the two chromosome types, the 24-plant had two surplus T-chromosomes and the 23-plant had one surplus M-chromosome. Since total numbers of chromosome arms (n.f.=nonble fundamental) in the karyotypes of the 23- and 24-plant were commonly 28, it has been speculated that reduction of two T-chromosomes and addition of one M-chromosome were occurred at once. This speculation suggested that a Robertsonian centric fusion (see Robertson, 1916) might occur between the two T-chromosomes to construct another new karyotype (Saruwatari and Shigenobu, 1989).

Concerning the karyotypic evolution in naturally grown *Zebrina* and *Cymbispatha* of the Commelinaceae, increase progress of M-chromosomes in number by cenric fusion would perform speciation has been discussed by several workers (Jones, 1978 ; Gracia,

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Table 1. Appearance frequencies of chromosome aberrations induced in the mother plant No. 13002124al of *Zebrina pendula* by irradiation of X-rays*

Dose (Gy)	No. of scions exposed to X-rays	No. of terminal and lateral shoots observed	No. of terminal and lateral shoots produced chromosome aberrations	Frequency of chromosome aberrations
3	5	19	2	10.5
9	5	21	8	38.1

*Other chromosome aberrations were found in adventitious root appeared directly from a cut-scion (see Figs. 1b and 2b).

1984). Following this fact, Jones (1978) hypothesized that *Zebrina* evolution would be progressed from: (1) 2n=28A(T) of tetraploid was produced from 2n=14A(T) of diploid by chromosome doubling; (2) and performed and repeated centric fusion to; and (3) then, led to the present karyotype. Thus, Jones' hypothesis (1978) and other interesting thoughts were experimentally traced and analyzed here.

Materials and methods

Our research strain 13002124al of *Zebrina pendula* Schnizl. called the mother plant was developed by Y. Shigenobu and his co-workers in Naruto University of Education and was transferred and was continuously cultivated in the experimental greenhouse, Hiroshima Jogakuin University for this experiment. This mother plant was a chromosomal mutant with 2n=23=7M+5A+9T+2f ("f" designated fragment)(see Fig. 2a) artificially induced and originated from the 23-plant by irradiation of X-rays. Ten cut-scions, each of which had five nodes and five leaves, were divided into two groups for exposure to X-rays at two different doses.

Irradiation of X-rays to the plants were made by the X-ray generator (MBR-1505R, Hitachi Medico) with 140 KV, 4 mA, 1 mm Al filter in the Atomic Energy Research Institute, Kinki University. The dose rate used was 23.6 Gy/min, and absolute doses used were 3 Gy and 9 Gy.

The groups exposed by the absolute doses of 3 Gy and 9 Gy were abbreviated in this description as "03" and "09", respectively and attached with the scion numbers 1 ~5. They were recorded after the mother plant. The Plant No. 13002124a1 indicated the mother plant and the first "1" meant a flower-shop in Fukuoka City of the plant-obtained location, the next number of three figures "300" meant the application dose of X-rays by Kojima (1992), the next number of one figure "2" meant number of scions, the next "12" meant X-ray exposure dose of 12 Gy by Kurasawa (1994), the next number of one figure "4" meant scion number, and the last "al" meant one of the lateral shoots used in this study occurred from the lower-most or the fifth node. In other words, "s" designated the roots occurred from the terminal shoot, and a, b, c,.....in alphabetical order designated roots occurred from lateral shoots in location order from the lateral shoots of the node closet to the base toward the upper part. If some lateral shoots were appeared from same node, they were symbolized 1, 2, 3.....in order. Then, root from certain lateral shoot in order and location of lateral shoot in same node were grouped together to be symbolized; *e.g.*, if the root was occurred from the lateral shoot of the node closet to the base and three lateral shoots appeared from the node, they were symbolized as "a1", "a2" and "a3". Then, roots from respective shoots after X-ray exposure were used to study and analyze karyotypes.

Chromosome observation was made by the standard, orthodox 2% aceto-orcein squash method after pretreatment in 2 mM 8-hydroxyquinoline for 5 h, fix in 3 : 1 mixture of ethanol and acetic acid at 10°C for 24 h and maceration in 2 : 1 mixture of 1N hydrochloric acid and 45% acetic acid at 60°C for 20 sec.

Results and discussion

Among 40 terminal and lateral shoots obtained karyotypes after irradiation of X-rays, ten or 25.0% showed chromosome aberrations (Table 1). Additionally, adventitious roots occurred from cut-scion also showed chromosome aberrations (Figs. 1b and 2b). In these chromosome aberration samples, three displayed increase number of M-chromosomes were described in detail as follows:

(1) The plant 13002124a1093c (Figs. 1b and 2b)

Adventitious root occurred from the third cutting-stem showed the karyotype of 2n=22=8M+5A+7T+2f. Comparing the karyotype of 2n=23=7M+5A+9T+2f (Fig. 2a) of the mother plant, that karyotype of this plant got decreased two T-chromosomes and increased one M-chromosome. Thus, that karyotype of the chromosome aberration displayed reduction of one chromosome from the chromosome number of the mother plant.

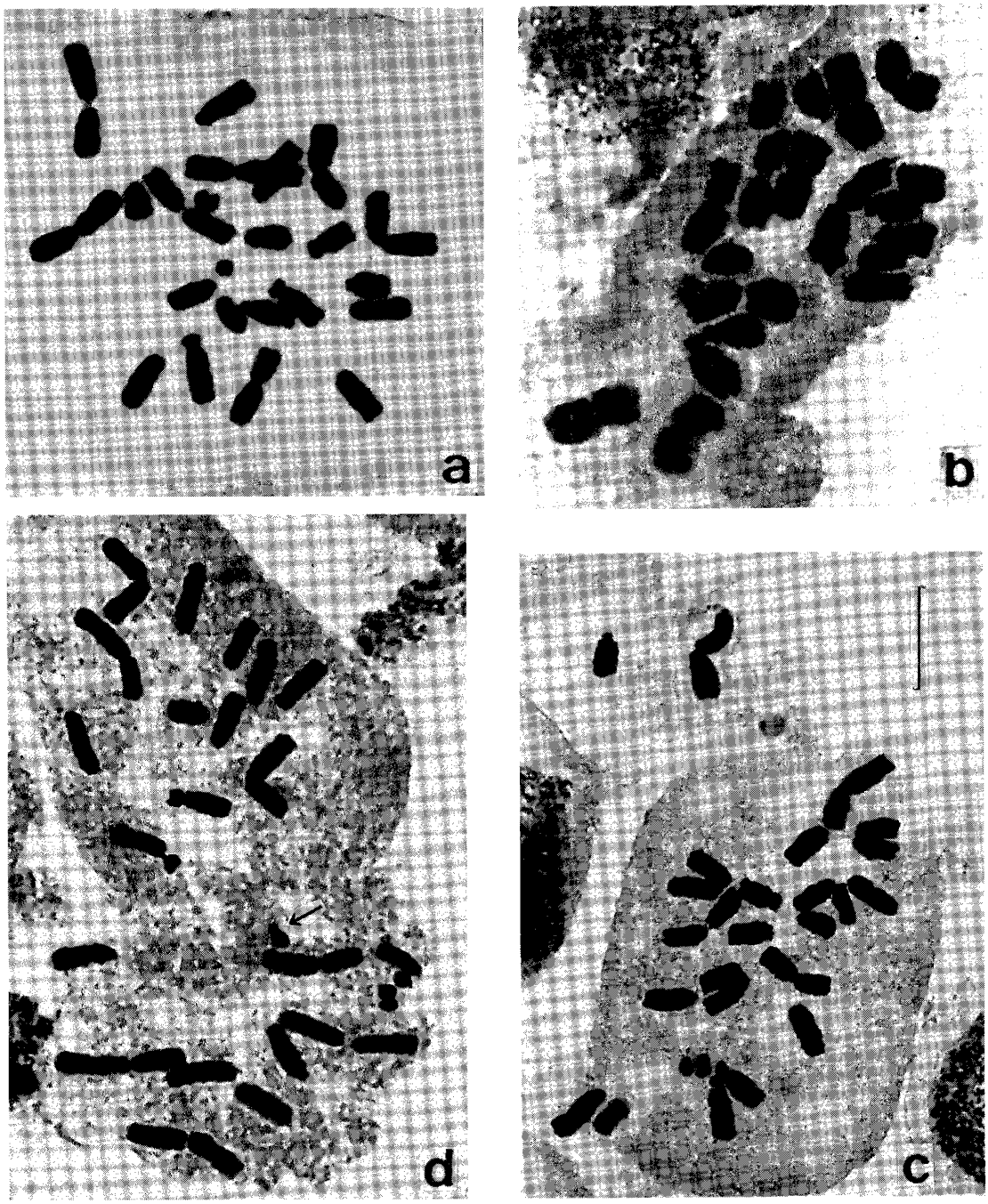

Figure 1. Chromosomes at mitotic metaphase in *Zebrina pendula*. a. Mother plant No. 13002124al. b-d. The karyotype with 8 M-chromosomes in an adventitious root from scion-cutting from the mother plant after irradiation by X-rays at 9 Gy dose. Arrow points a f-chromosome fragment. Bar=10 μm.

(2) The plant 13002124a1091b (Figs. 1c and 2c)

This individual plant had the karyotype of 2n=22=8M+4A+8T+2f which showed the same chromosome number to but different karyotype from those of the above 13002124a1093c. Comparing the karyotype of 2n=23=7M+5A+9T+2f (Fig. 2a) of the mother plant, the karyotype of this plant indicated the reduction of one A-chromosome and one T-chromosome and the addition of one M-chromosome. In other words, in the mother-plant cell, a centric fission could occur at the centromeric region of one A-chromosome. Then, the long arm of the A-chromosome could be fused to a T-chromosome at the centromeric region to form one M-chromosome. However, the short arm of the A-chromosome seemed to be fragmented and disappeared due to the absence of the centromere.

(3) The plant 13002124a1092d (Figs. 1d and 2d)

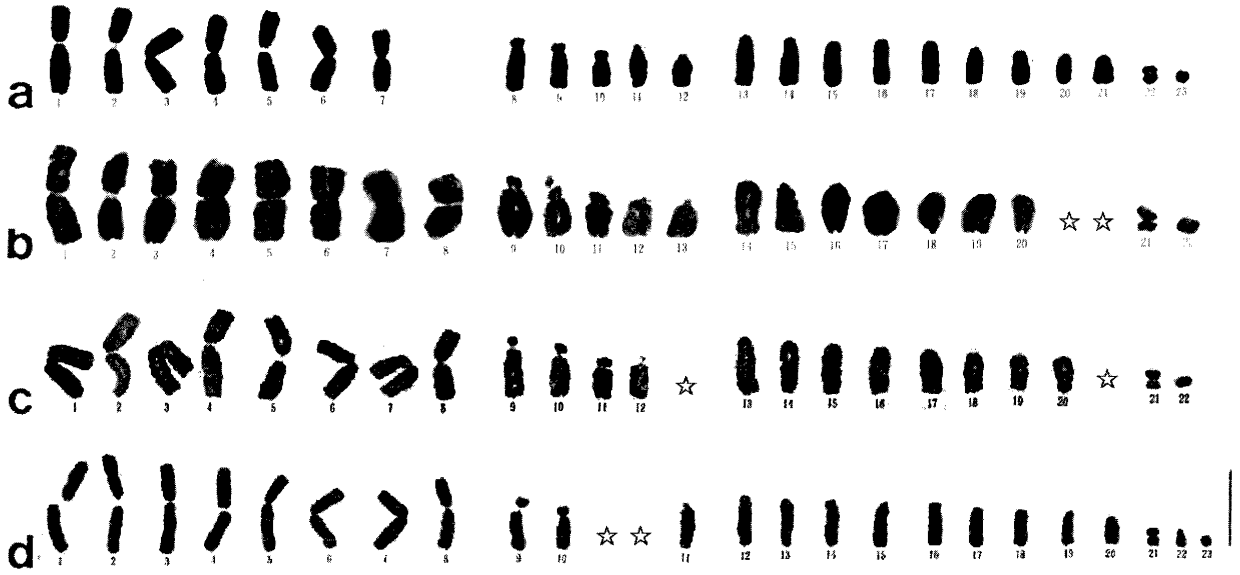

Figure 2. Karyotypes of the mother plant No. 13002124al which has 7 M-chromosomes (a) and the newly induced plants which have 8 M-chromosomes (b-d). a. Base karyotype of 7M+5A+9T+2f in the mother plant. b. Induced karyotype of 8M+5A+7T+2f; reduction of 2 T-chromosomes and addition of 1 M-chromosome. c. Induced karyotype of 8M+4A+8T+2f; decrease of 1 A-chromosome and 1 T-chromosome and addition of 1 M-chromosome. d. Induced karyotype of 8M+3A+9T+3f; decrease of 2 A-chromosomes and addition of 1 M-chromosome and 1 f-chromosome fragment. The star symbols indicate the lack positions of T- or A-chromosomes. Bar=10 μm.

This individual plant had the karyotype of 2n=23= 8M+3A+9T+3f of which the chromosome number was one chromosome larger than 2n=22 and which was different form that shown in the plant 13002124a1093c and the plant 13002124a1091b. Comparing the karyotype of 2n=23=7M+5A+9T+2f (Fig. 2a) of the mother plant, the karyotype of this plant indicated the reduction of one A-chromosome and one A′-chromosome which was categolized by extremely small short arm and the addition of one M-chromosome and one f-chromosome (fragmented chromosome). It was speculated that centric fission could occur at the centromeric region of two A-chromosomes, their two long arms could make fusion with each other at their centromeric regions to form one M-chromosome and then, their two short arms could make fusion with each other at their centromeric regions to form one f-chromosome. Thus, the present karyotype could be promoted by Robertsonian-type reciprocal translocation. Since the f-chromosome was obviously asymmetric in arm (see the arrow point in Fig. 1d; the 22nd f-chromosome), it could be produced by the centric fususion between the short arm of one A-chromosome and the extremely short arm of one A'-chromosome by the reciprocal translocation and it could be distinct from the existing fragmented chromosomes of 2f.

Thus, the three new karyotypes that have commonly 8 M-chromosomes (Fig. 2) were here induced commonly by the addition of M-chromosomes in number and reduction of T- or A-chromosomes in number due to the centric fusion by exposure of X-ray following our series of the experiments (Saruwatari and Shigenobu, 1989; Kojima, 1992; Shigenobu and Kojima, 1992; Shigenobu and Takamaru, 1993; Kurasawa and Shigenobu, 1996). On the other hand, the present study found the reduction of M-chromosomes in number or the addition of T- and A-chromosomes in number. Furthermore, the present chromosome mutants had never had M-chromosome numbers below 5 shown in the wild 23-plant with 2n=23=5M+8A+10T. Then, three (7.5%) plants showed the same karyotype as the wild 23-plant during the course of investigation. Thus, the chromosome speciation in *Zebrina pendula* could be progressed toward increase number of M-chromosomes above 5 M. In other words, *Zebrina*, which has chromosome evolution progressed toward reduction of chromosome numbers from 2n=23 down to 2n=20, suggested that the critical number "5" of M-chromosomes could be the lowest limit of the stabilization for its genome and number of M-chromosomes could be increased above 5, if the karyotype would artificially induced over 8 M-chromosomes. In this case, their chromosome numbers taken off the f-(fragmented) chromosomes were counted.

Gracia (1984) stated that among 85 individual plants of *Zebrina pendula* with n.f. 28 and four different karyotypes characterized by numbers of M-chromosomes

such as 4M, 5M, 6M and 7M collected in nature in Mexico, 73 individual plants or 85.9% or the largest population were distinct with 6 M-chromosomes and showed 2n=22=6M+5A+11T+1B and were seemed to be well-adapted to certain local habitat. According to Gracia's statement (1984), the Mexican nature in 1984 or a few years before seemed to hold the chromosome speciation step at the stage of 6 M-chromosomes. Those plants may maintain the lowest limit of 5 M-chromosomes for their genome stabilization and gradually progress chromosome evolution toward increase of M-chromosome numbers.

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