Study on Diffused Centromeric Nature of *Drosera* Chromosomes¹

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The *Drosera* chromosomes seemed to lack of single localized centromeres and thus, a distinct non staining gap exists between the sister chromatids along the entire length. This nature led some scientists to propose that *Drosera* chromosomes might possess diffused centromeres throughout the length (Kondo *et al.* 1976, Kondo and Lavarack 1984). However, no strong experimental evidence was available to prove the diffused-or poly-centromeric nature of *Drosera* chromosomes.

In mutation breeding Gamma radiation has been widely used in different plant materials having chromosomes with single localized centromeres. As a consequences several acentric chromosome fragments were resulted. Due to lack of centromeres these fragments produced laggards, anaphase bridge or micronuclei (Datta *et al.* 1986, Athma and Reddy 1986, Gautam *et al.* 1992, Ahmeed 1993). Thus, if the *Drosera* chromosomes have diffused centromeres, each artificially-induced fragment would contain centromeres and survive as a mitotically functional individual. Therefore, it is necessary to investigate whether or not the radiation-induced fragments of chromosomes in *Drosera* possess centromeres.

Gamma radiation is here exposed to *D. dichrosepala* Turz to induce chromosome fragments for confirming the validity of the hypothesis proposed by earlier workers (Kondo *et al.* 1976, Kondo and Lavarack 1984). Moreover, Cd-banding, Giemsa C-banding and fluorescent banding techniques are also applied here to elucidate the nature of fragment chromosomes.

Materials and methods

Gemmae of *Drosera dichrosepala* were purchased from Western Australia. After surface sterilization the gemmae were innoculated in hormone-free 1/2 Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962) supplemented with 0.2% gellan gum and 3% sucrose for *in vitro* culture. Germinated plants were subcultured in the same medium for mass propagation by multiple shoots. Plants were then subjected to Gamma radiation. Different doses of Gamma radiation applied to *D. dichrosepala* and the code used in this study for each dose were summarized in Table 1.

Orcein staining—roots obtained from both control and radiated plants were pretreated with 0.002 M hydroxyquinoline for 2 hr at 18°C followed by 15 min fixation in 45% acetic acid at 4°C. They were then hydrolyzed in a mixture of 1N hydrochloric acid and 45% acetic acid (2:1) at 60°C for 5 sec. The root tips were stained and squashed in 1% aceto-orcein.

Giemsa C-banding—the method proposed by Kondo and Lavarack (1984) was applied with a slight modification. Hydrolyzed root tips were squashed in 45% acetic acid. The cover slips were removed by the dry-ice method and the slides were air dried for at least 48 hr. They

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Table 1. Code, different doses of Gamma radiation and various consequences after radiation on *D. dichrosepala*

Code	Radiation doses (rad)	Number of individuals exposed	Number of individuals survived	Duration for new root formation (days)	Fragmentation
R-0 (control)	0	10	10	17	not seen
R-1	10	10	10	25	"
R-2	50	10	10	31	"
R-3	100	10	10	35	"
R-4	200	10	10	42	"
R-5	500	10	9	55	"
R-6	1,000	10	7	72	"
R-7	2,000	10	5	103	seen
R-8	5,000	10	4	127	"
R-9	10,000	10	1	149	"
R-10	20,000	10	1	182	"
R-11	35,000	10	0	—	—
R-12	70,000	10	0	—	—
R-13	100,000	10	0	—	—
R-14	300,000	10	0	—	—

were incubated in 5% barium hydroxide solution at 60°C for 5 min followed by 5 min rinsing in distilled water. The slides were then dipped in 2XSSC (0.3 M sodium chloride and 0.03 M trisodium citrate in aqueous solution) for 1 hr at 60°C, rinsed and air dried. The slides were stained with 3% Giemsa in 1/15 M phosphate buffer (pH 6.8) for 1 hr.

Centromeric banding (Cd-banding)—centromeres were stained with Cd-staining method (Hizume *et al.* 1992). The air-dried preparations were treated with Earl's saline (pH 8.5) at 85°C for 1 hr followed by rinsing in distilled water and stained with 3% Giemsa in 1/15 M phosphate buffer (pH 6.8) for 1 hr.

Fluorescent banding—methods proposed by Kondo and Hizume (1982) were followed with a little modification. Briefly, air-dried slides were preincubated in McIlvaine's buffer (citric acid-disodium hydrogen phosphate, pH 7.0) for 30 min. Slides were treated with 0.1 mg/ml distamycin A (Sigma) for 10 min followed by mild rinsing with the same buffer supplemented with 5 mM MgSO₄ and stained with 0.1 mg/ml Chromomycin A₃ (CMA) for 10 min. Same slides were destained in 45% acetic acid for 15 min and used for 4'-6 diamidino-2-phenylindole (DAPI) staining. Slides were dipped in McIlvaine's buffer (pH 7.0) for 30 min followed by 0.25 mg/ml actinomycin D (Sigma) treatment for 15 min. After rinsing in the same buffer for 10 min, the slides were stained with DAPI (0.1 µg/ml) for 7 min. Photographs were taken under a Nikon fluorescence microscope with BV filter cassette for CMA and UV filter cassette for DAPI stain.

Results

Different features regarding Gamma radiated plant materials are shown in Table 1. The control plants possessed 2n=12 chromosomes without any sort of abnormality (Fig. 1A). Among the 14 different doses of Gamma radiation, fragmentation occurred from R-7 upto R-10. Number of fragmented chromosomes were increased with the increasing of radiation doses (Figs. 1B-I). However, the plant materials radiated more than R-10 were completely died (Fig. 2). An abnormally big chromosome was found in every metaphase cells of R-7 and R-8 plants only that perhaps due to fussion because no such chromosome has been seen in the control plant (Figs. 1B arrowhead, 1C arrow).

Typical anaphase with regular disjunction and proper seggregation of the fragmented chromosomes were observed (Fig. 1E arrow). No micronucleus, lagging chromosome or

Fig. 1. Mitotic chromosomes of *D. dichrosepala* before and after Gamma radiation. A. Control, metaphase. B. R-7, metaphase. C. R-8, metaphase. D. R-9, metaphase. E. R-8, late anaphase. F. R-7, Cd-banding. G. R-9, C-banding. H. R-10, CMA staining. I. R-10, DAPI staining. Bar=10 μ m.

anaphase bridge was observed in any case.

After centromeric banding, a number of paired bands appeared on the sister chromatids of all chromosomes (Fig. 1F). More than one paired Cd-bands were also observed in the fragmented chromosomes (Fig. 1F arrow). In a few cases, some portions at the terminal regions of the regular chromosomes were stained completely (Fig. 1F arrowhead).

A number of dark and prominent C-bands were found in every metaphase cell. Bands usually appeared at the terminal regions of the regular chromosomes, however, all the

Fig. 2. Survival rate of *D. dichrosepala* after exposing to different doses of Gamma radiation.

fragmented chromosomes were entirely stained (Fig. 1G arrow).

Sequential staining with CMA and DAPI were carried out in the same preparations. Many CMA-positive bands were found. The fragmented chromosomes showed CMA-positive bands that correlated with those of DAPI-negative bands (Figs. 1H, 1I arrow). Only two DAPI-positive bands appeared each in a regular and a fragmented chromosomes which were also slightly stained with CMA (Figs. 1H, 1I arrowhead).

Discussion

The control *D. dichrosepala* plant possessed $2n=12$ chromosomes ranging from 2.2–3.3 μm in length (Fig. 1A). Kondo *et al.* (1976) counted $2n=18$ chromosomes with a chromosome length ranged from 1.7–2.0 μm for this species. Thus, the present result was completely different from that of previous report both in chromosome number and size. Since the chromosomes of this species could be broken easily into fragments by radiation, it might suggest that the chromosome number reported by Kondo *et al.* (1976) would be a result of spontaneous fragmentation of chromosomes. Moreover, the size of chromosomes might provide an additional support to this idea.

Cd-banding revealed the presence of diffused-or poly-centromeres, since numerous paired dark bands appeared on both the chromatids along the length. Paired Cd-bands were also found in the fragmented chromosomes (Fig. 1F arrow). Anamthawat-Jonsson *et al.* (1993) proposed that the movement of chromosomes depend on their centromere activity. In this study, regular anaphase was observed with a typical segregation of fragmented chromosomes revealed that centromeres of the fragmented chromosomes functioned actively during disjunction, and thus poleward movement was possible for them (Fig. 1E arrow). Therefore, it might be firmly postulated that *Drosera* chromosomes possessed diffused-or poly-centromeres and thus, each fragment contained centromeres and could survive as a minute chromosome. Moreover, the centromeres of the minute chromosome were quite functionable during kinetic activity at anaphase.

In this species C-bands appeared at the terminal regions of the regular chromosomes whereas fragment chromosomes were entirely stained (Fig. 1G) this suggested that i) fragment chromosomes might be derived from the fragmentation of the regular chromosomes at the terminal region (Fig. 1B arrow), and ii) fragment chromosomes were highly heterochromatic in nature.

Fluorescent banding revealed that most of the fragment chromosomes of this species were rich in GC base composition, since the DAPI-negative portions of them were correlated with that of CMA-positive ones (Figs. 1H, 1I).

Bimodality in *Drosera* karyotypes found sometimes was described to be caused by interspecific hybridization since the F1 hybrids showed bimodal karyotypes (Kondo and Segawa 1988). However, in this investigation, alteration of karyotypes was observed due to the fragmentation and fusion of chromosomes. Thus, spontaneous fragmentation and fusion of chromosomes might be considered as one of the possible reasons for promoting bimodal karyotype in *Drosera*.

Summary

Drosera dichrosepala was exposed to different doses of Gamma radiation. Fragmented and fused chromosomes were observed as a consequences. Diffused centromeres in every fragment chromosome was detected by centromeric banding (Cd-banding) and was supported by its typical disjunction and totally lack of lagging chromosomes or micronuclei from anaphase to telophase. C-banding revealed that the fragment chromosomes were highly heterochromatic and fragmentation might be occurred at the terminal regions of chromosomes. Fluorescent banding suggested that most of the fragment chromosomes were rich in GC base composition in the species. Alteration of karyotype due to Gamma irradiation also indicated that spontaneous fragmentation or fusion of chromosomes might be a possible factor for promoting the bimodal karyotype in this genus.

Key words : Diffuse centromere; C-banding; fluorescent banding; Cd-banding; *Drosera dichrosepala*; Droseraceae.

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