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Effects of Gamma-radiation and Chloral Hydrate on the Growth and Differentiation of the Gametophyte of two Terrestrial Ferns

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Irradiation of spores of *Lygodium flexuosum* with 25 k rads of γ-rays resulted in abnormal cell division and non-cordate gametophyte. At 45 k rads and above abnormal rhizoidal differentiation was observed. Twin protonema and multibranched protonema were formed at 65 and 85 k rads. One-, occasionally, two-celled protonema with giant cells and enlarged chloroplasts were noticed above 85 and 100 k rads. Tumorised protonema were produced at 65 k rads and above. Radiation had similar effects as chloral hydrate on *Ampelopteris prolifera* so far as inhibition of spore germination cell division and reduction in the length of protonema, rhizoids and their output, apical notch formation, differentiation of sex organs are concerned, Treatment with higher concentration of chloral hydrate (50–80 μg/ml) and higher doses of γ-radiation (30–85 k rads) resulted in multinotched gametophytes with abnormal chloroplasts. Protonemal abnormalities accompanied by the decrease in cell number were also enhanced. Abnormal rhizoids with swelling, septation, branching and chlorophyll pigments were commonly observed at 10, 20, 30 and 60 μg/ml concentrations, respectively. Antheridial initiation was delayed by 3 days and there was a gradual reduction in mean antheridial number with increased concentration. Initiation of archegonia started after 60 days of spore germination in prothalli treated with chloral hydrate, whereas in control it occurred after 35 days.

Key Words: Gamma-radiation, Chloral hydrate, Abnormality, Spore, Development, Protonema

Introduction

Knudson (1940) exposed spores of *Polypodium aureum* to X-rays and obtained gametophytes with abnormal chloroplast phenotypes. Aberrant form of the chloroplast were observed in irradiated spores of *Osmunda regalis* (Howard & Haigh 1968, Allen et al. 1973 and Allen & Haigh 1973). Dominant nuclear mutation, which determined chloroplast distribution in cells of both the gametophyte and sporophyte generations, was discovered by Haigh and

Howard (1970). Dodd and Ebert (1970a, b, 1972, 1973a, b) and Dodd and Schwartz (1975) reported radiobiological damage in the irradiated dry spores of *O. regalis*. Abnormal or tumour like gametophytes were observed from irradiated spores of *Pteridium aquilinum* (Partanen & Steeves 1956, Partanen 1960 and Partanen & Nelson 1961), *O. regalis* (Haigh & Howard 1973) and *Pteris vittata* (Palta & Mehra 1973). The present study deals with the effects of γ-irradiation on the growth and differentiation of the gametophyte of *Lygodium flexuosum*. Chloral hydrate, which had been shown to be mutagenic in *Antirrhinun majus* (Stubbe 1940), bacteria (Barthelmess 1956), *Drosophila* (Goldstein 1960) and *Vicia faba* (Amer 1973), was used to compare the effects of two kinds of mutagen.

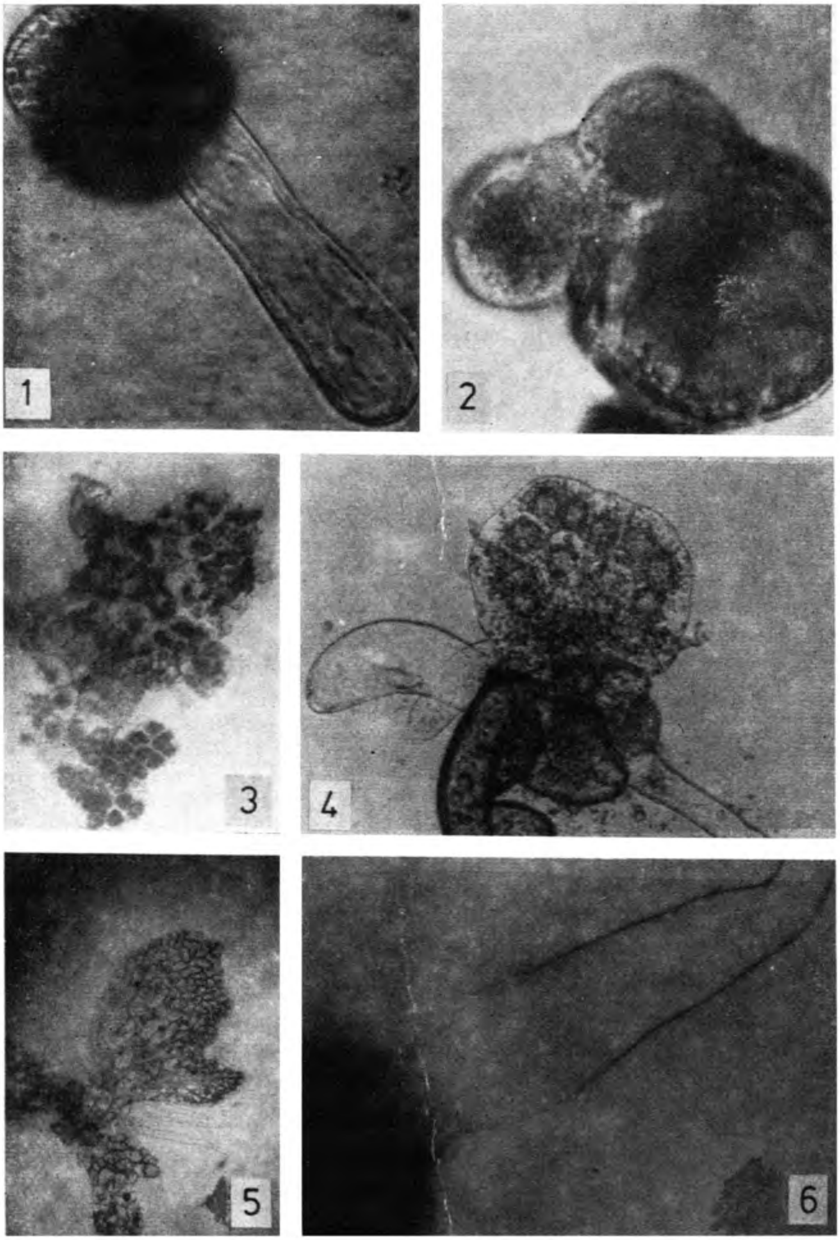
Materials and Methods

Spores of *Lygodium flexuosum* were collected from a forest of the Mirzapur district and those of *Ampelopteris prolifera* from the descendent of an original stock from the jungle of Gorakhpur district, both in Uttar Pradesh. These were properly stored in a desiccator which was kept cool. Five hundred mg of spores packed in polythene bags were irradiated with 5, 10, 15, 25, 35, 45, 65, 85, 100 k rads of γ-radiation from a ^{60}Co source of Ci emitting 1000 rads/20.9 sec at the National Botanical Research Institute, Lucknow. The irradiated spores were sown in petridishes containing 25 ml of autoclaved (15 lb/inch^2) nutrient medium (Klekowski 1969) with *p*H 5.8 and solidified with 1% agar. Likewise, chloral hydrate at different concentrations of 5, 10,20,30,40,50,60, 70 and 80 μg/ml was incorporated into nutrient medium and the surface sterilized (with 4% sodium hypochlorite) spores of *A. prolifera* were uniformly inoculated in the medium. The cultures along with the controls were maintained at 24±2°C under continuous white fluorescent illumination of 250–300 ft. C. Random samples of 100 germinating spores/developing prothalli were used for taking average.

Observations

Normal development: Tetrahedral and trilete spores of *L. flexuosum* germinated after 4 days of inoculation. Germination is polar and of the *Anemia*-type with prothallial development like that of *Adiantum* (Nayar & Kaur 1971). The gametophyte produced antheridia after 25 days of inoculation and became hermaphrodite within a week thereafter. Antheridia developed at the central region of the cordate prothalli and a few on the wings as well. The archegonia differentiated next to the apical notch with the neck pointing downwards. The spore of *A. prolifera* germinated after 6 days and the gametophytes initiated antheridial development after 30 days and turned hermaphrodite within a week thereafter. Antheridia were scattered near the rhizoids and the archegonia below the apical notch, pointing their necks away from them.

Effects of gamma-radiation and chloral hydrate: A number of radiation and chemical effects were realised in the gametophytic generation of the two ferns. At 45 k rads the effect of irradiation was manifested in inhibition, of which delay in mitosis was very prominent. Tumorous growth in protonema (2–15%) was remarkable at 65 k rads and at higher doses (figure 4). The percentage of spore germination and length of protonema of *A. prolifera* gradually decreased whereas abnormalities in protonema increased with the increase in concentra-

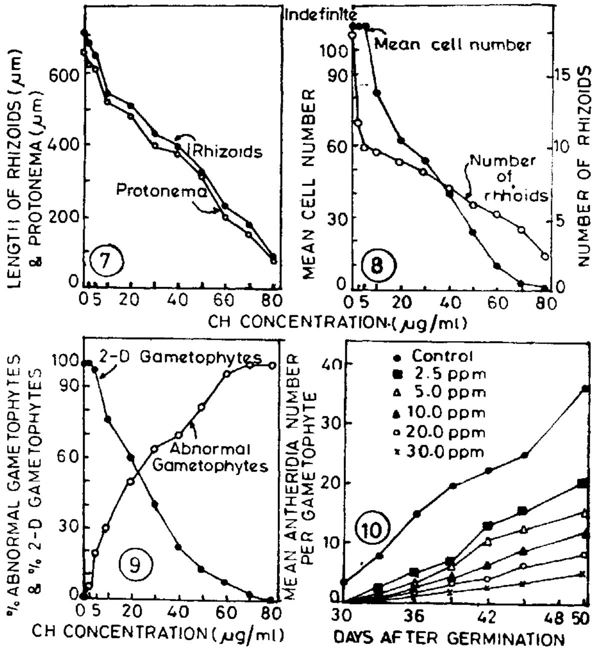


Figures 1–6 Effects of gamma radiation on the gametophytes of *Lygodium flexuosum;* **1**, Chlorophyllous and bulbous rhizoid at 85 k rad (×150); **2**, Abnormal two celled protonema (Chlrophyllous mutant) at 100 k rad, 16 days old (× 400); **3**, Abnormal multinotched gametophyte at 65 k rad (× 100); **4**, Abnormal gametophyte (globular) with secondary swollen rhizoid at 65 k rad, 16 days old (× 150); **5**, Multibranched gametophyte at 45 k rad, 19 days old (× 50); **6**, Swollen primary rhizoid at 85 k rad (× 100)

tions of the chemical used (figure 7). Abnormal protonemal structures such as two-celled protonema having degenerate chloroplasts or a cell with multiple rhizoids were frequently encountered at 70 μg/ml concentration of chloral hydrate (figure 9). Higher doses of radiation were detrimental and 85 k rads caused the formation of twin protonema. Complete cessation of cell division occurred at 100 k rads with 2–3-celled protonema or with a single-celled enormous protonema containing larger chloroplasts (figure 2). All such cells showed premature death due to rupture of cell wall, while one- or two-celled protonema of *A. prolifera* was observed at 80 μg/ml CH. This indicates a fall in 2-D filaments at higher concentrations (figure 9). With the increase in concentrations of chloral hydrate, the mean cell number per protonema decreased (figure 8). Multinotched and multibranched protonema were also observed at 65 and 45 k rads or at higher doses in *L. flexuosum* (figures 3, 5). The same pattern of protonemal growth was observed at different concentrations of chloral hydrate in *A. prolifera*.

Chlorophyllous, bulbous and branched rhizoids were noticed from 45 k rads onwards which constituted 10–15% of the total rhizoidal output (figures 1,4 & 6). Similar abnormalities were also recorded in *A. prolifera* at lower and at higher concentrations of the chemical. The length and number of rhizoids decreased and these were found to be dose-dependent in both the ferns.

Antheridial development remained unaffected and archegonia developed only under milder treatment in *L. flexuosum*, while in *A. prolifera*, antheridial initiation was delayed by three days. Chloral hydrate-treated prothalli started differentiating antheridia after 33 days of spore-



Figures 7–10 7, Effects of chloral hydrate on elongation of rhizoid and protonema; **8**, Effects of chloral hydrate on cell division and number of rhizoides; **9**, Percentage of abnormal and 2-D gametophytes of *A. prolifera* under influence of chloral hydrate; **10**, Effects of chloral hydrate on the differentiation of antheridia between 30–50 days of growth

germination, whereas in controls it took 30 days. The initiation of antheridia could be inhibited by employing higher concentrations of the chemical (figure 10). Development of archegonia started after 60 days in the prothalli treated with lower concentrations of the chloral hydrate as against 35 days in the controls.

Discussion

Twin protonema observed at 85 k rads was the result of unusual longitudinal division of the protonemal apical cell due to the disturbed growth pattern. Such cases have also been documented by Kato

(1957) and Palta and Mehra (1973). In addition, disturbance in biogenesis yielded multibranched protonema. Spores irradiated at 85 k rads and at higher doses failed to multiply but increased enormously in size. The contents of such giant cells bulged into spherical balloon-like objects owing to vacuolation in the cytoplasm, which is indicative of the loss of cellular control on the growth processes of the prothallus. Bloom (1948) ascribed the globular formation to nuclear swelling, whereas Partanen (1958) attributed it to induced mutation.

The present finding of decrease in the frequency of the prothalli changing from 1-D to 2-D growth pattern following increase in the doses of radiation and chemical is in conformity with the results of Hotta and Osawa (1958), Hotta (1960), Raghavan (1964, 1965 a, b, & 1968 a, b), Raghavan and Tung (1967), Mohr (1965), Nakazawa and Tanno (1965), Bergfeld (1965) and Davis (1968). Schraudolf (1967), Burns and Ingle (1968), Miller (1968) and Davis (1970), however, were unable to confirm the results.

Abnormal differentiations of rhizoids, such as swelling and branching, had some similarity with the rhizoidal anomalies found in *Goniopteris prolifera* (*A. prolifera*) by Mehra (1952) and *Dryopteris erythrosora* by Kato (1957) after treatment of the spores with colchicine. Chlrophyllous rhizoids produced by radiation and chloral hydrate probably resulted from disturbances in the pre-existing basic polarity. Palta and Mehra (1973) advocated that chlorophyllous rhizoids are formed due to inactivation of cytoplasmic factors followed by the failure of first mitotic division and thus leading to the transformation of the spore cell into a rhizoidal structure. While the inactivation of the cytoplasmic factors remains the main cause, we tend to affirm the totipotency of the cells (cf. Mehra 1952) which under unusual condition of

Table 1 *Growth characteristics of gametophytes of* Lygodium flexuosum (*L*) *Sw. after gamma-irradiation (rounded up till 16th days of germination)*

Treatment in k rad	Mean rhizoid No.	Mean rhizoidal length (μm)	Mean cell number	% of 2-D gametophytes	% of abnormal gametophytes
Control	15.6±0.08	896.4 ±3.59	indefinite	100	—
5	14.0±0.02	884.78±2.46	indefinite	100	5.8
10	13.8±0.02	863.2 ±1.76	indefinite	100	6.2
15	13.6±0.02	853.57±1.73	indefinite	100	8.3
25	12.6±0.01	819.5 ±1.28	indefinite	100	15.0
35	12.4±0.02	792.98±0.96	indefinite	100	45.2
45	12.0±0.01	705.5 ±1.09	70.5±.07	70	48.3
65	7.0±0.28	645.33±1.01	12.2±.01	33.3	80.6
85	1.4±0.05	365.2 ±0.74	4.0±.03	2.2	100.0
100	1.0±0.01	45.0 ±0.20	2.0±.03	—	100.0

± S. E.

stress can differentiate chloroplasts in them. Branching, chlorophyllous rhizoids and reduction in length and number of rhizoids were also observed by Singh (1974) in *Pityrogramma calomelanos*, Khare and Roy (1977) in *D. cochleata* and Niranjan and Roy (1980) in *Cheilanthes rufa* treated with maleic hydrazide.

It has been reported by several workers that formation of archegonia is not initiated unless the gametophyte could form a notch meristem because archegonia are positioned just below the apical notch. Due to this, prothalli treated with higher concentrations of the chemicals could not differentiate archegonia. Schedlbauer (1978) also observed inhibition of apical notch meristem formation in the gametophytes of *Ceratopteris thalictroides* by treatment with benlate.

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