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Effects of Gamma Radiation on the Growth and Differentiation of the Gametophyte in the Fern *Cheilanthes rufa* D. Don

A R S NIRANJAN and S K ROY

Department of Botany, Banaras Hindu University, Varanasi 221 005

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Gamma radiation dose above 20 Krads resulted in abnormal cell divisions and production of non-cordate gametophyte. Twin protonema and multibranched protonema bearing antheridia were observed at 80 and 50 Krad doses, respectively. One, occasionally two-celled protonema with giant cells and enlarged chloroplasts were noticed above 80 Krad doses. Tumorization in protonema occurred at 70 Krads and onward doses. Abnormal rhizoidal differentiation such as swelling, branching and their emergence near the tip of protonema was frequently recorded at 40 Krads; though the former two made their appearance even at 30 Krad dose. Chlorophyllous rhizoids were not uncommon above 50 Krad doses. The length and number of rhizoids and other abnormal features were found to be dose-dependent. Development of antheridia in irradiated prothalli was delayed by four days in comparison to control. No archegonia ever appeared in treated prothalli.

Key Words: 1–D=Linear growth, 2–C=Two-dimensional growth, Krad=Kilorad, Ft.–c.=Fort Candle

Introduction

Partanen and Steeves (1956) studied the effects of X- and γ irradiation on young gametophytes of *Pteridium aquilinum* and reported tumorous growth of protonematic cells. Haigh and Howard (1973) observed tumorous outgrowth in young gametophytes of *Osmunda regalis,* induced by X-rays. Inhibition of cell division and rhizoid differentiation, change in the site of rhizoid formation, induction of tumors and degenerative changes of cytoplasm in *Pteris vittata* were noticed by Palta and Mehra (1973) with X-rays. Dõpp (1937), Maly (1951), Breslavets (1951) and Mehra and Palta (1969) have observed chloroplast changes induced by X-, γ- and UV-radiation. Effects of UV irradiation on the developmental stages of gametophytes of *Pteris vittata, Osmunda japonica* and *Dryopteris varia* were observed by Kato (1964). The present study deals with the effects of γ radiation on the growth and differentiation of gametophytes of *Cheilanthes rufa* with particular emphasis on the cell division, rhizoidal output, mean cell number, abnormal growths and the development of sex organs.

Material and Methods

Spores of *C. rufa* were collected from Pithoragarh hills and stored in a desiccator in the laboratory. Dry spores packed in polythene bags were irradiated with 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 K rads of γ radiation from ^{60}Co source of 660 Ci emitting 1000 rads/20.9 seconds at N.B.R.I., Lucknow. The irradiated spores were dusted on 25 ml of autoclave-sterilized (15 lb/in^2) nutrient medium (Klekowski 1969) at 5.4 *p*H gelled with 1% agar in petridishes. The cultures along with one set of control were maintained at 24 $\pm$ 2°C under continuous white fluorescent illumination of 250-300 ft.-c. intensity, in a culture room. Measurements were taken by an ocular micrometer under a calibrated microscope and random samples of 100 plants were used for an average measurement.

Results

Normal development

Spores germinated after 6 days of sowing. Prothallial development is *Adiantum*-like (Nayar & Kaur 1971) (figure 1). The gametophytes produced antheridia 26 days after the germination and became bisexual within a week. Antheridia were scattered near the rhizoids, while archegonia just below the apical notch pointing their neck away from it.

The effects of γ-radiation

At 30 Krads the rate of cell division was considerably low and gametophytes thus formed did not develop apical notch (figure 2). Tumorous growth in protonema (5-20%) was noticeable at 70 Krads and above doses. Irregular emergence of rhizoids was observed at 40 Krads (figure 4), while branched and bulbous rhizoids were noticed at doses 30 Krads and above (figures 3 and 8). Chlorophyllous rhizoids appeared above 50 Krad doses (figure 9). Abnormal rhizoids were observable 20 days after germination and constituted 15% of the total rhizoids observed. At 80 Krad dose abnormal divisions led to the formation of twin protonema, with only a few observable cells (figure 6). Almost complete cessation of cell division occurred at 90 Krad dose where one, occasionally two-celled protonema with larger chloroplasts and cell dimension developed. In extreme cases, the enlarged chloroplasts were seen to come out of these giant protonema (figure 7) by rupturing the cell wall and ultimately leading to the death of such cells. Multibranched protonema bearing antheridia were observed at 50 Krad dose (figure 5). γ radiation decreased the length and number of rhizoids which were found to be dose-dependent (table 1). It is interesting to note that the formation of antheridia remained unaffected upto 50 Krad dose. Besides, initiation of antheridia in treated prothalli

Table 1 *Growth characteristics of gametophytes of* C. rufa *after γ- irradiation (20 days after germination)*

Treatment (Krads)	Mean cell number $\pm$ S.E.	Mean rhizoid number $\pm$ S.E.	Percentage of 2-D gametophytes	Percentage of abnormal gametophytes
Control	Indefinite	16.2$\pm$0.50	100	—
30	Indefinite	7.5$\pm$0.45	100	30
40	58.6$\pm$1.00	6.9$\pm$0.40	60	48
50	45.4$\pm$0.91	6.4$\pm$0.34	45	65
60	13.1$\pm$0.83	6.1$\pm$0.60	21	72
70	10.2$\pm$0.68	5.5$\pm$0.36	11	85
80	6.60$\pm$0.50	4.8$\pm$0.48	3	100
90	2.46$\pm$0.28	3.1$\pm$0.34	1.2	100
100	1.00$\pm$0.22	2.0$\pm$0.00	—	100

(invariably uniseriate) was observable 30 days after the germination of spores, while in control the same is noticeable only after 26 days of spore germination (table 2).

Table 2 *Mean antheridia number per gametophyte at different γ-radiation doses*

Days after spore germination	Control	30	40	50
26	2	—	—	—
30	6	1.5	—	—
34	13	3	1.75	—
38	18	7	4	2
41	29	12	9	5

(For each observation 50 gametophytes were observed)

Further, while archegonia did not differentiate in any of the treatments, apparently because of the lack of meristematic zone in the treated prothalli, antheridia did appear, though belated up to 50 Krads gamma doses.

Discussion

Tumorization

In spores irradiated at 90 and 100 Krad doses, some protonemal initials completely failed to divide and enormously increased in dimension because of their continued growth. These giant cells did not survive for quite longer. Palta and Mehra (1973) stated that when the spores of *Pteris vittata* were subjected to higher exposures of X-rays (75,600–10,800 R), spore cells often failed to undergo mitosis. In addition, their contents bulged into large spherical balloon-like structuers (giant cells) as a result of vacuolation in the cytoplasm. The tumor formation was found to be dose-dependent as also earlier reported by Partanen and Steeves (1956), Palta and Mehra (1973) and Haigh and Howard (1973). Haigh and Howard (1973) observed tumorous outgrowth in young gametophytes of *Osmunda regalis*, induced by X-rays and reported that radiation effect primarily responsible for tumor production is cell death and/or mitotic arrest. Single-celled giant globular or oblong cells were attributed to the nuclear swelling of spores (Bloom 1948). Partanen and Steeves (1956) described tumorous outgrowths from week-old prothalli of *Pteridium aquilinum* after X- and gamma exposures. Partanen (1958) further suggested that tumors were induced as a result of mutation. Thus, the present observations are well in conformity with those of Palta and Mehra (1973).

Slow growth

Doses beyond 40 Krads were effective in inhibiting the transformation of 1-D to 2-D phase. This could be ascribed to disturbed RNA or protein synthesis, because these have been argued to be connected in some way specifically in the initiation of 2-D growth (Hotta & Osawa 1958, Raghavan 1968). A general growth inhibition and its pattern at higher gamma doses (table 1) are in accordance with the reports of Allen and Haigh (1973) with X-rays (3 Krads) on *Osmunda regalis* and Singh (1976) with gamma radiation (40 Krads) on *Onychium siliculosum*. Palta and Mehra (1973) also reported that with the increase in X-ray exposure (75,600 R and above) the growth of the gametophyte showed a more pronounced decrease.

Branching of protonema

Twin protonema has been observed at 80 Krad dose due to disturbances in the normal growth pattern of the gametophyte as also observed by Kato (1957) in spores of *Dryopteris erythrosora* treated with concen-

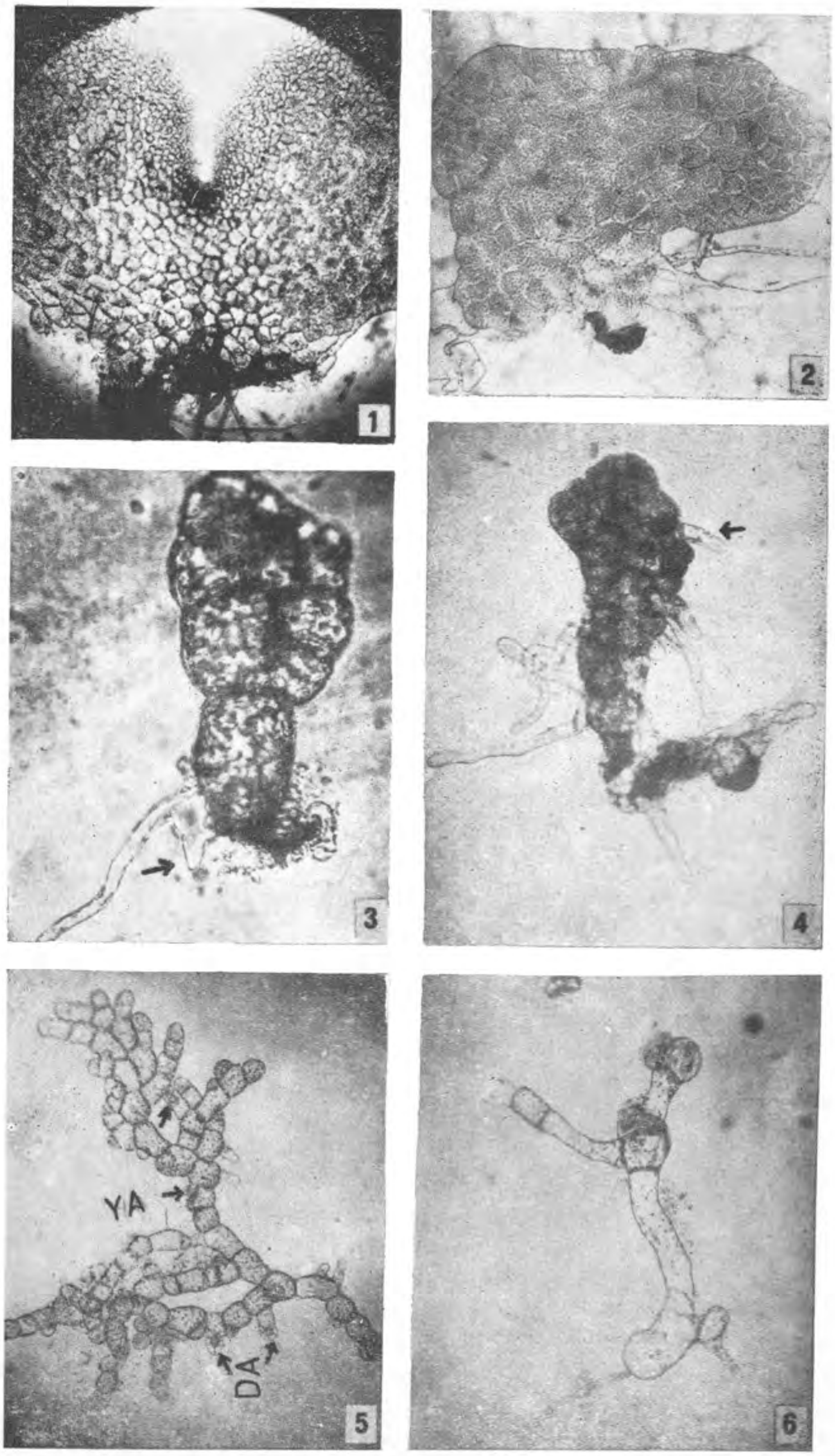
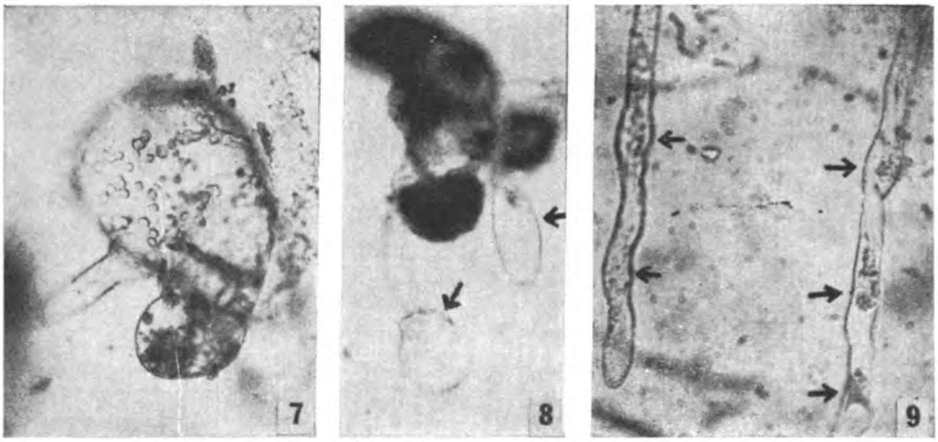


Figure 1–6 Effects of gamma radiation on the gametophyte of *Cheilanthes rufa*. *1*, Normal gametophyte (20 days old), (×50); *2*, Abnormal gametophyte without apical notch (20 days old) at 30 Krads (×310); *3*, Brached rhizoid at 60 Krads (×310); *4*, Emergence of rhizoid near the tip of protonema at 40 Krads (× 200); *5*, Multibranched protonema bearing antheridia at 50 Krads (×285) (YA=Young antheridia, DA=Dehisced antheridia); *6*, Twin protonema at 80 Krads (×-150)



Figures 7–9 *7*, Two-cealled protonema showing enlarged chloroplasts at 90 Krads (× 285); *8*, Bulbuous rhizoids at 80 Krads (× 200); *9*, Chlorophyllous rhizoids at 60 Krads; (× 400)

trations of tryptophan between 300–500 ppm. Palta and Mehra (1973) found that the two protonemal cells came out from the germinated spores as a result of X-ray treatment (100,800 R). Multibranched protonema observed at 50 Krads gamma radiation is in conformity with that noticed by Khare (1977b) in *Cheilanthes farinosa* exposed to UV light (87 ergs/mm^2/sec for 40 min). Kato (1964) observed extreme branching in *Pteris vittata* treated with UV exposures (30 μw/cm^2 for 2 min).

Rhizoids

Abnormal rhizoidal differentiation such as swelling, branching, their emergence near the tip of protonema and decrease in their length and number observed here are the features confirmatory to those recorded by Kato (1964) in *Pteris vittata* subjected to UV-irradiation (30 μw/cm^2 per 1 min), Palta and Mehra (1973) in *Pteris vittata* treated with X-rays (75,600 R) and Singh (1974a, b) in *Onychium auratum* exposed to UV light (51 ergs/mm^2/sec for 2 min). Abnormal swelling and branching of rhizoids were also obtained by Kato (1957) but with colchicine treatment given to the spores of *Dryopteris erythrosora*. Chlorophyllous rhizoids observed in the present study are presumably the result of disturbances in the pre-existing basic polarity. The inactivation of cytoplasmic factor followed by the failure of first mitotic division which could lead to the transformation of the spore cell into a rhizoidal structure was proposed to be the cause of chlorophyllous rhizoids (Palta & Mehra 1973). Similar results were also obtained by Khare and Roy (1977a) in the spores of *Dryopteris cochleata* treated with maleic hydrazide (10–22 ppm).

Sexuality

While archegonia formation was totally inhibited in all the gamma ray treatments, antheridial differentiation, though belated has been observed on irregular uniseriate branches of protonema up to 50 Krad dose. The observations on delay in antheridial differentiation are substantiated by earlier reports of Singh (1974b) and Khare (1977b) regarding the delayed development of sex organs in UV treated prothalli of *Onychium siliculosum* and *Cheilanthes farinosa*, respectively.

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