An Age-Dependent Change in the Response of Fern Gametophytes to Red Light

Abstract. When gametophytes of *Onoclea sensibilis* are grown in darkness a filament of a few cells develops which elongates at a constant rate. Low dosages of red light given at the beginning of growth increase the rate of elongation. After about 12 days in darkness, however, gametophytes show a decreased growth rate when given red light.

Spores of *Onoclea sensibilis* require light for germination. If sufficient light is given to insure germination and the spores are then placed in darkness, the gametophytes develop a characteristic form. No further cell divisions occur, and the gametophyte consists of a filament of one to three very elongated cells. Mohr (*1*) found that the gametophyte of *Dryopteris filix-mas*, growing on a mineral medium under continuous red light, produced a long filament of few cells, similar to but longer than plants growing in the dark. A different effect of red light was shown with gametophytes of *O. sensibilis* by Miller and Miller (*2*). Gametophytes germinated and grown for several days under white light and then transferred to darkness make only a small increase in area and cell number. Low dosages of red light (20 min per 24 hours, about 400 erg cm^{-2} sec^{-1}) permit growth and cell division in otherwise total darkness when sucrose is present in the medium; without sucrose red light is ineffective. The effect of red light is reversible by far red irradiation.

In Mohr's paper red light was shown to increase the "etiolated" character of the gametophyte, while Miller and Miller found a promotion by red light of the type of growth characteristic of light-grown gametophytes. We carried out experiments to determine whether the red-light effect reported by Mohr was found also with gametophytes of *O. sensibilis*. During the course of this work we determined that the nature of the effect of red light on filament elongation varies with the age of the gametophytes at the time red-light treatment is begun.

The method of spore sterilization and composition of the Knop's mineral medium used have been described (*2*). The light treatment for germination was given in an air-conditioned growth room at a temperature of 25° ± 2°C, with a photoperiod of 16 hours. Light was from white fluorescent tubes, about 400 ft-ca. Spores were exposed to light for 2 days on distilled H_2O, then transferred to Knop's solution with or without 1-percent sucrose and placed in an air-conditioned dark room (25° ± 2°C). Red light was given from one 15-watt red fluorescent tube at a distance of 10 cm. Growth measurements were made by projecting the images of gametophytes under a microscope onto a sheet of paper with an inclinable drawing mirror. The lengths of the gametophytes were traced on the paper and later measured with a flexible plastic rule. The actual size was calculated from the known magnification of the microscopic image.

In total darkness the filaments increase in length at approximately a constant rate for at least 3 weeks. In Knop's solution the growth rate is about 0.02 mm/day; the addition of 1-percent sucrose produces a higher rate of elongation, about 0.04 mm/day.

The effect of red light (15 min/6 hr) is dependent on the length of time the gametophytes have been growing in darkness. This is illustrated in Fig. 1, in which each point represents the average of measurements of 25 gametophytes. In this experiment gametophytes were grown on Knop's medium plus 1-percent sucrose. The solid circles represent growth in total darkness. At the times indicated by the arrows (0, 7, and 12 days in darkness) some of the gametophytes were started under the red light regime, which was continued for 4 to 5 days. The triangles show the elongation of red-light-treated gametophytes. If red light is given at the beginning of the growth period, the rate of elongation is considerably higher than that of the dark controls. After 7 days in darkness, red light has little effect on elongation, and after 12 days red light inhibits filament elongation compared with the dark-grown gametophytes. These effects of red light are independent of sucrose and take place as well on plain Knop's solution.

It is possible to study three effects of red light on gametophytes of *O. sensibilis*. If the gametophytes are treated, as in the present paper—2 days light, then darkness—one may produce an inhibition or promotion of filament elongation, depending on when the red light is given. If the gametophytes develop longer in white light, so that cell division in two planes has been initiated, red light then promotes cell division (*2*). Whether these effects are distinct or are aspects of the same process deserves further study.

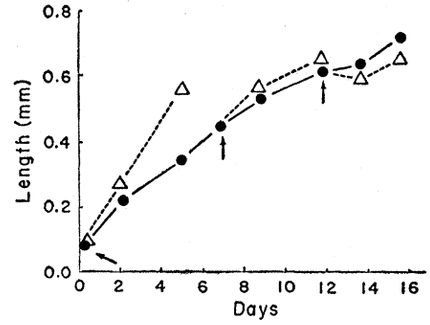

Fig. 1. Growth in length of dark-grown (circles) and red-light-treated (triangles) fern protonemata. Arrows indicate beginning of light treatment for samples at different ages.

The age-dependent promotion or inhibition of filament elongation has an interesting parallel in higher plants. Thomson (*3*, *4*) showed that red light increased the rate of elongation of very young internodes of oats and peas but inhibited the elongation of older internodes. One unresolved question pointed out by Thomson (*4*) is whether the increased rate of elongation of young internodes is a direct effect of red light or a correlative phenomenon resulting from the inhibition of the lower internodes. It is clear that the red light promotion of filament elongation is a direct effect, since the irradiation of young and old gametophytes is obligately separated in time. This feature is absent in irradiation studies of whole higher plants where many internodes of different developmental stages are present at the time of irradiation and during subsequent growth. If one is dealing with the same phenomenon in ferns and higher plants, the stimulation of young internode elongation by red light most probably represents a direct effect of light on the cells (*5*).

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References and Notes

1. H. Mohr, *Planta* **47**, 127 (1956).
2. J. H. Miller and P. M. Miller, *Am. J. Botany* **48**, 154 (1961).
3. B. F. Thomson, *ibid.* **38**, 635 (1951); **41**, 326 (1954).
4. ———, *ibid.* **46**, 740 (1959).
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