Short Communication

Rehydration of Phytochrome in Imbibing Seeds of Amaranthus retroflexus L.

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It is commonly recognized that light-sensitive seeds require a period of imbibition before full promotion of germination by a light stimulus can be attained. Yet little work describes the nature of these preliminary events. Of the several major limitations that may be involved, clearly the rehydration (or activation) of phytochrome and the system with which it interacts are the chief ones to consider. The object of this communication is to describe the rehydration of inactive phytochrome (Pr) in Amaranthus retroflexus L. seeds as determined by a method by which phytochrome rehydration can be physiologically separated from other events occurring during dark imbibition.

Recently, Tobin and Briggs (11) presented evidence for rapid rehydration of phytochrome in isolated embryos of Pinus palustris Mill. Using spectrophotometric methods, they detected low levels of phytochrome in dry seeds and much larger amounts 2 min after the addition of water. However, physiological changes in germination connected with the phytochrome system were not displayed until seeds had imbibed for approximately 3 hr.

Nyman (6) found that irradiation of dry seeds of Pinus sylvestris L. with red light could give subsequent promotion of germination. Recent studies by several authors using spectrophotometry traced the appearance and increase of phytochrome levels in various seeds (1, 4, 5, 7, 8). Some found phytochrome only in imbibed seeds (4, 5), while others could detect the pigment in dry seeds as well (1, 8). Kendrick et al. (4) found that water uptake of Amaranthus caudatus L. seeds followed the same pattern as the initial increases in phytochrome, but McArthur and Briggs (5) presented evidence that appearance of phytochrome in peas was not strictly correlated with water uptake. Most of these reports do not attempt to associate the early appearance of phytochrome in imbibing seeds with any physiological role. In a previous paper (9), we presented some evidence that rehydration of the inactive and active (Pfr) forms of phytochrome could be detected physiologically during prechilling of A. retroflexus seeds. This paper supports and extends these findings.

MATERIALS AND METHODS

Seeds of Amaranthus retroflexus L. (redroot pigweed), harvested in 1966 and stored at −10°C in sealed polyethylene containers, were used throughout. Duplicate lots of 100 seeds, sown on moist filter paper in Petri dishes, were used for each experimental treatment. After planting, the dishes were immediately enclosed in opaque, black cloth bags and placed in cabinets maintained at ±1°C of the specified temperature. Each treatment has been repeated at least four times.

Red light was obtained by passing light from cool white fluorescent tubes through two layers of red cellophane. At 1 m from the source the intensity was 0.6 mw cm⁻² of 600 to 680 nm radiation. Far red irradiations were from incandescent filament lamps filtered by two layers each of red and blue cellophane and 5 cm of water. Intensity of this source at 1 m was 0.75 mw cm⁻² in the 700 to 750 nm region. Energy distributions from these sources have been described (2).

At the conclusion of experimental treatments, germination was achieved by incubation at 35°C for 3 days.

RESULTS AND DISCUSSION

Experiments were conducted to examine the effect of various periods of dark imbibition at 10, 20, and 35°C on response to 5 min R.¹ These data (Fig. 1) indicate that, to promote complete germination following R irradiation, a minimal preceding period of approximately 6 days of dark imbibition at 10°C is required, while at 20 and 35°C, periods of approximately 4 and 1.5 days are required, respectively. Irradiations given at the conclusion of shorter periods of dark imbibition do not lead to complete germination. Dark imbibition periods may be considerably longer than the minimum required to allow full expression of the R stimulus in A. retroflexus seeds, without appreciable loss of sensitivity (3). These data, while only specifically applicable to A. retroflexus, differ from data for other light-sensitive species only in relatively minor ways. Apparently, there is more than one combination of temperature and period of dark imbibition allowing full germination after R irradiation. In other words, this kind of experiment indicates conditions which yield seeds physiologically ready to germinate completely after an input of Pfr but does not permit an analysis of the limiting factors which prevent complete germination prior to a certain minimal dark imbibition period.

A different approach to the problem was suggested from our earlier experiments. Thus, when A. retroflexus seeds were irradiated with 5 min of R 24 hr after the beginning of a 10°C prechilling and returned to 10°C for the remainder of the 6-day prechilling period, nearly complete germination would result when the seeds were subsequently transferred to 35°C (Fig. 1B in Ref. 9). Irradiations after 24 hr of prechilling also gave complete germination. Irradiations before 24 hr of prechilling induced less than complete germination, even though they were also held at 10°C for 6 days. Our previous publication (9) related these effects to rehydration of Pr. In contrast, data

¹Abbreviations: R and FR: red and far red illumination, respectively.
Fig. 1. Hours of dark imbibition at 10, 20, and 35 C required for a given level of germination of A. retroflexus seeds at 35 C following a 5-min R irradiation.

Fig. 2. Time course of rehydration as measured by changes in percentage germination of A. retroflexus seeds following imbibition at 10, 20, and 35 C for indicated number of hours.

reported herein (Fig. 1) show that 6 days of dark imbibition at 10 C are required before full expression of a subsequent light stimulus can be achieved. It is important to distinguish between the two types of experiments. In one (Fig. 1), a minimal dark imbibition period at 10 C is established which will subsequently allow full germination in response to R, indicating that all preliminary requirements to allow full germination have taken place. In the other (9), however, we show that after 24 hr sufficient rehydration of phytochrome has taken place so that R illumination will lead to full germination upon completion of prechilling and subsequent transfer to 35 C. Thus, we conclude that factors other than lack of transformable phytochrome limit complete germination after 24 hr, but prior to 6 days, of imbibition at 10 C. Prior to 24 hr of 10 C imbibition, however, rehydration of phytochrome is not sufficient for full germination (Fig. 1B in Ref. 9). It cannot be stated that rehydration of phytochrome was complete even in 24 hr, but enough so that saturating R illuminations would transform an amount to yield complete germination.

The choice of the 10 C imbibition temperature was also on the basis of previously observed dark decay characteristics of Pfr. We previously estimated (9) that the half-time for thermal inactivation of Pfr at 10 C is in the order of 20 days. Thus, any Pfr transformed by a R illumination during the early hours of imbibition would not appreciably decay during the remainder of the 6-day imbibition at 10 C.

From the previous experiments, we can now inquire into the effect of temperature on phytochrome rehydration. This can be done by imbibing the seeds at a designated temperature for various times, irradiating with saturating amounts of R (5 min), followed by a transfer to 10 C for 6 days, and by subsequent germination at 35 C. Data for imbibitions at 10, 20, and 35 C (Fig. 2) show that, whereas it takes approximately 24 hr of imbibition at 10 C before 5 min of R irradiation would induce complete subsequent germination, about 12 hr are required at 20 C, and only about 3 hr are required at 35 C. The rate of rehydration of phytochrome, therefore, doubles with each 10 C rise in temperature. In most cases, considerable promotion is evident after less than 1 hr of imbibition. The aforementioned times required for apparent rehydration of phytochrome are in the order of 10% of the dark imbibition period at the particular temperature required for full promotion of germination by R (Fig. 1).

The role of actual synthesis of phytochrome during these times must be considered. From data published elsewhere (10), we believe this factor to be negligible in these experiments. We estimated that phytochrome synthesis did not begin until about 24 to 48 hr of dark imbibition at 35 C, whereas in the present experiments, rehydration was complete (as physio-
logically measured) after 3 hr at 35 C. Kendrick et al. (4) could only detect phytochrome spectrophotometrically in A. caudatus seeds after 3 hr of imbibition at 25 C.

Since the rate of rehydration of phytochrome was found to be markedly dependent on temperature, experiments were performed to determine the rate of water uptake (fresh weight basis) as a function of temperature in A. retroflexus seeds. These data (Fig. 3) indicate a more rapid uptake of water at the higher temperatures during the initial hours of imbibition. Total water uptake was ultimately about 30% in all cases, however. An important feature of these data is that the time required for apparent complete rehydration of phytochrome and the uptake of approximately 17 to 19% water correspond quite closely at the three temperatures. Thus, in A. retroflexus seeds, rehydration of phytochrome is apparently complete when the seeds have imbibed about half of their ultimate water uptake, and the rate of rehydration is dependent on the temperature-controlled uptake of water.

It can be concluded, therefore, that the failure of irradiation to promote full germination in A. retroflexus seeds prior to the dark imbibition period which will allow full germination (Fig. 1) is not due to lack of transformable phytochrome once the seeds have imbibed about 17 to 19% water.

LITERATURE CITED