Dried seeds provide an opportunity to study the status of water as it affects biologically significant reactions in systems that survive desiccation. In previous studies we have reported the changes of physiological activities such as respiration and photosynthetic electron transport with water content in seeds in an attempt to define how water influences enzyme function, organelle integrity and metabolic activity (18-20). Phytochrome regulation of seed dormancy was used for this study to investigate the effects of water content on a photocontrolled reaction.

Diverse physiological activities can be correlated with the type of bound water (2, 12). Three types of bound water can be identified by the strength with which they sorb onto macromolecular surfaces. It is surmised that at moisture contents where the water is very tightly bound (region 1), most reactions are improbable due to the rigidity of the water and the macromolecules (3, 4, 10). At higher moisture levels, water becomes increasingly mobile so that diffusion and structural changes of macromolecules become increasingly possible (4, 12, 13). Thus, in region 2 of water binding, we see increased potentiation of physiological activity (2, 19, 22). By region 3, water has properties very similar to the bulk phase, and metabolic activity can reach its maximum within this region.

We have studied the red and far red light effects on dormancy release in lettuce seeds in order to determine whether photochemical reactions are less dependent on the state of water than 'thermal' chemical reactions. The photoconversion of Pr ↔ Pfr is photometrically detectible in imibed seeds (16, 17). Hsiao and Vidaver (5, 6) suggested that reversible phytochrome conversion required at least 15% water. More recently, it has been suggested that Pfr can be photoconverted at water contents as low as 8% (1, 9).

After lettuce seeds have been stimulated by red light, they can be dried to about 7% water with no apparent effect on germinability (5, 6, 21). The thermal reversion of Pfr to Pr in the dark can occur in partially hydrated seeds (about 30% water) (7).

In this paper, we have 'mapped' out the control of seed dormancy by red and far red light and dark reversion to dormancy at different moisture contents in an attempt to define explicitly the hydration levels at which the photo- and the thermal reactions are possible.

MATERIALS AND METHODS

Thermodynamic parameters describing the strength of water binding (ΔH of sorption) and the probability of reaction due to thermal motion (ΔG/KT) were calculated from sorption isotherms of desorbing seeds. These isotherms were determined by equilibrating lettuce seed at 5 and 25°C in chambers containing different saturated salt solutions to control relative humidity (11, 20).

Lettuce seeds (cv Waldmann's Green) at various water contents were given dark or continuous light conditions (cool-white fluorescent lamps) at 5°C. Each treatment was given to 200 seeds, and 2 replicates were used. Seeds were then irradiated with red light (660 nm) or far red light (730 nm) for 30 or 40 min, respectively, and then water was added for germination in the dark at room temperature. The wavelength of light was controlled by a Bausch and Lomb monochrometer with a tungsten light source. Percent germination after 72 h was used as an indicator of Pr ↔ Pfr conversion.

The rates of phytochrome photoconversion were estimated by irradiating seeds of various moisture contents for various lengths of time from 0 to 40 min and then adding water to allow germination to proceed. The rate of the dark reversion of Pfr was measured by irradiating seeds at 40% moisture for 15 min with red light and then storing them in the dark for 0 to 5 weeks at 5°C in different relative humidity chambers. After the storage period, seeds were moistened with distilled water and germinated in the dark for 72 h. The slopes of the germination curves in Figure 3 were used to estimate the rates of photoconversion depicted in Figure 4.
RESULTS

Thermodynamic data calculated from sorption isotherms of lettuce seeds indicate that there are three regions of water binding (Fig. 1A). Water is very tightly sorbed at moisture contents below 8%, with ΔH ranging between -3 and -8 kcal/mol. At moisture contents between 12 and about 18%, water is bound with intermediate strength; ΔH is about -1 kcal/mol. At moisture contents greater than 20% water is bound weakly (ΔH is about -0.4), and at about 32%, ΔH approximates 0.

The parameter ΔG/kT is a measure of the probability of reactions occurring due to the thermal energy of molecules. When ΔG/kT = -1, a reaction is said to be energetically possible, and therefore, spontaneous. The function of ΔG/kT, calculated for lettuce seeds from an isotherm at 25°C, decreases exponentially with higher water contents, and equals -1 at 8% water (Fig. 1B). Therefore, at water contents less than 8%, the energetics of thermal reactions are unfavorable.

Red light and far red light treatments at different water contents had dissimilar effects on the germination of lettuce seed (Fig. 2, A and B). When seeds that were equilibrated to various water contents in the dark were irradiated with red light (Fig. 2A), germination was not enhanced unless the seed water content exceeded 8%. Germination increased progressively as the water content of the seed increased, and was maximal when seeds of 18% water or more were irradiated with red light.

Seeds with low moisture contents (<8%) were sensitive to white light as evidenced by the germination increase from about 18 to 45% during the 2 weeks of equilibration under lighted conditions (Fig. 2B). The enhancement of germination acquired during storage in the light was reversed by irradiating seeds with far red light (Fig. 2B). The far red reversal occurred in all seed samples regardless of the moisture content and resulted in subsequent germination percentages that were very similar to seeds that had been equilibrated in the dark (Fig. 2A).

It is possible to infer the relative rates of Pfr → Pr photoconversion by estimating the initial slopes of time-course curves such as those in Figure 3A. The rates, plotted in Figure 4, showed similar trends to the total conversion data in Figure 2A. The rate was negligible in region 1 of water binding, and increased with increasing moisture content in regions 2 and 3. For seeds at the highest level of hydration (water contents greater than about 30%), Pr → Pfr photoconversion was apparently saturated within 15 min (Figs. 3A and 4). The authors recognize that this is a

Fig. 1. Thermodynamic parameters of water sorption in whole lettuce seed. A, Isosteric heat of sorption for the temperature range of 5 to 25°C; B, free energy at 25°C divided by Boltzmann’s constant and absolute temperature. The former parameter estimates the strength of water binding at a given water content, while the latter estimates the thermal energy within the system. The probability of thermal reactions is low when -ΔG/kT is greater than 1.

Fig. 2. Germination in lettuce after seeds of varying moisture contents are exposed to (A) red light for 25 min or (B) white light for 2 weeks, followed by far red light for 40 min. The points are the mean of two replicates and the error bars represent the maximum difference between the two replicates.

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rather slow response and suggest that the combination of a weak light dose and relatively dry tissue (compared to other studies) may be responsible.

The rate of Pfr → Pr photoconversion (Fig. 4) was estimated in a similar manner using data such as those in Figure 3B. The rates of conversions are seen to be essentially independent of the seed water content.

The dark reversion of Pfr → Pr was approximated by the change in germination after dark storage at various moisture contents (Fig. 5). The reversion was able to proceed at moisture contents of 8% or greater. Seeds dried to levels below 8% water maintained the red light enhancement of germination for at least 5 weeks. The drying process itself seemed to promote germinability, but this effect was lost after 1 week (Fig. 5).

**DISCUSSION**

These experiments show that the reversion to the dormant state can occur when lettuce seeds, at any hydration level, are exposed to far red light. We suggest, then, that the phototransformation of phytochrome from Pfr to Pr in lettuce seed is possible at all the water contents tested. Conversion of Pr to Pfr, as detected by release of dormancy in lettuce after exposure to red light, is restricted at water contents which correspond to region 1 of water binding; it becomes increasingly facile at water contents within region 2, and is maximal within region 3 (Fig. 2A). While dormancy release in very dry tissue was minimal after 25 min irradiation with red light, dormancy was broken somewhat, during a 2-week incubation time under white light as indicated by the higher germination levels (Fig. 2B). This suggests that either the conversion of dry Pr is very slow, or that dry Pr exists in a form which absorbs at a wavelength other than 660
nm as was suggested by Bartley and Frankland (1).

Our data suggesting differences in the photoconversion of phytochrome in the two directions may be related to the intermediates involved in phototransformation. Steps in the conversion of phytochrome as described by several researchers (1, 7, 9, 15) are:

\[
\begin{align*}
\text{red} & \quad \Pr \rightarrow \text{lumi-R} \rightarrow \text{meta-Ra} \rightarrow \text{Pfr} \\
\text{far red} & \quad \text{Pfr} \rightarrow \text{meta-Fa} \rightarrow \text{meta-Fb} \rightarrow \Pr.
\end{align*}
\]

The steps of \( \Pr \) to lumi-R and Pfr to meta-Fa are light induced, and the subsequent reactions are apparently conformational changes (1, 14). Meta-Fa is a stable intermediate, though a back reaction might occur slowly in the dark (1, 15). On the other hand, lumi-R is unstable and will convert within minutes to either Pfr or Pr depending on the environment (7, 8, 9). Since conformational changes require intramolecular mobility, and hence thermal motion, they should be restricted at water contents within the first region of water binding (Fig. 1). Photoresponses do not require thermal energy and so are likely to occur at all water contents. Thus, the rates of light absorption and initial photoconversions (Pr \( \rightarrow \) lumi-R or Pfr \( \rightarrow \) meta-Fa) should occur at all moisture contents. Since the lumi-R intermediate is unstable, the conversion of \( \Pr \rightarrow \text{Pfr} \) may be limited at low moisture contents whereas the reverse conversion can occur at any moisture content.

Differences in the measured rates of dormancy release or induction, via photoconversions of phytochrome, in seeds of different moisture contents (Figs. 3 and 4) probably reflect water influences on the balance between the rate of the conformational change and the rate of the back reaction. If we assume that the back reaction of the unstable lumi-R is relatively independent of moisture content, then the increased Pr \( \rightarrow \) Pfr conversion with increased moisture content would be due to the rising ability for the conformational change necessary for the conversion of lumi-R to Pfr. The dramatic increase in the rate of photoconversion in fully hydrated tissue suggests that the balance of the forward and back reactions was overwhelmingly in favor of the forward reaction, and photoconversion was completed within 15 min (time for saturating effect of red light (Fig. 3A)).

Far red light can reduce germination at all water contents. This is probably because meta-Fa is much more stable than lumi-R and similar amounts of thermal energy may be needed for either the forward or the back reaction (9).

The dark conversion of Pfr to Pr is influenced by moisture content. As in the work done by Vidaver and Hsiao (21), our data suggest that germinability induced by illumination can persist in dry seeds. However, the Pfr-mediated signal is eventually lost in wetter seeds (7). The rate of dark reversion, like the rate of photoconversion, is related to the hydration level, being ineffective in region 1. In region 2, the Pfr \( \rightarrow \) Pr reversion was progressive; in region 3, the reversion apparently occurred very quickly, within 2 weeks at 5°C. These results, consistent with the data in Figures 3 and 4, suggest that within the first region of hydration, conformational changes which require thermal energy are quite restricted, whereas within the second and third hydration regions such conformational changes are increasingly facilitated.

The levels of physiological activity detected in relatively dry biological tissue correspond fairly well to the three regions of water binding (2, 18–20). Physiological activity was restrained in region 1. Simple enzymic reactions become increasingly feasible with increasing water content in region 2. Integrated processes function within region 3 (2, 19, 20). It is believed that in region 1, water is so tightly bound that macromolecules are immobilized. The phototransformation of pigments, however, should occur even in region 1, since it is not a thermal reaction. We report here that the release of dormancy in lettuce seed by red light is restricted when the seed moisture content is within region 1, whereas the induction of dormancy by exposure to far red light can occur at any hydration level. Such an independence from the water content may be expected for photochemistry, since thermal energy is not required.

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