PHYTOCHROME IN SEEDS AND AN APPARENT DARK REVERSION OF P_r TO P_{fr}

J. BOISARD[1], C. J. P. SPRUIT[2] and P. ROLLIN[1]

[1] *Laboratoire de Physiologie Végétale, Faculté des Sciences de Rouen, 76 Mont-Saint-Aignan, France*
[2] *Laboratory of Plant Physiological Research, Agricultural University, Wageningen, The Netherlands, 263rd Communication*

INTRODUCTION

Some seeds are known to require light for germination (1). In some others, depending upon temperature, germination may occur in the dark and this may be completely repressed by far red irradiation during the initial phase of germination. It has been suggested that *a*, the presence of some P_{730} is required to induce germination, and *b*, that seeds, germinating spontaneously in the dark contain some P_{730}, presumably remaining from the period of seed maturation (2, 3, 4). To our knowledge, no direct spectroscopic observation of phytochrome in imbibed seeds, prior to the onset of visible germination (appearance of radicle) has so far been reported.

PHYTOCHROME IN SEEDS

We have studied the development of spectroscopically measurable phytochrome during the period following soaking of seeds of the dark germinating lettuce variety 'May Queen' (4), with the aid of a technique, described previously (5). Whereas in the dry seeds no absorption changes could be induced by either red or far red illumination, such photoreversible reactions could already be demonstrated a few hours after the start of imbibition. Although the absolute magnitude of the optical density changes induced by the actinic irradiation was small and amounted to only a few times the noise figure under the most suitable conditions of sample thickness and photomultiplier voltage, repeated measurements have shown the effect to be reliably due to the presence in the imbibed seeds of a photoreversible pigment. Preliminary measurements of the difference spectrum for the photoreaction, though of necessity of very limited accuracy, suggest that it cannot be very different from the known difference spectrum for

phototransformation of phytochrome. Maximum values for the optical density changes were observed around 660 nm and 730 nm. In lettuce seeds, the pigment concentration does not increase appreciably during the first sixteen hours, but rises sharply at about the time when visual germination becomes apparent, fig. 1.
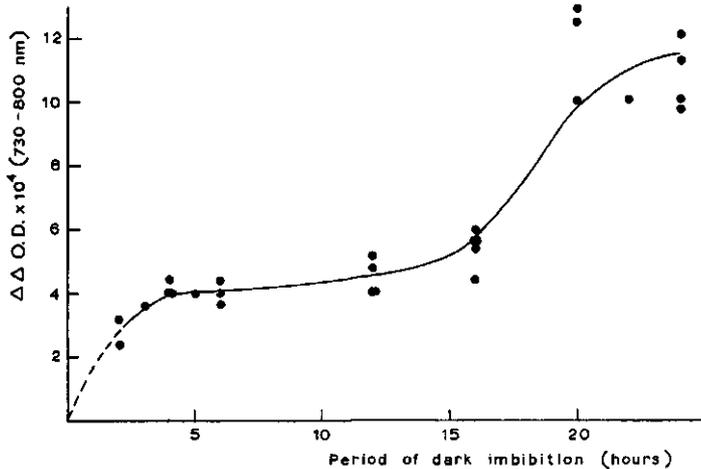

Fig. 1. Time course of phytochrome as ΔΔ O.D. between 730 and 800 nm following actinic irradiation, in lettuce seed 'May Queen'. The time scale represents hours after the start of dark imbibition. The limit of detectability was about 2×10^{-4} O.D.

The steep rise in total phytochrome after about 17 hours imbibition may not form a faithful reflection of pigment concentration in the seeds, as the optical properties and hence the effective path length for light absorption surely must change drastically when germination starts.

Similar observations have been made with seeds of *Nemophila insignis* and *Sinapis alba*.

DISCUSSION

The form of phytochrome in some seeds appears to differ in two respects from the pigment, normally found in etiolated plants. Firstly, we observed that at least a very considerable fraction of the pigment, present in fully dark imbibed seeds of *Lactuca* and *Nemophila* is present in the far red absorbing form. In contrast, phytochrome in etiolated seedlings is generally accepted to be entirely in the red absorbing form (6, 7). More surprising is the observation that, if the phytochrome in these seeds was converted tot he red absorbing form by far red actinic irradiation, there occurred what appears to be a rapid reversion to the far red absorbing form during a relatively brief subsequent dark period, ten minutes at room temperature being sufficient to effect a virtually complete transformation. Fig. 2 gives representative examples of such measurements for *Lactuca* and *Nemophila* seeds.
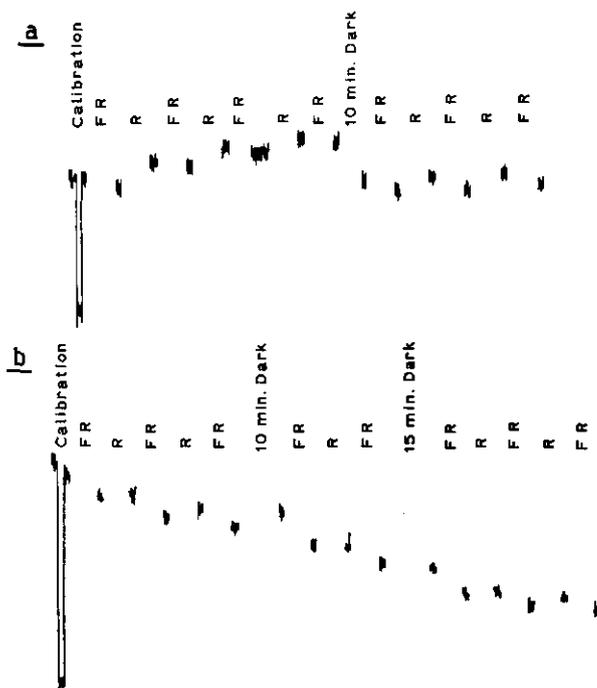
Fig. 2a. Seeds of *Lactuca sativa* 'May Queen' after 8 hours dark imbibition at room temperature. Copy of an original recording. Optical density changes upon alternate actinic irradiations with red (653 nm) and far red (737 nm). Sample thickness about 4 mm. Upwards: increased absorption at 730 nm. The calibration mark represents an absorption change of 4×10^{-3} O.D.

    b. Seeds of *Nemophila insignis* after 8 hours imbibition at room temperature. Both recordings demonstrate the initial presence of pigment in the far red absorbing form, and the reversion to this same condition of the red absorbing form after a short dark period.

In seeds of *Sinapis alba*, relatively large amounts of phytochrome could be detected, 4 hours after soaking. In most experiments, the pigment was mainly (50–100%) in the red absorbing form. Dark transformation of P_r to P_{fr} was also observed occasionally, but it was seldom as complete as in the other seed species, amounting to 20–50%. Only in a few experiments did we observe large initial P_{fr} values ($>80\%$) and large dark transformations. The reason for this variable behaviour is not yet clear.

The small magnitude of the optical density changes dictates extreme caution in the interpretation of these effects. In order to overcome possible interfering effects of spontaneous non-essential fluctuations and drift in optical density during the intercalated dark periods, we have repeated this type of experiment many times with essentially constant results. The most conclusive experiments are of the following type: After a terminal far red irradiation, the samples were left in the dark in the spectrophotometer (measuring beams shut off). After the required dark period, measurements were resumed and the effect of a further

actinic irradiation, either with red or with far red, was observed. The pigment was then found to be in the form that could be transformed by far red, not by red radiation, see fig. 2. There can be no question about the reality of the effect. It does not appear warranted at this moment, however, to conclude that this apparent dark reversion of seed P_r to P_{fr} forms a counterpart of the well-known dark reversion of P_{730} to P_{660} in etiolated plant parts and phytochrome solutions (vide infra) (9, 10). If the latter reaction should turn out to be an equilibrium reaction, its position is very far to the side of P_{660}. Moreover, the rate of transformation of P_{730} to P_{660} appears to be very much slower than the opposite reaction that we have now observed in seeds. One might suppose that the emergence in the dark of a pigment, transformable by far red light, is due to the formation of additional P_{fr} from an inactive precursor in the seeds. This possibility seems to be ruled out by the observation that a, this process occurs only after far red actinic irradiation, not after red, b, that the total quantity of phytochrome remains constant after such a dark period. The additional observation that this reversion process occurs in the dark at 0° with a speed that is not much less than at room temperature, also does not favour an interpretation in which biochemical reactions are involved. We cannot, as yet, completely exclude the possibility that in imbibed seeds, the light-induced interconversion of pigment includes some very slow steps and that in the usual irradiation cycles of one minute red – one half minute observation – one minute far red etc., only a fraction of the total amount of pigment is phototransformed. The remainder might then be present in the form of one or more relatively long-lived photo-inactive intermediates, which decay to P_{fr} during the 10-minute dark periods. The attractiveness of such an explanation is not enhanced, however, by the observation that an increase in the duration of the illumination periods does not lead to a detectable increase in the $\triangle\triangle$O.D. values.

We have been unable to observe this reversion reaction when the seeds had passed the phase of rapid increase in phytochrome content (fig. 1, $\pm$ 20 hours). After this phase, the pigment exhibits the normal reactions of phytochrome from etiolated plants.

Whatever the explanation for the observed apparent dark reversion of P_r to P_{fr} in imbibed seeds, there clearly is a sharp contrast with the behaviour of phytochrome in etiolated seedlings. In order to explain some of the physiological reactions of plants previously exposed to red light, it has been postulated (8) that the far red absorbing form of phytochrome, initially formed, partly reverts to the original red absorbing form in the dark. Such a dark reversion has been demonstrated spectroscopically in purified solutions of oat phytochrome (9) and in isolated organs of pea seedling, if the simultaneously occurring dark decay of P_{730} is prevented by removing oxygen (10). The apparent inverse reversion reaction now found in seeds, may explain why light suppression of germination in these seeds sometimes can only be brought about by giving far red throughout the whole sixteen hours following imbibition. It also explains why the phytochrome found in dark imbibed seeds can be in the far red absorbing form. In most plants, studied so far, P_{730} is not stable in the dark, however, and is sub-

4

ject to a temperature and oxygen dependent destruction (phytochrome decay) (7, 11, 12). Following a far red illumination, the pool size of phytochrome therefore decreases in the dark, with a half life of about one hour. Apparently, this reaction does not occur in the imbibed seeds, P_{fr} being stable there over periods of many hours. So, we must conclude that the phytochrome in seeds, before germination, in several respects behaves differently from the pigment found in etiolated plants.

Attempts to demonstrate phytochrome in seeds of tomato (var. Saint Pierre) were, so far, unsucessfull, presumably due to the extremely low concentration of the pigment in these seeds. In *Lactuca* var. Grand Rapids, the very dark colour of the seed coats prevented spectrophotometric measurements.

SUMMARY

In seeds of *Lactuca sativa* 'May Queen', *Nemophila insignis* and *Sinapis alba*, photoreversible absorption changes due to phytochrome, or a similar pigment, could be observed after a few hours imbibition. In dark-imbibed seeds of *Lactuca* and *Nemophila*, the greater part of the pigment was present in the far red absorbing form. After phototransformation to the red absorbing form, a relatively short period of darkness resulted in renewed transformability of the pigment by far red radiation. One way of interpreting this finding is to assume a dark transformation in these seeds of the red absorbing to the far red absorbing pigment form.

REFERENCES

1. H. A. BORTHWICK, S. B. HENDRICKS, E. H. TOOLE, V. K. TOOLE (1954), Bot. Gaz. **115**, 205–225.
2. J. BOISARD, thesis, Faculté des Sciences, Université de Rouen, Oct. 1968.
3. A. L. MANCINELLI, H. A. BORTHWICK and S. B. HENDRICKS (1966), Bot. Gaz. **127**, 1–5.
4. P. ROLLIN and G. MAIGNAN (1966), C. R. Acad. Sc. Paris, **263**, 756–759.
5. P. J. A. L. DE LINT and C. J. P. SPRUIT (1963), Meded. Landbouwhogeschool Wageningen **63–14**.
6. W. L. BUTLER, H. C. LANE and H. W. SIEGELMAN (1963), Plant Physiol. **38**, 514–19.
7. W. L. BUTLER, H. C. LANE (1965), Plant Physiol. **40**, 13–17.
8. S. B. HENDRICKS (1960), Cold Spring Harbor Symp. XXV, 245–248.
9. F. E. MUMFORD (1966), Biochemistry **5**, 522–524.
10. C. J. P. SPRUIT (1967), Meded. Landbouwhogeschool Wageningen **67–14**.
11. L. H. PRATT and W. R. BRIGGS (1966), Plant Physiol. **41**, 467–474.
12. C. J. P. SPRUIT (1965), Meded. Landbouwhogeschool Wageningen **65–12**.