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New evidence . . .

Light Dependency In Seed Germination

WHEN LAND is made weed-free by chemical control methods, particularly with contact herbicides, the weed-free condition is best maintained by not disturbing the soil. Such disturbance can lead to germination of a new crop of weed seeds that otherwise would remain dormant.

Seed dormancy is evidently an excellent survival mechanism, from the point of view of a plant species. There are several methods of breaking dormancy in various species, including chilling, freezing, abrasion, leaching, oxygen, and light. These studies involved only the light requirements.

Small seeds

A large percentage of small-seed species require both moisture and a small amount of red light in order to start the germination process. Such requirements are well suited to the needs of our typical weeds, which are invaders of disturbed soil. They guarantee that seeds will germinate after disturbance, but that a reservoir of viable ungerminated seeds will remain in soil below the top inch or two. Light requirement is typical of small-seeded, rather than of large-seeded species. The red wavelengths involved can penetrate about one inch of sandy soil, thus activating just those seeds that can manage to emerge.

A great deal is already known about some aspects of this light control. The red light is detected by a pigment, "phytochrome," that, although present only in minute concentrations within the seed, has nevertheless been extracted and purified recently by USDA researchers. When a molecule of phytochrome absorbs red light (660 millimicrons), it is converted to another form known as P_{fr}. This new form of the pigment now absorbs far-red light (735 millimicrons; a very deep and dull color), with a subsequent reversion to the original light-absorbing form, known as P_r. This back-and-forth interconversion has been demonstrated both with the purified phytochrome and with light-requiring seeds.

Grand Rapids lettuce seed is the classical material for such studies because it is one of the few agricultural varieties in which light requirement has not been bred out of the species during domestication. When Grand Rapids seed is kept moist, but completely in darkness, only 5% to 30% of the seeds germinate, no matter how long the germination test is carried out. For a given batch of seeds, the light-independent germination is very reproducible, if incubation temperature is kept constant. However, when these imbibed seeds are given a few minutes of either filtered red light, or room illumination containing red light, and then replaced in darkness, essentially complete germination occurs. If, soon after the dose of red light, another dose of filtered *far-red* light is administered, no stimulation of germination occurs. After the radicle protrudes through the seed coat, neither red nor far-red light has any further effect upon growth.

Red illumination

All evidence indicates that P_{fr} (the product of red illumination) is the active compound. But the steps leading from P_{fr} to germination are relatively unknown. Recent research by other scientists has shown that when oxygen was kept away from the seeds, P_{fr} had no effect. Seeds were given a dose of red light, and kept under nitrogen gas for several hours; then P_{fr} was converted to P_r with a dose of far-red light, and seeds were put back into ordinary air. These seeds behaved as though they had never been exposed to red light at all; in other words, they had failed to "escape" from the reversal by far-red even though it was given many hours after the red treatment. In control seeds kept in air after red treatment, "escape" from far-red reversal occurred in only 4 to 8 hours (about 80% escape).

This means that one of the steps leading from P_{fr} to germination can be blocked by lack of oxygen. However, anoxia is rather nonspecific and the use of more specific types of inhibitors is needed to pinpoint which reactions are involved. One such inhibitor, CIPC, is a carbamate herbicide related chemically to IPC, swep, and barban ("Carbyne").

EFFECT OF INHIBITORS UPON PHYTOCHROME-INDUCED INCREASE IN GERMINATION RATE

Inhibitor		Time of addition (hours)	Per cent germination*	
			R-8, FR-28	R-8, FR-R-28
Expt. 1				
None		..	96	96
CIPC	30 ppm	0	43	87
CIPC	30	3	27	90
A-2-C	30	0	88	87
A-2-C	30	3	88	99
Expt. 2				
None		..	84	94
Puromycin	500 ppm	0	82	90
PFPA	10	0	79	86
Expt. 3				
None		..	95	96
PFPA	30 ppm	0	71†	67†

* "R-8, FR-28" indicates seeds exposed first to red light after 8 hours of imbibition and then to far-red light 20 hours later. Similarly, "R-8, FR-R-28" indicates an additional exposure to red light after 28 hours, subsequent to leaching away of inhibitors. Dark-germinated seeds, not given any exposure to red light, gave 29% germination.

† 30 ppm PFPA caused severe inhibition of radicle elongation.

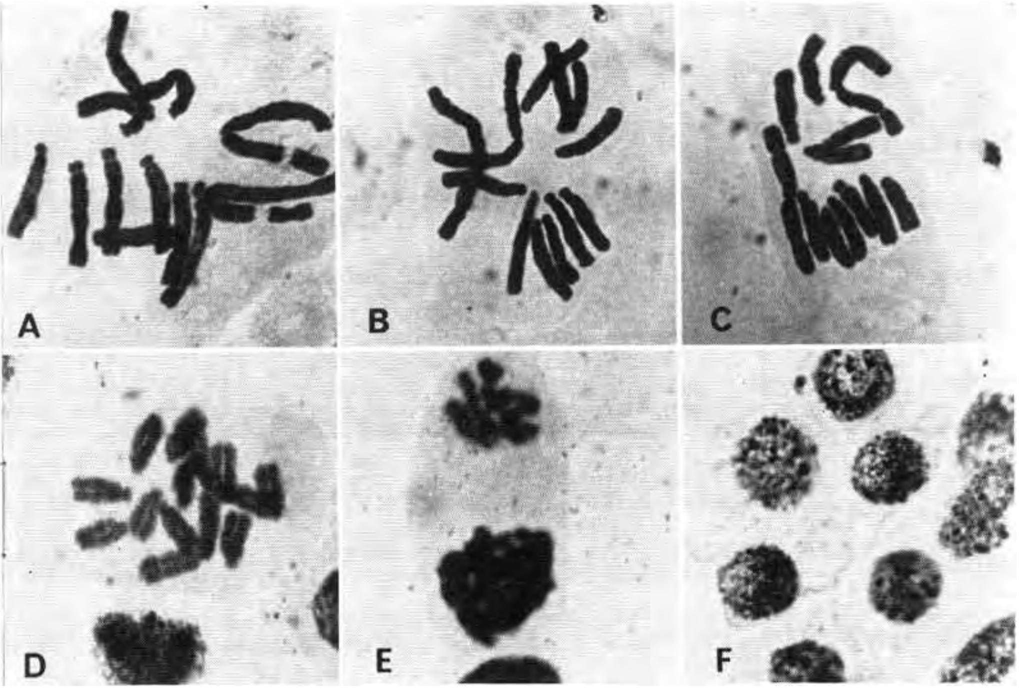

Figure 1. Contraction of mitotic chromosomes and nuclear "coagulation" of broad bean roots (Vicia faba) induced by 10 ppm IPC: A, no treatment; B, 1 hour; C, 2 hours; D, 3 hours; E, 4 hours (clumping and progressive disintegration); and F, 5 hours (complete disintegration and granulation).

It blocks protein synthesis and causes a dramatic shortening of mitotic chromosomes (figure 1)—perhaps by acting at a critical step in the process of protein synthesis.

Genes

The genes of the chromosomes in the nucleus are composed of DNA, but they direct the synthesis of proteins outside of the nucleus, in the cytoplasm. The intermediary here is known as "messenger RNA"—a sort of mirror image copy of DNA that acts like a blueprint to direct the types and sequence of amino acids to be incorporated into a protein. A good analogy would be to think of the DNA as a central filing system of designs drawn in India ink, and the messenger RNA as blueprint copies of the designs, so that other factors in the cytoplasm can assemble the nuts and bolts (amino acids) in the proper sequence.

Inhibitors

In these studies, the effects of substituting other inhibitors for anoxia were tested. The two most important inhibitors used were CIPC and PFPA. PFPA (parafluorophenylalanine) is a relative of the naturally occurring amino acid, phenylalanine. It confuses the issue at a stage after CIPC—at the point where amino acids are converted into protein. Two other inhibitors of protein synthesis were also used (puromycin and azetidine-2-carboxylic acid), but certain evidence suggests that they did not penetrate the seed coat. On the other hand, CIPC seems to prevent the synthesis or use of messenger RNA.

The results (see table) show that if seeds contained CIPC during the period when phytochrome was in the P_{fr} state, there was no light-stimulation of germination. But when PFPA or the other inhibitors were present, P_{fr} acted in some manner that subsequently led to germination after PFPA had been leached from the seeds—even though P_{fr} by this time was no longer present.

In the summary (figure 2), CIPC and anoxia are shown as preventing the synthesis of a messenger RNA required for eventual germination. P_{fr} is also required to cause synthesis of this messenger RNA. But if the "reading" of the messenger RNA blueprint is temporarily delayed by PFPA, azetidine-2-carboxylic acid, or puromycin, enough P_{fr}-induced messenger RNA accumulates during this period to cause eventual germination when the inhibitors are finally removed.

Seeds of some other lettuce varieties, as well as other plant species, do not normally require light for germination. But many such seeds can be made light-requiring by keeping them warm and moist. These conditions cause a functional anoxia, since respiration increases with elevated temperature, but diffusion of oxygen through intact seed-coats does not. During this high-temperature incubation, messenger RNA that was already present in the dried seed was destroyed without being used to make protein. When the seeds were cooled down, they required synthesis of new messenger RNA for germination, and this synthesis required the presence of P_{fr}.

Summary

In summary, seeds that require light to initiate germination are probably those which are deficient in a particular kind of messenger RNA. Phytochrome, when in the active P_{fr} state, stimulates the cell nuclei to release the messenger RNA. In turn, the messenger RNA causes the production of certain (unknown) enzymes that start the seed irreversibly on the road to germination.

The influence of phytochrome is not limited to seed germination. A remarkable variety of botanical phenomena show responses to red and far-red light. For instance, fall-blooming plants need a long night to convert their leaf buds to flower buds. The leaves are the receptor organs, and the leaves in this respect are sensitive to a red light interruption of the dark period; photosynthesis is only indirectly involved. Formation of red pigments (anthocyanins) in seedling turnips and corn requires red light. Even the formation of unsaturated fatty acids in linseed requires red light. It is possible that a similar mechanism, concerned with phytochrome-controlled synthesis of specific messenger RNA's, is involved in all cases.

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Figure 2. Postulated mechanism for phytochrome action.

$$\begin{array}{ccccccc} \text{Nuclear DNA} & \xrightarrow[\ \ \text{anoxia}\ \ ]{P_{fr}\ \ \text{CIPC}} & \text{messenger RNA} & \xrightarrow[\text{anoxia}]{\text{PFPA}} & \text{protein} & \longrightarrow & \text{germination} \\ & & \downarrow & & & & \\ & & \text{degradation of messenger RNA} & & & & \end{array}$$