

there may be flavin oxidases with relatively high oxygen affinities which can be involved in normal respiration.

It has been shown with potato tuber slices (15) and an Aroid spadix (17) that higher plant tissues having a cyanide and CO insensitive respiration can have a relatively high oxygen affinity, with a $1/K_m$ equal to 3×10^5 M. Although this value is higher than the affinity of many flavin oxidases, the results of the present study suggest that this group of enzymes cannot be ruled out as terminal oxidases for respiration on this basis alone. However, the evidence that these plant tissues contain a complete cytochrome system which participates in the normal respiration and which may be involved in cyanide-resistant respiration (17) must also be considered before a final conclusion is reached.

SUMMARY

The respiratory rate of *S. faecalis* cells and the activity of the extracted DPNH oxidase are essentially independent of the oxygen tension when the reaction systems are gassed with 5, 20 and 100 % O₂. The oxygen affinity ($1/K_m$) of 4×10^5 M is unusually high for a flavin oxidase, and the implications of this finding for the analysis of respiratory mechanisms are discussed.

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THE RELATIONSHIP OF THE KINETIN AND RED-LIGHT PROMOTIONS OF LETTUCE SEED GERMINATION¹

CARLOS O. MILLER

DEPARTMENT OF BOTANY, INDIANA UNIVERSITY, BLOOMINGTON, INDIANA

Certain plant growth effects produced by application of kinetin strikingly resemble effects resulting from exposure to red light (2, 3, 4, 5). For example, both kinetin and red light promote the expansion of etiolated bean leaf disks and the germination of lettuce seeds. On the basis of such observations, suggestions have been made that there is a close relationship between biochemical activities of kinetin (6-furfurylamino-purine) and reactions controlled by red light. The experiments reported herein have been performed with the hope of learning the nature of this close relationship if it actually exists.

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In the works cited above, the plant material was at some time exposed to at least a small amount of light. Although there was no evidence that such light affected the controls, the possibility that weak light has a very strong influence in the presence of kinetin was not eliminated. Parts of our own experiments were performed under a dim, green light which did not noticeably influence the germination of lettuce seeds wetted with water; the assumption was made, therefore, that it represented a control condition essentially equivalent to darkness. We now know that this is not so; weak light has very large effects on germination provided kinetin has been supplied to the

seeds. Furthermore, the strong promotion of germination by kinetin previously reported (3) must have been considerably dependent upon exposure to small quantities of light. This relationship, of course, does not necessarily apply to results obtained by other investigators with other plant materials. The conclusion concerning lettuce seed germination is supported by the data represented in figure 1. In this and all other experiments reported in this paper, Grand Rapids lettuce seeds (harvested in 1955) were sprinkled into Petri dishes onto pads consisting of three sheets of Whatman no. 1 filter paper wetted with 5 ml of either water or 5×10^{-5} M kinetin. These dishes were kept in absolute darkness at $25 \pm 1^\circ \text{C}$ for 16 hours, next exposed to red light for the indicated times, and then returned to darkness. The amount of germination was determined 48 hours later, a seed being regarded as germinated if any part of the embryo protruded through the seed coats. This criterion of germination was selected because kinetin, at the concentration used, inhibits radicle elongation. At lower concentrations, the inhibition is not very marked but the percentage of germination is smaller. Nevertheless, experiments in which only 5×10^{-6} M kinetin was used revealed the same relationships between the kinetin and light effects as observed with higher concentrations; in these experiments, the radicles emerged before any other parts of the embryos. The red light was obtained by filtering radiation from a 15-watt standard daylight fluorescent tube through two sheets of DuPont red cellophane. The exposures were made at a distance of 205 cm from the surface of the tube and, as measured by a thermopile (black body), the amount of energy at the point of exposure was 98 ergs/sq cm \times sec. Although kinetin did promote germination to some extent in absolute darkness, it was much more effective if the seeds were briefly exposed to the weak light.

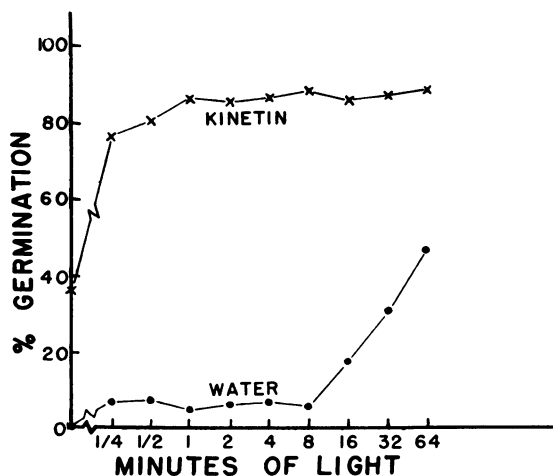


FIG. 1. Promotion of lettuce seed germination by red light in the presence or absence of kinetin. Light exposures at an intensity of 98 ergs/sq cm \times sec and for the indicated times. 5×10^{-5} M kinetin was used.

TABLE I
INFLUENCE ON LETTUCE SEED GERMINATION OF COMBINED TREATMENTS WITH KINETIN, RED LIGHT, AND FAR-RED LIGHT

LIGHT TREATMENT	% GERMINATION *	
	ON WATER	ON KINETIN
Darkness	1	34
Red	42	89
Far-red	1	65
Red then far-red	3	64

* Approximately 120 seeds/treatment. 5×10^{-5} M kinetin used.

Only a small percentage of the seeds imbibed on filter paper containing only water responded to the short periods of illumination; with the same exposures, most of the kinetin-soaked seeds germinated. A permissible interpretation of the curves is that kinetin increases the efficiency with which the photoreaction brings about germination.

Further evidence supporting this interpretation has been obtained in attempts to inhibit germination by exposure to far-red light. In our previous report, we stated that far-red does not reverse the kinetin promotion. In the present studies, using a filter system more refined than the one previously employed, we have learned that far-red reverses — but only partially — the promotion caused by exposing kinetin-soaked seeds to red light; and, furthermore, far-red actually promotes germination of kinetin-soaked seeds which otherwise have been kept in the dark! Thus, in a typical experiment (table I), germination of kinetin-soaked seeds illuminated with a high-intensity red light for one minute was 89 % as compared to 34 % for similarly soaked seeds kept in the dark. Exposure to far-red for 15 minutes starting one minute after the end of the red-light exposures decreased the 1st value to 64 % but increased the 2nd value to the same level. The far-red light source used here consisted of one 200-watt tungsten lamp and a filter of two layers of DuPont red cellophane combined with a layer 1 cm thick of a 0.2 % solution of Poirrier's blue. Exposures were made at a distance of 45 cm from the loose end of the tungsten bulb. Energy values were not obtained for this setup, but it was established that exposures of 0.5 minute gave the same results as obtained with exposures of 15 minutes for either promotion or inhibition. Thus, results presented here represent saturation exposures. With this filter system, no transmission was detected in a Beckman DU spectrophotometer in the range 450 to 730 $m\mu$, was less than 0.2 % in the range 400 to 440 $m\mu$ and was 4 % at 750 $m\mu$. A possible interpretation of these observations is based upon the fact that the action spectra for promotion and for inhibition of germination overlap and the likelihood that in the region of overlap the two pigments are in equilibrium (1). It may be that some molecules of the pigment involved in promotion are activated at

wavelengths of 730 $m\mu$ or longer (as already suggested, 1) and, in the presence of kinetin, quite strongly promote germination. Thus, information obtained by exposures to far-red irradiation also can be interpreted to mean that kinetin increases the sensitivity of the seeds to promotion by red light.

Much can be learned about the relationship being considered here by determining just when kinetin must be supplied to the seeds in order to bring about the increase of sensitivity to red light. In the experiments described above, the kinetin solutions were applied to dry seeds at the beginning of the tests; that is, light exposures were made only after the kinetin solution had been imbibed by the seeds. As a method of supplying the kinetin after the photoreaction had occurred, seeds were imbibed with water for 16 hours, exposed to weak red light, and then transferred to appropriate solutions. These seeds showed little response to kinetin. Thinking that this lack of response might be due to failure of kinetin to get into the seeds prompted the following procedure: Seeds were imbibed on water-soaked filter paper in the dark for 16 hours and then some were exposed to bright red light for 8 minutes. Next, the seeds were immediately blotted and dried over calcium chloride in a desiccator kept in darkness at $25 \pm 1^\circ \text{C}$. Three days later, the dried seeds were transferred to Petri dishes containing filter paper pads wetted with either water or $5 \times 10^{-5} \text{M}$ kinetin. After two additional days in darkness, the germination percentages were determined. Results of a typical experiment are presented in table II. It is quite obvious that kinetin has increased the germination response of the seeds even though supplied after the light reaction was completed. This fairly well eliminates the possibilities that the compound promotes germination by participating directly in the photoreaction or by promoting reactions preparatory to the photoreaction.

Therefore, if kinetin participates directly in a chain of reactions of which the light reaction is also a part, it is very likely that the involvement occurs after photoreaction. Our endeavors to examine this possibility by measuring the influence of kinetin on the rate of dark reactions which immediately follow the photoreaction (1) have yielded no clearly interpretable results. For this reason, the experiments are not described here. It is the author's feeling,

TABLE II
EFFECT ON LETTUCE SEED GERMINATION OF KINETIN
SUPPLIED AFTER EXPOSURE TO RED LIGHT

LIGHT TREATMENT BEFORE DRYING	% GERMINATION *	
	ON WATER	ON KINETIN
Dark	3	29
Red light	28	94

* Approximately 120 seeds/treatment. Seeds imbibed on water, given light treatments, dried, and later germinated. $5 \times 10^{-5} \text{M}$ kinetin used.

however, that all data thus far obtained are consistent with the idea that kinetin does not participate directly in the photoreaction nor in the reactions which immediately precede or follow it. Furthermore, since kinetin at its optimal concentration cannot completely substitute for the light reaction, it cannot be the product of the photoreaction. Although it should be emphasized that these conclusions do not eliminate the possibility of the kinetin and red light actions occurring fairly close together in the same metabolic meshwork, it may be wise for the moment to study each effect separately and intensively.

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