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# Respiration of Grand Rapids lettuce (*Lactuca sativa* L.) seeds in relation to chemical and photocontrol of germination

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A 10-min irradiation with red light (R) of 'Grand Rapids' lettuce (*Lactuca sativa* L.) seeds increased respiratory rates ($QO_2$ and $QCO_2$) over those of far red (FR)-irradiated seeds within 2 to 3 hr. Differences in respiration between R- and FR-irradiated seeds became more pronounced with time. The respiration, in darkness at 25°C, of seeds air dried after germination promoting impregnation treatments with 1.0 mM $GA_3$+0.5 mM kinetin in acetone was stimulated slightly by the 6th hour after planting and markedly by the 12th and 24th hour. Results with seeds in $GA_3$+kinetin dissolved in water were similar. ABA, which inhibits germination and seedling growth, prevented stimulation in respiration of light-induced seeds. ABA also repressed respiration when used with $GA_3$+kinetin. The germination and growth retardant, isopropyl N-(3-chlorophenyl) carbamate (CIPC), did not affect the respiration of either R- or FR-treated seeds. A comparison of the effects of chemical and light treatments on respiration indicate that chemical and photocontrol mechanisms are not identical.

Relationships between respiration and seed vigor have been reported for many crops (*9, 13, 14, 17*). Lettuce seed respiration was promoted by light treatments promotive to germination (*4, 7*) and inhibited by germination inhibitors (*3*).

Light responses of seeds may be influenced by temperature (*15*) and by treatment of the seeds with cytokinins, gibberellin, abscisic acid (*5*) and coumarin (*3*). Khan (*5*) proposed that, in seeds, cytokinins permit $GA_3$ activity in the presence of ABA.

Use of organic solvents (*6, 10, 11*) permit impregnation of dry lettuce seeds with germination stimulators and inhibitors prior to imbibition. Although it has been suggested that organic solvents and the chemicals they carry do not reach embryo tissues (*1, 16*), light-requiring lettuce seeds impregnated with $GA_3$ germinated in darkness whereas others impregnated with ABA failed to germinate in light (*6*).

Germination of light sensitive seeds may be either initiated or prevented by light. The far-red absorbing form of phytochrome, $P_{fr}$, initiates germination and the red absorbing form, $P_r$, prevents germination (*2*). We report the effects of

Abbreviations: ABA, abscisic acid; CIPC, isopropyl N-(3-chlorophenyl) carbamate; $GA_3$, gibberellic acid; R, red light, 600–660 nm; FR, far-red light, 700–750 nm.

$P_{fr}$ and $P_r$ actions and of germination promotors and inhibitors on respiration of 'Grand Rapids' lettuce seeds.

## Materials and methods

Seeds of lettuce (*Lactuca sativa* L., cv. Grand Rapids) stored at −18°C in sealed glass jars at about 8% seed moisture were used throughout.

Respiration was measured sequentially with a Gilson differential respirometer for up to 24 hr after the start of imbibition. Results are expressed as $\mu$l $O_2$ uptake or $CO_2$ evolution $hr^{-1}$ 100 $seeds^{-1}$. Specific conditions of vessel size, amount of solution and number of seeds per vessel, temperature, aeration, etc., are given with the appropriate figures and tables. Seed numbers from 25 to 100 per reaction vessel and vessel size (e.g., 5- or 15-ml) did not influence apparent respiratory rates of lettuce seeds, and $O_2$ content of the flasks was not allowed to decrease by more than 5% of the original concentration. Because radicle emergence started between the 12th and 16th hour, readings on the light-promoted seeds were not usually made beyond the 12th hour.

Germination was tested on replicates of 50 or 100 seeds per petri dish. The seeds were planted on two filter papers with 7.5 ml water or test solution. Germination was scored after 28 and 48 hr at 20°, 22° or 25°C. Germination in the respiratory vessels was scored at the 25th hour. Seeds were held in total darkness except during specified light treatments. Seeds were irradiated with red light (R) at ambient temperature (about 25°C). The light was from 2.44-m, slimline, T8 cool-white fluorescent lamps filtered through 2 layers of red cellophane. The broad band R radiation (600–660 nm) at seed level of 1 m was 0.6 mW $cm^{-2}$. For irradiation with far-red (FR) at ambient temperature, light from three 300-watt incandescent flood lamps was filtered by 2 layers each of red and blue cellophane and 10 cm of water. The broad band FR (700–750 nm) radiation at seed level of 1 m was 0.75 mW $cm^{-2}$. In experiments given in Tables 2 and 3 unfiltered fluorescent light of about 430 lux was used intermittently for about 20% of the time.

Seeds were impregnated for 4 hr with 1.0 mM $GA_3$+0.5 mM kinetin, 0.2 M ABA, and 0.2 M ABA+1.0 mM $GA_3$+0.5 mM kinetin in acetone according to Meyer and Mayer (*10*) and Khan (*6*). The seeds were then decanted and air-dried overnight. In some tests aqueous solutions of $GA_3$, kinetin, and ABA were added to the petri dishes or respiration vessels.

## Results and discussion

Seeds given continuous R during the first 3 hr of imbibition had greater respiratory rates than did seeds given 5 min of FR at the end of a 3-hr dark imbibition (Table 1). Likewise, seeds given continuous R during the first 12 hr of imbibition had greater respiratory rates than those of the dark controls (Table 1). The above results show a definite promotive effect of R on seed respiratory rates 4 to 5 hr after the start of imbibition. This is well in advance of radicle emergence, which was not evident until after 12 hr. The increase in respiration of R-treated seeds agrees with results of Hagen et al. (*4*) for $O_2$ uptake.

Table 1 *Influence of light treatments on respiratory rates of Grand Rapids lettuce* (Lactuca sativa *L.*) *seeds*

| Hr after planting | Oxygen Uptake ($\mu$l $O_2$ $hr^{-1}$ 100 $seeds^{-1}$) | |
|---|---|---|
| | Air | Air |
| | (0–3 hr R)[a] | (0–3 hr dark, 5 min FR)[a] |
| 4–5 | 34 | 28 |
| 6–7 | 29 | 22 |
| 8–9 | 31 | 21 |
| Germination, 48 hr | 99%[b] | 3% |
| | (0–12 hr R)[c] | (0–12 hr dark)[c] |
| 12.5–13.5 | 38 | 31 |
| 14.5–15.5 | 47 | 36 |
| 16.5–17.5 | 53 | 39 |
| Germination, 48 hr | 99%[b] | 51% |

[a] Each 5-ml vessel at 20°C contained 0.3 ml $H_2O$ and 25 seeds on a paper ring. Data are means of 5 reps.

[b] Germination on filter papers in petri dishes at 20°C, with same light conditions as for respiration. Means are of 3 reps of 50 seeds each.

[c] Each 15-ml vessel at 20°C contained 0.5 ml $H_2O$ and 50 seeds on a paper ring. Data are means of 5 reps.

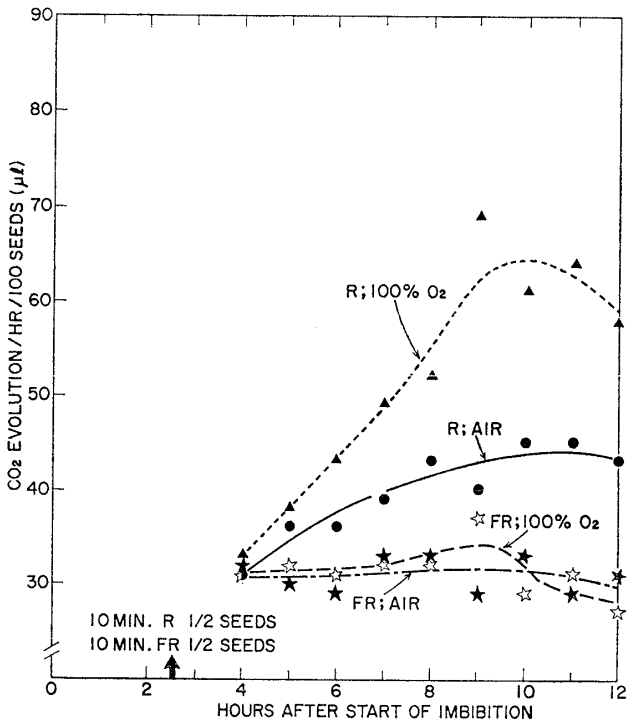


Fig. 1. *Respiration as $CO_2$ evolution of R- and FR-treated Grand Rapids lettuce* (Lactuca sativa *L.*) *seeds.* Seeds were imbibed in water and given 10-min R or FR at 2.5 hr. $CO_2$ evolution was measured at hourly intervals between 4 and 12 hr after wetting. Each 15-ml flask contained 0.4 ml $H_2O$ and 100 seeds on a filter paper ring. Measurements were at 22°C in air or in 100% $O_2$. Data points are means of 4 replicate samples. --▲--, R in 100% $O_2$; -●-, R in air; ---☆---, FR in 100% $O_2$; ---★---, FR in air.

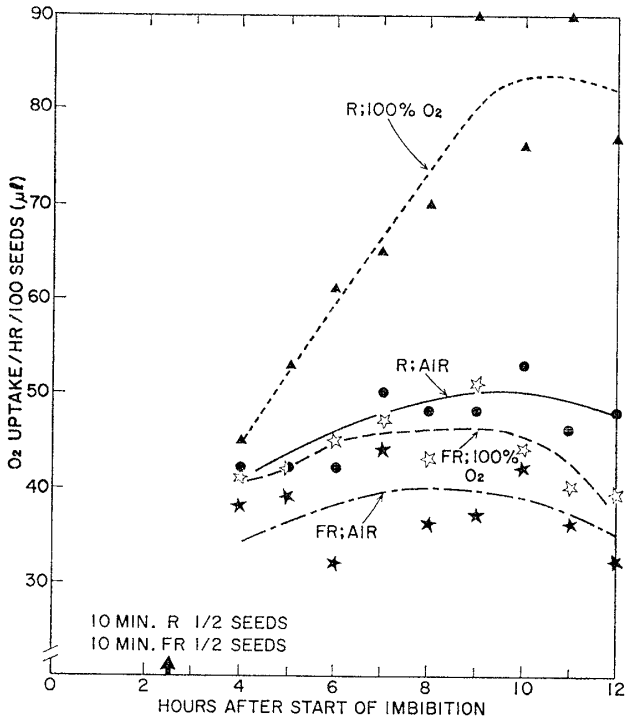


Fig. 2. *Respiration as $O_2$ uptake of R- and FR-treated Grand Rapids lettuce seeds.* Same as Fig. 1, except respiration was measured as $O_2$ uptake, and data points are means of 5 replicate samples.

Tests were conducted to determine how soon differences in respiratory rates could be detected after the light treatments. When seeds were exposed to 10 min of R or FR 2.5 hr after wetting, differences in respiration were observed within only 2.5 hr after the R treatments for $CO_2$ evolution (Fig. 1) and 3.5 hr for $O_2$ uptake in air (Fig. 2). Differences between R- and FR-treated seeds became more pronounced with time. These results show that changes in respiratory rates are among the first changes to be observed after germination-influencing light treatments, and they extend earlier observations on $O_2$ uptake (*4*) to include $CO_2$ evolution. Likely, conditions that favor the onset of germination (R), increase respiratory rates because of the heightened metabolic activity of the seeds.

If $O_2$ were rate limiting for respiration due to diffusion barriers, then differences in respiratory rates between promotive and inhibitory light treatments might be partially masked. We therefore, measured respiration in 100% $O_2$ as well as in air to reduce possible diffusion barriers. Preliminary tests showed that 100% $O_2$ did not inhibit germination. Differences in $O_2$ uptake between R- and FR-treated seeds were observed sooner in 100% $O_2$ than in air and, with R-treated seeds, became much more pronounced with time (Fig. 2). Rates of $CO_2$ evolution of FR-treated seeds increased neither in 100% $O_2$ nor with time (Fig. 1). Respiration of the FR-treated seeds decreased after 8–10 hr. Hagen et al. (*4*) and Leggatt (*7*) observed a similar break in respiration of germination-inhibited seeds.

Respiration in darkness of seeds impregnated with $GA_3$+kinetin was greater than that of the controls by the 6th hour (Table 2). Germination of the impregnated seeds in darkness was also greater than that of the untreated controls (Table 3). Respiration of the acetone controls was similar to that of untreated controls except

Table 2 *Influence of impregnation with chemicals that promote and inhibit germination on respiration of Grand Rapids lettuce* (Lactuca sativa *L.*)

| Treatment | Oxygen uptake ($\mu$l $O_2$ $hr^{-1}$ 100 $seeds^{-1}$)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Light[b] | | | | Dark | | | | |
| | 2hr | 4hr | 6hr | 12hr | 2hr | 4hr | 6hr | 12hr | 24hr |
| Control, no treatment | 38 | 46 | 44 | 51 | 36 | 46 | 37 | 39 | 49 |
| Acetone control | 41 | 47 | 46 | 60 | 37 | 44 | 34 | 34 | 42 |
| 1.0 mM $GA_3$+0.5 mM kin | 43 | 50 | 51 | 63 | 40 | 44 | 44 | 72 | 197 |
| 0.2 M ABA | 42 | 50 | 47 | 49 | 44 | 43 | 40 | 60 | 35 |
| 0.2 M ABA+0.5 mM kin +1.0 mM $GA_3$ | 44 | 49 | 47 | 50 | 39 | 42 | 39 | 60 | 49 |
| | $CO_2$ evolution ($\mu$l $CO_2$ $hr^{-1}$ 100 $seeds^{-1}$)[a] | | | | | | | | |
| Control, no treatment | 33 | 41 | 36 | 48 | 22 | 34 | 31 | 34 | 34 |
| Acetone control | 34 | 41 | 37 | 56 | 25 | 35 | 31 | 39 | 27 |
| 1.0 mM $GA_3$+0.5 mM kin | 34 | 41 | 44 | 58 | 30 | 35 | 37 | 58 | 159 |
| 0.2 M ABA | 35 | 42 | 39 | 44 | 28 | 34 | 32 | 44 | 20 |
| 0.2 M ABA+0.5 mM kin +1.0 mM $GA_3$ | 41 | 43 | 41 | 45 | 24 | 33 | 32 | 43 | 39 |

[a] Respiration measurements were in air on 50 seeds per 15-ml flask with 1.0 ml $H_2O$ at 25°C. Impregnation treatments were: acetone control, 1.0 mM $GA_3$+0.5 mM kinetin, 0.2 M ABA, and 0.2 M ABA+1.0 mM $GA_3$+0.5 mM kinetin. Data are means of one experiment of 3 replicates (light) and two experiments of 3 replicates each (dark).

[b] Unfiltered fluorescent light of about 430 lux 20 percent of the time.

for a possible slight stimulation at 12 hr in light (Table 2). Acetone slightly increased germination in darkness and did not affect the response of the seeds to R. The respiration of seeds impregnated with 0.2 M ABA was the same as for controls

Table 3 *Influence of promoting and inhibiting chemical treatments on Grand Rapids lettuce* (Lactuca sativa *L.*) *seed germination*[a]

| R Treatment (Min) | Seed bed | Hr of reading | Percent Germination | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Impregnation with chemicals[b] in acetone | | | | | Chemicals[b] dissolved in water | | |
| | | | Control (no treatment) | Acetone control | $GA_3$+ kinetin | ABA | ABA+ $GA_3$+ kinetin | Control (no treatment) | $GA_3$+ kinetin | ABA |
| 0 | Resp. vessel | 25 | 15 | 33 | 88 | 0 | 1 | 15 | 53 | 0 |
| 0 | Petri dish | 28 | — | — | — | — | — | 22 | 86 | 0 |
| 0 | Petri dish | 48 | 16 | 30 | 100 | 16 | 86 | 24 | 96 | 0 |
| Int. | Resp. vessel[c] | 25 | 90 | 96 | 93 | 0 | 7 | 90 | 89 | 0 |
| 2 | Petri dish[d] | 28 | — | — | — | — | — | 95 | 92 | 0 |
| 2 | Petri dish | 48 | 100 | 98 | 98 | 84 | 87 | 98 | 96 | 0 |

[a] Germination values are the means of 4 replicates of 100 seeds each (Petri dishes) or 6 replicates of 50 seeds each (Resp. vessel).

[b] Concentrations were 1.0 mM $GA_3$, 0.5 mM kinetin, and 0.2 M ABA.

[c] Seeds in the respirometer flasks were exposed to intermittent, unfiltered white fluorescent light of about 430 lux and were at 25°C. Each 15-ml flask contained 1.0 ml water or test solution.

[d] Seeds in Petri dishes were exposed to 2 min R after about 3 hr dark imbibition and held at 25°C.

Table 4 *Influence of 30 ppm CIPC on respiration of Grand Rapids lettuce* (Lactuca sativa *L.*) *seeds under germination promotive (R) and inhibiting (FR) conditions*

| | Oxygen uptake ($\mu$l $O_2$ $hr^{-1}$ 100 $seeds^{-1}$)[a] | | | |
|---|---|---|---|---|
| | Continuous FR 0–2.5 hr; 10-min R | | Continuous FR 0–2.5 hr | |
| Hr after planting | $H_2O$ | CIPC | $H_2O$ | CIPC |
| 4 | 48 | 46 | 46 | 48 |
| 5 | 41 | 41 | 40 | 42 |
| 6 | 48 | 50 | 41 | 42 |
| 7 | 49 | 47 | 43 | 44 |
| 8 | 44 | 44 | 34 | 35 |
| 9 | 55 | 53 | 41 | 44 |
| 10 | 52 | 52 | 41 | 42 |
| Germination, 48 hr[b] | 99% | 99% | 6% | 3% |

[a] Each 15-ml flask at 22°C contained 0.4 ml $H_2O$ and 100 seeds on paper rings. Data are means of 5 replicates for FR+R and of 4 replicates for FR.

[b] Seeds, 100 per Petri dish, were germinated on blotters with 9 ml $H_2O$ at 22°C. Seedling growth was inhibited about 50% by 30 ppm CIPC; germination at 24 hr was inhibited by CIPC.

in light and, except for 12-hr, in darkness. No germination occurred up to 25 hr (Tables 2 and 3). However by 48 hr, germination was the same as for the controls in darkness and only 16% lower than that of controls for R-treated seeds.

In agreement with the R-treatments, chemicals promotive to germination increased respiratory rates. In contrast to R-treatments, chemical treatments only slightly increased respiration by 6 hr and effects were not well defined until 12 hr. Although both R and chemical promotors stimulated respiration by 6 hr and well before germination, it is not clear whether the greater delay after treatment in effects with chemicals indicates a different site of action, delay caused by chemical diffusion to active sites, or the inability of seeds to express differences in respiratory metabolism before 6 hr.

In aqueous 0.2 M ABA solution, no germination occurred at 25°C during the 48-hr test period in either light or darkness (Table 3). The greater inhibitory effect on germination of ABA in aqueous solution compared with that of the impregnation treatment may indicate either that the impregnation treatment only brings the test chemical to the outer seed tissues as suggested by Anderson (*1*) and Triplett and Haber (*16*) or that ABA in impregnated seeds is diluted by excess water in the substrate. However, impregnation with ABA did suppress germination over the period respiration was measured. ABA used with $GA_3$+kinetin also suppressed their promotion of respiration (Table 2) and germination (Table 3). In 48 hr, the dark germination of seeds impregnated with ABA+promoters was the same as the light germination of ABA impregnated seeds and was not promoted by R. Germination of seeds in aqueous solutions of $GA_3$+kinetin and of ABA was similar to that of seeds with the corresponding impregnation treatments, that is respiration (data not shown) and dark germination (Table 3) were stimulated by $GA_3$+kinetin and inhibited by ABA.

It has been suggested that phytochrome ($P_{fr}$) acts by derepressing an inactive

gene, and that no stable intermediate or product mediates between $P_{fr}$ and gene derepression (*8*, *12*). Experiments by Mann and Jordan (*12*) showed that R did not promote Grand Rapids lettuce germination above dark controls (about 30%) when the germination inhibitor, isopropyl N-(3-chlorophenyl) carbamate (CIPC), was present. CIPC presumably blocked the action of $P_{fr}$ on gene derepression. With the above mechanism we would not expect a general difference in metabolism (e.g., in respiratory rates) between R and control seeds in the presence of CIPC. However, when seeds imbibed under FR in water or in 30 ppm CIPC were given 10 min R at 2.5 hr, CIPC had no pronounced effect on $O_2$ uptake of either R-stimulated or FR-inhibited seeds (Table 4). The failure of CIPC at germination-delaying concentrations to suppress the R stimulation of respiration would appear to contradict the above mechanism.

Germination has occurred by 24 hr, so differences in respiratory rates between the various treatments by this time may be the result, rather than the cause of, the effects on germination. Differences in respiratory rates at 12 hr or earlier precede germination, although metabolic processes leading to radicle protrusion have started. Effects of the germination-stimulating $GA_3$+kinetin impregnation treatments on respiration are evident by 6 hr but are not well defined before 12 hr. In contrast R treatments stimulated respiration within 2 to 3 hr after exposure. This difference between chemical and light treatments indicates that the mechanisms by which they control germination might not be identical. In both cases, however, effects on respiration occur well in advance of germination response.

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