Factors Affecting the Induction of Secondary Dormancy in Lettuce

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ABSTRACT

The relationship between the temperature at which germination of 50% of the seeds is inhibited in the light (GT50 Light) and secondary dormancy was investigated in three cultivars of Lactuca sativa L. Seeds were incubated for varying periods under non-germinating conditions and subsequent germination in response to red light (R) was determined over a wide range of temperatures. Dark incubation at 32 C reduced the GT50 Light of cv. New York but did not affect germination at temperatures below 24 C. Dark, 32 C incubation had no effect on the GT50 Light of cv. Great Lakes. In cv. Grand Rapids, dark incubation at 15, 24, 32, or 35 C initially reduced the GT50 Light. However, longer incubations induced a secondary dormancy, i.e., the seeds became unable to germinate at all temperatures in response to R given after the high temperature incubation. A single exposure to R at the beginning of a 32 C incubation slowed the induction of secondary dormancy. Repeated exposures to R prevented the induction of secondary dormancy, but did not prevent a decline in the GT50 Light. GA3 mimicked the effect of repeated R.

The differences in the germination behavior of the three cultivars suggest that there may be qualitative differences in the germination mechanism of these cultivars. This research demonstrates the significance of monitoring germination at a range of temperatures to avoid misinterpretation of the data.

Lettuce (Lactuca sativa L.) seed germination is strongly temperature-dependent. As the temperature rises above the optimum (18), germination declines sharply, often falling from 100% to near 0% with an increase of only 2 or 3 degrees C (23). The temperature at which there is a 50% reduction in germination has been termed as upper temperature cut-off point (31) or simply, GT50 (10). When seeds are exposed to temperatures above GT50, they become thermomorphic. In lettuce, GT50 typically occurs in the range of 25 to 30 C, but the precise temperature may differ among cultivars (24, 32), and among different lots of the same cultivar (26).

Numerous studies have shown that the promotive or inhibitory effects of chemical or physical factors on germination can be related to shifts in the GT50 (11, 12, 20–24). A brief exposure to R2 raises the GT50 in both light-sensitive (e.g. Grand Rapids) and so-called light-insensitive cultivars such as Great Lakes (11, 20). For descriptive purposes, lettuce may be thought of as having two GT50, one for germination in the dark (GT50 Dark) and one for germination in the light (GT50 Light) (20). The germination-modifying effects of GAs, KIN, ABA, and solutions of high osmotic pressure have been shown to be related to changes in one or both of these GT50 (21, 24).

Incubation of fully imbibed seeds under conditions not suitable for germination is known to reduce their germination potential. High temperature incubations can induce a light requirement in dark germinating lettuce seeds (1, 2), i.e., the seeds become photodormant. This effect has been attributed to a decline in the GT50 Dark (11, 12). In the light sensitive cv. Grand Rapids, prolonged dark, or high temperature incubations may also decrease photosensitivity until the seeds become unresponsive to R, i.e. secondarily dormant (3, 4, 9, 27, 31, 34, 35).

In most previous studies of secondary dormancy, its onset has been monitored at only one temperature, thus, little is known about the changes in GT50 Light during the induction of secondary dormancy. In the present study, changes in germination have been monitored over a wide range of temperatures in the examination of the effects of interactions among light, temperature and growth regulators on the induction of secondary dormancy in three lettuce cultivars.

In this paper, high-temperature incubation refers to the lowest temperature capable of suppressing germination to zero in both light and dark treatments; and secondary dormancy refers to the seeds that become incapable of germination in response to R at any temperature.

MATERIALS AND METHODS

Plant Materials. Achenes of L. sativa L. cvs. Grand Rapids (lot I), New York, and Great Lakes were obtained from the Robertson Seed Co., Edmonton, Alberta. Another lot of Grand Rapids (lot II) was obtained from the Carolina Biological Supply Co., Gladstone, OR. All seeds were examined before use and damaged, abnormally small, or off-color seeds were discarded.

Germination Studies. For each replicate approximately 100 seeds were distributed in 9-cm plastic Petri dishes containing 2 layers of Whatman No. 1 filter paper and 5 ml distilled H2O or test solution. Seeds were incubated under non-germinating conditions provided by darkness or supraoptimal temperatures, depending on the cultivar. High temperature incubations were conducted at the lowest temperature capable of suppressing germination to 0% under both light and dark germination conditions. This was 35 C for cv. Grand Rapids (I) and 32 C for all other seed lots (Table I). For dark incubations Petri dishes were wrapped in a single layer of aluminum foil. In some experiments seeds were exposed to R during the course of a high temperature incubation. In these cases the Petri dishes were sealed with parafilm to prevent them from drying out. At the end of the incubation period all Petri dishes were opened to room air and any seeds that had germinated were removed. Following a light or hormone treatment all samples were wrapped in aluminum foil and placed in growth cabinets adjusted to temperatures of 15, 20, 22, 24, 26, 28, 30, 32 or 35 C. Temperatures within the Petri dishes were measured with a copper/constantan thermocouple and were found to vary less than 1 degree C.
than ± 0.25 C. Germination, as measured by radicle emergence, was determined after 5 days in cv. Great Lakes. In all other seed lots germination was determined after 3 days in seeds kept at 20 to 35 C, and after 5 days in seeds at 15 C. No further germination occurred after these times. All treatments were represented by at least three replicates. Most experiments were repeated at least once.

Light Sources. All manipulations of the seeds were conducted in a darkroom under a green safelight consisting of two 15-w cool-white fluorescent tubes wrapped in a triple layer of No. 39 'primary green' Cinemoid (Rank Strand Electric Ltd., London, England). Red irradiation (7.5 x 10^{14} Q.m^{-2}.s^{-1}) was provided by filtering the light from four 15-w cool-white fluorescent tubes through a double layer of No. 15 'ruby' Cinemoid. Far red light (1.1 x 10^{14} Q.m^{-2}.s^{-1}) was obtained by filtering the light of four 75-w incandescent bulbs through 10 cm water and one layer each of No. 5 'orange' and No. 20 'deep blue' Cinemoid. All light treatments were for 5 min.

RESULTS

Preliminary Experiments. Seeds of three different lettuce cultivars were imbibed at temperatures ranging from 20 to 35 C either in light or darkness (Table I). At 20 C, the seeds of cvs. Grand Rapids (II) and New York were light-sensitive as compared to cvs. Grand Rapids (I) and Great Lakes that germinated fully in both light and dark. In all seed lots, as temperatures increased, dark germination levels declined more rapidly than germination in response to R. Thus at 30 C, R promoted germination even in the so-called light insensitive cultivar, Great Lakes. Similar results have been obtained by other workers (29).

In another experiment, the effects of high temperature incubation (35 C) on the germination of cv. Grand Rapids (I) in the dark, or after exposure to R, were examined (Figs. 1 A and B). High temperature incubations progressively suppressed subsequent dark germination at all temperatures ranging from 15 to 26 C. Germination in response to R was also severely suppressed at 24 and 26 C. Both the GT50 Dark and the GT50 Light declined during high temperature incubation. The seeds became photodormant at 15 and 20 C while simultaneously becoming unresponsive to R at 24 and 26 C. These results demonstrate the importance of conducting germination studies over a wide range of temperatures. In the earlier reports, when after exposure to high temperatures the subsequent germination was monitored only at one temperature, high temperature incubations of less than 24 h were often found to have no effect on the germination of Grand Rapids seeds, in response to R (5, 35). The dangers of monitoring germination at a single or a few arbitrarily chosen temperatures have been stressed by Reynolds (20). In all further experiments germination was determined at eight temperatures ranging from 15 to 32 C.

Response of Different Cultivars to High Temperature Incubation. The effects of high temperature incubations on the GT50 Light of three lettuce cultivars are shown in Figure 2. Prolonged incubations had little effect on the GT50 Light of cv. Great Lakes. In contrast, both cv. New York and cv. Grand Rapids showed considerable declines in their GT50 Light, particularly within the first 0.5 days of incubation. Two days of incubation reduced the GT50 Light of cv. New York to approximately 25 C; longer incubations had little further effect on the GT50 Light. In cv. Grand Rapids (I), incubations longer than 1 day reduced germination at all temperatures and eventually led to the total suppression of germination. After 8 days of high temperature incubation, GT50 Light in cv. New York was 24.5 C as compared to 28.5 C in cv. Great Lakes. In cv. Grand Rapids (I), germination declined to below 20% after 8 days of high temperature incubation. Repeated or prolonged exposures to R had no additional effect on germination in any cultivar. Prolonged high temperature incubations

Table 1. The Influence of Light and Temperature on the Germination of Several Varieties of Lettuce

<table>
<thead>
<tr>
<th>Variety</th>
<th>Light Conditions</th>
<th>20</th>
<th>26</th>
<th>30</th>
<th>32</th>
<th>35</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Rapids (I)</td>
<td>R</td>
<td>100</td>
<td>100</td>
<td>85</td>
<td>25</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>100</td>
<td>68</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grand Rapids (II)</td>
<td>R</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Great Lakes</td>
<td>R</td>
<td>100</td>
<td>100</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>R</td>
<td>94</td>
<td>97</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>60</td>
<td>38</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
</tbody>
</table>
also affected the time course of germination. Eight days of high temperature incubation delayed emergence by up to 24 h in all three cultivars.

Effect of Incubation Temperature on Dormancy Induction. The effect of incubation temperature on the rate and pattern of dormancy induction in cv. Grand Rapids (II) is shown in Figure 3. Dark germination was less than 5% at all incubation temperatures. The decline in GT50 Light was temperature dependent. Twelve h at 32 C reduced the GT50 Light to approximately 25 C. Two days at 24 C and 4 days at 15 C caused a similar decline in the GT50 Light. Dormancy induction appeared to occur in two stages at all three incubation temperatures. Initially, germination was reduced only in the vicinity of the GT50 Light; longer incubations eventually suppressed germination at all temperatures.

The complete suppression of germination occurred more rapidly in lot II (Fig. 3 B) than in lot I of cv. Grand Rapids (Fig. 2 C). This was true even when lot II was incubated at much lower temperatures (24 C, as compared to 35 C for lot I). Within the same seedlot the rate of dormancy induction was temperature dependent, being more rapid at higher incubation temperatures.

Role of Light and Growth Regulators. Secondary dormancy can usually be broken by a combination of R plus growth regulators such as KIN, ethylene or GA3 (7, 27, 28, 34, 35). In our own experiments, a higher level of germination could be restored in secondarily dormant seeds by R + KIN than by R + GA3 (Fig. 4). In the absence of R, neither GA3 or KIN had any promotive effect on germination (data not shown).

In all the experiments discussed above, secondary dormancy was induced by dark incubation. To determine the effect of light on the induction of secondary dormancy, seeds were exposed to R during the course of a high temperature incubation (Fig. 5). Compared to the dark incubation control, both initial R and repeated R treatments slowed the decline in the GT50 Light. Prolonger high temperature incubations eventually suppressed germination at all temperatures in seeds receiving a single exposure to R (initial R) but not in seeds receiving repeated exposures to R (repeated R). Previous reports (e.g. 33) and data presented in Figure 6 indicate that the promotive effects of a single exposure to R are completely lost within 48 h at high temperatures, presumably through the thermal reversion and/or destruction of Pfr. To determine if the escape reactions of the phytochrome system were involved in prevention of the second stage of dormancy induction, seeds receiving repeated R were exposed to FR instead of R at the end of the high temperature incubation. Zero germination oc-

Fig. 2. Effects of high temperature incubation on the GT50 Light of cv. Great Lakes, New York, and Grand Rapids (I). Seeds were incubated at 32 C (Great Lakes, New York) or 35 C (Grand Rapids [II]) for periods of 0.5 to 8 days, exposed to R and transferred to the indicated temperatures. Untreated seeds (Od) were imbibed 1 h at 24 C before R irradiation and then transferred to germination temperatures. Each point represents the mean of 4 samples. The treatments can be summarized as follows: Preincubation (0 to 8 days) at 32 or 35 C → R (5 min) → Incubation at 15 to 35 C.

Fig. 3. Effects of dark incubation at 32, 24, or 15 C on the GT50 Light of cv. Grand Rapids (II). The procedure was the same as in Figure 2 except dark incubations were at 32, 24, and 15 C. For untreated seeds (Od) each point represents the mean of 10 samples. All other points represent the mean of 4 samples. The treatments can be summarized as follows: Preincubation (0-11 days) at 32, 24 or 15 C → R (5 min) → Incubation at 15 to 32 C.
SECONDARY DORMANCY INDUCTION IN LETTUCE

Fig. 4. Germination of secondarily dormant seeds in response to KIN or GA₃. Seeds of cv. Grand Rapids (II) were incubated for 4 days at 32°C and transferred to fresh Petri dishes containing distilled H₂O, 100 μg/ml GA₃, or 10 μg/ml KIN. All samples were then exposed to R and transferred to the indicated temperatures. Each point represents the mean of 4 samples. The treatments can be summarized as follows: Preincubation (4 days) at 32°C → H₂O, GA₃ or KIN → R (5 min) → Incubation at 15 to 30°C.

Secondary dormancy is known to occur in a number of light sensitive species, for example, Chenopodium album L., Rumex crispus L. and L. sativa L. (3, 15, 32). In lettuce, secondary dormancy has been reported in cv. Grand Rapids, and several European cultivars. Some cultivars appear to be immune to the induction of secondary dormancy (32). From the present study it is clear that if the term secondary dormancy is reserved for those seeds that become unable to germinate at any temperature in response to R, then only cv. Grand Rapids became secondarily dormant. High temperature incubations reduced the GT₅₀ Light of cv. New York, however.

The difference in germination behavior between Grand Rapids and Great Lakes ‘type’ cultivars is thought to be quantitative, rather than qualitative, with respect to temperature (8). The fact that secondary dormancy can be induced in some cultivars but not in others suggests that there may be qualitative differences among the germination mechanisms of these cultivars. One possible difference that deserves further study relates to the effects of supraoptimal temperatures on the phytochrome system. Our results and earlier studies (13, 25) indicate that the escape reactions of the phytochrome system do not proceed to completion in seeds of cv. Grand Rapids held at high temperatures (above 30°C). In contrast, Negm et al. (19) clearly showed that escape from FR reversibility does occur at 35°C in cv. Great Lakes (Mesa 629). The reason for these conflicting results is not readily apparent.

The induction of secondary dormancy in cv. Grand Rapids appeared to occur in two stages, i.e. a decline in the GT₅₀ Light, followed by the suppression of germination at all temperatures. Exposing seeds to R or GA₃ during the course of a high temperature incubation prevented the second stage of dormancy induction, but did not prevent a decline in the GT₅₀ Light. This fact, combined with the observation that high temperature incubation reduced the GT₅₀ Light of cv. New York without inducing secondary dormancy, suggests that separate processes may control the two stages of dormancy induction. The present data do not rule out the possibility that these two stages of dormancy induction are the result of the same or similar processes carried to different degrees of completion.

The two stages of dormancy induction resemble the ‘toxic’ and ‘inhibitory’ effects of germination inhibitors on lettuce seed, described by Reynolds (21, 22). Extremes of pH reduced germination at all temperatures, an effect described as toxic. Low concentrations of ABA suppressed germination by reducing the GT₅₀. This was termed ‘true’ inhibition. The suppression of germination by ABA involved both effects, with ABA being inhibitory at low
Great Lakes and Grand Rapids incubated at high temperatures. Vidaver and Hsiao (35) concluded that the induction of secondary dormancy in cv. Grand Rapids was independent of the phytochrome status of the seed. However, their study failed to take into account the rapid thermal reversion and/or destruction of Pfr that occurs in seeds held at high temperatures (33). The induction of secondary dormancy occurred more rapidly in lot I of Grand Rapids than in lot II. This probably relates to the fact that lot I exhibited a high level of dark germination at temperatures up to 26 C (Fig. 1), while dark germination levels were negligible in lot II at all temperatures above 15 C. Presumably, Pfr levels are higher in dark germinating seeds.

The induction of secondary dormancy appears to involve an effect on the phytochrome system itself. In lettuce cv. Grand Rapids (13), *R. crispus* L. (6, 30) and *C. album* L. (16), prolonged dark incubations decrease photosensitivity until all three species become unresponsive to R, or secondarily dormant. The induction of secondary dormancy in *R. crispus* did not affect phytochrome transformations, or appear to involve a decrease in total phytochrome. In lettuce, Pfr is present in secondarily dormant seeds exposed to R, but it cannot function to promote germination unless a growth regulator such as KIN is also present (27, 28). Duke et al. (6) concluded that the decline in photosensitivity and eventual imposition of secondary dormancy in *R. crispus* was related to decreased levels of 'X', the component with which Pfr interacts. If this is the case then the protective effect of R or GA3 could involve preventing the loss or disorganization of the X factor.

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SECONDARY DORMANCY INDUCTION IN LETTUCE

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