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A Thermo-labile Process in Dark Germination of New York Lettuce Seeds

By

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A thermo-labile process, that determines the level of dark germination of New York lettuce seeds, was separated from the other miscellaneous processes during germination. It began 2 hr after the start of imbibition, and completed several hr before the radicle protrusion at 15°C. The optimum temperature of the process was 15°C, and the activity of the process was zero at 28°C. Secondary dormancy may be induced by the reversible inactivation of the process at high temperature. It was suggested that the physiological role of the process is the generation of the growth potential of embryonic axes in order to overcome the restraint imposed by seed coats.

It is well known that photo-sensitive lettuce seeds would germinate at low temperature even under far-red light and not at all at high temperature even under red light (*3*). Some workers (*3*, *4*), attempted to investigate the promotive effect at low temperature separately from the inhibitory effect at high temperature. We proposed previously that the temperature effects on the germination of lettuce seeds, such as the effects at low and high temperature, would be explained only with a thermolabile factor other than the phytochrome system (*5*). Secondary dormancy is another effect of temperature on the germination. Many workers attempted to account for the mechanism of secondary dormancy, but could not clarify it (*1*, *2*, *8*, *9*, *10*, *11*). The following two facts suggest that secondary dormancy may be explained by the action of the thermo-labile factor: (1) The sensitivity curve to red light does not decline at 3° and 15°C, but declines slowly at 25°C and sharply at 35°C (*3*); (2) the product of the phytochrome system would be changed to the metabolite(s) essential for germination only through a thermo-labile reaction (*5*). A substantial nature of the thermo-labile factor has not been studied in detail, but we reported that the disappearance of small fat bodies during the early stage of imbibition (*6*) and the increase of the growth potential of embryonic axes necessary to overcome the restraining force imposed by seed coats (*7*) might be regulated by the thermo-labile factor.

The following observations were carried out to separate the process controlled by the thermo-labile factor from the other miscellaneous processes during the germination. Such observations are necessary to investigate in detail the role of the thermo-labile factor in germination and the relationship between the factor and secondary dormancy.

Seeds of lettuce, *Lactuca saliva* L. var. New York 515, were obtained from Takii seed Co., Kyoto in March 1974. Experiments were carried out in 1974. For the

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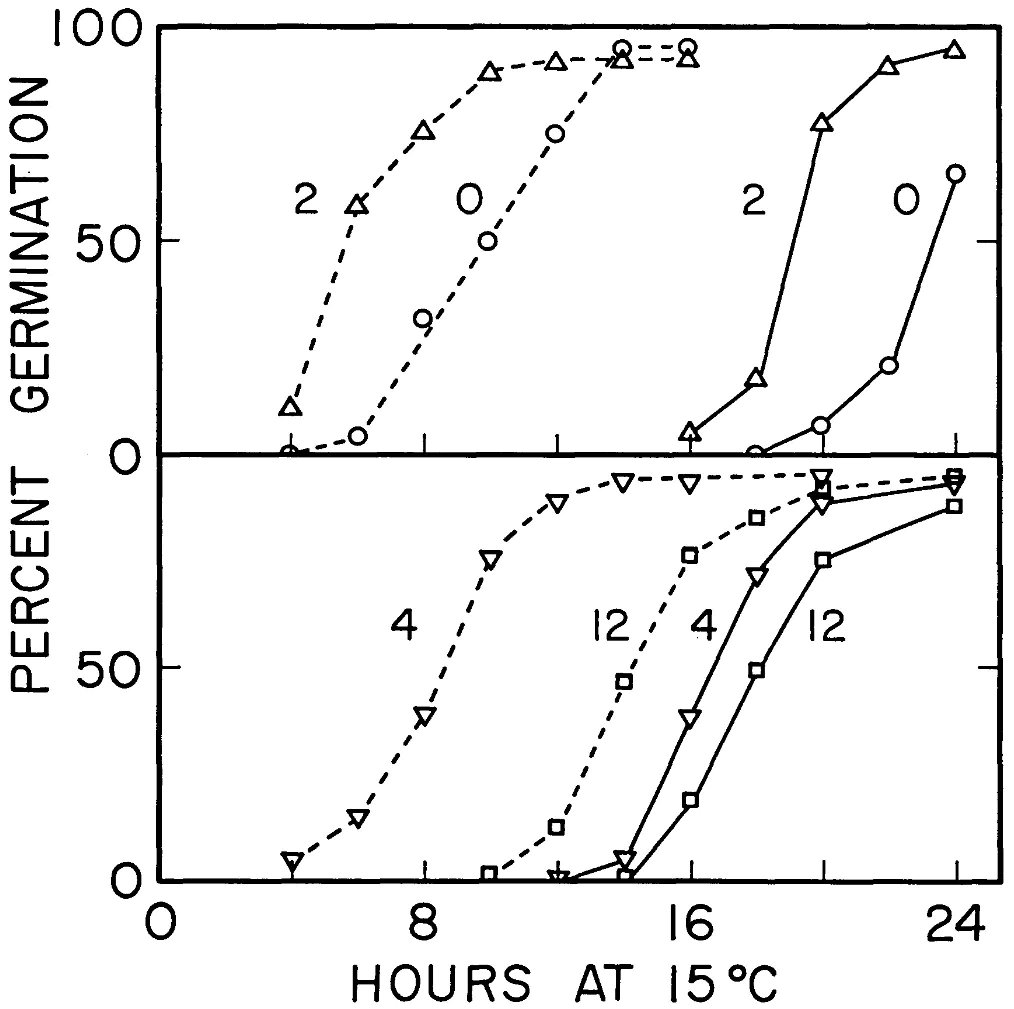

Fig. 1. Effect of preincubation at 30°C on the progress of the thermolabile process (dashed lines) and of the actual germination (solid lines). Numbers on curves indicate hours of the preincubation at 30°C.

germination tests, about a hundred seeds were placed in a 7-cm petri dish on a filter paper saturated with 3 ml of distilled water. The dishes were immediately placed in light-tight cardboard boxes in all experiments. The percentages of germinated seeds were counted 48 hr after the start of imbibition. Temperature was controlled within ± 1°C during germination tests.

A preliminary experiment showed that preincubation for the initial 2 hr at 30°C followed by incubation at 20°C promoted the germination, whereas prolonged preincubation at 30°C over 2 hr dealyed the germination. These results indicate that the first 2 hr of imbibition is not a thermo-labiie phase (Phase I). Phase I is followed by a thermo-labile phase (Phase II). It was reported previously that the level of dark germination was determined by the duration at low temperature (*5*). The progress of reaction(s) in Phase II, that determines the level of dark germination, therefore, can be estimated by plotting the germination level against the duration at low temperature (a escape curve from the thermo-labile process; dashed lines in Fig. 1). Escape from the thermo-labile process was completed within about 12 hr at 15°C in the case of 2-hr preincubation at 30°C (Fig. 1). The radicle protrusion, however, begins at 16th hr. About 13 hr intervened between the escape curve from the thermo-labile process (the dashed line) and the actual germination curve (the solid line) at 50%

level of germination (Fig. 1). This intervening duration (Phase III) is not thermo-labile, for the germination level shown on the dashed line was determined by incubating the seeds at 30°C.

Fig. 1 shows that prolonged preincubation at 30°C delayed the recovery of the thermo-labile process at 15°C and shortened the duration of Phase III. Actual germination was not delayed by the preincubation for 12 hr, but it was already shown that prolonged preincubation beyond 24 hr at high temperature dealyed not only the recovery of the thermo-labile process but also the actual germination at low temperature (*5*). These results indicate that secondary dormancy is induced by the reversible inactivation of the thermolabile process at high temperature. The shortening of the duration of Phase III by the preincubation at 30°C indicates that the reaction(s) in Phase III can proceed before the end of the thermo-labile process (Phase II).

Effects of temperature on Phase I, II, and III were determined in the next experiments. Seeds were imbibed at various temperatures (10° to 40°C) for the first 2 hr, and transferred to 15°C. All of the seeds initiated to germinate from 18th to 20th hr after the start of imbibition, but the initial rates of germination were found to be affected by the temperature treatments for the first 2 hr. Therefore, the increased germination rates (%) from 20th to 22th hr were divided by 2 (=22–20) hr, and the values were plotted against temperatures (Curve I in Fig. 2). The optimum temperature of Phase I was about 30°C. It is noticeable that Phase I is active even at 40°C almost in the same order as 20°C.

Relative efficiencies of Phase II at various temperatures were determined as follows: seeds were imbibed at 30°C for 2 hr and transferred to various temperatures (10° to 30°C) for various hours (2 to 32 hr) followed by incubation at 30°C, and the escape curves from the thermo-labile process were determined as mentioned above. The durations from 2th hr to 50% level of germination were affected apparently by the temperature treatments (10° to 30°C) and therefore the reciprocal hours of the durations were plotted against temperatures (Curve II in Fig. 2). The escape curve at 26°C stopped at about 30% level of germination, and that at 28°C was 0% for 32 hr. The optimum temperature of Phase II was 15°C, and the activity of Phase II at 28°C was zero.

Effect of temperature on Phase III was determined as follows: seeds were imbibed at 30°C for 2 hr and transferred to 15°C for 6 hr followed by the incubation at various temperatures (10° to 40°C). The germinated seeds were counted at definite intervals, and the duration of Phase III was estimated by determining the duration from 6th hr after the transfer to 15°C to actual germination of 50 percent. The germination level of the seeds treated at 35°C stopped at about 15%, and that at 40°C was zero for 48 hr after the start of imbibition. Relative rate of progress in Phase III may be shown by the reciprocal hours of the duration at various temperatures. The results were shown in Fig. 2 (Curve III). The optimum temperature of Phase III was 25°C. The effectiveness of Phase III at 40°C was zero.

Ikuma and Thimann (*3*) analyzed the germination process in Grand Rapids by changing the conditions during imbibition, such as temperature, light, and O_2 atmosphere, and reported that the germination comprises 4 phases, i.e. preinduction, induction, postinduction phase, and phase of visible germination. The induction phase was designated as a phase in which the reversible reaction of the phytochrome functions

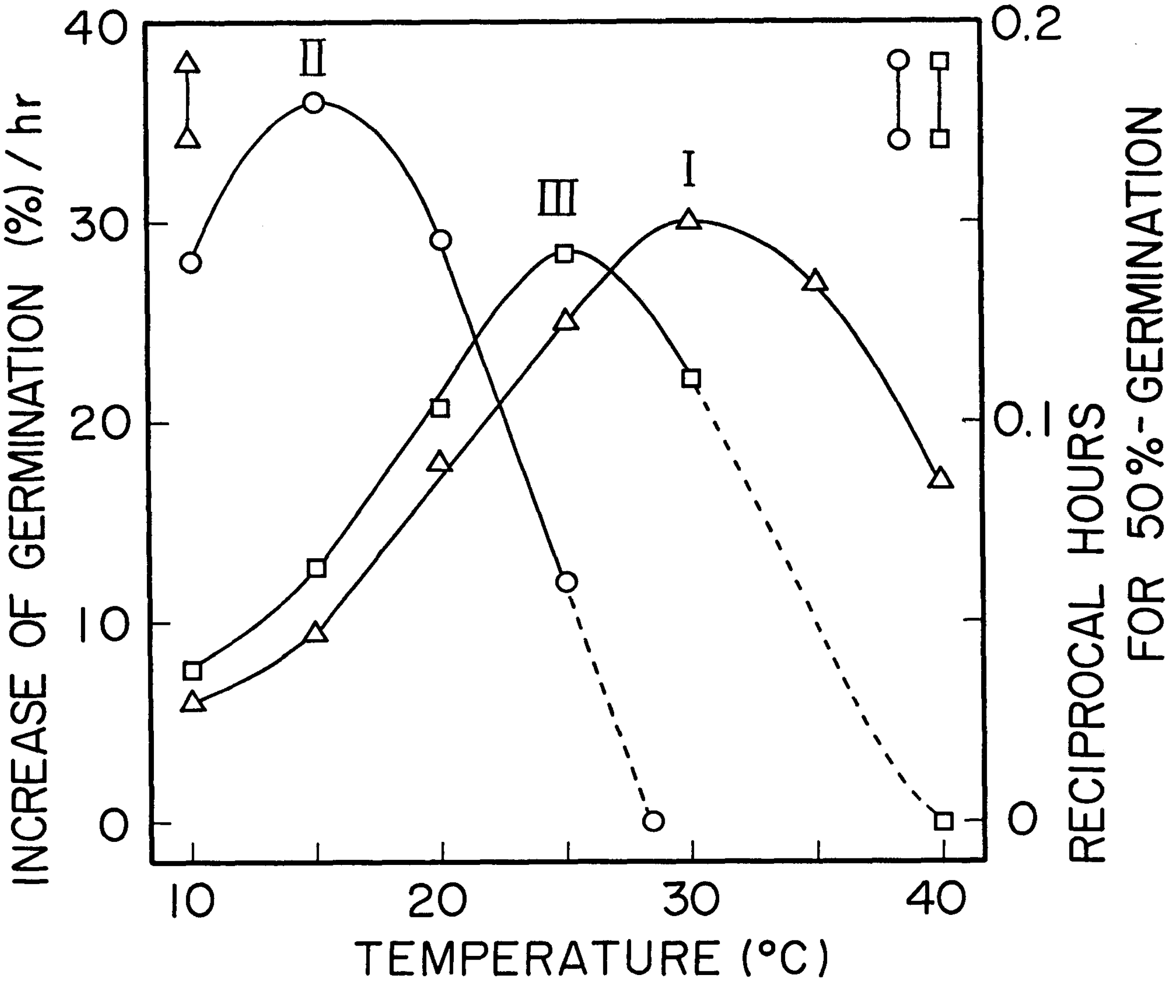

Fig. 2. Effect of temperature on Phases I, II, and III in the dark germination of lettuce seeds. See text for the estimation method of each curve.

maximally. Since the maximum effect of red light was observed during 1.5 to 2 hr after the start of imbibition, Phase I in the present paper will correspond roughly to the preinduction and the induction phase. The postinduction phase, therefore, corresponds to Phases II and III in the present paper.

We reported recently that the growth potential of embryonic axes of New York lettuce was 0.24 M at 35°C and 0.54 M at 20°C in the dark, and that the restraining force of the seed coats was equivalent to 0.4 M mannitol (*7*). It may be interpreted that the generation of the growth potential in embryonic axes at 20°C consists of two compotents; a thermo-stable one (0.24 M) and a thermo-labile one (0.3=0.54–0.24 M). Since both the thermo-labile component of the growth potential and the thermo-labile prcoess (Phase II) are the essential step to germination, it may be suggested that the role of Phase II in germination is the generation of the growth potential over the level of 0.24 M. If that is a case, Phase III would correspond to the other miscellaneous processes containing the thermo-stable increase of the growth potential leading to elongation of embryonic axes within the seeds coats. It is reasonably accepted that the elongation of embryonic axes (i.e. Phase III) can proceed during the prolonged preincubation at 30°C (see Fig. 1).

The sequence of Phase I, II, and III may be summarized as in Fig. 3. This diagram indicates the following results obtained above. (1) A thermo-labile phase (Phase

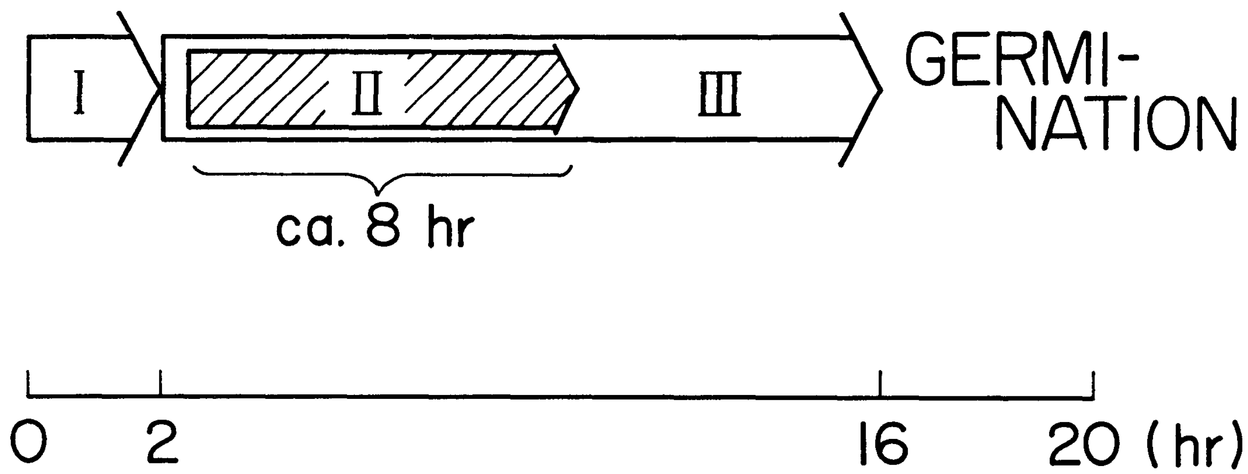

Fig. 3. Three phases characterized with different optimum temperatures shown in Fig. 2. Phase I is a thermo-stable phase for the first 2 hr of imibibition. Phase II is a thermo-labile process that determines the level of dark germination of lettuce seeds. Phase III is the other miscellaneous processes leading to elongation of embryonic axes within the seed coats. Time scale is given at optimum temperature of each phase.

II) that determines the level of dark germination follows a thermo-stable phase (Phase I) for the first 2 hr of imbibition. (2) The other miscellaneous processes leading to elongation of radicles within the seed coats is Phase III. A part of the processes in Phase III would proceed before the end of Phase II, but Phases I and III alone can not generate the growth potential enough to rupture the seed coats.

In the present report, the thermo-labile process could be separated to some extent from the other miscellaneous processes during dark germination. The shapes of Curves I, II and III in Fig. 2 are very similar, whereas their optimum temperatures are different. It will be a most simple explanation that the mechanism of the decrease of activity in Phase II at the higher side of temperature is the same as those in Phases I and III. That is a reason why we are investigating the thermo-labile factor as the regulating element of Phase II.

References

1) Berrie, A.M.M.: The effect of temperature and light on the germination of lettuce seeds. *Physiol. Plant.* 19: 429–436 (1966).
2) Borthwick, H.A. and W.W. Robbins: Lettuce seed and its germination. *Hilgardia* 3: 275–301 (1928).
3) Ikuma, H. and K.V. Thimann: Analysis of germination processes of lettuce seed by means of temperature and anaerobiosis. *Plant Physiol.* 39: 756–767 (1964).
4) Scheibe, J. and A. Lang: Lettuce seed germination: evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temperature effect. ibid. 40: 485–492 (1965).
5) Takeba, G. and S. Matsubara: Analysis of temperature effect on the germination of New York lettuce seeds. *Plant & Cell Physiol.* 17: 91–101 (1976).
6) Takeba, G. and S. Matsubara: Rapid disappearance of small fat bodies during the early stage of imbibition of lettuce seeds. ibid. 18: 1067–1075 (1977).
7) Takeba, G. and S. Matsubara: Measurement of growth potential of the embryo of New York lettuce seed with mannitol concentration in various combinations of temperature, red light, and hormones ihid. 20: in press (1979).
8) Van Staden, J.: Changes in endogenous cytoskinins of lettuce seed during germination. *Physiol. Plant.* 28: 222–227 (1973).

9) Vidaver, W. and A.I. Hisao: Secondary dormancy in light-sensitive lettuce seeds incubated anaerobically or at elevated temperature. *Can. J. Bot.* .53: 2557–2560 (1975).

10) Villiers, T.A.: Seed aging: chromosome stability and extended viability of seeds stored fully imbided. *Plant Physiol.* 53: 875–878 (1974).

11) Wareing, P.F. and P.F. Saunders: Hormones and dormancy. *Ann. Rev. Plant Physiol.* 22 : 261–288 (1971).