Physiological Analysis of Growth Retardation of Rice Seedlings Caused by Low Temperature

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Crops of tropical and subtropical origin are known to suffer growth damages, so-called chilling injury, under the temperature above 0°C. The critical temperature is reported to be 12–10°C.[5] The rice plant is not exceptional, but it suffers diversified damages such as low temperature damage in the nursery stage,[2] growth retardation of seedlings by low temperature after transplanting,[3] sterility caused by low temperature at the panicle formation stage,[7] and poor ripening due to low temperature after heading. The temperature range causing these damages, except the seedling damage in the nursery stage, is generally 20–15°C, which is higher than the critical temperature for the chilling injury.

In view of the fact that these damages differ each other in external appearance, but have the critical temperature range almost in common, it would be possible to find out a mechanism which is basically common, if not completely, by examining the mode of occurrence of these damages at a cell level or at a level of specific metabolic system. Therefore, the present study attempted to examine the mechanism of growth retardation of transplanted seedlings due to low temperature and also to reveal the physiological mechanism of low temperature damages in growth and related metabolic systems.

Growth retardation of transplanted seedlings due to low temperature as affected by water stress

In cool areas of Japan, initial growth of rice crop is often retarded by low temperature. Particularly, the retardation of rooting and growth of transplanted seedlings is a serious problem because it exerts adverse effects on later growth. Regarding the growth of seedlings, many studies have so far been done, and the importance of physiological activity related to energy metabolism has been pointed out.[9] On the other hand, the importance of morphological characteristics of seedlings at the time of transplanting is emphasized in the actual practice of rice cultivation.[4] Consequently, it is presumed that water stress might participate in the growth of seedlings, as a factor other than low temperature. Therefore, effect of water stress under a low temperature condition was examined in relation to morphological characteristics of seedlings.

As shown in Table 1, spindly seedlings, which had been grown lankily, were very poor in rooting and growth under a low temperature, 15°C, after transplanting. Water stress given to the seedlings by exposing them to wind accelerated the adverse effect of the low temperature, giving no rooting at all. Water stress caused by placing seedlings in hypertonic aqueous solution of polyethyleneglycol also accelerated the effect of low temperature on rooting (Fig. 1). These results suggest that low temperature and water stress exert an inhibitive effect on seedling growth by a multiplier action. To promote growth of transplanted seedlings under low temperature, water stress must be avoided as far as possible.

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Table 1. Comparison of growth between vigorous and spindly rice seedlings after transplanting

Seedlings	Increase of dry weight (mg/10 seedlings)			Number of newly appeared roots per seedling			Length of the longest root newly appeared (cm)		
	23°C	15°C	15°C[3)]	23°C	15°C	15°C[3)]	23°C	15°C	15°C[3)]
Vigorous[1)]	193	145	111	9.8	3.0	0.7	8.6	1.7	0.4
Spindly grown[2)]	113	62	26	8.4	0.5	0	7.1	0.1	—

Note: Determinations were done 7 days after transplanting. Number of newly appeared roots and length of the longest root newly appeared were the average of 10 seedlings.
1)..... Seedlings grown outdoors in May (leaf age; 3.5, plant height; 11.9 cm, shoot dry weight; 15.5 mg).
2)..... Seedlings subjected for 6 days to high temperature (27/20°C day-night temperature) and low light intensity (shading by covering with acryl case) prior to transplanting (leaf age; 3.7, plant height; 12.6 cm, shoot dry weight; 11.9 mg).
3)..... Transplanted seedlings were exposed to wind generated by a fan in the daytime.

Effect of low temperature on growth and physiological activities

Effect of temperature on growth of plumules and radicles of germinated seeds was examined. As given in Fig. 2 and 3, the Arrhenius curves for plumules and radicles declined gradually with the lowering of temperature from 30° to 18°C and then the curves began to fall sharply at the temperature 18°C, and again began to drop further at 12°C. Thus, each curve showed two discontinuities, at 18°C and 12°C.

To make clear the cause of these two discontinuities, respiratory rate, which is closely related to growth of seedlings, was measured. As shown in Fig. 4, the Arrhenius curve of respiration of germinated seeds declined almost linearly with the lowering of temperature from 30° to 9°C. Activity of cytochrome c oxidase, an enzyme playing an important role in respiration, also showed a linear decline in the same temperature range as above, showing no discontinuity as observed in growth (Fig. 5). In addition, content of ATP (adenosine triphosphate), a high-energy substance produced by respiration, was found almost constant, irrespective of temperature, within the temperature range from 25° to 6°C (Fig. 6). From these results, it is difficult to consider that the growth retardation due to low temperature is directly caused by the lowering of respiratory activity under low temperature. Some other factors may be involved.

Abnormality occurs in nitrogen metabolic system of rice plants treated by low temperature, 18°–12°C. Namely, the content of soluble nitrogen such as free amino acids, amides, etc., in leaf blade is increased markedly. In the fractionation pattern of Sephadex column the relative proportion of high-molecule protein fraction is decreased while that of low-molecule fraction is increased[1)]. Decrease in activity of protein synthesis is considered a cause for such abnormal pattern in nitrogen metabolism occurring under low temperature.

Therefore, effect of temperature on ^{14}C-leucine incorporation into protein fraction of seedlings was examined. As shown in Fig. 7, the amount of incorporation was almost similar at any temperature within the temperature range from 26° to 18°C, whereas it decreased markedly with the lowering of temperature below 18°C. The similar result was also obtained for the incorporation of ^{15}N into protein fraction by the experiment using ^{15}N-ammonium sulfate (Fig. 8). The temperature, 18°C, at which the activity of protein synthesis began to decrease, coincides with the discontinuity of growth rate of plumules and radicles shown in Fig. 2 and 3.

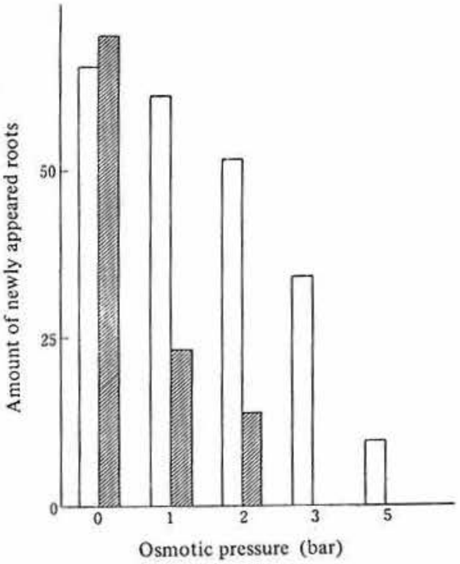


Fig. 1. Effect of osmotic pressure of medium solution on rooting of rice seedlings

Note: Roots were decapitated at 0.5 cm from their base, and grown in the hypertonic aqueous solution of polyethyleneglycol 300 at 25°C (□) and 15°C (■) for 5 days and 15 days respectively. Amount of newly appeared roots = (number of roots) × (length of the longest root).

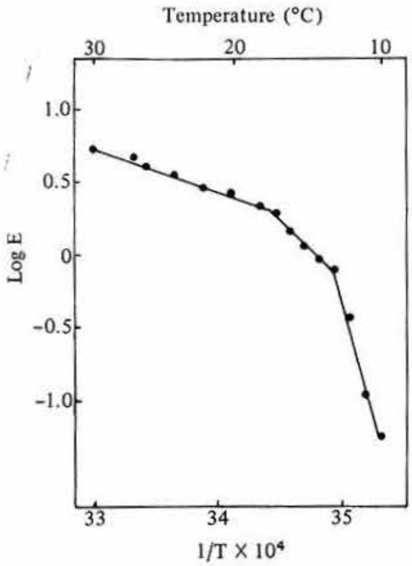


Fig. 2. Arrhenius plot of elongation rate of plumule

Note: E: Elongation rate of plumule (mm/day).

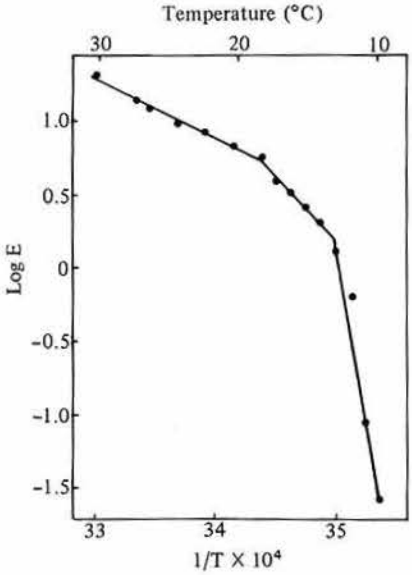


Fig. 3. Arrhenius plot of elongation rate of radicle

Note: E: Elongation rate of radicle (mm/day).

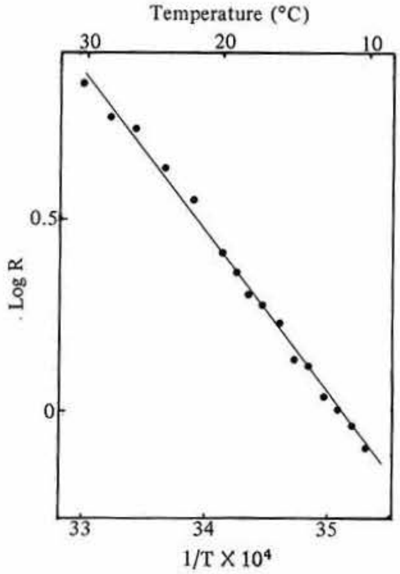


Fig. 4. Arrhenius plot of respiration rate of rice seedlings

Note: R: Respiration rate (O^2 uptake ml/hr/DW in g).

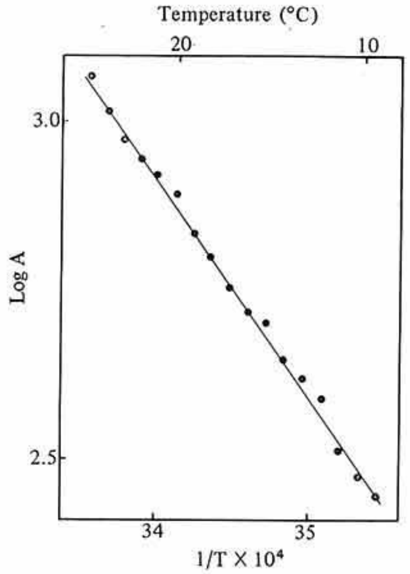


Fig. 5. Arrhenius plot of activity of cytochrome c oxidase from radicle of rice seedlings

Note: A: Activity of cytochrome c oxidase (O_2 uptake nmole/hr/100 μl of enzyme solution).

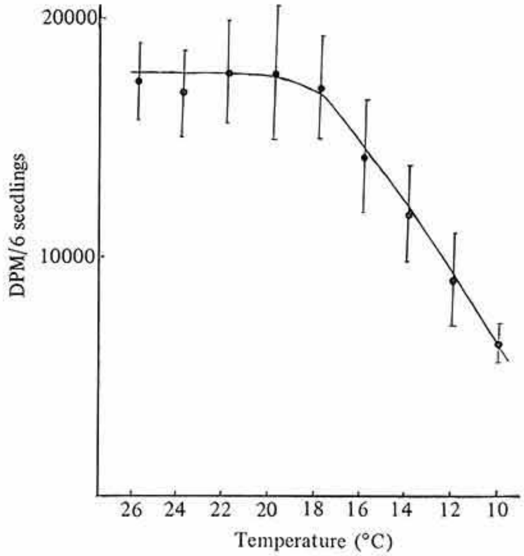


Fig. 7. Effect of temperature on ^{14}C-leucine incorporation into protein fraction of rice seedlings

Note: DPM = counts of ^{14}C-leucine incorporated into protein fraction per min.

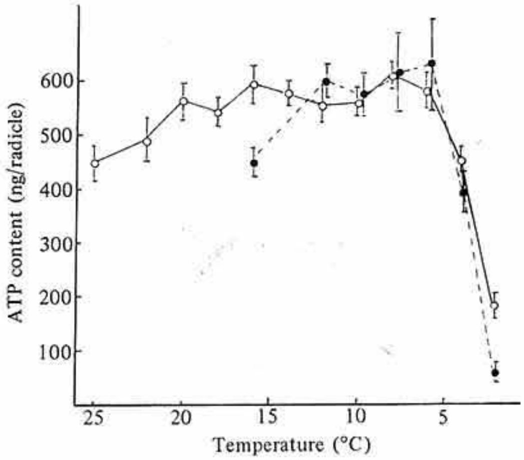
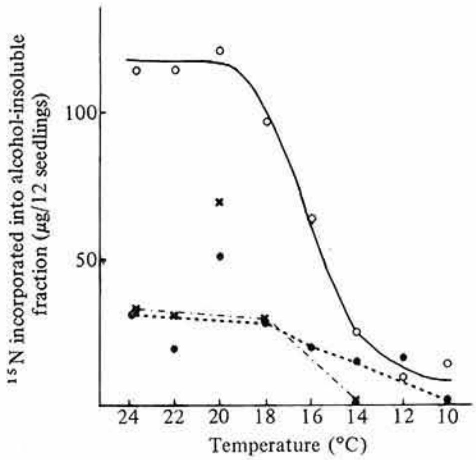


Fig. 6. Effect of temperature on ATP content in radicle of rice seedlings

Note: Solid line and broken one indicate temperature treatment for 24 hr and 120 hr, respectively.



Note: Seedlings were divided into new leaf (6th ○—○), old leaves (below 5th leaf ●---●) and roots (×-·-×).

Fig. 8. Effect of temperature on ^{15}N incorporation into protein fraction of rice seedlings of 6-leaf age.

This fact seems to offer a clue to clarify physiological mechanism of growth retardation under low temperature.

Discussion

Growth retardation of rice plants under low temperature is basically caused by inhibition of physiological activities due to low temperature, although there is a case where water stress is involved as shown for growth of transplanted seedlings. To reveal this mechanism, it is regarded desirable to investigate the cause of the occurrence of the two discontinuities at 18° and 12°C on the Arrhenius curves of plumule and radicle growth.

The temperature, 12°C, coincides with the critical temperature at which crops of tropical and subtropical origin suffer chilling injury. As to the mechanism of chilling injury occurrence, it is widely[6] accepted that transition of lipids from liquid phase to solid phase or decrease in fluidity is the cause, and that they depend upon the ratio of unsaturated fatty acids/saturated fatty acids. Like other crops of tropical origin, which are liable to suffer chilling injury, rice plants are known to have a low ratio of unsaturated/saturated fatty acids[8]. It seems possible, therefore, that the chilling injury is involved in the occurrence of discontinuity at 12°C.

On the contrary, it is interesting that the temperature for the other discontinuity, 18°C, coincides with the critical temperature for various low temperature damages including cool weather damage of panicle sterility type. The mechanism for the discontinuity at 18°C, however, seems to be different from that for chilling injury. In this respect, the present study revealed that incorporation of ^{14}C of amino acid or ^{15}N of ammonium sulfate into protein was inhibited at about 18°C, due to decreased activity of protein synthesis. From this result, it is supposed that the inhibition of protein synthesis begins almost coincidentally with the incipient metabolic abnormality inducing growth retardation at 18°C.

However, as the mechanism of protein synthesis is complicated, involving nucleic acid metabolism, more detailed investigation will be needed.

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