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High Temperature - Induced Changes in Germination, Seedling Vigour and the Metabolic Activities in Rice Seeds

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Abstract. The effects of temperature treatments on rice seeds in the early imbibitional phase were studied with respect to changes in germination percentage, seedling growth in terms of root and shoot lengths, water uptake, respiration and hydrolytic enzymes, *viz.* α-amylase, adenosine triphosphatase (ATPase) and phytase. As compared with the control, the treatment for 30 min at 50 °C caused a variable degree of increase in all these characters and a positive correlation existed between seedling vigour and hydrolase activities. The treatment for 30 min at 60 °C, on the other hand, elicited a retarding influence on these characters. As regards the enzyme activities, the damaging effect of 60 °C could, however, be visualized only after 72 h of germination which was preceded by an enhancement during the early hours.

Previous reports indicated that conditions during the early stages of germination could predetermine the subsequent growth of plants (WOODSTOCK and POLLOCK 1965, HIGHKIN and LANG 1966). Striking correlations were noticed between respiration rates in the early imbibitional phase and the subsequent rates of seedling growth. Measurements of the rates of respiration were used to predict and forecast later seedling growth and to estimate the extent of damage by heat or cold in the early imbibitional phase (WOODSTOCK 1965, WOODSTOCK and FEELEY 1965). Although seedling or cotyledon respiration may serve as an index of the overall metabolic activity, respiratory mechanism cannot be regarded as the only spot of injury.

In a study on the chemical basis of temperature responses in plants, KETELLAPPER and BONNER (1961) observed a partial prevention of injury caused by unfavourable temperature by the application of essential metabolites, which led them to suggest that environmental factors influence growth by affecting specific biochemical events. Although this citation does not pinpoint any particular aspect of metabolism, it is equally possible that the activity of different hydrolyzing enzymes of endosperm responsible for supplying the respiratory substrates to the embryo are likewise affected by

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the heat treatment. In order to test this possibility, the present investigation was undertaken with rice seeds by applying less severe heat treatments which might stimulate or reduce vigour without loss of viability.

Material and Methods

Pure line rice seeds *Oryza sativa* L. (cv. Bhasamanik), after surface sterilization with 0.1% mercuric chloride were subjected to 50 °C and 60 °C for 30 min in the early imbibitional phase. Seeds of the treatment and the control sets were imbibed with water in a submerged condition for a total period of 3 h. At the start of the experiment, they were placed in water, the temperature of which was raised quickly to 50 °C and 60 °C and maintained for the initial 30 min of imbibition. Following such heat treatments, they were shifted to water at a normal room temperature of 30 °C and kept there for the remaining 2½ h of imbibition. In contrast to this, the seeds kept immersed for 3 h constantly at 30 °C were designated as control. They were then spread over moist filter paper in Petri dishes and allowed to germinate in a dark humid atmosphere at 30 °C for the rest of the experimental period. The first sampling was made from 24 h imbibed seeds and subsequent samplings at intervals of 24 h up to 4 days.

Respiration was measured from oxygen absorption at 30 °C following Warburg's manometric technique as described by UMBREIT *et al.* (1957).

Three enzymes, *viz.* α-amylase, adenosine triphosphatase (ATPase) and phytase, were assayed from the crude homogenate of the tissue extract. Extractions were made at 0 to 4 °C in a refrigerated centrifuge.

α-amylase was assayed according to the method described by BERNFELD (1955) and modified by DURE (1960). Phosphatase activities were measured according to YOUNG and VARNER (1959) and the phosphate released was determined following the method of Fiske and Subbarow (1925). Protein was determined by the Folin-phenol reagent (LOWRY *et al.* 1951).

Results and Discussion

Rice seeds were found to decline in germination and vigour during a period of one year after harvest in December. In order to ascertain the effects of temperature on germination, the present experiment was started in the following August when germination after 96 h was reduced to about 80 per cent.

TABLE 1

PERCENTAGE GERMINATION OF RICE SEEDS held at 50 °C and 60 °C for 30 min during initial imbibition period before transfer to 30 °C

Germination [h]	Temperature during initial imbibition period		
	Control 30 °C	50 °C	60 °C
48	64	75	36
72	75	82	50
96	79	88	65

The effect of temperature treatments in the early imbibitional period on the germination percentage is shown in Table 1. Germination was improved by 50 °C treatment and the difference from the control was evident only after 48 h when the sign of germination by the protrusion of embryo was first noticeable. Germination was largely depressed in samples at 60 °C and the effect was more severe in the early phase and less in the later phase. In this

treatment, there remained at the end of the experiment an approximately 15 per cent difference from the control which had evidently lost viability.

Although there was little significant change in water uptake by heat treatments, the differences could still be measured (Table 2). Starting from a 3 h soaking period, water uptake was increased by 50 °C treatment in all the

TABLE 2

PERCENTAGE WATER CONTENT OF RICE SEEDS germinated at 30 °C after exposure to 50 °C and 60 °C for 30 min during initial imbibition period

Germination [h]	Temperature during initial imbibition period		
	Control 30 °C	50 °C	60 °C
3	12	15	11
24	25	28	23
48	46	48	44
72	53	57	50
96	70	78	68

succeeding stages while there was inhibition at 60 °C. The root and shoot lengths presented in Table 3 indicate that 50 °C improved the seedling vigour from the beginning and the promotion of shoot growth was much more than that of

TABLE 3

SHOOT AND ROOT LENGTHS [cm] OF RICE germinated at 30 °C after exposure to 50 °C and 60 °C for 30 min during initial imbibition period

Germination [h]	Plant part	Temperature during initial imbibition period		
		Control 30 °C	50 °C	60 °C
48	Shoot	0.68	0.94	0.54
	Root	2.05	2.30	1.63
72	Shoot	1.21	1.70	1.04
	Root	3.15	3.91	2.91
96	Shoot	1.82	2.23	1.60
	Root	4.00	4.41	3.60

the root length. At 60 °C, both shoot and root lengths were inhibited more or less to the same extent and this inhibition remained throughout the experiment.

Respiration rates also followed the same trend of effects of temperature as on germination and seedling growth (Table 4). Coincident with temperature induced changes in shoot and root lengths, there was promotion in the rate of

O_2 consumption at 50 °C in all the stages of the test period. At 60 °C, on the other hand, respiration rates declined. Differences between treated and control sets were detected within 2 hours of imbibition but no visible sign of germination by protrusion of embryo was observed until after 48 h.

WOODSTOCK and JUSTICE (1967) reported that irradiation treatments of corn seeds inhibited seedling growth and respiration rates also and the treatment which stimulated seedling growth also stimulated the respiration rates. The injurious effect of high temperature in the early imbibitional period on the subsequent seedling vigour has been reported by WOODSTOCK and FEELEY (1965). Treatment with 45 °C was found by them to cause heat damage to a corn variety, while in the present experiment the increase in seedling vigour and respiration at 50 °C was evident. This is presumably due

TABLE 4

RESPIRATION RATES OF RICE germinated at 30 °C after exposure to 50 °C and 60 °C for 30 min during initial imbibition period. Respiration expressed as O_2 uptake per seedling [$\mu l\ h^{-1}$]

Germination [h]	Temperature during initial imbibition peiod		
	Control 30 °C	50 °C	60 °C
3	3.46	3.87	3.17
24	3.65	4.15	3.66
48	8.39	8.81	7.09
72	11.10	11.85	9.31
96	14.51	15.74	11.32

to the fact that the response to any treatment is a function of the dose times the duration through which it is applied. The minimum duration of heat treatment in their experiment was 2 h, much longer than the 30 min. employed in this experiment.

The temperature relation of the seedling growth can be visualized on the basis of the auxin content of the rice seeds during germination. The auxin concentration in the endosperm is supraoptimal for embryo growth, as the elimination of a fraction of the endosperm results in optimal growth until it reaches a limiting value (SIRCAR *et al.* 1955). In the present experiment, the auxin content might have been inactivated by the heat treatment of 50 °C below that of the control to a level which encourages enhanced embryo growth. 60 °C treatment presumably lowered the auxin content below the optimal level to depress the seedling growth.

The increase in seedling vigour at 50 °C is accompanied by an increase in the α-amylase, ATPase and phytase activities (Fig. 1). The promotive effect became evident at 24 h and the differences from the control were fairly wide up to 72 h. At 96 h, however, the residual effects of temperature treatment became less pronounced and the enzyme activities almost equalled those of the control.

It is of interest to note that at the higher temperature of 60 °C, enzyme activities showed a dual response. Up to 48 h, there was an enhancement over

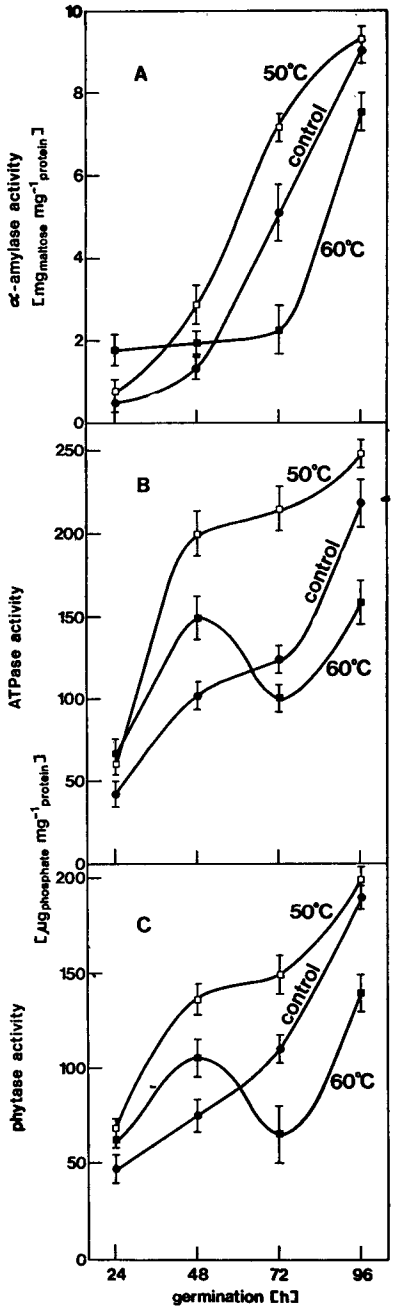

Fig. 1. Effect of high temperature during initial inbibition period on α-amylase activity (maltose released per mg protein), on ATPase activity (phosphate released per mg protein), on phytase activity (phosphate released per mg protein) of rice seeds during germination.

the control and the deleterious effect was measurable only after 72 h. The apparent disparity between enhanced hydrolase activity and depressed growth rates during the first 72 h of germination suggests that there may be other sites of temperature injury. Although the present data do not lead to any definite conclusion, it is possible that the respiratory machinery is liable to be damaged because a close parallelism exists between respiration and growth rates. WOODSTOCK and GRABE (1967) suggested that the growth effects of the temperature treatments are presumably mediated through an impaired mitochondrial activity.

MARCUS *et al.* (1966) found that the rapid activation of the protein synthesizing capacity of ribosomes occurs during the initial 20 min. imbibition and concluded that this activation may be an energyrequiring process. In the present study, temperature treatments were made during the first 30 min. of imbibition and it is suggestive that the protein synthesizing capacity is either stimulated or inhibited depending on the intensity of treatments with its concomitant after-effects.

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JAYANTI MUKHERJEE, S. MUKHERJI a S. M. SIRCAR, Botanické oddělení University, Calcutta, Indie: **Změny indukované vysokou teplotou v klíčivosti, energii klíčení a metabolické aktivitě semen rýže.** — Biol. Plant. **15** : 65—71, 1973.

Byl sledován vliv teploty na semena rýže v počáteční fázi botnání: na klíčivost, růst klíčních rostlin, příjem vody, dýchání a na aktivitu hydrolytických enzymů, tj. α-amylázy, adenosintrifosfatázy (ATPázy) a fytázy.