Effect of High Temperature Pre-treatment on Elongation of the Mesocotyl of Rice Seedlings : III. Seed water content and mesocotyl elongation

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https://doi.org/10.5109/22836

出版情報：九州大学大学院農学研究院紀要. 17 (3/4), pp.275-281, 1973-10. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 17, 275-281 (1973)

Effect of High Temperature Pre-treatment on Elongation of the Mesocotyl of Rice Seedlings

III. Seed water content and mesocotyl elongation

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(Received April 18, 1973)

The stimulatory effect of a 40°C treatment of seeds on mesocotyl elongation increased with the increased water content of the seeds after immersion and a maximum mesocotyl length was obtained at a seed water content of about 27%, regardless of the temperature during immersion of the seeds. When seeds were dried after immersion, little stimulatory effect of the 40°C treatment on mesocotyl elongation was observed. In the plants grown from seeds which were treated at 40°C in an oxygen free atmosphere of nitrogen gas, only a slight stimulation of mesocotyl elongation by the high temperature pre-treatment was observed. Furthermore, stimulation of mesocotyl elongation by the high temperature pre-treatment of seeds was nearly the same in treatments in which embryos were detached from the endosperm before or after the 40°C treatment. From these results, it seems probable that an aerobic respiratory metabolism may be required in the embryo during the 40°C treatment of seeds for the stimulation of mesocotyl elongation by this treatment.

INTRODUCTION

During the course of investigation on the stimulation of mesocotyl elongation by a high temperature pre-treatment of the seeds of rice before sowing, it was found that the stimulatory effect gradually increased, passed through a maximum, and finally fell off, as the duration of a period of seed-immersion at 25°C before the 40°C treatment increased (Inouye et al., 1970a).

Using an aseptic culture method, this work was carried out to obtain more detailed information on the water content of seeds at the time of the high temperature pre-treatment.

MATERIALS AND METHODS

Hoyoku, a late maturing cultivar of paddy rice of the *japonica* type, was used. Unless otherwise stated, preparation of basal culture medium, methods of disinfection of the husked seeds, immersion of seeds in distilled water and treatment of the seeds at 40°C were identical with the techniques previously described (Inouye et al., 1970b).

After the high temperature pre-treatment, two seeds were sown in each

glass tube (18 x 250 mm) containing the culture medium. These tubes were wrapped with light-proof paper and placed in the dark at a constant temperature of 25 "C. At 14 days after sowing the length of the mesocotyl and coleoptile was measured. Each experimental treatment consisted of about 10 tubes.

Respiration rates of the seeds at 30 °C were measured by their oxygen uptake using a Warburg apparatus. In this experiment, 50 seeds were placed outside the centre well in each flask and 0.5 ml 20 % KOH were placed in the centre well. Respiration was measured over a period of one hour.

Water content of the grain was calculated after drying the grain at 105 °C for about 5 hours.

RESULTS AND DISCUSSION

Effect on mesocotyl elongation of the temperature of immersion of the seeds before the high temperature treatment

After disinfection, the seeds were immersed in sterile distilled water at 5, 15 or 25°C for various periods in darkness. After immersion, the water adhering to the surface of the seeds was removed with sterilized filter paper, and the seeds were treated at 40 °C for 10 days in the dark. After this high temperature pre-treatment, two seeds were sown in each tube containing culture medium. The results are presented in Fig. 1.

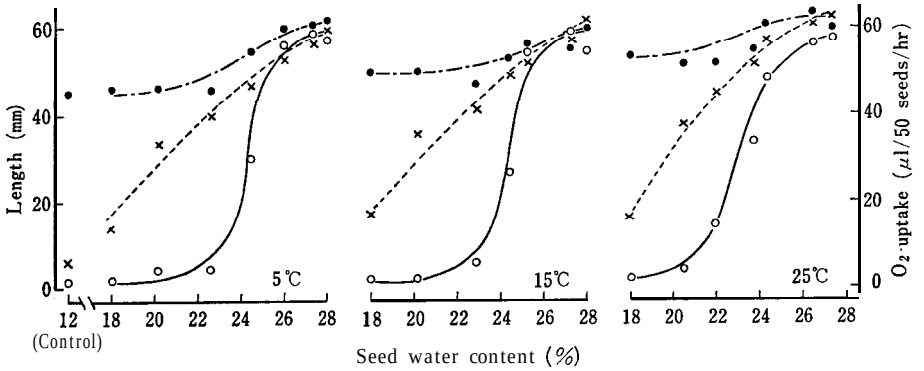

Fig. 1. Effect on mesocotyl elongation of the temperature of immersion of the seeds before high temperature treatment (40°C for 10 days). Open circles: Mesocotyl; Closed circles : Coleoptile ; Crosses : O_2-uptake (μl/50 seeds/hr) at 30°C.

The temperature at which the seeds were immersed had no effect. When the seeds contained less than about 20% water prior to the treatment at 40 °C there was no stimulation of mesocotyl growth. However, when the seeds contained more than 22 % water the stimulatory effect of the high temperature increased with increasing water content and reached a maximum at about 27 % water content. In these seeds the mesocotyl length of the treated plants was about forty times as long as that of control. Among the seeds with a water from about

22 to *27* %, average mesocotyl length of treated plants increased by about 10 mm with every increase of 1 % of water.

On the other hand, respiration rates of seeds increased with increasing seed water content, and was similar for all of the immersion temperatures. When seeds contained similar volumes of water, the respiration rates of the seeds at *30* °C was almost the same, irrespective of the immersion temperatures.

Effect of *drying seeds* **after immersion on mesocotyl elongation**

After immersion of the seeds for 14 hours at 25 "C, water adhering to the surface of seeds was removed with sterilized filter paper and the seeds were dried in a desiccator under reduced pressure for various periods. The seeds were then treated for 10 days at 40°C in the dark. Two seeds were sown in each tube containing culture medium. Results are given in Fig. 2.

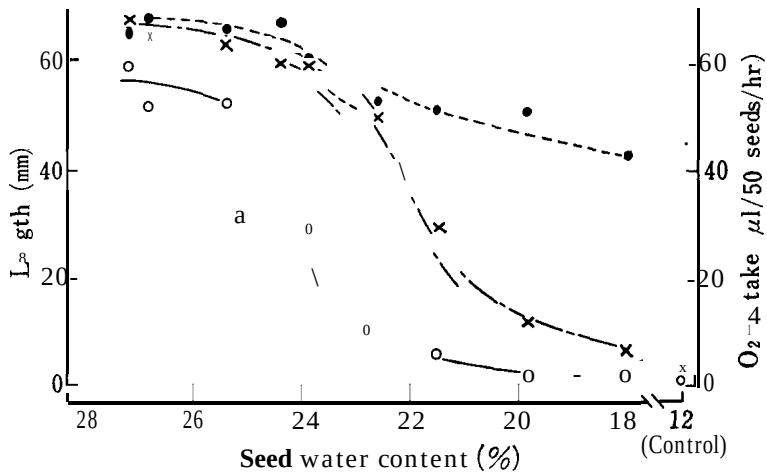

Fig. 2. Effect of drying seeds after the immersion on mesocotyl elongation (40°C for 10 days). Open circles : Mesocotyl; Closed circles : Coleoptile ; Crosses : O_2-uptake (μ1/50 seeds/hr) at 30°C.

Stimulation of mesocotyl elongation by the high temperature pre-treatment was almost the same in seeds with a water content from 27 % (treated control) to 26 %. When the water content of the seeds was less than 26 % mesocotyl length decreased with decreasing water content. On average the mesocotyl length of treated plants with a water content between 25-22 % decreased about 10 mm with every 1 % decrease in seed water content. The mesocotyl length of plants from seed with a water content of about 20 % was the same as that of the untreated control. The effect of drying the seeds on coleoptile elongation was not so obvious as in the mesocotyl, although the effect of the high temperature pre-treatment was also decreased by drying the seeds. At a seed water content of about 18 %, coleoptile length of the treated plants was the same as that of the untreated control.

Respiration rates of the seeds decreased with the decrease in water content.

Thus, the decreasing stimulatory effect by the high temperature pre-treatment on mesocotyl elongation was in parallel with the decreasing respiratory rates of the seeds.

Effect on mesocotyl elongation of drying the seeds during the high temperature treatment

After immersion of seeds for 14 hours at **25 "C,** seed water content reached about **27** %, and the seeds were then treated at **40** ℃ for 0, **2, 4, 6, 8** and 10 days. The seeds were dried in a desiccator under reduced pressure for about 2 hours, until seed water content fell to about 18 %, and each treatment was divided into

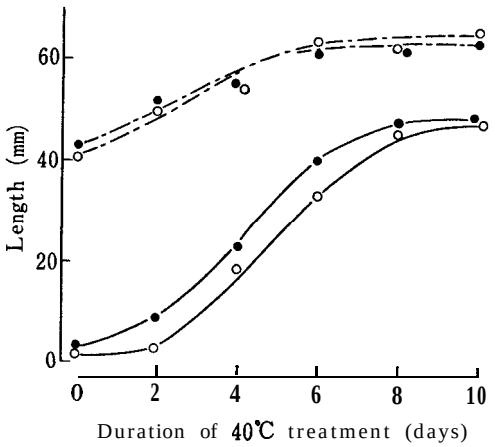

Fig. 3. Effect on mesocotyl elongation of drying seeds during the high temperature treatment (40°C). Open circles : After the 40℃ treatment, the seeds were dried until seed water content falls to about 18 % and were sown immediately ; Closed circles : After the drying, seeds were treated at 40°C an additional period, to make a total of 10 days ; Solid lines : Mesocotyl ; Broken lines : Coleoptile.

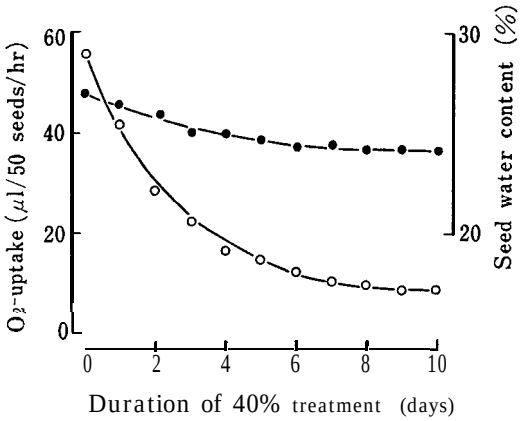

Fig. 4. Changes of respiration rates of seeds and seed water content during the high temperature treatment (40°C for 10 days). Open circles : O_2-uptake (μl/50 seeds/hr) at 30°C; Closed circles: Water content of seeds (%).

two groups. One group was sown immediately after drying and the another was treated at 40 ℃ for an additional period, to make a total of 10 days. Two seeds were sown in each tube containing culture medium. Results are shown in Figs. 3 and 4.

When seeds were dried within 6 days after the start of the 40 ℃ treatment, a slight and additional stimulatory effect of the treatment on the elongation of the mesocotyl was observed. However, the seeds which were dried after treatment at *40* ℃ for 8 or more days, little effect of drying was observed. Drying the seeds had similar effect on both the elongation of the coleoptile and the mesocotyl.

The respiration rate of the seeds decreased rapidly after the 40 ℃ treatment until 6th day, it only decreased slightly after the 7th day. Although seed water content decreased gradually when the number of days at 40 ℃ was increased, it did not change significantly on and after the 6th day from the start of the treatment (Fig. 4).

Effect on mesocotyl elongation of oxygen and nitrogen gases during high temperature treatment of seeds

As previously described, high temperature pre-treatment of seeds increased mesocotyl elongation and this was associated with increasing respiratory rates at the beginning of *40* ℃ treatment. From this result it may be deduced that the stimulatory effect of the high temperature pre-treatment on mesocotyl elongation depends upon the increased respiratory activity of the seeds during the 40 ℃ treatment.

In order to make this point clear the following experiment was conducted. After immersion of the seeds for 14 hours at *25 "C,* water adhering to the surface of seeds was removed and about *25* seeds were put into each empty glass tube (22 x 80 mm). The tubes were closed with a rubber stopper fitted with a stop cock. Air was removed from the tubes through the stop cocks with a water vacuum pump, and either oxygen or nitrogen gas was injected into the tube through the cock. Then the tubes were treated at *40* ℃ for **10** days in the dark. After this latter treatment, two seeds were sown in each tube containing culture medium. Results are given in Table 1.

Table 1. Effect of oxygen and nitrogen during high temperature treatment of seeds on mesocotyl elongation (40°C for 10 days).

Condition	No. of plants	Average length (mm)	
		Mesocotyl	Coleoptile
O_2	21	54±23.3	58±17.0
N_2	20	5± 2.7	36±12.0
Air	13	51±13.0	56±13.0
Control	21	1± 0.8	41± 7.9

As shown in Table 1, stimulation of mesocotyl elongation was similar whether the seed was treated at 40 ℃ in air or in oxygen. When seeds were treated at 40 ℃ in nitrogen, that is in an oxygen free atmosphere, only a slight stimulation of mesocotyl elongation by the high temperature pre-treatment was observed.

These results show that an aerobic respiratory metabolism in seeds during the 40°C treatment is essential for stimulation of mesocotyl growth by the high temperature pre-treatment.

Removal of endosperm and mesocotyl elongation

In order to assess the effect of removal of the endosperm on the stimulatory effect of the high temperature pre-treatment of seeds on mesocotyl elongation, the following experiments were designed.

a) After immersion of seeds for 14 hours at 25 “C, water adhering to the surface of seeds were removed with filter paper and seeds were treated at 40 ℃ for 10 days in the dark. The embryo was then detached from the endosperm with a razor and two embryos were sown in each tube. b) After immersion of the seeds for 0, 2, 4, . . . 12 or 14 hours in distilled water at 25 “C, the embryo was detached and placed on moistened filter paper for 14, 12, 10, . . . 2 or 0 hours,

Table 2. Effect of removal of endosperm on mesocotyl elongation **(40°C** for 10 days),

(a)

Time of removal of endosperm	No. of plants	Average length (mm)	
		Mesocotyl	Coleoptile
Before 40℃	**24**	23±8.2	29±12.3
After 40°C	16	21±9.4	27±11.5
Control	20	1±0.6	28± 2.6

(b)

Duration* (hr)	No. of plants	Average length (mm)	
		Mesocotyl	Coleoptile
2	24	24±12.6	24± 14.1
4	20 21	27±26±14.3 12.6	28±11.21±13.41
6	21	27±11.8	24±12.9
8			
10	24	25± 10.4	27± 12.9
	24	26±12.4	31±11.1
12	26		
14		21±23± 9.5 8.2	27±11.529±12.3
Control	20	1± 0.6	28± 2.6

*Duration of immersion of seeds at 25°C before the time of removal of endosperm. Thereafter, embryo was placed on moistened filter paper, the time held at 25°C making a total of 14 hours.

the time held at 25 ℃ making a total of 14 hours. Water adhering to the surface of the embryos was removed with filter paper and embryos were treated at 40 ℃ for 10 days in the dark. Two embryos were then sown in each tube of culture medium.

In these experiments, the culture medium contained 2 % sucrose besides White's minerals and agar. Results are given in Table 2 – (a) and – (b).

As shown in Table 2 -(a), the stimulation of mesocotyl elongation by the high temperature pre-treatment was nearly the same whether the embryo was detached from the endosperm before and or after the 40 ℃ treatment.

Furthermore, stimulation of mesocotyl elongation by the high temperature pre-treatment was almost the same in all of the treatments, irrespective of seed water content on the time of detaching the embryo from the endosperm before the 40 ℃ treatment (Table 2 – (b)).

From the above result, it seems probable that the stimulatory effect on mesocotyl elongation by the high temperature pre-treatment of seeds before sowing may be caused mainly by the metabolic changes of the embryo when containing a suitable amount of water.

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