STUDIES ON THE BOTANICAL CHARACTERISTICS OF GENUS DIOSCOREA

III. Effect of sucrose, boric acid and temperature on pollen germination of Chinese yam (*Dioscorea opposita* T. cv. Nagaimo)

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Introduction

In our previous paper, it was reported that the existence of female plants of Chinese yam cv. Nagaimo was recognized and dioecism of cv. Nagaimo was confirmed[7]. However in Sapporo, even if male and female plants of cv. Nagaimo are planted mixed with each other and crossed by open pollination, it is very difficult in many cases to obtain seed under the ordinary circumstances[8]. It is considered that clarification of the cause of the problem is very important and the investigation should be carried out from both sides—on male and female plants.

Ability of pollen to germinate is one of the most important characters with male plants, however, germination of the pollen of genus Dioscorea has not been examined in detail. On the point of view mentioned above, the authors have examined pollen germination in cv. Nagaimo in order to clarify the problem of fruiting and seed formation. According to various experiments, it has been recognized that sugars and boron are most important to accelerate pollen germination in various kinds of plants[1,3,5]. In the present paper, the authors described the effect of sucrose, boric acid and temperature on pollen germination in cv. Nagaimo in the test using synthesized medium.

Materials and Methods

In 1980, male plants of cv. Nagaimo were planted at the experimental farm of Hokkaido University in Sapporo. At about ten o'clock in the morning of August 4th and 18th, anthers were picked from the male

flowers and brought to the laboratory. Then pollen grains in anthers were placed on the liquid medium. As the quantity of the pollen in an anther is very little and a pollen grain is very small and sticky in Chinese yam, the pollen was scratched and collected carefully with needles. It was recognized that pollen used in these experiments would be fertilizable, because those were stained by aceto carmin. In this report, the following two experiments were carried out.

(1) *Experiment 1*; *Effect of sucrose and boric acid*

In this experiment, twenty types of liquid media were prepared by additions of 0, 2, 5, 10 and 15% of sucrose combined with 0, 30, 100 and 300 ppm of boric acid. In these media, 300 ppm of $Ca(NO_3)_2 \cdot 4H_2O$, 200 ppm of $MgSO_4 \cdot 7H_2O$ and 100 ppm of KNO_3 were included for supplying inorganic ions with reference to the basal medium developed by BREWBAKER and KWACK[2]. The pollen was germinated in humid condition at 25°C. As to observation of the germination 70 to 100 pollen grains in a field of vision were observed and the manipulation was repeated three times. Pollen with the tube as long as the major axis of a pollen grain was regarded as germinating. Also, the tubes of ten pollens selected at random in a field of vision were measured and the procedure was repeared three times.

(2) *Experiment 2*; *Effect of temperature*

Pollen was placed on the medium with 5% of sucrose, 100 ppm of boric acid and three kind of inorganic ions according to the result of Experiment 1. The pollen germinated at five different temperatures —10, 15, 20, 25 and 30°C— under fluorescence light. The method of observation was the same as in Experiment 1.

Results

(1) *Effect of sucrose and boric acid*

The germination percentage were only 10 to 13% in the absence of sucrose, regardless of concentration of boric acid, and below 40% in the absence of boric acid. But in the media containing 30, 100 and 300 ppm of boric acid combined with 2, 5, 10 and 15% sucrose, the percentage of pollen germination was 60 to 70% (Fig. 1).

As to tube elongation, the tubes did not elongate in the media to which either sucrose or boric acid was not added. Tubes elongated rapidly to become uniformly longer on the media with 5% of sucrose, and longest especially on the medium with 5% of sucrose and 100 ppm boric acid. On

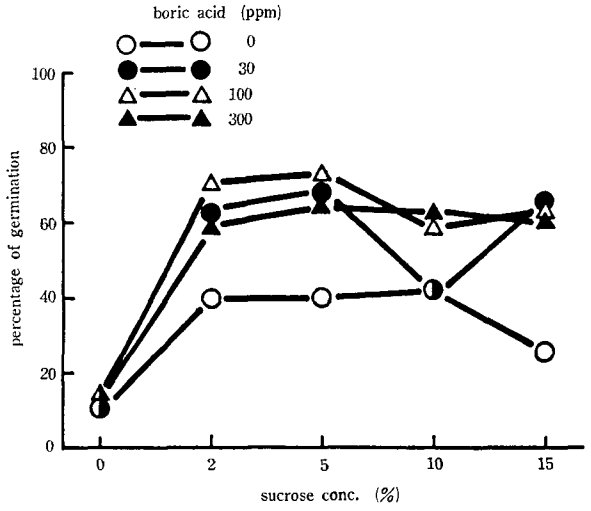

Fig. 1. Effect of sucrose and boric acid on pollen germination (25°C, 12 hours)

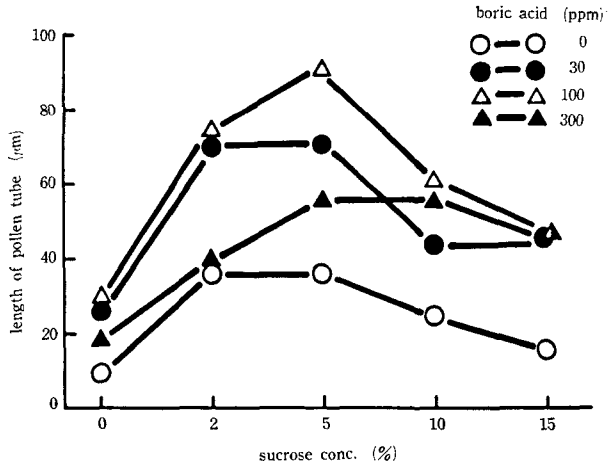

Fig. 2. Effect of sucrose and boric acid on elongation of pollen tube (25°C, 12 hours)

the contrary, elongation was inhibited on the media with 10 or 15% of sucrose (Fig. 2, Fig. 3).

In the present experiment, the inhibition of tube elongation and the thickening of tube membrane was observed at high concentration of sucrose (10 and 15%). Although substances composing the tube membrane were synthesized at a cap block in the pollen tube tip, the tube can not elongate

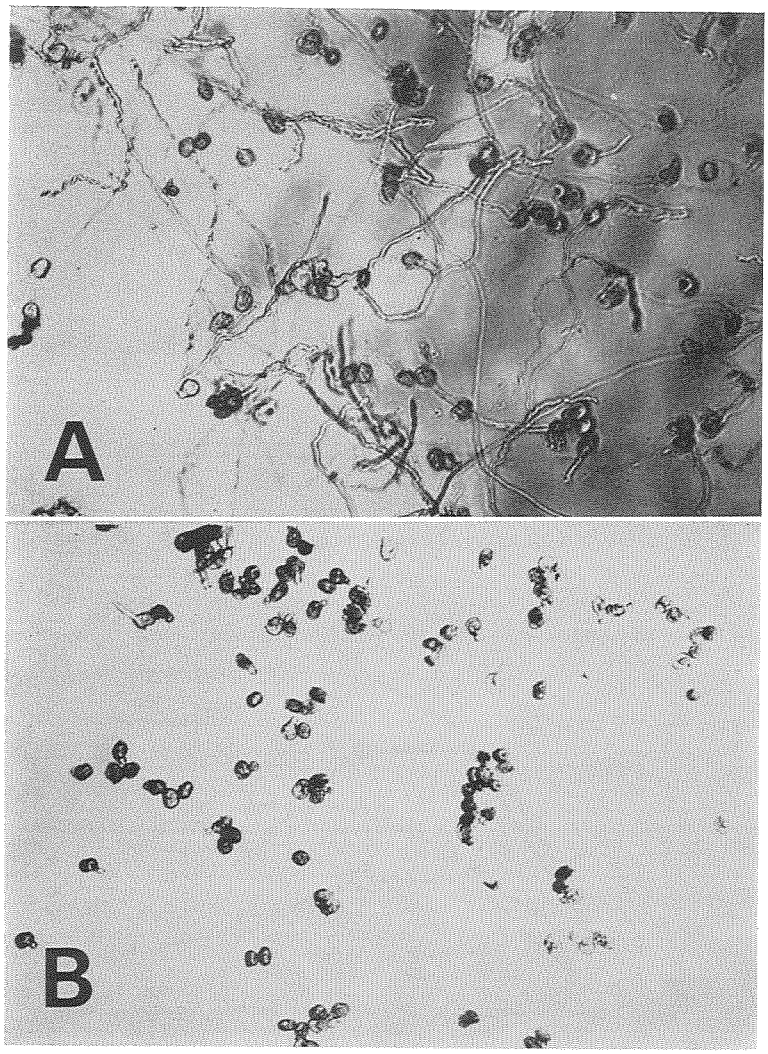

Fig. 3. Elongation of pollen tube.
A: sucrose 5% and boric acid 100 ppm
B: sucrose 0% and boric acid 0 ppm
(25°C, 12 hour)

smoothly, because of the deficiency of water absorption and low turgor pressure.

Plasmoptysis was not observed in any treatment, even in the media with none or low concentrations of both sucrose and boric acid.

(2) *Effect of temperature*

The percentage of pollen germination was 29% at 25°C and 39% at 30°C after 3 hours of incubation, and finally reached 70% at both temperatures. At 20°C, it took a long time to begin to germinate and the percentage of germination was 36% after 6 hours. At 10 and 15°C, it was only 20% to 30% after 24 hours (Fig. 4).

After 3 hours of incubation, pollen tubes elongated by 35 μm at 30°C, 25 μm at 25°C, 18 μm at 20°C, but scarcely elongated at 15°C and 10°C.

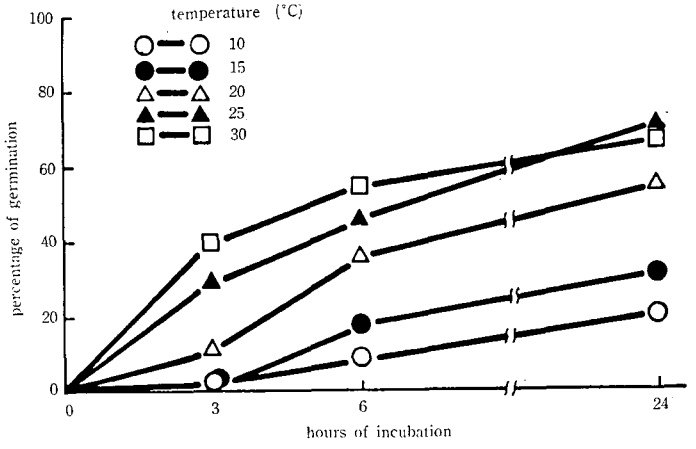

Fig. 4. Effect of temperature on pollen germination (medium; sucrose 5%, boric acid 100 ppm)

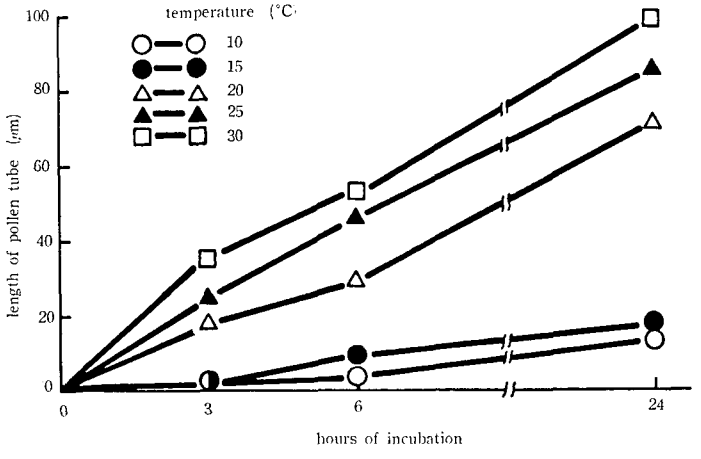

Fig. 5. Effect of temperature on elongation of pollen tube (medium; sucrose 5%, boric acid 100 ppm)

After 24 hours, tubes elongated by more than 70 μm at 20°C, 25°C and 30°C. Whereas the length was only 17 μm at 10°C and 15°C. (Fig. 5).

Discussion

In Experiment 1, the medium which contained 5% sucrose and 100 ppm boric acid is suitable for pollen germination of cv. Nagaimo. In this medium, the germination percentage was highest and pollen tubes were longest. In agreement with various experiments by other workers, this study confirmed that boron enhanced the formation of tube membrane. It is well known that the interrelation between the turgor pressure in pollen and the rate of formation of pollen tube membrane is significant in the normal germination and tube elongation. No plasmoptysis was seen on the medium containing none or low concentrations of sucrose, regardless of the absence or presence of boric acid. The authors can not give a clear-cut explanation of the problem, which intend to clarify the cause of it.

In relation to temperature effects on pollen germination, it is shown that higher temperature is optimal in plants flowering in the summer[6]. In addition, the native land of genus Dioscorea opposita is a subtropical zone in southern China[4]. From this study, it is confirmed that pollen germination in cv. Nagaimo is better at high temperature than low temperature within the range of 10 to 30°C.

Through these experiments, it is recognized that the pollen of cv. Nagaimo has the ability to germinate consequently and the failure of pollen to germinate has no cause of poor fruiting and seed formation.

Summary

It is very difficult to produce seeds with viability enough to germinate and have the capability of developing into an intact plant in Chinese yam cv. Nagaimo under ordinary circumstances. For the purpose of clarifying this cause, the germination of pollen was examined through a test using synthesized liquid media to study the effects of sucrose, boric acid and temperature. Percentage of pollen germination was more than 60% in the media with 2 to 15% sucrose and 30 to 300 ppm boric acid. The pollen tube was longest in the medium with 5% sucrose and 100 ppm boric acid. The medium with 5% sucrose and 100 ppm boric acid is most suitable for pollen germination of cv. Nagaimo. The suitable temperature for germination is 25°C and 30°C, and germination and pollen tube elongation were inhibited by low temperature. It is recognized that pollen of cv. Nagaimo has viability

enough to germinate.

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