

## Effects of Gibberellin and Cytokinin Treatments on the Promotion of Germination in Papaya, *Carica papaya* L., Seeds

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### Introduction

When the intact seeds of papaya were made to be germinated in the constant temperature (30°C), the seeds scarcely germinated after 20 to 30 days, as reported in the previous report<sup>6)</sup>. But both the treatments of removal of the arils (gelatinous covering) and of drying of seeds in the room-temperature, promoted germination; in case when both the treatments mentioned above were done at the same time, a considerably higher germination percentage was observed. Besides this, the pre-treatment under 15°C promoted germination conspicuously in case of the removal of the arils. In the present experiments, the effects of gibberellin (GA) and cytokinin (benzyladenine, BA) on the promotion of germination, immediately after the seeds were collected and dried, were investigated.

### Materials and Methods

#### [Exp. A] Effects of GA-treatments on the germination of seeds

(A-1) Treatments done immediately after the seeds were taken out of a fruit

A native papaya fruit from the Formosa cultivated in Yaku Island, was used. Although the papaya fruit was ripe (the fruits were ripe in all the experiments after this), the upper half portion of it was slightly greenish. Seeds were taken, immediately, out of the fruit and were manually removed of the arils from testae. With no drying, the seeds were soaked into GA-solution in the concentrations of 0, 10, 50, 100, 500, 1000 ppm for 20 hours, respectively. GA-solution was made by dissolving 1000 mg of GA in 30 ml of acetone, and then the solution was diluted into 1000 ml of water; and 1000 ppm of the GA-solution was diluted into the respective concentrations used in this experiment. The number of seeds used was 80 pieces, respectively. After GA-treatments, the seeds were slightly washed by water and were planted in the germination-beds made from river-sand in the plastic pots. All the pots were watered and placed in a dark room, at the constant temperature (30°C). The germination was counted 15 times, at the interval of two days, for 30 days after sowing.

(A-2) Treatments done after the seeds were dried for 13 days in the room-temperature

A native papaya fruit of Yaku Island, and another fruit cultivated in Yaku Island but was native of Hawaii — these two kinds of fruits were mixed and used for this experiment. Seeds were removed of the arils and were dried for 13 days in the room-temperature. The concentrations of GA-treatments done for the dried seeds and the other procedures were similar to those described in Exp. A-1. The number of seeds used was 50 pieces, respectively.

(A-3) Treatments done after the seeds were dried for 27 days in the room-temperature

Seeds from the fruit from Ibusuki in Kagoshima Prefecture were used in this experiment. The seeds dried for 27 days without removal of the arils were soaked into GA-solution in the concentrations of 0, 50, 100, 500, 1000 ppm for 20 hours, respectively. The procedures after the treatments were similar to those described in Exp. A-1. The number of seeds used was 200 pieces, respectively.

**[Exp. B] Effects of BA-treatments on the germination of seeds**

(B-1) Treatments done immediately after the seeds were taken out of a fruit

Seeds used were from a fruit from Ibusuki in Kagoshima Prefecture. The seeds were removed of gelatinous covering, and were soaked into BA-solution in the concentrations of 0, 10, 50, 100, 500, 1000 ppm. The method to make BA-solution is to add 0.2 ml of hydrochloric acid in 100 ml of water, with the dissolution of 1000 mg of BA in the hot solution (below 40°C), and to dilute the solution in 1000 ml of water, together with the dilution of 1000 ppm of BA-solution into the respective concentrations used in this experiment. The treated seeds were sown in the germination-beds containing river-sand, in the plastic pots. All the pots were watered and placed in a dark room at the constant temperature (30°C). The germination was investigated 15 times at the interval of two days for 30 days after sowing. The number of seeds used was 130 pieces, respectively.

(B-2) Treatments done immediately after the seeds were taken out of the fruit

Three fruits were from Hawaii. The seeds taken out of the three fruits were mixed together, removed of the arils, and those seeds were divided into the group of 200 pieces for each treatment of BA-solution. With no drying, the 200 pieces were soaked into BA-solution, in the concentrations of 0, 1, 5, 10, 50, 100, 500, 1000 ppm for 20 hours, respectively. The other procedures were similar to those described in Exp. B-1.

(B-3) Treatments done after the seeds were dried for 25 days in the room-temperature

Three fruits used here, were from Hawaii. The seeds were mixed together, removed of the arils, and dried for 25 days in the room-temperature. The number of seeds for each BA-treatment was 120 pieces. The concentration of BA-treatment and the other procedures were similar to those described in Exp. B-2.

## Results

(Exp. A-1) As shown in Fig. 1, in the GA-treatments immediately after the seeds were taken out of the fruit, the germination of each plot containing control began 6 days after the sowing. Only 5% of the germination was counted in the control plot, even on the last day of the germination-investigation. On the contrary, the germination percentages were 39 and 60% in 500 and 1000 ppm of the GA-treated plots, respectively; considerable promotions of germination were noted. The increase of germination in 500 and 1000 ppm of the treated plots continued to the later days after sowing, that is, for 14 days in case of 500 ppm, and for 22 days in case of 1000 ppm, after sowing; and the germination percentages were noted to be higher than in other plots. Germination percentages in the plots of 10, 50, and 100 ppm were lower than those of 500 and 1000 ppm on the last day of the germination-investigation, but their germination percentages were higher than the control-plot.

(Exp. A-2) The author showed, in the previous report<sup>6)</sup>, that the germination percentage was improved in case of the dried seeds. But in this experiment, drying for 13 days promoted

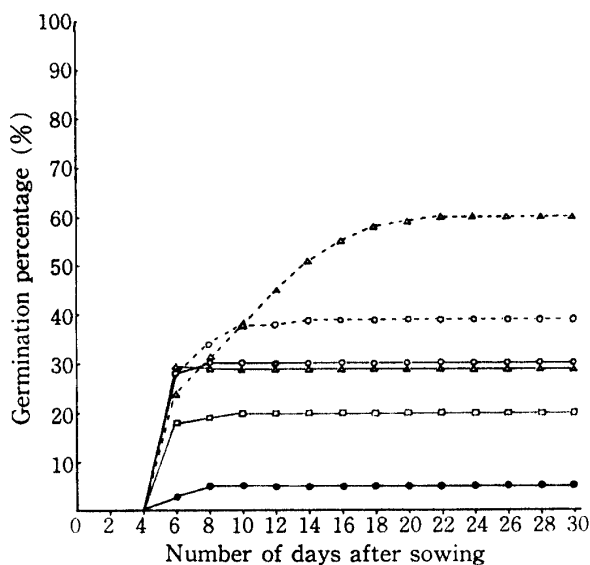


Fig. 1. The effects of GA-treatments of various concentrations on the germination in papaya-seeds (Treatments were done immediately after the seeds were taken out of the fruit and the arils were removed.).

----Δ---- 1000 ppm    —△— 50 ppm  
 ----○---- 500 ppm    —○— 10 ppm  
 —□— 100 ppm        —●— Control

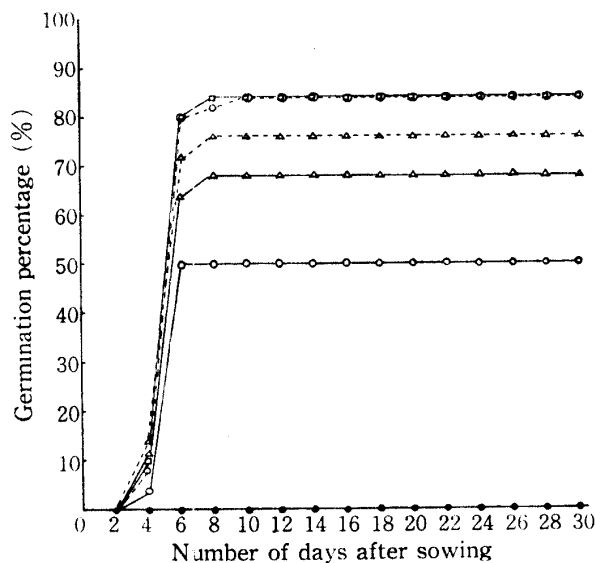


Fig. 2. The effects of GA-treatments of various concentrations on the germination in papaya-seeds (Treatments were done after the arils were removed and the seeds were dried for 13 days in the room-temperature.).

----Δ---- 1000 ppm    —△— 50 ppm  
 ----○---- 500 ppm    —○— 10 ppm  
 —□— 100 ppm        —●— Control

no germination in the control plot, i.e. the germination percentage was 0% on the last day of the investigation (Fig. 2). The germination percentages were good in the order of 100, 500, 1000, 50, 10 ppm. All GA-treatments were effective for the promotion of germination. In case of the dried seeds removed of the arils, the GA-treatments in the lower concentrations (100 and 500 ppm) were noted to be slightly superior to that in the higher one (1000 ppm) in the germination percentage. This result was different from that in Exp. A-1.

(Exp. A-3) In case of the dried seeds kept for 27 days in the room-temperature without removal of the arils, the germination in the respective treatments containing control was good. In the earlier days after sowing, 500 and 1000 ppm of GA-treatment showed germination percentages higher than those in the other cases. The germination in the control scarcely occurred 12 days after sowing, but since then, showed a steep increasing curve of germination. In the 500 and 1000 ppm of the concentration, a steep rise in the germination-curve was indicated, 10 days after sowing (Fig. 3); and their treatments enhanced, considerably, the germination-rate. On the last day of germination-investigation, all the treated plots containing the control, came to be nearly equal in the germination percentages.

(Exp. B-1) In the BA-treatments done immediately after the seeds were taken out of the fruit, and were removed of the arils from testae, the germination-rate was promptest in the control plot, and was less in promptitude in all the treated plots in the earlier days after sowing; germination percentages noted 8 days after sowing, were 31% in the control plot, 2% in 10 ppm of the treated plot, 1% in 50 ppm, 0% in 500 and 1000 ppm (Fig. 4). Germination percentages in 10, 50, 100 ppm of the treated plots came to be almost equal to the control (78%) on the last day of the investigations, but in the higher concentrations of 500 and 1000 ppm, those were suppressed con-

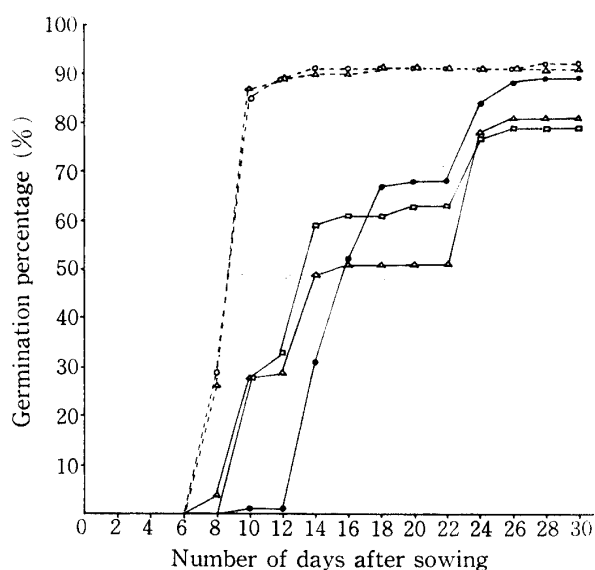


Fig. 3. The effects of GA-treatments of various concentrations on the germination in papaya-seeds (Treatments were done without removal of the arils and after the seeds were dried for 27 days in the room-temperature.).

----△---- 1000 ppm    —△— 50 ppm  
 ----○---- 500 ppm    —●— Control  
 —□— 100 ppm

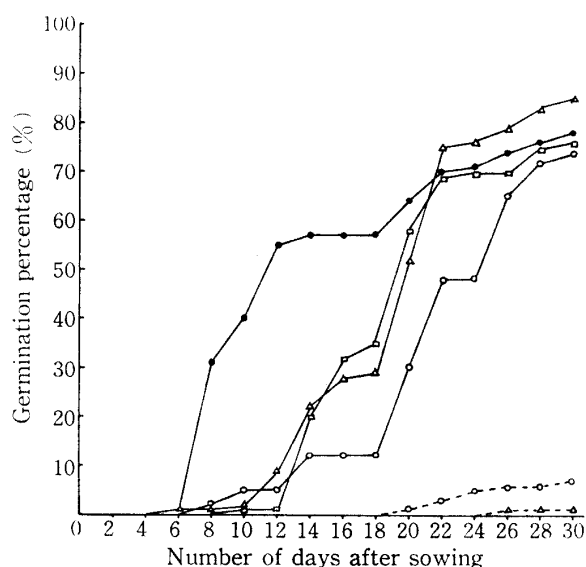


Fig. 4. The effects of BA-treatments of various concentrations on the germination in papaya-seeds (Treatments were done immediately after the seeds were taken out of the fruit and the arils were removed.).

----△---- 1000 ppm    —△— 50 ppm  
 ----○---- 500 ppm    —○— 10 ppm  
 —□— 100 ppm        —●— Control

spicuously; and was 7% and 1%, respectively. Generally speaking, the BA-treatments were not effective for the promotion of the germination in the papaya-seeds. In this experiment, only the removal of the arils with no drying made the germination in the control (water treatment) promoted considerably.

(Exp. B-2) In 1, and 5 ppm of the lower concentration in BA-treatment, the germination-rate was prompter than in the control (0%); germination percentages were 50 and 53%, 6 days after sowing, respectively. The germination in the control began 8 days after sowing, and the considerably high germination percentage (68%) was noted on that day. 10 days after sowing, the germination in the control and in 1, 5, and 10 ppm of the treated plots, showed the same curves, the increase of germination was scarcely indicated till the last day of the investigations, and about the same germination percentages were counted on the last day (Fig. 5). On the contrary, the germination was suppressed conspicuously in 50, 100, 500, and 1000 ppm of the BA-treated plots every investigation-day. In this experiment, the germination was suppressed in 50 and 100 ppm of the treated plots, and this result was different from those showing no suppression in Exp. B-1. In this experiment, a considerable germination in the control was noted in case of the removal of the arils, with no drying.

(Exp. B-3) 1, 5, and 10 ppm of the lower concentration in BA-treatments enhanced slightly the germination-rate in the earlier days after sowing (Fig. 6). In 1, 5, and 10 ppm of the treatment and the control, prompt germination occurred 12 days after sowing. But 50 and 100 ppm of the treatments inhibited the germination more than the control; and 500 and 1000 ppm of the treatments inhibited it particularly. Generally speaking, from these results, BA-treatments were ascertained to have no effect on the promotion of germination.

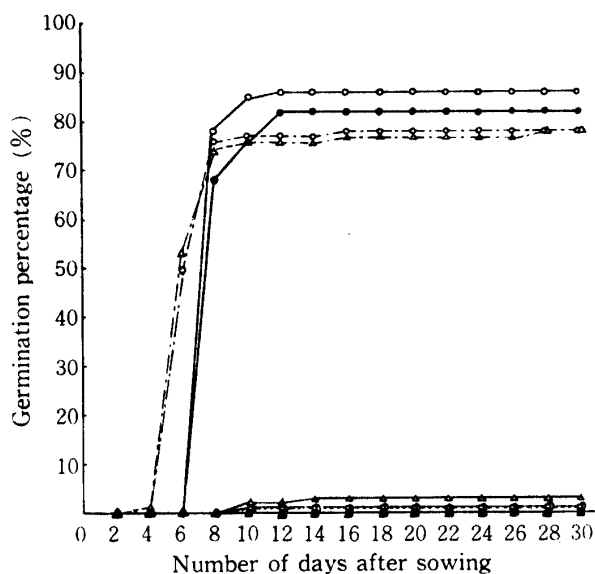


Fig. 5. The effects of BA-treatments of various concentrations on the germination in papaya-seeds (Treatments were done immediately after the seeds were taken out of the fruit and the arils were removed.)

---▲--- 1000 ppm    —○— 10 ppm  
 ---○--- 500 ppm    ---△--- 5 ppm  
 ---□--- 100 ppm    ---○--- 1 ppm  
 ---△--- 50 ppm    ---●--- Control

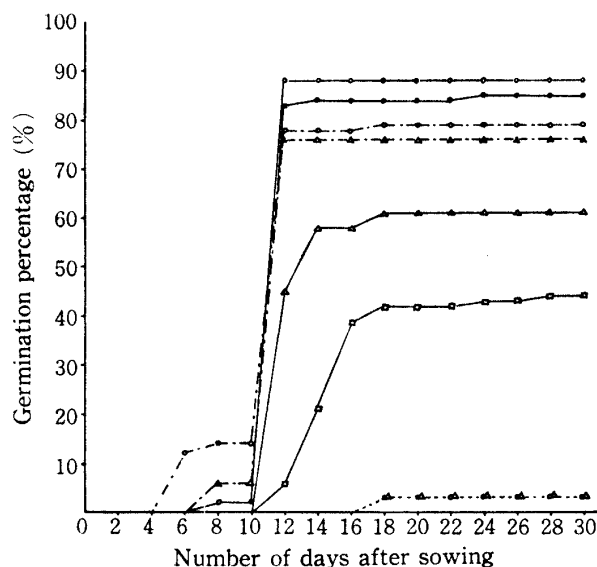


Fig. 6. The effects of BA-treatments of various concentrations on the germination in papaya-seeds (Treatments were done after the arils were removed and the seeds were dried for 25 days in the room-temperature.)

---△--- 1000 ppm    —○— 10 ppm  
 ---○--- 500 ppm    ---△--- 5 ppm  
 ---□--- 100 ppm    ---○--- 1 ppm  
 ---△--- 50 ppm    ---●--- Control

### Discussion

The effects of gibberellin (GA) treatments carried out up to now on the promotion of germination in papaya seeds, were various in the results. Lange<sup>2,3</sup> obtained the result that the germination was promoted by GA-treatment, but Ramirez<sup>4</sup> and Seth<sup>5</sup> did not obtain such result. Chacko et al.<sup>1</sup> also reported firstly that no significant effect of promotion by GA-treatments on the germination in papaya seeds was noted, though the germination-rate was enhanced by GA-treatments; and secondly, that 500 ppm GA was the optimum concentration for the promotion of germination. The authors carried out the present experiments in order to ascertain the effect of GA-treatment on the germination in papaya-seeds, done with removal or without removal of the arils (gelatinous sarcotestae), immediately after the seeds were taken out of the fruit or after the seeds were made dry in the room-temperature. One of the authors already reported<sup>6</sup> that the removal of the arils brought the promotion of germination and that the seeds dried in the room-temperature had the promoting effect on germination, too. Also one of the authors had recognized the presence of growth-inhibitors in the gelatinous sarcotesta and in the seed, containing testa (unpublished report). The authors assumed that the removal of the arils might be resulted in the partial decrease of growth-inhibitors, and that the drying of seeds (with or without the removal of the arils) might induce the inactivity of growth-inhibitors. The arils were removed immediately after the seeds were taken out of the fruit (this practice helped surely the absorption of GA-solution), and their seeds were soaked into various concentrations of aqueous GA (Exp. A-1). The effect of

the germination-promotion was observed in all the concentrations of GA, and its effect was better, particularly in 500 and 1000 ppm of the higher concentrations. This result was assumed, by the authors, to have been derived from the fact that GA antagonized to growth-inhibitors in the seed containing testa, and negated the inhibiting effect of the growth-inhibitors. It was supposed by the authors that, the fact that the germination was scarcely observed in water treatment of the control, was resulted from the fact that the seed was kept in the dormant state by the growth-inhibitors. In the Exp. A-2, the promotion-effect of germination was not observed as in the previous report<sup>6)</sup>, though the seeds were removed of the arils and were dried for 13 days in the room-temperature. It was assumed that such result was brought in, on account of the shorter period of the drying days. The presence of individual-difference for the germination of the papaya-seeds was assumed by some authors' experiments to be due to the differences in the native land, and in the harvest-time of the fruit, and in the degree of ripeness. Although 1000 ppm GA-treatment was best for the germination in the Exp. A-1; 100 and 500 ppm GA-treatments of the concentration lower than 1000 ppm were best in case of the dryness in the room-temperature in the Exp. A-2. This result was assumed to be due to the fact that the growth-inhibitors in the seeds containing testae were decreased, in certain degrees, by the drying of the seeds. The high germination-percentages in all the GA-treated plots and the control, were shown on the last day after sowing in the Exp. A-3. This result was assumed to be due to the fact that the dryness of such a long period as 27 days inactivated the growth-inhibitors in the gelatinous covering and in the seed, even if the gelatinous covering was attached to the testa. Furthermore, the fact that 500, and 1000 ppm of the higher concentration enhanced the germination-rate, was assumed to be due to that, although the seeds were dried, the growth-inhibitors still were left considerably in the seeds, and accordingly, 500 and 1000 ppm GA made the inhibiting effect of their growth-inhibitors negative. And moreover, although any datum of the experiment carried out by the authors was not recorded in this report, in case of the removal of the arils and of the dryness for 25 days in the room-temperature, 50 and 100 ppm GA-treatments and the control were almost equal in the germination curves. This was assumed to be due to the fact that the removal of gelatinous covering and the dryness of such a long period as 25 days made the growth-inhibitors decreasing and inactive; therefore, the better germination was obtained in the control, even if, without GA-treatments. From these three experiments (Exp. A-1, 2, 3), the effects of germination-promotion of the GA-treatments were confirmed; and in a certain case (Exp. A-3) the germination-rate was enhanced by GA-treatments.

Although, in the Exp. B-1, 50 and 100 ppm of BA-treatments showed no suppression in the later days of the investigations, in the Exp. B-2 and Exp. B-3, the treatments suppressed germination in comparison with the control. The reason why these results were brought forth, is obscure. And in both of the Exp. B-2 and Exp. B-3, BA-treatments of the lower concentration (1 and 5 ppm) enhanced, somewhat, the germination-rate, the cause of which was left unascertained.

### Summary

In order to ascertain the effect of GA- and BA-treatments, without dryness or with dryness, of the papaya-seeds (also with or without removal of the arils), on the promotion of germination, the present experiments were carried out.

1) Generally speaking, the GA-treatments of the various concentrations were effective for the promotion of germination, but in a certain case, the effect enhanced only the germination-rate.

2) The effect of BA-treatments of the various concentrations was not effective for the promotion of germination; and higher concentrations in BA-treatments were rather suppressive to the germination. But in the lower concentrations of BA-treatment, the germination-rate was enhanced slightly in the earlier days after sowing.

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