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REGULATION OF SEED DORMANCY IN CHERIMOYA (*Annona cheromola* Mill.)

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Abstract: Exogenously applied growth promoting and inhibiting substances regulated seed dormancy in cherimoya. Growth promoting substances, such as gibberellic acid (GA_3) and/or kinetin increased germination percentage; on the other hand, growth-inhibiting substance such as abscisic acid (ABA) induced dormancy. Seeds treated with GA_3 at the concentration of 10^{-4}M also enhanced germination. The application of GA_3 and/or kinetin antagonized the effect of ABA. Effect of day/night temperature on seed germination also tested, 30°C/25°C and 25°C/20°C showed 80% and 60% germination respectively, on the otherhand no germination was observed at low temperature (20°C/15°C). At the temperature 37°C/32°C, hundred percent radical was protruded but radical tips turned brown and died within a week.

Key words: Dormancy; Radical; Testa; Germination; Growth regulators.

Introduction

The cherimoya is ranked first among the annona fruits in the tropical and subtropical areas (Oches *et al.*, 1966). The cherimoya appears to have originated in the cool, dry highlands of Ecuador and Peru. The fruit was introduced to India, Egypt and other countries at the early date. Testa of unstratified seeds contains high concentration of ABA. According to Liep and Crame (1966), seeds with intact integument germinate more slowly than those without integument. Therefore, the effect of ABA could be reduced in unstratified seeds by removing the integument. The ABA level in the seed can be reduced by both high and low temperatures (Bonamy and Dennis, 1977). Seeds of cherimoya possess an undeveloped rudimentary embryo that requires an extended period of high temperature for breaking the dormancy. This situation could be overcome by treating the dormant seeds with GA_3 and cytokinin (Sankhla and Sankhla, 1968) and the action of cytokinin are both antagonistic to the inhibitory effect of ABA and complementary to the action of GA_3. Therefore, the regulation of seed dormancy by temperature for germination, applied growth regulators at different concentrations and testa removal were investigated through the experiment.

Materials and Methods

Ripe cherimoya fruits (cv. Big Sister) were harvested from polyethylene shaded culture house of Wakayama Fruit Tree Experiment Station, Wakayama, Japan. Seeds were sampled and washed in running tap water several times, dried up and preserved at room temperature for two weeks.

Before giving treatments, seeds were dipped in 70% ethanol and washed several times with sterile water. For each treatment, twenty-five seeds were placed on one layer of Whatman 2 filter paper in a petri dish (each petri dish contained five seeds) where 5 to 10 ml sterile water was added. The petri dishes were sealed with parafilm to reduce the evaporation. The filter papers were further wetted by sterile water inside the laminar hood when they were about to dry.

The treatments used were (a) temperature (37°C/32°C, 30oC/25oC, 25°C/20°C and 20°C/15°C), (b) removal of testa (without and with removal of testa), (c) Growth regulators (GA_3, ABA and kinetin @ 10^{-3}, 10^{-4} amd 10^{-6}M) and (d) Combinations of growth regulators (GA_3+Kin, GA3+ABA, Kin+ABA and GA_3+Kin+ABA @ 10^{-4}M).

Temperature treatments were given by placing the petri dishes at 37°C/32°C, 30°C/25°C, 25°C/20°C and 20°C/15°C. For testa removal, seeds were soaked in sterile water for 24 hours and testa was removed with the aid of forceps without damaging the endosperm tissues. For the treatment of growth regulators, 225 seeds were equally divided into three groups. The first group was treated with three concentrations of GA_3 viz. 10^{-3},10^{-4} and 10^{-6}M (twenty-five seeds for each concentration). The second and third groups were treated with ABA and kinetin, respectively at the same concentrations as GA_3. The cultures were maintained at 30°C/25°C

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day/night temperature.

To investigate the antagonistic effect of GA_3 and/or kinetin to ABA, a single concentration (10^{-4}M) of these growth regulators was used. For GA_3 + kinetin, GA_3 + ABA and kinetin + ABA treatments, 50 and 25 seeds were soaked for 24 hours in GA_3 and kinetin solution respectively. Out of 50 seeds, 25 were transferred to ABA and the rest 25 seeds were transferred to kinetin solution for another 24 hours. Seeds, those were soaked earlier in kinetin were transferred to ABA solution and treated for further 24 hours. In the case of treatment GA_3 + kinetin + ABA, 25 seeds were soaked for 24 hours in a solution containing GA_3 and kinetin and then transferred to ABA solution for another 24 hours.

Data collection: The cultures were examined at two days interval to record data on germination. Standard deviation was calculated according to Mian and Mian (1984).

Results

The petri dishes were maintained at day/night temperature of 37°C/32°C, 30°C/25°C, 25°/20°C, and 20°C/15°C. Testa rupture as well as radical protrusion was not observed at the lowest temperature of 20°C/15°C within 60 days of culture. The highest percentage of effective germination (80%) was found at 30°C/25°C (Fig.-1). At the temperature of 37°C/32°C, 100% radical protrusion was observed, but the radical tip became brown within a week and further growth was not observed at this temperature. Maximum radical growth rate was obtained at 30°C/25°C followed by 25°C/20°C (Table 1). Radicals protruded at 25°C/20°C and 30°C/25°C were healthy; their growth was satisfactory and transferred in the soil when the radical length was about 3 to 4 cm.

Table 1. Effect of temperature and testa removal on radical growth rate per day of cherimoya seeds

Treatment	Growth rate ± SD (mm)
Temperature (37°/32°C)	-
Temperature (30°/25°C)	5.3 ± 0.08
Temperature (30°/25°C)	5.1 ± 0.03
Temperature (30°/25°C)	-
Testa removal	408 ± 0.05

Response of growth promoters such as GA_3 and kinetin and growth inhibitor like ABA was examined at the concentrations of 10^{-3}, 10^{-4} and 10^{-6}M. The effect of GA_3 and kinetin become visible after 10 and 12 days respectively. Seeds treated with ABA obtained only 10% radical protrusion after 45 days of incubation at 10^{-4}M (Fig.-2). Among the concentrations, both GA_3 and kinetin 10^{-4}M were found suitable for dormancy breaking. At this concentration GA_3 obtained 100% and kinetin 85% germination, respectively within 30 days of culture (Fig. 2). The highest radical growth rate, 6.9±0.23 mm was obtained for GA3 + kinetin followed by (6.0±0.9 mm) for GA_3 at 10^{-4}M while kinetin at this concentration produced 3.9±0.3 mm radical length per day (Table 2).

Table 2. Effect of different concentrations and combinations of growth regulators on radical growth rate per day of cherimoya seeds

Treatment	Growth rate ± SD (mm)
GA_3 10^{-3} M	6.0 ± 0.15
GA_3 10^{-4} M	6.0 ± 0.09
GA_3 10^{-6} M	5.7 ± 0.01
Kinetin 10^{-3} M	4.2 ±0.07
Kinetin 10^{-4} M	3.9 ± 0.03
Kinetin 10^{-6} M	3.9 ± 0.02
ABA 10^{-3} M	-
ABA 10^{-4} M	2.5 ± 0.00
ABA 10^{-6} M	2.5 ± 0.00
GA_3 + Kinetin (10^{-4} M)	6.9 ± 0.23
GA_3 + ABA (10^{-4} M)	5.3 ± 0.03
Kinetin + ABA (10^{-4} M)	4.8 ± 0.20
GA_3 + Kinetin + ABA (10^{-4} M)	5.6 ± 0.11

The removal of testa from cherimoya seeds affected germination percentage as well as radical length. Testa removed seeds achieved 80% germination, whereas, intact seeds obtained 65%. The response of testa removal was 25 days earlier than intact seeds. The radical length was 4.8±0.05 mm and 2.6±0.19 mm (Table 1) for testa

removal and intact seeds respectively.

Both GA_3 and kinetin antagonized the effect of ABA. Fig. 3 shows the percentage of radical protrusion at different combined treatments of GA_3, kinetin and ABA. Combination of GA_3+kinetin obtained the highest germination percentage, followed by GA_3+ABA (Fig.-3). The first radical protrusion was observed after 10 days of incubation at GA_3+kinetin. Seeds treated with ABA achieved only 10% germination.

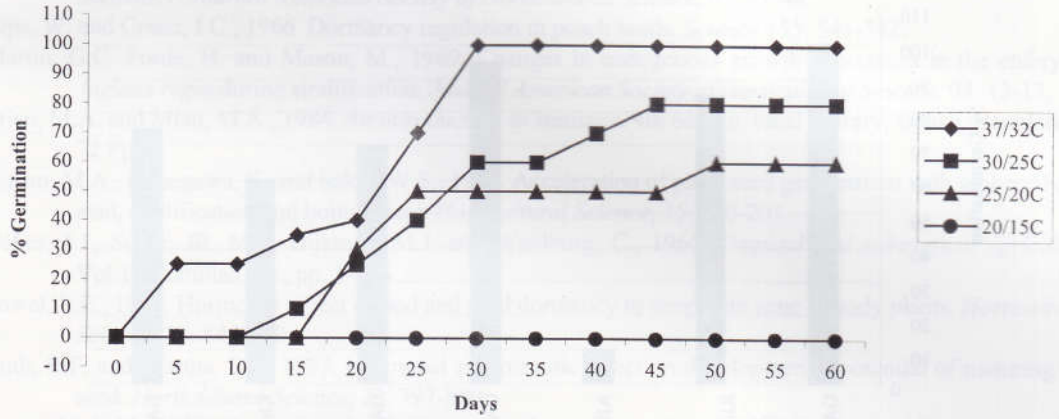

Fig 1. Germination percentage of cherimoya seeds at different day/night temperature as quantified by radical protrusion.

Discussion

The cherimoya seeds consist of thick outer covering (testa) on a brownish white endosperm, which, in turn, contain a small and undeveloped embryo (Sanewski, 1957). This type of embryo requires an extended period and higher temperature for germination (Hudson *et al.*, 1990). Exogenously applied growth regulators can control dormancy. Khan (1971) and Powel (1987) stated that specific growth promoting and inhibiting compounds are involved directly in controlling seed development, dormancy and germination. In the present study, germination of cherimoya was stimulated by the treatment with growth promoting component, GA_3 and kinetin.

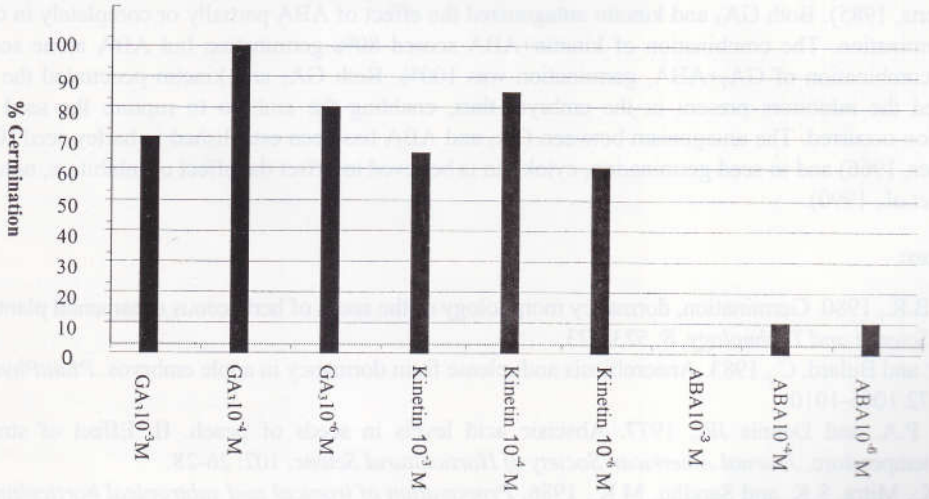

Fig. 2. Germination percentage of cherimoya seeds at various concentrations of growth regulators as quantified by radical protrusion.

Gibberellic acid accelerates the seed germination in plum (Nagao *et al.*, 1980) *Trollius ledebouri* (Hepher and Roberts, 1985). Treatment of *Annona squamosa* seeds with GA_3 increased the percentage of germination (Bose *et al.*, 1986). Abscisic acid, the naturally occurring compound is an important growth regulator for germination (Walton, 1980), which prevents "precocious germination" (Ruth and Martha, 1987), but high inhibitor levels

have been considered responsible for the lack of development in rudimentary embryo (Atwater, 1980). High concentration of ABA obtained lower germination in peach seed after removing the testa (Daniel and Martin, 1972). In the present study, radical were protruded only 10% after 45 days. Hence, ABA might be leached out from the seed resulted germination. Abscisic acid may antagonize the effect of GA_3 by inhibiting enzymes involved in the pathway of gibberellin synthesis (Wareing and Saunders, 1971). Presoaking the seeds in water might lead to the subsequent increased uptake of subsequently applied GA_3.

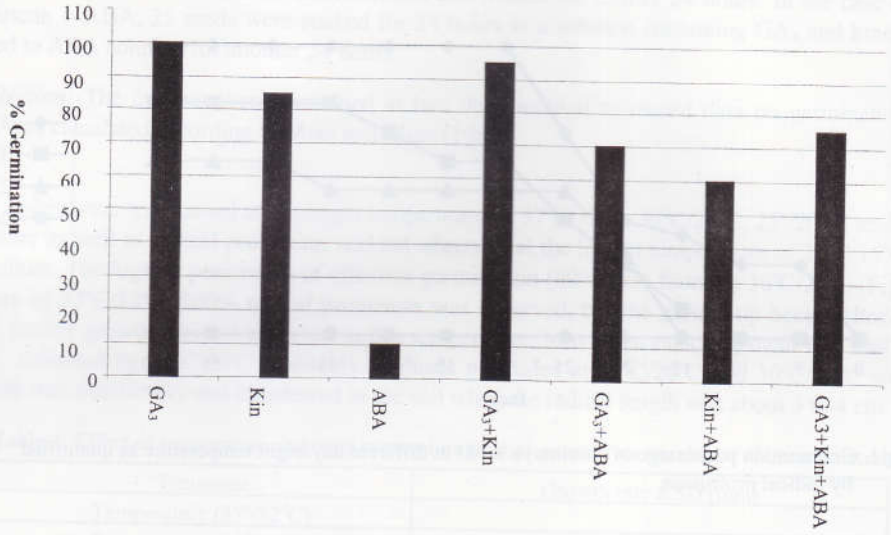

Fig.-3. Germination of cherimoya seeds treated with different growth regulators and their combinations at 10^{-4} M.

The percentage of germination was higher for testa-removed seeds than those of intact seeds did. Seeds have high concentration of ABA in the testa that might reduce the embryo development as well as endosperm enlargement causing less seed germination. Freshly harvested walnut (Martin *et al.*, 1969), plum (Lin and Boe, 1972) and apple (Barthe and Bulard, 1983) have high concentration of ABA in testa and lesser amount in the cotyledon. Physical contact of the testa with the embryo axis has been sufficient to inhibit germination (Hepher and Roberts, 1985). Both GA_3 and kinetin antagonized the effect of ABA partially or completely in cherimoya seeds germination. The combination of kinetin+ABA scored 80% germination but ABA alone scored only 10%. In combination of GA_3+ABA, germination was 100%. Both GA_3 and kinetin penetrated the testa and neutralized the inhibitors present in the embryo, thus, enabling the embryo to rupture the seed coat and germination occurred. The antagonism between GA_3 and ABA has been established in barley seed (Chrispeels and Varner, 1966) and in seed germination, cytokinin is believed to offset the effect of inhibitors, notably ABA (Hudson *et al.*, 1990).

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