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COMMUNICATION I

Breaking Dormancy in Kentia Palm Seeds by Infusion Technique

ABSTRAK

Dalam penyelidikan ini, tiga pelarut organik iaitu diklorometan, dimetil sulfksida (10%) dan aseton, telah digunakan untuk merawat biji benih sebelum dicambahkan untuk membuat penilaian terhadap kesan pengangkutan asid gibberelik (GA_3) terus kepada embrio bagi mengalakkan percambahan yang lebih awal. Tiap-tiap pelarut mengandungi 100 milligram asid gibberelik. Keputusan telah menunjukkan bahawa pelarut-pelarut organik ini berkesan terhadap pengangkutan asid gibberelik kepada embrio biji benih Palma Kentia, untuk mengalakkan percambahan. Tanda percambahan pertama in vitro *dapat diperhatikan selepas dua bulan dikulturkan. Kemunculan plumul dapat diperhatikan selepas empat bulan dicambahkan dalam pasir. Dalam tempoh sembilan bulan kajian, rawatan menggunakan diklorometan didapati amat berkesan. 85 peratus biji benih bercambah dalam pasir steril manakala 90 peratus bercambah* in vitro. *Ada kemungkinan bahawa rawatan ini berfaedah untuk mempercepatkan percambahan spesies palma yang menunjukkan dormansi sebelum percambahan.*

ABSTRACT

In this investigation three organic solvents, namely dichloromethane, dimethyl sulfoxide (10%) and acetone each containing 100 mgl^{-1} gibberellic acid (GA_3) were used to treat the seeds prior to germination, to assess the effecitiveness of these solvents in transporting GA_3 directly to the embryo for triggering an early germination. The results indicate that organic solvents appear effective in transporting the GA_3 to the embryo of Kentia Palm seeds to improve germination with no deleterious effects. The first sign of germination in vitro *was observed after 2 months in culture, while the emergence of the plumule in sand was observed around the 4th month of planting. Over the 9-month study period, for the treatment where dichloromethane was used, 85% of the seeds planted in sterile sand had germinated while in* in vitro, *90% of the seeds had germinated. It is probable that this approach may prove beneficial in improving the germination time in palm species that exhibit long periods of dormancy before germination.*

INTRODUCTION

Kentia palm *(Howea forsteriana),* a slow growing elegant ornamental, has currently a strong world demand as an indoor plant. This species of palm is endemic to the lowlands of Lord Howe Island, off the coast of New South Wales, Australia (Pickard, 1983). The sale of Kentia palm seedlings and seeds form a main source of revenue for the island (Lamont, 1985). The germination of seeds of this palm has been reported to take from 6 months to 3 years (Jones, 1984). This prolonged germination period has resulted in the short supply of this plant material.

Dormancy in palms seeds at maturity has been reported for many palm species (Jones, 1984; Wagner, 1982; Wan and Hor, 1983). The use of GA_3 to stimulate germination of seeds which are physiologically dormant is also well documented (Abu Dahoub *et al.* 1975; Albert, 1970; Anon, 1976, Chandra and Chauhan, 1976; Hartman and Kester, 1975; Krishnamurthi, 1973; Martin, 1968; Venator, 1972). Germination studies carried out by Lamount (1985) on Kentia palms indicated that seeds treated with 250 mgl^{-1} GA_3 in aqueous solution improved germination by about 7% over the control (i.e. without GA_3 treatment). How-

ever, the reduction in time period for germination after GA_3 treatment has not been elaborated upon by the author. A similar study by Wan and Hor (1983) on oil palm seeds indicated that improved germination (7% over the control) was obtained when seeds were treated with an aqueous solution containing 500 mgl^{-1} GA_3, followed by a heat treatment at 40°C for 60 days.

The impermeable hard seed coat in most palm species is one of the main causes for dormancy. The rate of water imbibition by these seeds is very slow and may take several days or weeks. This may be a reason why full promotive effects of GA_3 on germination are not significantly expressed. Much of the GA_3 suspended in water probably settles on the surface of the seeds and only a very low percentage of this GA_3 may reach the embryo. This small amount may not be sufficient to elicit a rapid biological response for triggering an early germination in the seeds.

In this study the use of organic solvents to introduce GA_3 directly to the embryo for improved germination was investigated.

MATERIALS AND METHODS

Mature dried seeds, light green or yellow in colour were obtained from Lord Howe Island. The seeds were depericarped, bulked and thoroughly mixed to ensure homogenity before subjecting them to the various treatments. After bulking, 20 seeds were taken at random for moisture determination and another 20 seeds were cut open and the embryos excised for a viability test using the tetrazolium method.

A total of 5 treatments were studied. As the number of seeds procured was limited, only 40 seeds were employed for each treatment. They were:

a. Immersing in water (control);
b. Immersing in water containing 100 mgl^{-1} GA_3;
c. Immersing in dichloromethane containing 100 mgl^{-1} GA_3,
d. Immersing in 10% dimethyl sulfoxide (DMSO) containing 100 mgl^{-1} GA_3 and
e. Immersing in acetone containing 100 mgl^{-1} GA_3.

The 10% DMSO was prepared using distilled water. For treatment (b) the GA_3 at 100 mgl^{-1} was dissolved directly in water. For treatments (c), (d) and (e) the GA_3 was dissolved in a minimal amount of absolute alcohol and dispensed into the organic solvents.

Five hundred ml of each of the solutions was prepared and 40 seeds were immersed into each of the solutions. They were then placed in an air conditioned room (21° – 22°C) for 12 hours. At the end of 12 hours imbibition, the seeds for treatments (a) and (b) were removed and divided into 2 lots of 20 seeds. One lot from each treatment was planted in sterilized sand in two replicates (i.e. 10 seeds per replicate). The other lot of 20 seeds was sterilized in 20% commercial Clorox for 30 minutes and placed in a sterile sealed container containing sterile distilled water which just covered the seeds. Incubation period in water was 6 days after which the seeds were aseptically cultured on a sterile water – agar (6%) medium. For treatments (c), (d) and (e), after the 12 hour soaking period, the seeds were removed and allowed to dry in an air conditioned room for 30 minutes followed by another 6 hours of vacuum drying in a vacuum desiccator. After removal from the desiccator, the seeds were then allowed to further dry in an air conditioned room for 24 hours. By this time most of the organic solvents had evaporated from the whole seeds. The seeds in each of the treatments were then divided into 2 lots of 20 seeds. One lot from each treatment was then planted in sterilized sand in 2 replicates while the other lot was surface sterilized, kept in sterile distilled water for 6 days and cultured aseptically as for treatments (a) and (b).

The seeds cultured in *in vitro* were maintained at 28° – 29°C in a temperature-controlled room under 12 hour photo-period of 8000 to 10 000 lux intensity provided by fluorescent light. For seeds planted in sand, the sand was moistened to about 60% of its water holding capacity and the seeds were maintained in a germination room at ambient temperature (29° – 30°C) and relative humidity 60–70%. The duration of the experiment was 9 months.

RESULTS AND DISCUSSION

The moisture content of the seeds was 19.58 ± 0.43%, wet weight basis. The tetrazolium test showed a 100% viability indicating that the seeds were alive at the start of the experiment.

The *in vitro* method gave more rapid and

higher germination than the in-sand method over the 9-month study period. In *in vitro*, germination commenced from the second month *(Fig. 1)*.

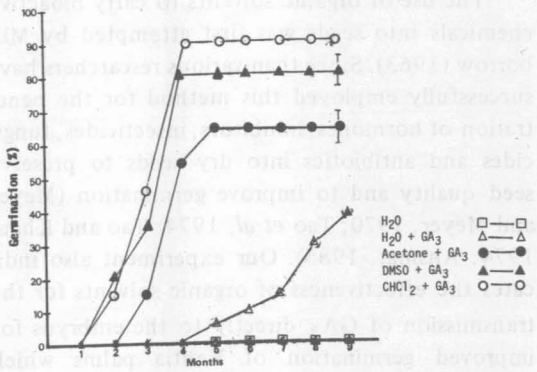

Fig. 1: Cumulative germination percentage over a period of 9 months for seeds in the various treatments, grown in vitro. *Bars indicate standard deviation.*

Seeds treated with dichloromethane and 10% DMSO showed the first signs of germination at 2 months of culture, followed by the treatment with acetone. Seeds imbibed with GA_3 in water showed the first signs of germination at the fifth month of culture. Seeds in the control treatment (water alone) showed no signs of germination over the entire 9 month period of study. At the end of 9 months, 90% of the seeds treated with dichloromethane, 80% of the seeds treated with 10% DMSO and 60% of the seeds treated with acetone had germinated and produced good shoot and root systems; compared to 40% with GA_3 in water and 0% in only water. *Plate 1a* shows a germinating seed and *Plate 1b* shows seed development in the five treatments studied, photographed at 7 months in culture.

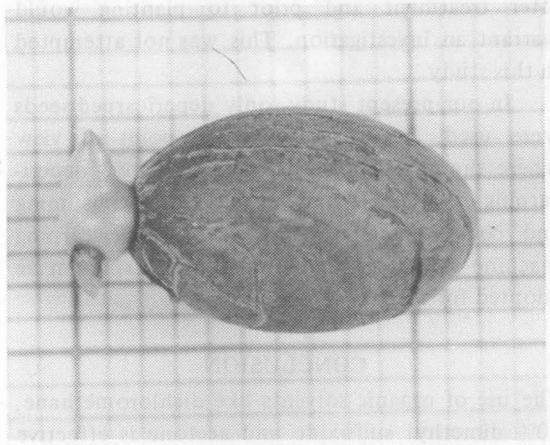

Plate 1a: A germinating seed of the Kentia Palm

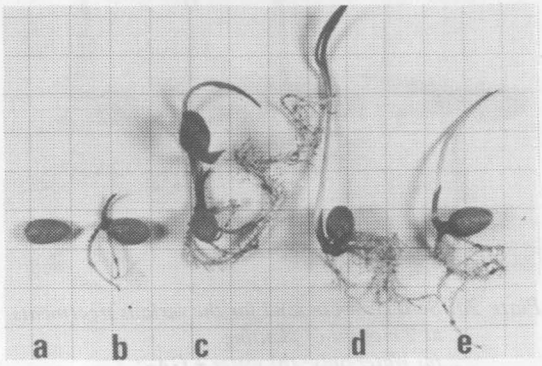

Plate 1b: In vitro *grown seeds in the various treatments at 7 months of culture*
(a) Water only; (b) Water + GA_3;
(c) Dichloromethane + GA_3;
(d) DMSO + GA_3 (e) Acetone + GA_3

In sand *(Fig. 2)*, the first emergence of the plumule was observed at the fourth month of planting. This was observed in the treatment where dichloromethane was used. In the fifth month, plumule emergence was also observed in treatments where 10% DMSO and acetone was used. By the end of the ninth month, 85% of the seeds treated with dichloromethane and DMSO, 50% treated with acetone had normal seedlings compared to 20% with GA_3 in water and 0% with water only. *Plate 2a* shows the development of plants in sand from the various treatments at 9 months of growth. *Plate 2b* shows normally developing seedlings in polybags transplanted from both the *in vitro* culture and from sand.

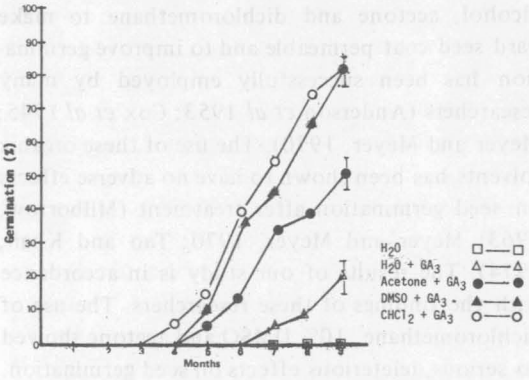

Fig. 2: Cumulative germination percentage over a period of 9 months for seeds in the various treatments, grown in sand. Bars indicate standard deviation.

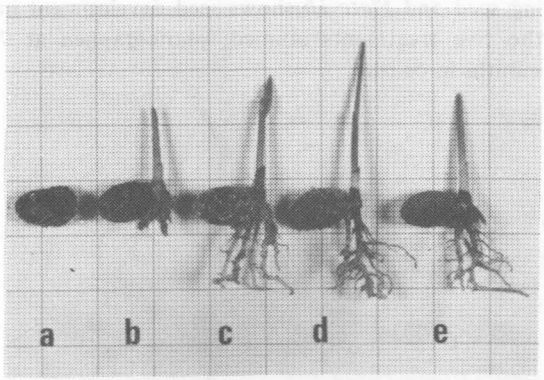

Plate 2a: Seed grown in sand for the various treatments at 9 months of planting
(a) Water only; (b) water + GA_3;
(c) Dichloromethane + GA_3;
(d) DMSO + GA_3 (e) Acetone + GA_3

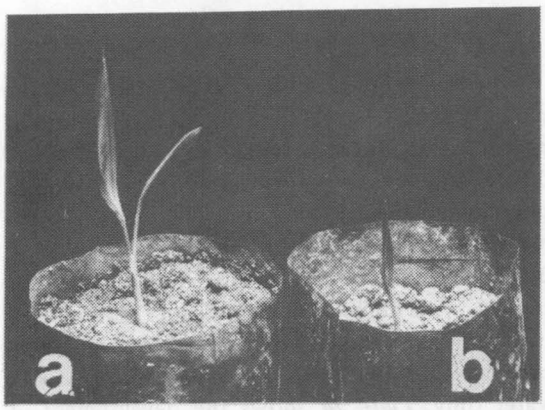

Plate 2b: Seedlings growing in polybag
(a) Seedling germinated in vitro
(b) Seedling germinated in sand

The use of organic solvents such as ethyl alcohol, acetone and dichloromethane to make hard seed coat permeable and to improve germination has been successfully employed by many researchers (Anderson *et al* 1953; Cox *et al* 1945; Meyer and Meyer, 1970). The use of these organic solvents has been shown to have no adverse effects on seed germination after treatment (Milborrow, 1963; Meyer and Meyer, 1970; Tao and Khan, 1974). The results of our study is in accordance with the findings of these researchers. The use of dichloromethane, 10% DMSO and acetone showed no serious deleterious effects on seed germination. On the contrary, they showed a promotive effect where an early germination was encouraged. In both *in vitro* and sand studies, dichloromethane appeared the best solvent for improved germination followed by 10% DMSO and acetone *(Figs. 1 and 2)*.

The use of organic solvents to carry bioactive chemicals into seeds was first attempted by Milborrow (1963). Since than various researchers have successfully employed this method for the penetration of hormones, inhibitors, insecticides, fungicides and antibiotics into dry seeds to preserve seed quality and to improve germination (Meyer and Meyer, 1970; Tao *et al*, 1974; Tao and Khan, 1974; Ahsmat, 1985). Our experiment also indicates the effectiveness of organic solvents for the transmission of GA_3 directly to the embryos for improved germination of Kentia palms which otherwise have a long dormant period of up to 3 years. The optimum concentration of GA_3 that will trigger an early germination within the shortest time period, when transmitted directly to the embryos through organic solvents, was not attempted in this study. The reason being the limited supply of seeds available for study. An investigation in this direction may yield some worthwhile information on further shortening the germination period in this palm species.

The seeds cultured *in vitro* started germinating about 2–3 months ahead of those planted in the sand. The probable reason for this difference could be the extra 6 days of imbibition that these seeds received over those planted in the sand. In sand, imbibition of the seeds would be slower as compared to seeds immersed directly in water. The probability of speeding up germination in sand if seeds were imbibed in water for about six days after treatment and prior to planting would warrant an investigation. This was not attempted in this study.

In our present study, only depericarped seeds were used. From a commercial point of view where thousands of seeds are germinated, depericarping every single seed would be time consuming and cost ineffective. However, a depericarping machine similar to that used for oil palm can be adopted for use.

CONCLUSION

The use of organic solvents like dichloromethane, 10% dimethyl sulfoxide and acetone is effective in transporting GA_3 to the embryo of Kentia palm seeds to improve germination. This approach

may prove beneficial in improving the germination time in palm species that exhibit long periods of dormancy before germination.

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