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# PHYSIOLOGY OF SEED GERMINATION IN *PITTOSPORUM RESINIFERUM* HEMSL.

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## ABSTRACT

The study investigated the influence of exogenous and endogenous factors in the germination of the seeds of *Pittosporum resiniferum Hemsl.* The apparent cessation of any appreciable inbibition after an initial rapid phase, inspite of the the adequacy of organic nutrient reserves in the seed and the failure of excised embryos to develop in a medium supplemented with essential inorganic nutrients and sucrose seeds suggests that mature seeds undergo an innate form of dormancy.

Seeds germinated only under sterile conditions in a solid (agar) medium. Germination was optimal when the seed coats were removed and the medium was supplemented with 0.01% sucrose. The bioassay of water-soluble extracts of the seed coat and endosperm indiciated the presence of growth inhibitors which could not be antagonized by the inclusion of IAA, $GA_3$, or kinetin in the germination medium.

## Introduction

Early reports on *Pittosporum resiniferum* Hemsl., common known as petroleum nut, dealt mainly with its taxonomy and distribution. Because of its high levels of dihydroterpenes and heptane in oil extracts from fruits, recent studies have focused on the mass propagation of the species (Noble, 1978, and Noble and Orallio, 1983). Although the use of marcots (Noble, 1978; Ronquillo and Tañgan, 1983; Veracion and Bersamin, 1983) has been successful, these would hardly meet propagule requirements on a commercial scale.

The very low germination rates of 3-5% obtained under field conditions have been attributed to seed immaturity (Noble, 1978; Veracion, and Costales, 1982). Although Veracion and Costales (1982) and Noble and Orallo (1983) reported a 59-75% germination rate when seeds collected from ripe fruits were immediately used, they failed to specify the physico-chemical or environmental condition during their germination studies.

Hence the work described here was undertaken to investigation the effects of various environmental and physiological factors on the germination of *P. resiniferum* seeds.

## Materials and Methods

The seeds used in the study were taken from mature fruits of *P. resiniferum* plants in their natural habitat, specifically in Luisiana (Laguna province), Baguio City, and Atok (Benguet province). Only seeds measuring at least four mm in length were used for uniformity. Germination as scored by the emergence or extrusion of the radicle was noted daily for six months. Experiments were undertaken at the Botany Research Laboratory where the ambient temperature was maintained at 28-32°C.

To minimize microbial infestation, germination experiments were conducted under highly sterile conditions. Intact seeds were surface-sterilized with 2.0% $HgCl_2$ aqueous solution and scarified seeds with 0.02% $NaHClO_3$ and rinsed repeatedly with sterile water before germination. Petri dishes, filter paper (Whatman No. 42), and test tubes were autoclaved at 15 psi for 15 minutes. Only sterile distilled water was used. Seed transfers and water additions were undertaken in a sterile chamber.

*Imbibition*

Imbibition was examined in intact seeds (control), in seeds scarified by chipping the basal coat, and in seeds whose mucilage adhering to the seed coat was removed by starch. Each treatment was replicated three times with 50 seeds in each replicate. Previously weighed seeds were placed in test tubes containing five ml of distilled water. The seeds were weighed every 24 hours for 10 days. Before weighing, the water adhering to the seed coat was removed with sterilized absorbant tissue paper. Weight increment was taken to reflect water uptake. The water in each test tube was changed daily.

*Germination in distilled water*

To assess the effect of scarification, the basal or entire seed coat of the seed was chipped off. Scarified seeds were germinated in sterile petri dishes lined with moist filter paper.

To determine the influence of temperature on the germination of seeds under continuous light or dark conditions, three ambient temperature regimes were set-up, namely, 15-17°C, 22-25°C, and 28-32°C. Intact seeds were germinated in sterile petri dishes lined with moist filter paper. In the dark set up, petri dishes were wrapped with carbon paper and the addition of water and examination of the seeds were done under weak green light.

Freshly harvested seeds stored for one, two, four, six and eight weeks in a dessicator were used in determining the viability period of the embryo. Seeds were germinated in petri dishes lined with moist filter paper.

In the above experiments, there were three replicate in each treatment and 50 seeds in each replicate. Each dish was moistened to saturation point daily with sterile water. Light provided by cool 40-watt flourescent tubes with an intensity of 40-45 $\mu$Ein $m^{-2}s^{-1}$ at the level of the dishes or cups.

In another experiment, freshly harvested seeds were germinated in a sterile soil medium composed of loam, sand and humus (3:1:1) where a 59-75% germination rate had been reported (Noble and Orallo, 1983; Veracion and Costales, 1982). A hundred seeds were germinated in plastic cups filled with the soil medium. Each cup contained five seeds and was irrigated to saturation point with distilled water every other day to six months.

*Effect of Exogenous Growth Regulators*

To determine the influence of growth-promoting substances, seeds were germinated in petri dishes lined with filter paper moistened with 10 ml of aqueous solutions of 10, 50 and 100 ppm of indoleacetic acid (IAA), gibberellic acid ($GA_3$) and kinetin. Seeds germinated in dishes moistened with sterile water served as the control group. Each treatment was replicated three times with 50 seed in each petri dish.

*Seed and embryo culture*

To determine the effect of supplementary organic and inorganic nutrients on the germination of intact and decoated *P. resiniferum* seeds, $NH_4NO_3$ (5.6 m/l) and four concentrations of sucrose, namely, 1.0, 2.5, 5.0, 10, and 20 g/l were added to enrich the solid basal medium of Raghavan and Torrey (1964). The same basal medium but without sucrose served as the control. The germination response in a liquid medium was also studied using Raghavan and Torrey's (1964) formulation but without agar. The pH of the liquid or solid medium was adjusted to 5.5 with 0.01 N NaOH before autoclaving for 15 minutes at 15 psi. In both media, seeds germinated in the absence of sucrose served as the control. Seeds were also germinated in a plain agar medium to observe the effect of the absence of supplementary nutrients.

Ten ml of each of the three culture media were poured into sterile test tubes for the liquid or petri dishes for the solid medium. The test tubes were stoppered with sterile cotton wads.

In this experiment, both intact and decoated seeds were used. Intact seeds were first surface-sterilized with 2.0% $HgCl_2$ solution for 20 minutes and rinsed several times with sterile distilled water. Seeds with excised seeds coats were surface-sterilized with 0.02% hypochlorite solution for two minutes and rinsed with sterile distilled water. Three replicates per culture medium were prepared and each replicate had 30 seeds.

To determine the responses of the embryo to the three culture media, embryos were excised asceptically and then embedded on the solid medium in sterile petri dishes or floated in the test tubes with the liquid medium. Twenty embryos were placed in each of the three replicates of each culture medium.

The cultures wre maintained in the laboratory at 28-32°C and were given a daily 12-hour illumination period (Raghavan and Torrey, 1964). Cool-white floures-

cent tubes and incandescent bulbs provided a light intensity of about 40-45 $\mu$Ein $m^{-2}s^{-1}$ at the level of the test tubes and petri dishes.

*Morpho-anatomical features of the seed*

Intact seeds and newly germinated seeds were dissected with a sharp razor blade and the size of the embryo was measured under the stereomicroscope. The entire length of the embryo from the tip of the radicle to the tip of the cotyledon and the lengths of the root-hypocotyl axis and the cotyledon were noted.

Some seeds sectioned using the paraffin method (Sass, 1958) for histological studies. The seeds were soaked in 1.0% $HNO_3$ for two weeks to soften the seed coat, fixed in formalin-alcohol-acetic acid, washed for 12 hours in flowing water, dehydrated in a graded tertiary-butyl alcohol series and then embedded in paraffin. Serial sections were stained with safranin-fast green. Tissues of the embryo were then examined under the microscope.

*Endogenous Inhibitors*

Seed coats and endosperms of freshly harvested seeds were excised. Water-soluble substances were extracted using the methods of Battle and Whittington (1969) to determine the presence of endogenous inhibitors. The crude extract was then diluted into a a series of concentrations equivalent to 125, 83, 63, and 42 mg/ml.

Wheat (*Triticum aestivum* var. Trigo I) seeds were used in the bioassay because of its high germination rate, short germination period and sensitivity to inhibitors (Evenari, 1949). Seeds were germinated in petri dishes lined with filter paper moistened with the series of concentrations of the extract. Fifty seeds were placed in each petri dish and each concentration was replicated twice. Germination rates and radicle extension were measured daily for five days. Seeds germinated in distilled water served as control.

## Results

*Imbibition*

The significant increases in the fresh weight of seeds in the different treatment groups would reflect a rapid initial imbibition rate after 24 hours (Figure 1). Seeds scarified by chipping off the basal coat apparently imbibed the least amount of water. Only the control or untreated as well as the starched seeds, however, continued to imbibe water until the third day, after which no further increments in fresh weight were observed until the tenth day. Imbibition rate was highest in the intact seeds.

*Effects of environmental and physical factors and exogenous growth regulators*

No germination was observed in any of the experiments subjecting intact seeds to scarification, different temperature regimes, storage periods, and plant

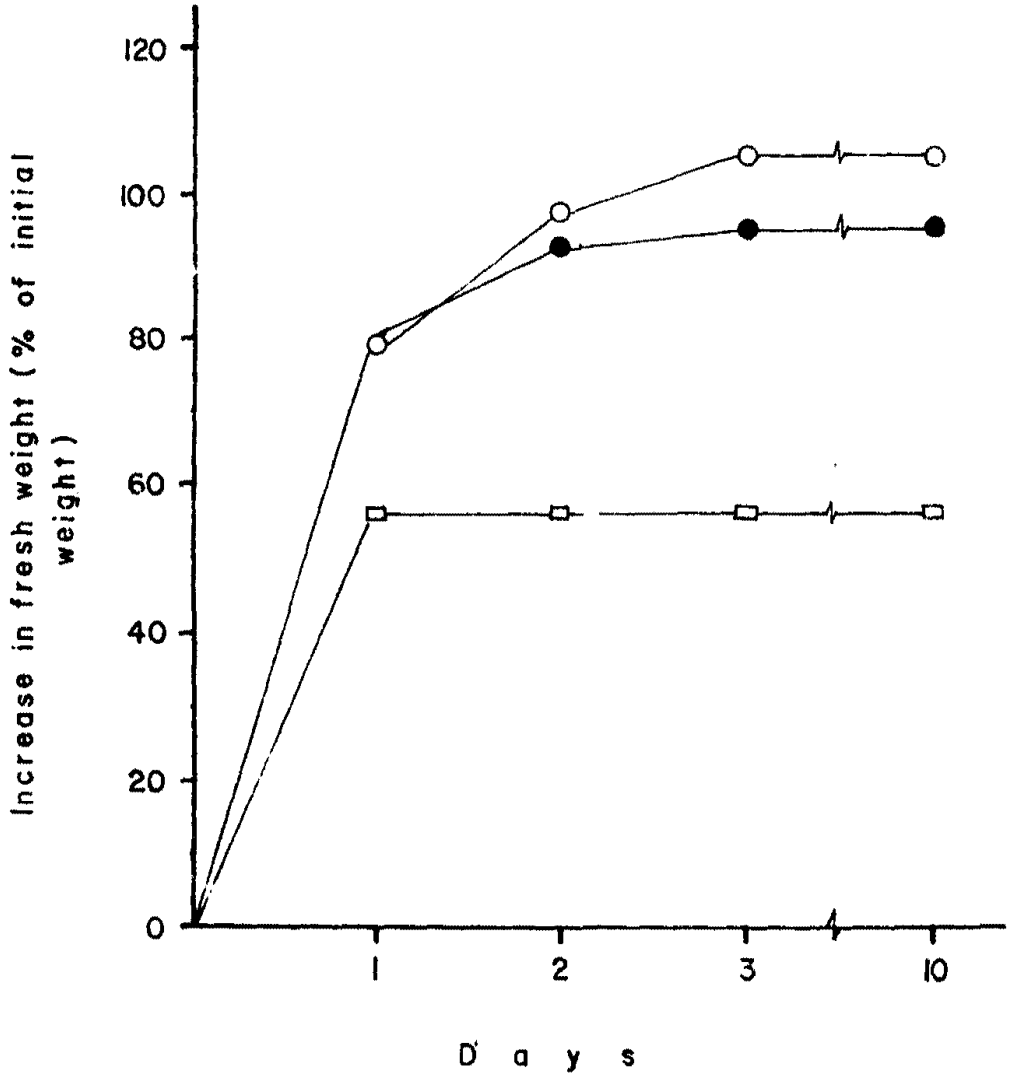



Figure 1. Increase in fresh weight of *P. resiniferum* seeds germinated in distilled water (–○– control; –●– starched; –□– scarified).

growth regulators and germinating them in a loam-seed-humus medium during the entire experimental period.

*Germination of seeds on different culture media*

The germination responses of intact and decoated seeds to liquid and solid media in the presence or absence of sucrose are summarized in Table 1. Seeds failed to germinate in the liquid medium even when it was supplemented with different sucrose levels. Germination occurred only in the agar-based solid medium where seeds with excised seed coats had a higher germination rate than those with intact seed coats.

Table 1. Effect of a liquid or solid (agar) medium supplemented with different levels of sucrose on the germination of *P. resiniferum* seeds with or without seed coat

| *Media* | *Sucrose Content (g/l)* | *Average Percentage Germination* | | *Average Germination Time (Days)* | |
|---|---|---|---|---|---|
| | | *With Seed Coat* | *Without Seed Coat* | *With Seed Coat* | *Without Seed Coat* |
| Liquid culture medium (without agar) | 0 | 0 | 0 | – | – |
| | 0.10 | 0 | 0 | – | – |
| | 0.25 | 0 | 0 | – | – |
| | 0.50 | 0 | 0 | – | – |
| | 1.00 | 0 | 0 | – | – |
| | 2.00 | 0 | 0 | – | – |
| Solid culture medium (with agar) | 0.00 | 0.0 | 44.0 | 1 | 74.4 |
| | 0.10 | 18.9 | 55.1 | 85.0 | 83.1 |
| | 0.25 | 30.0 | 44.3 | 120.8 | 82.8 |
| | 0.50 | 0.0 | 24.0 | 1 | 82.2 |
| | 1.00 | 0.0 | 21.6 | 1 | 86.4 |
| | 2.00 | 0.0 | 2.4 | 1 | 27.0 |
| Agar only | 0.00 | 0.0 | 20.3 | 1 | 74.0 |

[1]No germination was noted.

The presence of sucrose in the solid medium also affected the germination response of decoated seeds. While the highest germination percentage (55.1%) was observed in the medium containing 0.10% sucrose, germination rates declined with increasing sucrose concentration in the medium. Thus, when the sucrose content was increase to 1.0 and 2.0%, the germination percentage decreased to 21.6 and 2.4%, respectively. The control group had a 44.0% germination rate. A 20.3% germination rate of decoated seeds was noted in a plain agar medium.

Intact seeds germinated only when the solid medium contained 0.10% and 0.20% sucrose, with 18.9 and 30.0% rates, respectively. In the absence of sucrose and at higher sucrose concentrations in the solid medium, no germination was observed even after six months.

While it took intact seeds 120 days on the average ot germinate in the solid medium containing 0.25% sucrose, the mean germination period was shortened to 85 days when the sucrose content was only 0.10% (Table 1). Decoated seeds grown on a solid medium with 2.0% sucrose had the lowest germination percentage but the lowest mean germination period of only 27 days. The average germination time of other decoated seeds grown under lower sucrose level ranged on the average from 74-86 days.

*Growth of excised embryos in liquid and solid media*

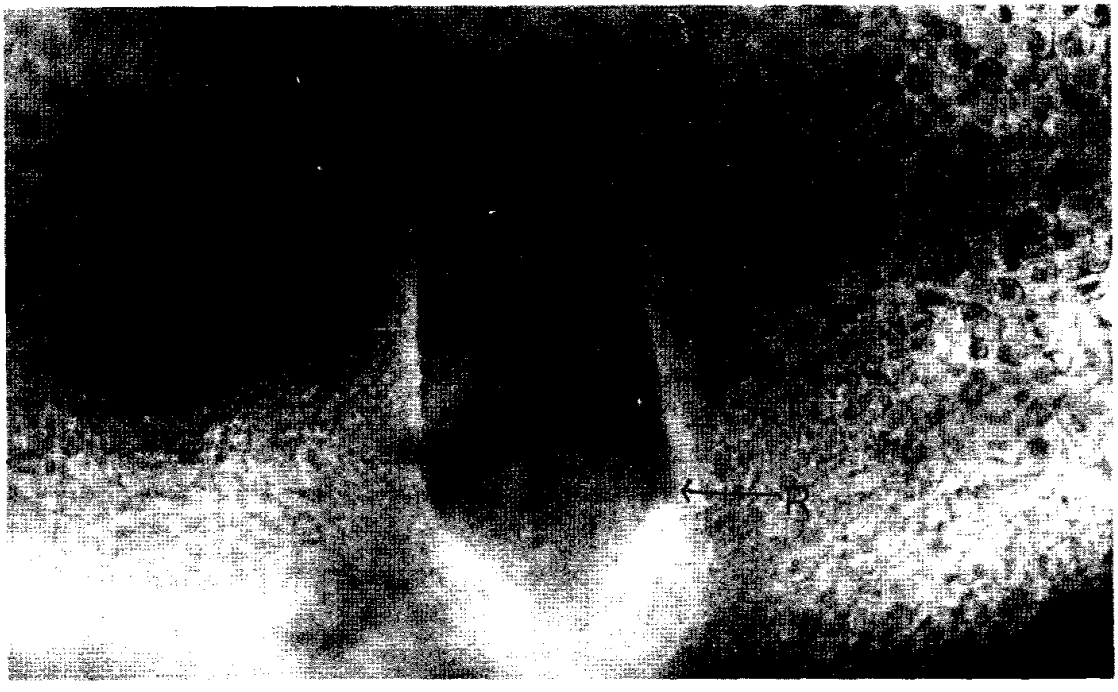
When excised embryos were cultured in liquid and solid media, no enlargement or growth was evident even after one month. The addition of varying levels of sucrose into the medium also did not influence the growth of the embryo.

*Seed mophology before and after germination*

Microscopic examination of larger seeds showed the presence in the hollow space near the hilum of a small torpedo-shaped embryo with a differentiated root-hypocotyl axis and cotyledon (Figure 2). Its shoot apex, however, has apparently not given rise to the epicotyl. The seed is albuminous with an endosperm occupying most of the volume of the seed. The embryonic length from the tip of the radicle to the tip of the cotyledon is only 0.60 mm on the average and extends from one-third to one-fourth the width of the seeds (Table 2). The hypocotyl-root axis is

Table 2. Changes in the size of the embryo during germination

| | *Average Length (mm)* | |
|---|---|---|
| | *Initial* | *Germinated* |
| Embryo | 0.60 | 4.05 |
| Hypoctyl-root axes | 0.34 | 2.07 |
| Cotyledon | 0.29 | 1.98 |

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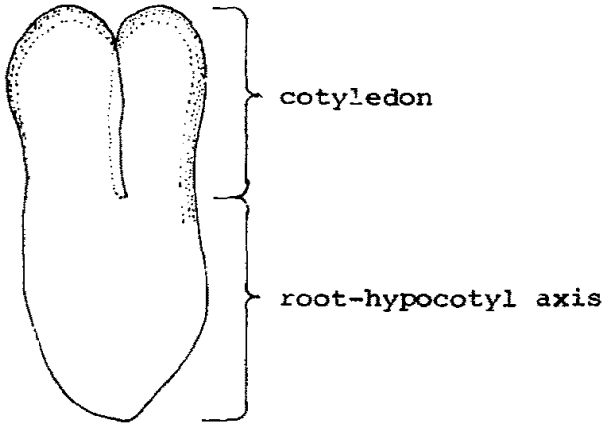


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Figure 2. Photomicrograph under LPO (A) and diagrammatic representation (B) of *P. resiniferum* embryo (C=cotyledon; R=root-hypocotyl axis).

about 0.34 mm in length while the cotyledon is 0.29 mm long. The presence of a distinct procambium, ground meristem, and protoderm would suggest that the tissues of the embryo are already organized when mature fruits are shed.

After germination in the solid culture medium, embryos measured approximately 4.05 mm in length. The hypoctyl-root axis was about 2.07 and the cotyledon 1.98 mm long.

*Bioassay of endogenous growth inhibitors*

Although there were no significant differences in the germination percentage of wheat seeds in the distilled water and seed extract media, there was apparently a significant delay in the average germination time of seeds in the seed coat extracts, except at the lowest concentration (Table 3). Although the average germination time of wheat seeds in the endosperm extracts was longer than that in ditilled water, the differences were not statistically significant.

After 24 and 48 hours, growth or extension of the radicle, however, was apparently inhibited in seeds germination in the aqueous extracts of both the whole seed and endosperm (Table 4). The inhibition was apparently more severe in the seed coat extracts.

Table 3. Germination responses of *Triticum aestivum* L. var Trigo I to crude seed coat and endosperm extracts of *P. resiniferum* seeds

| *Extract* | *Extract Concentration (mg extract/ml distilled* | *Average Germination Time (Days)* | *Percentage Germination* |
|---|---|---|---|
| Control | 0 | 1.5 | 86 |
| Seed coat | 125 | 2.2* | 88 |
| | 83 | 2.2* | 88 |
| | 63 | 2.2* | 86 |
| | 42 | 2.0 | 85 |
| Endosperm | 125 | 1.9 | 90 |
| | 83 | 1.9 | 86 |
| | 63 | 1.9 | 86 |
| | 42 | 1.5 | 84 |

*Significantly different at $P < 0.05$ (ANOVA).

Table 4. Root of *Triticum aestivum* L. var Trigo I seedlings germinated in different concentration of seed coat and endosperm extract of *P. resiniferum*

| *Extract* | *Root Length (mm) After Day 1* | *Day 2* |
|---|---|---|
| Control | 10.1 ± 3.6 | 25.6 ± 7.1 |
| Seed coat extract | | |
| 125 mg/ml | 4.3 ± 2.2* | 10.4 ± 3.2* |
| 83 mg/ml | 4.1 ± 1.8* | 10.3 ± 2.5* |

Table 4 (Continued)

| *Extract* | *Root Length (mm) After Day 1* | *Day 2* |
|---|---|---|
| 63 mg/ml | 4.4 ± 1.9* | 9.4 ± 3.6* |
| 42 mg/ml | 5.6 ± 2.4* | 11.4 ± 3.0* |
| Endosperm extract | | |
| 125 mg/ml | 4.3 ± 1.4* | 9.9 ± 3.3* |
| 83 mg/ml | 6.1 ± 2.7* | 13.5 ± 2.5* |
| 63 mg/ml | 7.9 ± 2.1* | 15.5 ± 3.6* |
| 42 mg/ml | 9.0 ± 3.5* | 16.8 ± 2.8* |

*Significantly different from control at $P < 0.05$.

## Discussion

The high water intake by *P. resiniferum* seeds could be attributed to the presence of mucilagenous substances on the seed coat. Seeds with mucilage or mucilage-like substances tend to absorb more water due to the hydrophillic nature of these materials (Mayer and Shain, 1974; Bewley and Black, 1978). The removal of the mucilage may account for the lower water uptake of starched seeds. The relatively lower increase in fresh weight of the scarified seeds might reflect a leaching out of substances from the seed into the medium (Flentje and Saksena, 1964; Larson, 1968).

The cessation of water uptake after a rapid initial imbibition in the intact, scarified, and starched seeds would suggest that the seed of *P. resiniferum* possibly undergoes in innate form of dormancy since it has been reported that non-viable seeds and those with an innate form of dormancy do not further imbibe water or increase their fresh weight after a rapid initial water uptake (Shull, 1916; Esashi and Leopold, 1968; Koller, 1972; Villier, 1972; Bewley and Black, 1978). Germinating seeds usually increase their fresh weight further after an initial imbibition stage (Wareing, 1969; Bonner, 1968). The failure of intact seeds to germinate under favorable temperature and light regimes further supports the idea that the seed has an innate form for dormancy since it apparently requires more than the favorable environmental conditions or factors that would normally induce a seed with only an imposed type of dormancy to germinate (Wareing 1969).

Noble and Orallo (1983) and Veracion and Costales (1982) reported a 59-75% germination rate when seeds from newly harvested mature fruits were used and germinated in a medium composed of loam, sand and humus (3:1:1), but failed to describe the environmental conditions under which their experiments were undertaken. Using the same medium and likewise freshly harvested seeds, we however, failed to induce a similar germination response. Furthermore, using seeds stored

for different periods did not affect their non-germination. Hence our data seemingly fall to support the possibility of an innate short viability of *P. resiniferum* seeds (Noble and Orallo, 1983; Veracion and Costales, 1982).

Since the removal of the seed coat, partially or wholly, through mechanical or chemical means did not also stimulate the seed to germinate, the seed coat could not be the primary factor or the only factor contributing to the dormancy of the seed. The coat of some seeds could hinger water uptake, limit oxygen diffusion and resist embryo expansion (Taylorson and Hendricks, 1977). Swollen mucilages on the seed coat could also lengthen the diffusion pathway of oxygen in the seed and, thus, also inhibit seed germination (Negbi, *et al.*, 1966; Witztum *et al.*, 1969; Mayer and Shain, 1974). Excision of the seed coat or scarification usually removes this barrier imposed by the seed coat and allows the seed to germinate (Taylorson and Hendricks, 1977).

Exogenously added growth regulators were also ineffective in stimulating seed seed germination. It has been reported that growth promoters such as gibberellins and cytokinins could induce seeds with an innate form of dormancy to germinate (Khan, 1975). Webb *et al.*, (1973, 1973a), however, did not observe any influence of growth promoters in the germination of *Acer saccharum* and *A. pseudoplatanus* seeds which also undergo an innate form of dormancy. Apparently the endogenous levels of growth promoters in the seeds of *P. resiniferum* may not be a limiting factor in their germination.

Germination occurred only when seeds were grown on asceptic culture media with agar as a solidifying agent. No germination was, however, observed in liquid media. A similar significant inhibition of sugar beet seed germination had been noted (Heydecker and Chetram, 1971). More successful embryo cultures had been obtained also on agar than liquid surfaces (Narayanaswami and Norstog, 1964; Rappaport, 1954) apparently because of reduced oxygen levels in the latter (Norstog, 1961).

The relatively lower germination rate of seeds in the agar medium without sucrose would, however, indicate that the endosperm might not be lacking in organic nutrients. It is then possible that inorganic rather than organic nutrients may be limiting the growth of the embryo. *Cattleya mollie* and *C. trianaei* seeds absolutely require exogenously supplied ammonium and nitrate ions in the nutrient medium for germination (Raghavan and Torrey, 1964).

The lower germination rates observed with sucrose concentrations higher than 0.10% might be due to the higher osmotic pressure of the medium. A high osmotic pressure of the culture medium could inhibit embryonic growth (Rappaport, 1954). During germination the movement of water into the seed decreases as the water potential of the medium is reduced by the presence of osmotic substances (Bewley and Black, 1978).

Failure of the excised embryo to grow on a culture, medium containing all essential inorganic and organic nutrients suggests that, aside from nutrient reserves, the endosperm probably contains other substances necessary for embryo growth.

Extracts of seeds from which embryos have been removed occasionally enhanced the *in vitro* germination and growth of isolated embryos (Narayanaswami and Norstog, 1964; Rappaport, 1954).

Microscopic examination of the *P. resiniferum* seed revealed that the embryo is extremely small and torpedo-shaped, which feature characterizes an immature embryo in *Capsella.* (Raghavan and Torrey (1963). The excised embryo from the seeds of *P. resiniferum* fruits that had alrady been shed still undergoes some enlargement before germination. This development pattern is similar to that of the *Fraxinus* seed which has a physiologically immature embryo (Steinbauer, 1937). Although the *Fraxinus* embryo attains its maximum size and is fully differentiated with a hypocotyl, cotyledon and epicotyl at maturity while fruits are still attached to the plant, it still undergoes considerable growth in the water before it can germinate (Steinbauer, 1937; Wareing 1969; Villiers, 1972). In the case of *P. resiniferum* seeds, an increase of about six to 6.5 times in the size of the embryo was noted upon the onset of germination when the radicle emerged from the seed. It would appear then that the *P. resiniferum* embryo is still immature when ripe fruits are shed. This also strongly suggests an apparent state of innate dormancy undergone by the seed.

The wheat-seed germination bioassay of *P. resiniferum* seed extracts indicated the presence of water-soluble growth inhibitors in both the seed coat and endosperm. Evenari (1949) reported that radicle or root growth inhibition by seed extracts are indicative of the presence of germination inhibitors. The sugar beet seed germinates best when the inhibitors present in the seed cluster are washed of (Heydecker and Chetram, 1971). The presence of such inhibitors might also explain the dormancy as well as the long germination time of the *P. resiniferum* seed. The greater reduction in root growth of wheat seedlings treated with seed coat extracts would suggest the presence of higher levels of endogenous inhibitors or more potent inhibitors in these tissues than in the endosperm, which may account for the lower germination rates and the longer germination time of intact seeds. The observed failure of IAA, $GA_3$, and kinetin in initiating germination may also suggest at the least that these inhibitors are not rendered ineffective by these exogenously applied growth promoters.

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