GERMINATION OF SEEDS OF Myrciaria Cauliflora (MART.) BERG.(MYRTHACEAE)[1]

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ABSTRACT- Germination of seeds of *Myrciaria cauliflora* (Mart.) Berg. is indifferent to light. After 12 days of incubation almost 100% of germination was obtained at 30 ^{O}C. Moisture content of the seeds was associated to the germination process. Below 30% moisture, seeds failed to germinate. These seeds are short-lived even at high moisture levels. Leakage of electrolytes and organic solutes suggested membrane deterioration as the main factor responsible for the loss of viability. Storage conditions which avoid dehydration of the seeds are necessary to extend viability.

Additional index terms: recalcitrant seed, seed moisture, seed viability.

GERMINAÇÃO DE SEMENTES DE JABUTICABA

RESUMO- A germinacao de sementes de jaboticaba *(Myrciaria cauliflora* (Mart.) Berg.) é indiferente à luz. Após 12 dias cerca de 100% de germinação foi obtida à temperatura constante de 30 ^{O}C. O teor de umidade das sementes foi associado ao processo de germinação. Abaixo de 30% de umidade as sementes não germinaram. Estas sementes tem longevidade curta mesmo com altos níveis de umidade. Perda de eletrólitos e substâncias orgânicas sugerem uma deterioração de membranas como fator principal pela perda da viabilidade. Condições de armazenamento que evitem a desidratação das sementes são necessárias para prolongar a viabilidade.

Termos adicionais para indexação: semente recalcitrante, umidade, viabilidade.

INTRODUCTION

In the Brazilian flora, the Myrthaceae family is represented by at least 1200 species. From these, about 200 species produce edible fruits (Hoehne, 1979). *Myrciaria cauliflora*, although known for more than a hundred years for its edible fruits, is scarcely cultivated in this country. Its natural habitat is in the tropical forest of Brazil (near the tropic of Capricorn). This species produces fruits several times during the year and although the fruits are shed under the tree, the presence of germinating seeds or seedlings is seldom encountered.

Seeds which can be dried and in which longevity is increased by decrease in temperature and moisture content have been described as orthodox seeds. However, there is another group of species which produce seeds which normally never dry out on the mother plant. They are shed in moist condition and indeed they are killed if the moisture content is reduced below some relative high critical level: these seeds have been described as recalcitrant (Roberts, 1973a).

Recalcitrant seeds are produced mainly by two types of plants: by those growing in aquatic environments where seeds would not normally dry out and by perennial plants which produce seeds in a relative humid environment. It seems that those recalcitrant which have short period of viability are restricted to humid tropical areas where the environment is suitable for seedling growth all year round. Thus, a study was initiated to determine some of the factors involved in the germination process of *M. cauliflora*.

MATERIAL AND METHODS

Seeds (average mass of an individual seed = 278 ±19 mg) of *Myrciaria cauliflora* (Mart.) Berg. collected from trees around Campinas (22^{o} 54'S 47^{o} 5'W) were used throughout the experiments. Seeds were taken from the fruits and the pulp removed with sawdust. They were then washed with distilled water and dried to remove surface water. Whole fresh fruits and clean seeds (about 50% moisture content on fresh matter basis) were stored in polyethylene bags in incubators at 10 and 25 ^{0}C and sampled periodically for germination. Samples of 100 seeds were stored in 3Ox4Ocm polyethylene bags with the following characteristics: thickness = 80 µm; diffusion of 5g water vapour.m^{2}.day^{-1} and 2500cm^{3} O_2.m^{2}.day^{-1} at 38 ^{o}C and 90% relative humidity.

Moisture content

Seeds were placed in open Petri-dishes in a ventilated chamber (28 ± 2 ^{O}C) for 24 to 48h for fast dehydration or kept in perforated polyethylene bags in the laboratory for

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one month for slow dehydration. Ten samples of 10 seeds each were weighed periodically until the desired moisture content was obtained. Dry weight of the seeds was obtained after 48h in a ventilated oven (80 ^{o}C). Seed moisture content was expressed on fresh matter basis.

Germination

In order to determine the optimum conditions for germination, seeds were removed from fresh fruits and placed in 9 cm Petri- dishes on filter paper wetted with distilled water and kept in incubators for 12 days in continuous light or darkness at constant temperatures (with exception of one experiment in which were tested 20, 25 and 30 ^{o}C, most of the experiments were carried on at 30 ^{o}C). A seed was considered germinated after the protrusion of the radicle (about 3 mm). Six replicates of 10 seeds each were used per treatment.

Efflux of electrolytes and organic substances

Three replicates of 10 seeds each were placed in beakers with 50 ml deionized water at 25 ^{o}C and the efflux of electrolytes recorded using a conductivimeter (cell constant 1 cm^{-1}) according to Simon & Harum (1972). For the efflux of organic substances a 4 ml aliquot was taken and determined using a spectrophotometer at 264 nm as described by Pollock (1969).

Statistical analysis

For the direct comparison of two means the Student *t* test was used at P<0.05. For more than two means the data were subjected to an analysis of variance after transformation of the percentages to arcsin. When *F* was significant the LSD was calculated by the Tukey test at P<0.05.

RESULTS

Germination of the seeds

Although no significant differences were found among the treatments (only light treatment at 25 ^{0}C), the highest germination percentages were obtained when seeds were incubated at 30 ^{o}C in darkness (Table 1).

TABLE 1- Effect of temperature and light on germination of seeds of *M. cauliflora* (48.84 ± 1.3% moisture content). Results after 12 days of incubation.

Treatments		Germination %
	light	80ab
20 ^{o}C	darkness	80ab
	light	70b
25 ^{o}C	darkness	82ab
	light	95ab
30 ^{o}C	darkness	100a

Different letters differ significantly at 5% level (Tukey test).

Seeds with different moisture content

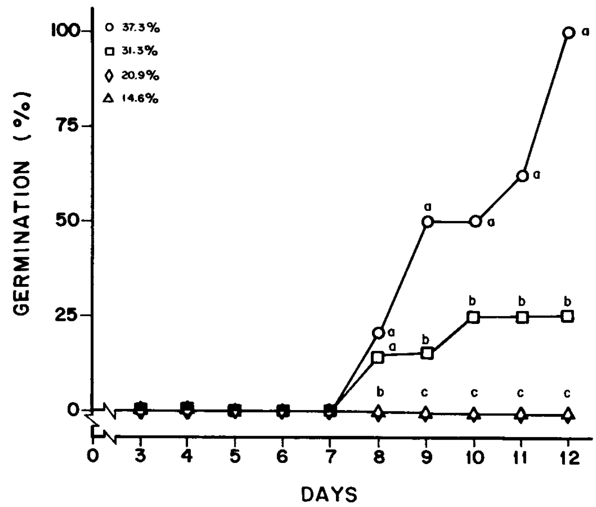

FIGURE 1- Germination of seeds of *M. cauliflora* with different moisture contents (%). Different letters mean a significant difference at 5% level, among different treatments (Tukey test).

Seeds with 37, 31, 20 and 14% moisture content were incubated at 30 ^{o}C in darkness (Fig. 1). The germination process was associated with the seed moisture content. Lowering the moisture content to 31% reduced germination and below this point, seed failed to germinate after 12 days. These results were obtained from seeds dehydrated for 48 h in a ventilated chamber. Similar results were recorded for slow-dehydrated seeds (about one month in perforated polyethylene bags: control, non-dehydrated seeds (40.84 ± 0.4% moisture content) resulted in 100% germination after two weeks; after four weeks of incubation, seeds with 20.7% moisture content produced 2.5% germination and seeds with 13.0% moisture content failed to germinate (0% germination). By the end of the study, non-germinated seeds with low moisture content were dissected under a stereoscopic microscope. The embryonic axes of these seeds were completely decayed. The tetrazolium test for viability according to Delouche et al (1962) was inadequate for these seeds.

The red color of the seeds probably due to the presence of anthocyanin makes impossible the visualization by the tetrazolium test. Extraction of the pigment by methanol:HCl 1% gave a peak of absorbance in the spectrophotometer at 530 nm and a TLC developed with butanol:acetic acid:water (5:4:1) or methanol:HCl 1:1 showed a pink spot that turned bluish when exposed to ammonia vapor.

Efflux of electrolytes and organic solutes

There was a negative relationship between moisture content and efflux independent of the storage temperature (10 and 25 ^{o}C); the higher the moisture content, the lower the efflux of electrolytes and solutes from the seeds (Fig. 2A and B and Table 2).

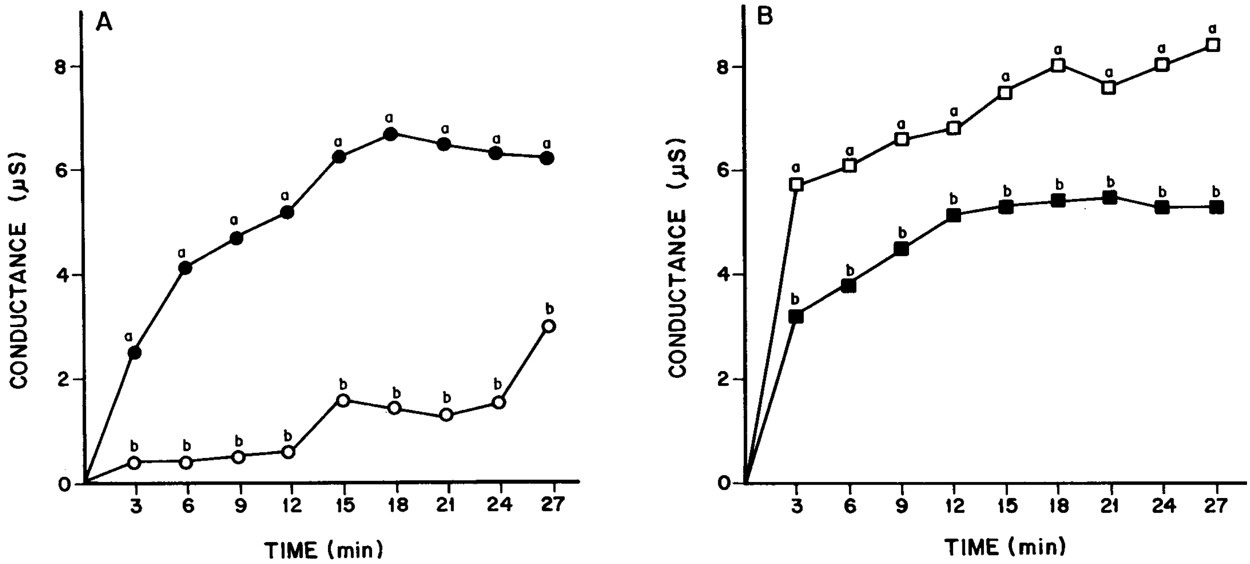

FIGURE 2- A. Efflux of eletrolytes from seeds of *M. cauliflora* with different moisture contents. ●=24%; ❍= 35%, stored one week at 25 ^{o}C. B. Efflux of electrolytes from seeds of *M. cauliflora* with different moisture contents. ■=30%; ❑=23%, stored one week at 10 ^{o}C. Different letters show significant difference at 5% level between treatments (Student "t" test).

TABLE 2- Efflux of organic solutes from seeds of M. cauliflora stored at different temperatures and different moisture content.

Temperature oC	Moisture Content %	Absorbance 264nm
	27 ± 0.9	0.322a
10	45 ± 1.5	0.192b
	28 ± O.8	0.109A
25	34± 1.0	0.083B

Figures followed by different letters differ significantly at 5% level. Small letters for 10 ^{O}C and capital letters for 25 ^{O}C.

Storage of the seeds

Seeds taken from fruits stored at 10 and 25 ^{o}C were short-lived. Even with a very high moisture content (above 50%) after 20 days of storage the germination was 10%, and after 40 days the seeds did not germinate, independent of storage conditions. Stored seeds also did not possess long viability (after 200 days) even when the moisture content was very high (about 50%). The percentage of germination of seeds stored at 25 ^{o}C decreased sharply after 40 days of storage and from 60 days onward no germination was detected after 10 days of incubation. For the seeds stored at 10 ^{o}C, similar patterns were found up to 60 days although lower percentages of germination were obtained when compared to the seeds stored at 25 ^{o}C. From 60 to 200 days of storage at 10 ^{o}C, the germination remained at low values (Fig. 3).

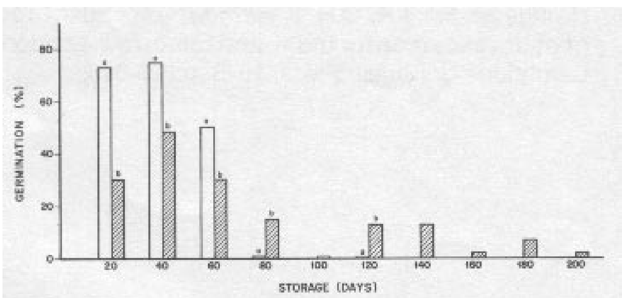

FIGURE 3- Germination of seeds of *M. cauliflora* during storage: open bars = seeds stored at 25 ^{o}C; hatched bars = seeds stored at 10 ^{o}C. Different letters mean a significant difference at 5% level (Student *t* test).

DISCUSSION

The results of seeds germination with different moisture contents showed a typical behavior of recalcitrant seeds (Roberts, 1973a). Independent of the drying rate, seed viability was strongly reduced by low moisture content. Such seeds need a high moisture content to maintain their viability. They are also known as non- orthodox seeds (Bewley & Black, 1985). Some economically important tropical species are known to have recalcitrant seeds such as *Teobroma cacao*, *Hevea brasiliensis*, *Persea american*a, *Mangifera indica* and *Cocos nucifera* (King & Roberts, 1980a; Barrueto, Pereira & Neves, 1986; Corbineau & Come, 1988).

Because these recalcitrant seeds deteriorate so rapidly, special care needs to be taken in the methods adopted for handling, storing, packing and germination. Storage of recalcitrant seeds is still a problem. Even stored with a high moisture content, the life-span of the seeds is short. Traditional methods of storage are inadequate and in some cases low temperatures cause irreversible damages (Roberts, 1972; King & Roberts, 1980b). In *M. cauliflora*, storage of seeds inside the fruit proved not to be adequate. Percentages of germination were very low after 20 days of storage (less than 10%). Similarly no germination was detected when seeds from fruits collected one month after been shed from the trees were tested for germination (data not shown).

The temperature of storage seemed to be not so important. A reduction in germination after storage at 10°C for 20 to 60 days and a higher conductivity in the leachates from seeds stored at 10 °C than at 25 °C could suggest a chilling injury. Nevertheless, at 10 °C the germination was maintained for a much longer period (200 days) but at very low values.

The lower germination after 80 - 200 days storage at 25 °C than at 10 °C in polyethylene bags could be a result of lack of oxygen. Seed respiration is expected to be much higher at 50% moisture content at 25 °C than at 10 °C. Although seed respiration was not measured the number of seeds per bag and the gaseous diffusion rates of the polyethylene used do not make certain an anoxious environment to the stored seeds.

The redish color of the seeds of *M. cauliflora* makes impossible the use of the tetrazolium test for viability. In this case, solute efflux might provide better evaluation of seed viability.

Seeds with high moisture content showed a negative relationship between moisture content and efflux of electrolytes and organic solutes. This strongly suggests irreversible damages to membranes (Roberts, 1973b). Other morphological and biochemical alterations are also known such as chromosomes, ribosomes and oxidative enzymes (Villiers, 1974; 1975).

From the ecological point of view bats seem to be mainly responsible for the dispersal of this species. They usually split out the seeds after eating the fruits. Such seeds. if they fall on humid ground as commonly found in a tropical forest, can germinate successfully.

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