

DENSITY SEPARATION OF GERMINATED SEEDS OF *MELALEUCA LEUCADENDRA* (L.) L. (MYRTACEAE)

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RESUMO

A seleção das sementes germinadas *Melaleuca leucadendra* (L.) L. (Myrtaceae) com três dias de idade foi obtida através da separação por densidade com o uso de solução de 60% (p:v). Este método foi eficiente para a separação de 100% das sementes germinadas com a produção de plântulas normais, eliminando assim as sementes não germinadas que libera substâncias ativas responsáveis pela inibição do alongamento do hipocótilo de alface.

Palavras Chave: seleção de sementes, sementes viáveis, separação por densidade

ABSTRACT

The improvement of germinated seeds of *Melaleuca leucadendra* (L.) L. (Myrtaceae) was obtained by density separation of three-day old seedlings in 60% sucrose solution (w:v). This method demonstrated to be efficient for separation and yield 100% of normal seedlings eliminating non-germinating seeds which liberate dark becoming substances responsible for inhibition of lettuce hypocotyl elongation.

Key words: seed selection, viable seeds, density separation

INTRODUCTION

The selection of germinated seeds from non-germinated seeds by density separation has been described by Taylor et al. (1978) in *Capsicum annum* L. and *Apium graveolens* L. by the use of sucrose solutions. Taylor and Kenny (1985) proposed density separation as a routine procedure for the improvement of germination of seeds of *Brassica oleracea* L.

Jordão et al. (1988) reported the selection of viable *Hormidium coriaceum* seeds by density separation with discontinuous sucrose gradient. This technique showed to be efficient for the enrichment of the low proportion of viable lots of this orchid seeds.

Some seeds liberate phytotoxic compounds which are responsible for the inhibition of seed germination of another species and sometimes of the own species (Maguire, 1977).

This fact was observed in seeds of *Melaleuca leucadendra* when seedlings maintained with non-germinated seeds presented slight inhibition of seedling growth.

Melaleuca leucadendra (L.) L. yields cajeput oil and present timber useful (Willis, 1973). According to Blake (2004) cajeput oil is noted for having the following actions and properties: analgesic, antimicrobial, antineuralgic, antiseptic for internal use, antispasmodic, cicatrizing, emollient, pediculicide, pulicide, rubefascent, sedative and sudorific. The cajeput oil is a volatile oil distilled from leaves of *Melaleuca leucadendra* and contains 50-60% of eucalyptol (cineol), L-pinene, terponeol, valeric, butyric benzoic and other aldehydes (Anonymous, 2004). Castro et al. (2005) working with economic viability of cultivation and essential oil extraction of *Melaleuca alternifolia* Cheel, another species with economic potential, calculated the annual profit and observed the low probability for the activity to become economically unviable. Because the low number of viable seeds of *Melaleuca leucadendra*, the improvement of germinated seed quality by density separation was carried out and the results are presented in this paper with proposal of this seed selection to improve the seed batches by plant growers.

MATERIAL AND METHODS

Seeds of *Melaleuca leucadendra* (L.) L. (Myrtaceae) used in this work were collected at the Campus of the University at Rio Claro, São Paulo, Brasil. The collected fruits were allowed to open at 25°C and the seeds were collected and cleaned with the aid of a sieve. A seed batch was allowed to imbibe in distilled water at 25°C for a period of 48h and submitted to a discontinuous sucrose gradient prepared by pipetting 1ml each of 10, 20, 30, 40, 50 and 60%(w/v) sucrose solutions inside centrifuge tubes. After loading the seeds on top of the gradient the tubes were centrifuged at 3500 rpm for 15 seconds and after the seeds were collected with the aid of micropipette and seeds were counted under a stereomicroscope. 10mg of seeds, approximately, were allowed to imbibe and germinate in distilled water for three days at 25°C at continuous fluorescent white light ($8.93\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\text{nm}^{-1}$). After this period seeds were collected with the aid of an automatic pipette and loaded on top of the 60% (w/v) sucrose solution. After approximately 15 seconds, to avoid damage (Taylor and Kenny, 1985), the seedlings were collected and washed at least 10 times with distilled water and put inside 90mm Petri dishes with two layers of filter paper imbibed with Hoagland nutrient solution. To follow the development, the seedlings were transferred to a 11 x 11 cm plastic boxes filled with washed sand and Hoagland nutrient solution.

For the analysis of the inhibitory effect of leached browning substances, 100mg of seeds of *Melaleuca leucadendra* were allowed to imbibe for 24 hours in 90mm diameter Petri dishes containing 8mL of distilled water and then 50 seeds of *Lactuca sativa* L. cv. Grand Rapids (Ferry Morso, California, USA) were spread on the filter paper put on the *M. leucadendra* seeds. The germinated seeds were scored after 72 hours germination period at 25°C under continuous fluorescent white daylight ($8.93\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\text{nm}^{-1}$). The hypocotyl and root lengths were measured with the aid of a Mitutoyo paquimeter. At least 30 seedlings per treatment were counted and the results are expressed as mean±standard error.

RESULTS AND DISCUSSION

The seeds of *M. leucadendra* used in this work exhibited very low percentage of viable seeds (16.8%), and could be classified in two categories: i. clear empty unviable seeds and ii. dark viable seeds. Van der Moezel and Bell (1987) studied 10 species of *Melaleuca* but they only reported the relative percentage germination between the species which becomes impossible to determine the absolute values.

Jordão et al. (1988) reported that viable orchid seeds present low density due to the presence of hydrated cells and unviable seeds present high density because they present empty cellulosic tegument. Due to these properties *Hormidium coriaceum* seeds were separated by density.

Apparently, in *M. leucadendra* the unviable seeds present only the clear empty tegument with different density when compared to the viable seeds, with low density by the presence of hydrated cells of the embryo. When seeds were submitted to a discontinuous sucrose gradient, 56.3% of viable seeds and 74.7% of unviable seeds located at the bottom of the tube (Table 1). Although this technique was recommended for separation of seeds of *Hormidium coriaceum*, *Epidendrum ibaguense* and a hybrid (*Laelia perrinii* x *Cattleya gutata*) x *Cattleya loddigesii* (Lopes et al., 1987) it does not work for seeds of *Melaleuca leucadendra* which present viable seeds with varieties of densities. When germinated seeds (seeds with 1mm length root) mixed with non germinated seeds were loaded, without centrifugation, on the 60% sucrose solution, the germinated seeds were floating at the over the sucrose solution (Table 2). The period of 15 seconds was enough to separate germinated seeds and caused no damage to the seedling since normal development was observed. This treatment was enough to select 100% of germinated seeds located on top of the sucrose solution and discarding the empty seeds which liberate dark becoming substances responsible for slight inhibition of the seedling development which go to the bottom of the tube.

Table 1. Density separation, of viable and unviable seeds of *Melaleuca leucadendra* (L.) L., after centrifugation, at 35000 rpm for 15 seconds, in a discontinuous sucrose gradient.

| Interphase of sucrose concentration (% , w/v) | Viable seeds (%) | Unviable seeds(%) |
|---|------------------|-------------------|
| 30-40 | 0 | 0 |
| 40-50 | 12.50 | 8.20 |
| 50-60 | 31.25 | 17.10 |
| Bottom | 56.25 | 74.70 |

The slight inhibitory effect of leached substances on the hypocotyl length of lettuce seedlings was observed (control = 1.94 ± 0.05 cm; with *M. leucadendra* seeds = 1.05 ± 0.08 cm) while no effect was observed on root length (control = 2.66 ± 0.21 cm; with *M. leucadendra* seeds = 2.81 ± 0.05 cm). Thus, with this treatment

of selection of germinated *M. leucadendra* seeds, the inhibitor of hypocotyl elongation leached from those seeds can be discarded.

For seeds of *Eucalyptus pilularis* it is recommended the use of 1 kg sucrose dissolved in one Liter of water for separation of seeds which float on this solution from chaff and other impurities which go to the bottom (Gunn, 2001). As recommended for selection of seeds of *Eucalyptus pilularis* in a program of plantation of *M. leucadendra* the seedlings separation by density, could be used as a routine practice since commercial sugar can be used to obtain the 60% sucrose solution.

Table 2. Separation of germinated seeds of *Melaleuca leucadendra* (L.) L. by floating of 60% sucrose solution (w:v).

| Seeds position | Germinated seeds (%) | Non germinated seeds (%) |
|----------------|----------------------|--------------------------|
| floating | 96% | 0% |
| Bottom | 4% | 100% |

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