

Application of Seed Treatments to Increase Germinability of Cardamom (*Elettaria cardamomum*) Seeds under *in vitro* Conditions

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Abstract

Cardamom (*Elettaria cardamomum*) has great commercial value as a spice in Sri Lanka. Due to the presence of hard seed coat, cardamom has low seed germination potential. Therefore the present study was conducted to study the potential to improve the seed germination by applying different mechanical, physical and chemical treatments and to develop a cost effective and rapid seed germination method in *in vitro* conditions.

Complete Randomized Design (CRD) with ten replicates was used for the study. The seeds of cardamom were treated with 40 different seed treatments and surface sterilized by using 20% clorex. MS basal medium was used without plant growth regulators and solidified by 0.6% agar. Anova (DMRT) test showed that there were significant effects at $p < 0.05$ level on seed germination of cardamom seeds with different seed treatments.

According to the results 50% HNO_3 for 15 min exposure was the best treatment to obtain higher number of germinated seeds (90%) within 6 weeks upto 0.6 cm height. The second and third best treatments were 75% HNO_3 for 15 min and 75% HNO_3 for 10 min which showed 80% and 65% seed germination percentages respectively. This experiment showed chemical treatment (HNO_3) is the best to induce seed germination of *E. cardamomum* in *in vitro* conditions. The results obtained in this study will be important for plant breeders and farmers who cultivate these species commercially.

Keywords: Cardamom, seed germination, in-vitro condition, MS basal medium

Introduction

Cardamom (*Elettaria cardamomum*: Zingiberaceae) known as the “Queen of Spices”, is a perennial herbaceous plant with a pseudo stem and thick irregular shaped rhizomes (add a reference). It is an expensive spice, price being exceeded only by saffron and vanilla. It is indigenous to southern India and Sri Lanka. Many historical texts mention cardamom seeds have been used as a flavouring agent in many culinary preparations and in applications in medicine and industry.

The world's total production of cardamom is around 35,000 metric tons annually and major countries which produce cardamom are Guatemala, India, Sri Lanka, Tanzania and Vietnam. In Sri Lanka, total production of cardamom is 61 metric tones. Cardamom can be found in central hill country of Sri Lanka where elevation is 600 meters above mean sea level. Kandy, Matale, Kegalle, Nuwara Eliya, Rathnapura and a part of Galle are the major growing districts. In Sri Lanka total extent under Cardamom cultivation is 2,794ha (Progress Reports of DEA 2009).

The major problems reported related to seed germination of cardamom is the hard seed coat, due to the presence of mucilaginous layer. Seeds of cardamom variety *Malabar* were found to exhibit dormancy due to their hard seed coat (Radhamani *et al.*, 1991). Radhamani *et al.*, (1991) have reported various physical and chemical treatments for breaking seed dormancy. Cardamom seeds are considered recalcitrant, with relatively low retention of germinability during storage. The mature seeds of cardamom contained a high percentage of water at harvest, and germinability at harvest was around 62%, confirming the recalcitrant nature of the seeds. Seed germinability was completely lost at moisture levels below 50% (dry wt. basis). Drying at 20°C retained some degree of germinability for 7 days. In terms of storage, sealed polythene containers and low temperature (5°C) were helpful for maintaining some degree of germinability approximately 15 days (Sangakkara, 1990). Cardamom is generally susceptible to a large number of viral diseases such as Foorkey, Chirkey and other fungal and bacterial diseases and the control of such diseases in plantation crops is very difficult. Viral diseases are not be transmitted through seedlings as the seeds are free from viruses. Healthy *in vitro* seedlings may give better establishment and easy fruiting of the crop. Every year demand for cardamom increases in the world but production of cardamom does not increase as compared to the demand. Cardamom is propagated through suckers or seedlings however the latter method is preferable in order to get rid of the virus 'katte' (http://www.krishiworl.com/html/condi_spices1.html).

With the increasing demand for quality plants, it has become necessary to conduct research to identify methods to reduce time taken for the seed germination and for constant supply of healthy plants. Therefore the present work was designed to find solutions for the problem, associated with the germinability of Cardamom.

Materials and Methods

Experimental Site

Experiments were conducted at Laboratory in the faculty of Agriculture, University of Ruhuna. In these experiments, mature seeds of cardamom were used as explants.

Seed collection and preparation

Fully matured fresh fruits of *E. cardamomum* were taken from healthy mother plants at Deniyaya research station. Fruits were dried under sunlight for 2-3 days and pericarp was removed to separate the seeds. After that seeds which were uniform size, color, absence of abnormalities, external damages and pest attacks were selected.

General experiment procedure and treatments

Selected seed lot was divided into groups and applied 40 treatments (Table 01). Each treatment was replicated ten times with 10 seeds for each treatment and the experiment was repeated 2 times.

Table 01: Seed treatments of *Elettaria cardamomum*

Treatments		
	Control	T ₁
Mechanical	Rubbed with coarse sand	T ₂₉
	Rubbed with two sand papers	T ₃₁
	Rubbed with sand wash – mix with wood ash – dry in shade	T ₃₉
	Water soak 12hrs	T ₃₀
Physical	Dipped in 100°C water 1,2,3,4,5 min	T ₃₂ , T ₃₃ , T ₃₄ , T ₃₅ , T ₃₆
	Hot water 50°C for 30 min	T ₃₈
Chemical	Wrapped in wet gauze	T ₄₀
	75% HNO ₃ (5, 10, 15 min)	T ₂ , T ₃ , T ₄
	50% HNO ₃ (5, 10, 15 min)	T ₅ , T ₆ , T ₇
	25% HNO ₃ (5, 10, 15 min)	T ₈ , T ₉ , T ₁₀
	75% CH ₃ COOH (5, 10 min)	T ₁₁ , T ₁₂
	50% CH ₃ COOH (5, 10 min)	T ₁₃ , T ₁₄
	25% CH ₃ COOH (5, 10 min)	T ₁₅ , T ₁₆
	50% HCl (5, 10 min)	T ₁₇ , T ₁₈
	25% HCl (5, 10 min)	T ₁₉ , T ₂₀
	25% H ₂ SO ₄ (5, 10 min)	T ₂₁ , T ₂₂
	50% H ₂ SO ₄ (5, 10 min)	T ₂₃ , T ₂₄
	80% Alcohol (15, 30 min)	T ₂₅ , T ₂₆
	50% Alcohol (15, 30 min)	T ₂₇ , T ₂₈
	Cholorox 10% 10 min	T ₃₇

Preparation of medium and planting seeds

Seeds were treated with above mentioned seed treatments separately and rinsed

with distilled water. After that seeds were washed using 70% (v/v) alcohol for 1 min. Then seeds were soaked with Clorox solution (sodium hypochlorite) 20% (v/v) and added 2 drops of Tween-20 for 20 minutes in the laminar air flow cabinet. After that seeds rinsed with sterile distilled water at 4-5 times. MS basal medium was solidified by 0.6% agar was autoclaved for 21 min at 121°C after adjusting the pH to 5.8. Finally seeds were kept on the solidified MS basal media containing tubes/petridishes for germination and growth.

Experimental layout and data analysis

Treatments were undertaken in Complete Randomized Design (CRD) to minimize experimental errors and to make better estimation of treatment effect. Analysis of Variance was done at the 0.05 significant levels by using SAS package (v6.12) (SAS Institute, Cary, NC 1995) and the mean separation was done by using Duncan’s New Multiple Range test (DMRT).

Results and Discussion

The hard, stony, seed coat and presence of mucilaginous layer over the seeds were reported to be responsible for the delaying of germination in seeds of *Elettaria* species . Breaking the dormancy of the seeds, providing optimum conditions for germination and facilitating water absorption were objectives of treatments of this study.

There is a significant difference in germination of *E. cardamomum* seeds under different seed treatments at (P < 0.05) level.

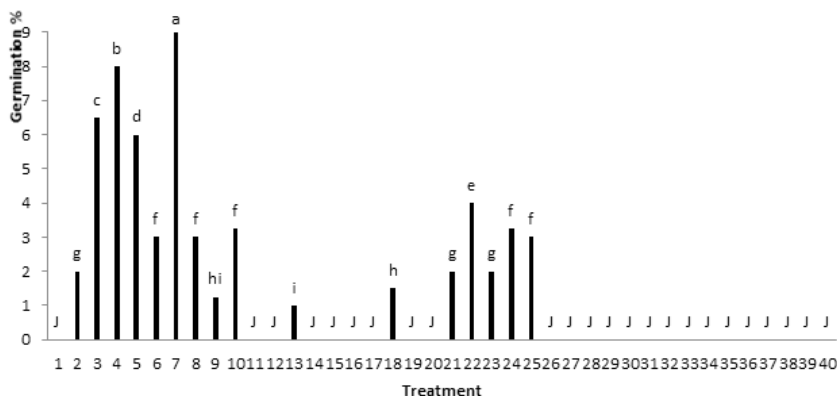


Figure 1a: Seed germination percentage of *E. cardamomum* seeds under different seed treatments in *In vitro* conditions. Means followed by the same lower case letters in each bar are not significantly different at 5% level in Duncan’s Multiple Range Test.

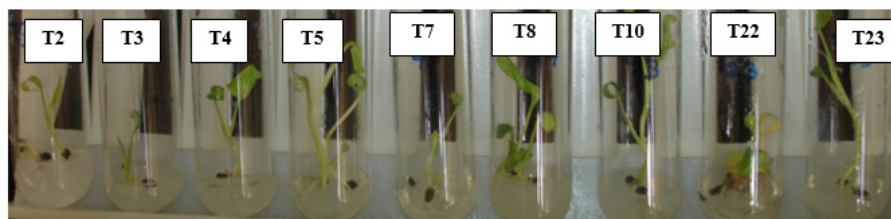


Figure 1b: *In vitro* seed germination of *E. cardamomum* under different seed treatments after 30 days.

According to the results the highest percentage (90%) of germinated *cardamom* seeds was observed in 50% HNO_3 for 15 minutes (T7). The second best treatment was 75% HNO_3 for 15 minutes (T4), and it displayed 80% germination percentage. The third best treatment was 75% HNO_3 for 10 minutes (T3), and germination percentage was 65% (Figure 1a and 1b). Seed germination was not observed in control (T1) during 15 weeks. Fourth and fifth best seed treatments were 25% H_2SO_4 for 10 minutes (T22) and 50% HNO_3 for 5 minutes (T5) that showed 40% and 60% germination percentages respectively. However T6, T8, T10, T24 and T25 showed around 30% seed germination but they were not significantly different. Treatments T2, T21 and T23 were not significantly different showing around 20% seed germination. Treatment T9 and T18 showed around 10% seed germination. Some treatments such as control (T1), T11, T12, T14, T15, T16, T17, T19, T20, T26 to T40 did not germinate even one seed.

According to Hartmann 1997, Copeland and McDonald 1995 mechanical treatments are the ideal for breaking seed coat dormancy. However according to the results of this research the chemical treatments are the best for breaking seed coat dormancy. According to Radhamani *et al.*, (1991) treatments of seeds with sulphuric acid (25%) for ten minutes and absolute alcohol (80%) for 30 minutes were most effective treatments in breaking seed dormancy. However findings could not be confirmed in this study. According to this study, mechanical methods give low germination percentages compared to chemical methods. This could happen due to two reasons; mechanical methods could not properly break the hard seed coat and it could injure the embryo. Some treatments didn't show even a single seed germination and it may be due to high temperature and high concentrations of chemicals that might have caused death of embryos. According to these results Acetic acid treatments, HCl treatments, Alcohol treatments and hot water treatments produced less favourable outcomes.

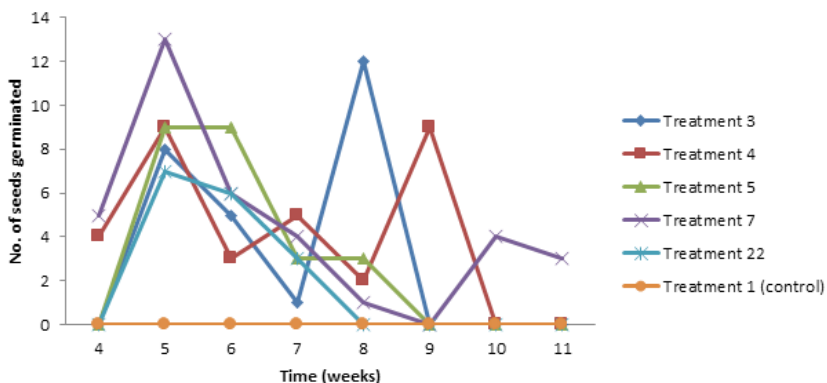


Figure 02: How seed germination patterns among the most effective treatments of *E. cardamomum*

According to figure 2 the highest number of seed germination (13.5 seedlings out of 15 seeds) was recorded by T7. It showed high germination at the beginning (4th week - 5 seedlings) as compared to the others. After the 4th week, germination increased rapidly and reached to maximum during 5th week. Treatment 7 showed highest germination within a short period of time, comparing to the other treatments and it was good to get plants within a short period of time. Treatment 3 and T4 were appropriate for production of higher number of plants (12 and 9 seedlings respectively) but they took comparatively long time (8 and 9 weeks respectively) period for seed germination. Treatment 4 was also suitable for produce plants within a short period of time because it produced 4 seedlings within four weeks period. Control (T1) did not initiate seed germination during the whole period (12 weeks).

Water absorption was greatly facilitated by chemically damaged seed coat. Jones (1995) observed during first four weeks for most seeds, water absorbed through hard seed coat act as a mechanical barrier to initiate germination. But with the water absorption gradually soften the seed coat and induce germination according to Copeland and McDonalds 1995. It has revealed that seeds of *E. cardamomum* were found to exhibit dormancy due to their hard seed coat (Radhamani *et al.*, 1991). It is evident that the dormancy reported in seeds of *Elettaria* species results from the impermeable seed coat. Any procedure which enables the seeds to imbibe moisture promotes germination (Robert *et al.*, 2011). By absorbing enough water, enzymatic activities enhance the rapid germination (Mayer and Mayber 1982). Robert *et al.*, (2011) observed low seed germination percentages of *Elettaria* species because of hard seed coat that act as a physical barrier for germination. Mayer and Mayber (1982) observed gradual increasing of seed germination due to absorbing water due to enhance the enzymatic activities for the rapid germination.

Conclusion

50% HNO₃ for 15min was the best treatment for *E. cardamomum* and it showed 90% seed germination percentage within 5 weeks. This experiment showed chemical treatments are superior to other methods for germination of *E. cardamomum* seeds.

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