DETERMINATION OF *EX VITRO* AND *IN VITRO* SEED GERMINATION OF *Celastrus paniculatus* WILLD. - AN ENDEMIC MEDICINAL PLANT AS AFFECTED BY DIFFERENT SEED TREATMENTS

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ABSTRACT

Celastrus paniculatus Willd. is an endangered forest climber where propagation and conservation is important and urgently needed due to its restricted distribution and high medicinal value. The current study was planned to develop seed propagation of C. paniculatus through ex vitro and in vitro means. Ripen seeds were pretreated with 100% Hydrocloric acid (HCl) for 10, 20 and 30 minutes and 2, 4 and 6 mg/L Gibberelic Acid (GA3) over night. One set of seeds were sown in sand and sand: coir dust (1:1) media ex vitro and another set was surface sterilized and established on MS, WPM and B5 media either incorporating 1 mg/L GA_3 or α-Napthaline Acetic Acid (NAA). All the experiments were designed according to factorial Completely Randomized Design (CRD). Results revealed that ripen seeds can be germinated ex vitro with 40 % germination rate in sand medium after treating with 100% HCl for 10 minutes and 2 mg/L GA_3 over night. Surface sterilized seeds using 3% Topsin M 70 (Thiophanate methyl 70% w/w) for 30 minutes, 100% Clorox (5.25% NaOCl) for 30 minutes and 70% ethanol for 2 minutes were germinated, in vitro on B5 medium containing 1 mg/L GA_3 with 85% germination after pretreating with 100% HCl for 10 minutes. Therefore, it is clear that in vitro seed germination is much higher than ex vitro seed germination.

KEY WORDS: Deciduous, *Ex vitro*, *In vitro*, Pretreatments, Seed germination, Surface sterilization

INTRODUCTION

Celastrus paniculatus Willd (Family: Celestraceae), locally known as Duhundu, is an important medicinal, deciduous, forest climber natively growing mostly in the hilly regions of northern India at an áltitude of 1250 m. The plant

has a distribution range in the sub Himalayan regions, hilly parts of Bombay, south of Gujarat, central India, Madras, Sri Lanka, Burma and Malay (Mehta et al., 2002)

Phytochemical investigations have revealed the presence of celastrine and paniculatin with the dark brown colour seed oil naming Celastrus or Malkangini oil. The oil has tranquilizing, sedative, anticonvulsant activities besides being a central muscle relaxant, anti-inflammatory, antipyretic, antiemetic, antiallergenic, analgesic and adaptogen with memory enhancing properties. In folk medicine, the seeds are boiled and taken as a tea. In Indian traditional system of medicine, Duhundu is used as an appetizer, laxative, emetic, aphrodisiac, brain tonic but is also good for cough, asthma, leprosy, gout, various fevers, headaches, leucoderma and rheumatism. The bark is reported to have abortifacient activity. It is one of the components of the drug "Mentat syrup" recommended for memory enhancing and mental disorders. More recently, it has been reported to be an antidepressant. Leaf sap is a good antidote for opium poisoning (Rekha et al., 2005)

Propagation studies on this valuable species are lacking and hindered due to its wild natural habitat in Sri Lanka. However, it is reported that ripen seeds can store in dry sand before sowing (Sheat, 1948). After three months cold stratification and removal of aril, higher germination rates were observed (Dirr and Heuser, 1987). Layering and rooting of cuttings are also practiced (Sheat, 1948). Detailed information of propagation of this plant is rare to find and therefore current study was planned to identify the possibility of propagating *C. paniculatus ex vitro* and *in vitro* for conservation and multiplication purposes of this valuable endangered medicinal plant.

MATERIALS AND METHODS

All the experiments were carried out at the Tissue culture laboratory, Department of Crop Science, Faculty of Agriculture, Mapalana, Kamburupitiya.

Ex vitro germination

Matured pods were collected from natural forest at Rathnapura (Low Country Wet Zone) and after purification, seeds were stored in a refrigerator under 4^0 C temperature. As pre seed treatments one set of seeds were treated with 100%

HCl solution with 10, 20 and 30 minutes exposure time durations and subsequently soaked overnight in water. Another set was treated with three concentrations (2, 4 and 6 mg/L) of Gibberelic acid (GA_3) solutions over night. Treated seeds were transferred to sand and sand: coir dust (1:1 ratio) potting media and placed under normal laboratory conditions (12hrs. light/12 hrs dark; 28± 2 ^{0} C temperature) for germination. Number of germinated seeds was counted weekly up to four months.

In vitro germination

Surface sterilization

Seeds were washed thoroughly using soap and water (v/v) and kept under running tap water for half an hour. Then surface sterilized with 3% fungicide (Topsin) solution for 30 minutes, four different concentrations (25%, 50%, 75% and 100%) of Clorox for three different exposure time durations (10, 20 and 30 min) followed by 70% ethanol for 2 minutes. Surface sterilized seeds were treated either using 100% HCl solution with 10, 20 and 30 minutes exposure time followed by overnight soaking or three concentrations (2, 4 and 6 mg/L) of GA_3 solutions overnight. Numbers of non-contaminated seeds were recorded up to a month.

Culture establishment

Surface sterilized seeds were cultured on MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1980) and B5 (Gamborg et al., 1968) media with and without activated charcoal either with 1 mg/L GA_3 or 1 mg/L NAA. Number of germinated seeds and quality of the seedlings were recorded up to four months.

Experimental design and statistical analysis

All the experiments were designed according to Factorial Completely Randomized Design (CRD). Data were collected weekly and analyzed using SAS statistical software.

RESULTS AND DISCUSSION

Ex vitro germination

There was an interaction effect of seed treatments and potting media on germination of *Celestrus paniculatus* seeds ($P \leq 0.05$). Pretreated seeds showed significantly higher germination compared to zero germination of non treated seeds. Pretreated seeds with 100% HCl solution for 10 minutes exposure time gave significantly higher germination (40%) in both media of sand and sand: coir dust (1:1). When the exposure time increased to 20 minutes, germination was decreased to 36% in sand medium but no germination was recorded in sand: coir dust (1:1) media. When exposure time increased further (up to 30 minutes), seeds were not germinated at all in both media (Figure 1).

Seeds treated over night with GA_3 were not germinated in sand: coir dust (1:1) medium irrespective to the GA_3 concentration. When sand is used as the potting medium, germination percentages were decreased with increasing concentrations of GA_3. Seeds treated with 2 mg/L GA_3 gave 40%germination followed by 28% germination with 4 mg/L GA_3 and no germination with 6 mg/L GA_3 in sand media (Figure 2). Even though the observations were taken up to 4 months, non of the seeds were germinated after 8 weeks.

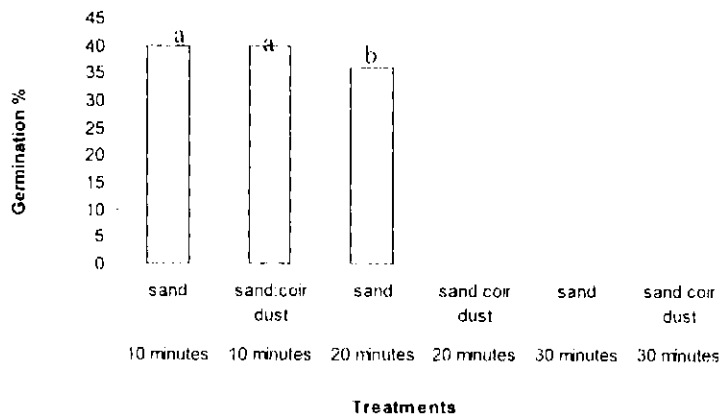

Figure 1. *Ex vitro* **germination % of seeds treated with HCl after 8 weeks (CV = 11.56 LSD = 2.91, Means represented by the same letter are not significantly different at $P \leq 0.05$ significant level)**

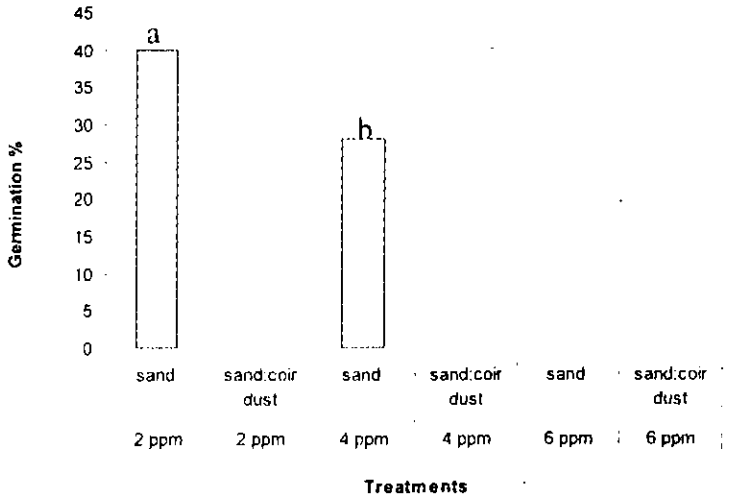

Figure 2. *Ex vitro* **germination % of seeds treated with GA_3 after 8 weeks CV = 16.10 LSD = 2.37, Means represented by the same letter are not significantly different at P≤ 0.05 significant level).**

It was recorded that three months' cold stratification and removal of aril lead to a higher germination rate (Dirr and Heuser, 1987), but during the current study both were not practiced due to the miniature size of the seeds. However, HCl treatment achieved better results with 10 minutes exposure time as it may damage hard seed coat of the seeds. Further experiments are needed to identify lower concentrations of HCl and lower than 10 minutes exposure times to achieve higher germination rates.

In vitro germination

Surface Sterilization

The interaction of Clorox concentration and exposure time significantly affect on non- contamination rates of *C. paniculatus* seeds (P≤ 0.05). 100% non-contamination was resulted when seeds treated 100% Clorox solution for 30 minutes (Figure 3). In addition, these seeds were dipped in 3% Topsin solution up

to 30 minutes before introducing to Clorox solution. After washing out of Clorox, seeds were again dipped in 70% ethanol solution for 2 minutes. However surface sterilized seeds were not germinated during one month period of observation.

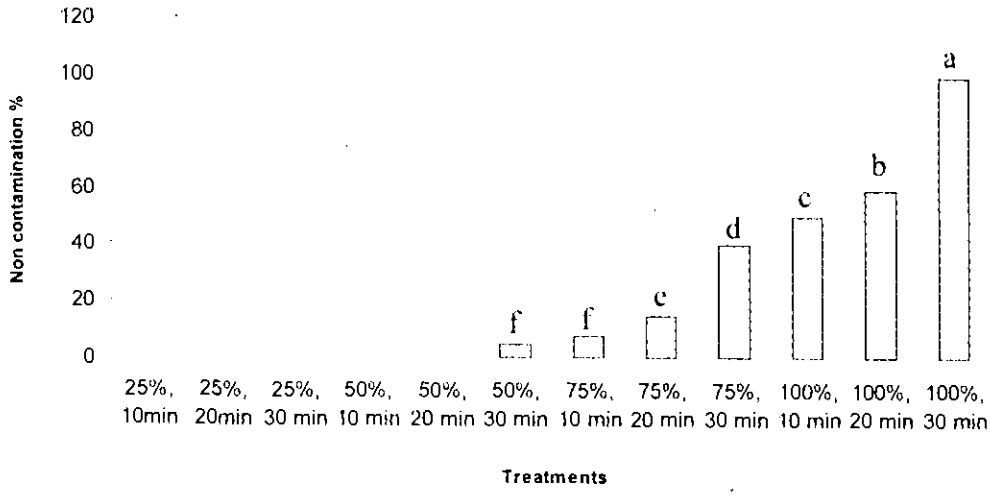

Figure 3. Non contamination percentages of seeds treated with different Clorox concentrations after 4 weeks (CV = 9.51 LSD = 5.50 Means represented by the same letter are not significantly different at P≤ 0.05 significant level).

When applying *in vitro* techniques it is a must to surface sterilize the plant parts which are going to be cultured on artificial nutrient media. Since plants and plant parts are in contact with a wide range of fungi and bacteria, it is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissue (Conger, 1987). NaOCl is the most common chemical agent used to sterilize plant tissues which is more effective and less expensive (Prasad, 1999). Some woody tissues such as buds, twigs and seeds were cleaned by immersing them briefly in a 70 % ethanol solution (Conger, 1987). The results of the current study proved the necessity of both Clorox and ethanol for surface sterilization to avoid contaminations during *in vitro* culture establishment of *C. paniculatus*.

In vitro establishment

Seeds pretreated with 100% HCl solution for 10 minutes gave 85% germination while 68% and 61% germination were achieved with 2 mg/L GA_3 and

100% HCl for 20 minutes pretreatments (Figure 4). When compared to *ex vitro* germination, (Figures 1 and 2) higher seed germination rates were observed when they were cultured *in vitro*. It has been recorded that three month cold stratification leads to a higher germination rate (Dirr and Heuser, 1987). The temperature of the culture room was 23 ± 2 °C, which was somewhat lower value than room temperature. For the surface sterilization, higher concentration of Clorox (100%) up to 30 minutes exposure time followed by 70% ethanol was used. Due to above chemicals the thick seed coat may be softened and even may be damaged, which may facilitate water absorption and subsequently induce the *in vitro* germination of *C. paniculatus* seeds. Abnormal seedlings were not observed when seeds were germinated; therefore the used chemicals for the surface sterilization had not effect on the quality of the produced seedlings. Even though observations were taken up to 4 months, none of the seeds germinated after 12 weeks.

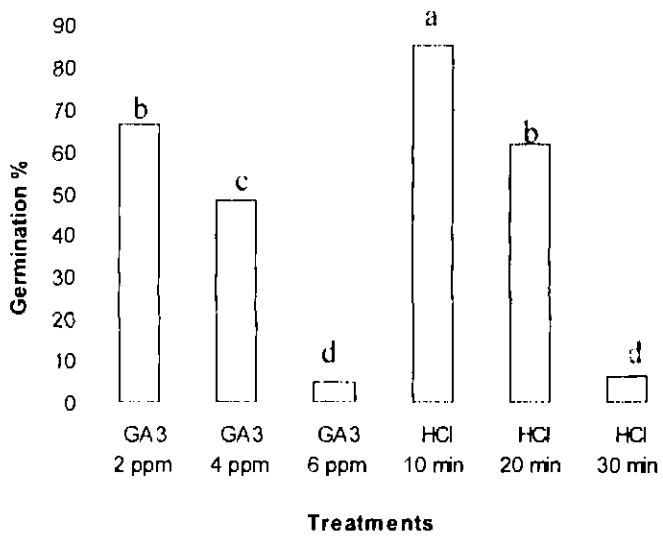

Figure 4. *In vitro* germination rates of seeds with different pretreatments after 12 weeks (CV = 8.39 LSD = 2.06, Means represented by the same letters are not significantly different at P≤ 0.05 significant level).

Surface sterilized and pretreated seeds cultured on B5 medium containing 1 mg/L GA_3 gave significantly higher (P≤ 0.05) germination when compared to WPM, MS and the same medium containing 1 mg/L NAA (Figure 5).

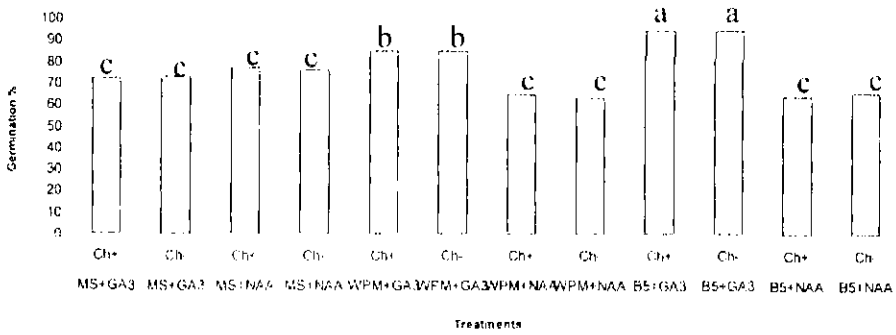

Figure 5. *In vitro* germination rates of seeds on different culture media after 12 weeks (CV = 10.36 LSD = 1.30, Means represented by the same letter are not significantly different at P≤ 0.05 significant level).

The success in cell, tissue and organ culture technology is related to the selection or development of the culture medium. The decision on using type of media for the metabolic needs of the culture cells and tissue is a major factor of success in plant regeneration process (Kumar, 2001). Activated charcoal is being used to provide dark conditions which facilitate rooting of some cultures and it acts as an absorbent in tissue culture media (Razdan, 2003). However, during the current study, dark conditions provided by activated charcoal did not enhance seed germination of *C. paniculatus* at P≤0.05 significant level (Figure 5).

Growth regulators must be added selectively to culture media. The type of growth regulators and concentration used will vary according to the culture purpose. There are several types of plant growth regulators each having a well defined effect on growth and development (Kumar, 2001). For seed germination GA_3 plays a main role to break seed dormancy and some auxins stimulate growth of the root apex (Taiz and Zeiger, 2002). During the current study among the three media tested, B5 was the best for seed germination of *C. paniculatus*, if the medium contains 1 mg/l GA_3.

CONCLUSION

Ripen seeds of *C. paniculatus* can be germinated *ex vitro* with 40 % germination rate in sand medium after treating seeds with 100% HCl for 10

minutes and 2 mg/L GA_3 solution for overnight during the period of 8 weeks and no germination was recorded after 8 weeks. Seeds can be surface sterilized by dipping in 3% Topsin M 70 (Thiophanate methyl 70% w/w) for 30 minutes followed by 100% Clorox (5.25% NaOCl) solution for 30 minutes and 70% ethanol for 2 minutes. Surface sterilized seeds were germinated, *in vitro* on B5 medium containing 1 mg/L GA_3 with 85% germination after pretreating with 100% HCl for 10 minutes within 12 weeks period and no germination was recorded after 12 weeks.

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