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Effects of Some Factors on the *in Vitro* Seeds Germination of *Oxytenanthera Abyssinica* (A. Rich.) Munro.

KEYWORDS: Oxytenanthera abyssinica seeds, germination, viability, temperature, storage, coats

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ABSTRACT *In this work, different parameters were tested for optimizing in vitro Oxytenanthera abyssinica seeds germination. Maximum seeds germination was achieved (100%) when these were dehusked and exposed to 28°C. For intact seeds, the coats played an inhibitory effect on in vitro germination (0%). A germination percentage 70% was achieved when the seeds were treated with sulphuric acid at 15 mn. For temperature storing, seeds viability was maintained till 8 years. The seedlings were successfully acclimated with 100% of plantlets survival.*

1. Introduction

Bamboos appear among the plants that are more valued in the world, especially in Asia and particularly those involved with rural economy (Sharma, 1987). In Senegal, the regions of Tambacounda, Kolda and Ziguinchor contain the most important populations of Oxytenanthera abyssinica. In these regions where the pluviometry varies from 700 to 1500 mm per year, the ecological conditions are optimal for better growth and development of natural populations of this bamboo (Sène, 1998). Given the importance that this species renders, the anthropic pressure is also too strong. Thus, culms of these bamboos are valued and serve as raw material for various uses. According to the stage of culm-development, the local inhabitants use them to build houses, furnitures and various other articles. The state of Senegal earns 500 million of CFA francs per year as revenue for the export of bamboo (Sène, 1998). Considering the importance of bamboo in Senegal, various research works were carried out for acquiring the basic knowledge (Sonko, 1995; Ndiaye, 2001, 2006b; Ndiaye et al., 2006a, 2008, 2013, 2014). However, bamboo flowering is regarded as a bad omen by many rural people, especially where the flowering incidence is accompanied by an increase in rodent population. It is believed that flowering of bamboo brings disasters like famine and other natural calamities, which has compelled the rural people to destroy the clumps after or during blooming (Mohan-Ram and Hari Gopal, 1981; John and Nadgauda, 2002). Bamboo is also known for its monocarpic habit, i.e., flowering once before culms death (McClure, 1966). In addition, the period between two flowerings can vary from 1 to 120 years depending on the species, some of which have never flowered. Thus, regeneration of bamboos through seeds is difficult due to the unpredicted vegetative cycle and the short duration of viability of seeds. Limitations in seeds viability, unpredicted flowering, warrant an urgent method for an alternative approach for developing efficient protocol for good germination.

The present paper describes the influence of some parameters on the germination of Oxytenanthera abyssinica seeds for maintaining the genetic diversity due to the sexual reproduction.

2. Material and methods

2.1. Plant material

The Oxytenanthera abyssinica seeds were collected from clumps located in the area of Mako (Kedougou, Senegal: latitudes 12°30 and 16°30 N and longitudes 11°30 and 17°30 W), during 2003 Juin 15. Due the limited availability and low viability, the seeds were immediately stored in freezer at 4°C.

2.2. Methods

2.2.1. Disinfection

We have used two types of seeds: dehusked seeds and non-dehusked seeds (intact seeds). For raising aseptic cultures, one group of the dehusked seeds were surface sterilized using an aqueous solution of mercuric chloride (0.1%) for 10, 15, and 20 minutes and the second group was double sterilized using mercuric chloride (0.1%) (20 mn) and bleach (10 mn). The same method of disinfection

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was used for the intact seeds, but the time of mercuric chloride of the double sterilization was 30 mn.

2.2.2. Influence of temperature on seeds germination
For checking the optimal temperature of germination, three temperatures (28, 35 and 42°C) were tested.

2.2.3. Influence of coats on seeds germination
To test the influence of seeds coats on percentage of germination at 28°C, two types of seeds were used:

- dehusked seeds; and

- intact seeds.

2.24. Influence of sulfuric acid scarification and hot water on seeds germination
The intact seeds were soaked in sulfuric acid 95% for 10 mn and 15 mn. The same times were tested for soaking the seeds in boiling water. The germination temperature was 28°C.

2.2.5. Influence of storage duration at 4°C on seeds germination
To study the influence of storage duration at 4°C, the seeds collected were immediately stored in freezer. The germination capacity of the seeds was tested over time at 28 °C.

2.2.6. Medium and culture conditions
The culture jars containing 60 ml gelled water, were autoclaved at 120°C for 20 mn. The seeds were inoculated in the jars, each jar containing 20 seeds. All the experiments were repeated four times, each repetition comprising 20 seeds. Cultures were maintained in dark chamber for germination.

3. Acclimatization
After germination, the seedlings were washed with luke warm water to remove adhered agar and the basal seedlings were dipped in the bavistin solution before transferring to the plastic pots containing sand, soil and well rotten Farm Yard Manure (1:1:1) for hardening. The seedlings were kept under green house with proper watering for 4 months. The number of acclimated plants was 33.

4. Data and statistical analysis
After inoculation, the percentage of seeds germination was recorded each day for 10 days.

The Newman-Keuls test at 5% was used for the statistical analysis of data (% of germination) within each experiment.

Photo 01. Oxytenanthera abyssinica spikelets

Photo 02. Intact Oxytenanthera abyssinica seeds

Photo 03. Dehusked Oxytenanthera abyssinica seeds

5. Results and discussion

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5.1. Disinfection

The figures 01 and 02 illustrate the effect of disinfection duration on percentage infection of Oxytenanthera abyssinica seeds after 15 days.

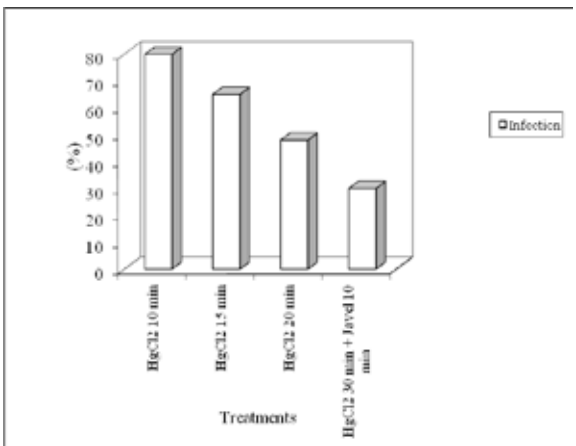

Figure 01. Percentage of in vitro infection of intact Oxytenanthera abyssinica seeds

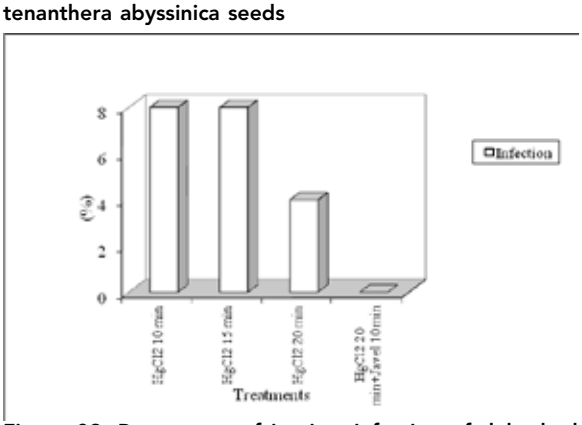

Figure 02. Percentage of in vitro infection of dehusked Oxytenanthera abyssinica seeds

Due to the variation of disinfection duration, the percentages of infection decreased. Thus, for the dehusked seeds, the percentage of contamination was 4% at 20 mn. This percentage was nil with the double disinfection (figure 01). Despite the double disinfection, the percentage of infection of the intact seeds was important (figure 02). This high infection rate (30%) of the intact seeds may be due to the presence of coats that protect the microorganisms against the lethal effect of the disinfectants.

5.2. Influence of temperature on seeds germination

The figures 03 and 04 illustrate the effect of temperature on in vitro germination of intact and dehusked seeds after 8 days.

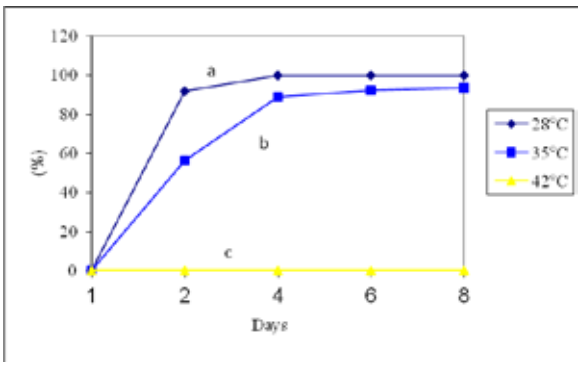

Figure 03. Effect of temperature on in vitro germination of dehusked Oxytenanthera abyssinica seeds

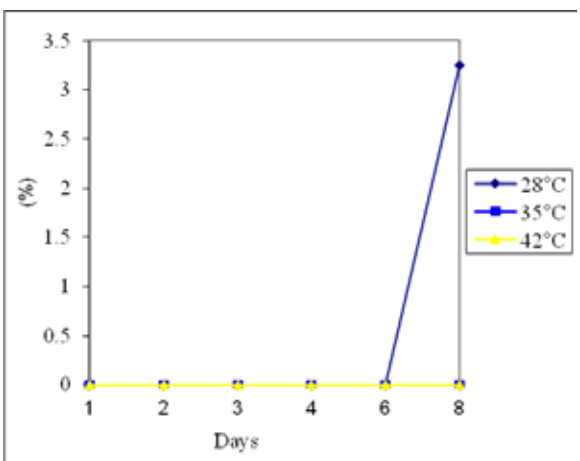

Figure 04. Effect of temperature on in vitro germination of intact Oxytenanthera abyssinica seeds

In the presence of optimum temperature (28°C), 100% dehusked seeds germinated during the first 4 days and 3.24% intact seeds, 8 days after inoculation. The temperature of 28°C was found to be better than 35°C and 42°C, for promoting the germination of seeds. When the temperature increased, the germination rate of the seeds decreased. According to Côme (1970), the high temperatures increase the oxygen needs of the embryo; may be this situation was responsible of the germination percentage decreasing. The high temperatures (45°C) could destroy the enzymes involved in the germination process and/or kill the embryo. Seeds germination of Ottelia alismoides was very low at 15°C and 30°C (Liyan et al., 2013).

5.3. Influence of seed coats on germination

The figure 05 shows the inhibitory effect of teguments on in vitro seeds intact germination after 8 days.

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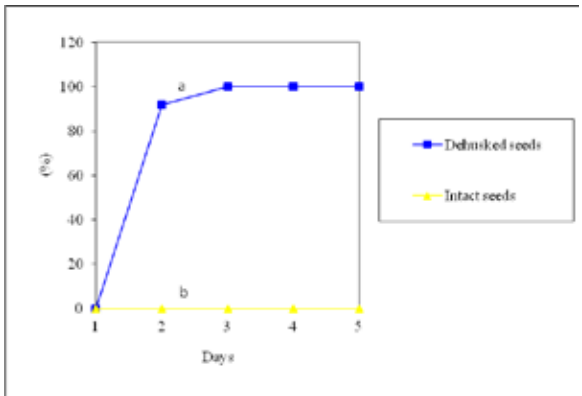

Figure 05. Influence of coats inhibitor action on in vitro germination of intact Oxytenanthera abyssinica seeds

Despite the optimum temperature, the percentage of intact seed germination was nil; in contrast, 100% dehusked seeds germinated (figure 05). The inhibition of the intact seeds germination may be related to the presence of inhibitory substances and/or the water and oxygen impermeability of the coats. The oxygen lack can inhibit germination; according to Thornton (1935), the embryo covered its coats needs to germinate, an atmosphere fifty to sixty times more oxygen than an isolated embryo. Gopi and Sood (2008) obtained 68.89% germination with dehusked seeds of Dendrocalamus strictus.

5.4. Influence of scarification and hot water soaking on germination

The figure 06 illustrates the effect of acid and boiling water action on in vitro intact seeds germination after 5 days.

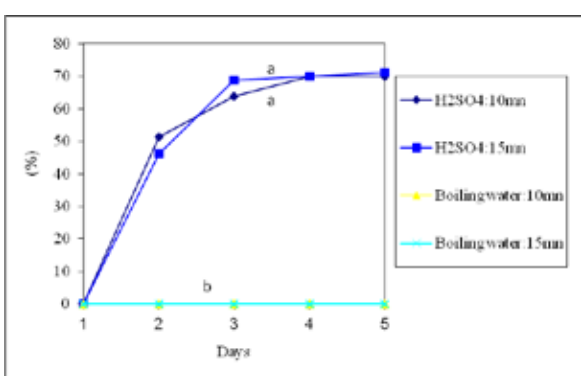

Figure 06. Effect of acid and boiling water soaking on germination of Oxytenanthera abyssinica seeds

The acid soaking for 10 to 15 mn gave highest germination rate in 4 days. But the percentage obtained at 10 mn which showed no significant difference with 15 mn (Figure 06). After the fifth day, the percentage of germination was always equal to 70%. The present investigation indicated that the seed coats had a drastical inhibitory effect on germination. Contrary to the positive acid effect, the boiling water action for 10 to 15 mn was not efficient for promoting the germination. Even in the past, many scientific studies were made on in vitro seed germination in different types of bamboos for large-scale propagation (White, 1963; Mascarenhas et al., 1975; Mehta et al., 1982; Bag et al., 2000; Sood et al., 2002).

5.5. Influence of storage duration on germination

The figure 07 shows the results of the effect of storage duration at 4°C on in vitro seed germination.

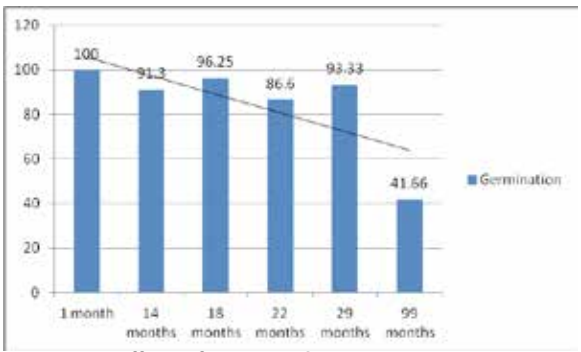

Figure 07. Effect of storage duration at 4°C on Oxytenanthera abyssinica seeds germination

Among different storage durations to the seeds, germination percentage varied from 60-100% in 10 days. Hundred percent germination was seen in the freshly collected seeds. Despite, the short duration of viability of bamboo seeds, the germination rate was rather important (41.66%) after 8 years of seeds storage at 4°C. The irregularity of the germination percentage noticed during the experiences, may be due to the heterogeneity of the seeds groups. With Phyllostachys meyeri seeds collected in December 2005, Shinjiro (2008) observed 75% of germination within 2 weeks. The Bambusa tulda seeds stored for 4 months at 0°C showed only 50-60% germination and the germination rate declined further with an increase in seed storage time and 4% of the in vitro raised seedlings were albinos (Sanjay, 1990). Gopi and Sood (2008) observed that the seeds viability of Dendrocalamus strictus was six months only and continuously deteriorated up to one year. Roberts (1972) concluded that depletion of essential metabolites, including loss factors were responsible for loss in seed viability. Hugo et al (2014) obtained 50% of final germination within 5 days at 30°C with one year seeds of Digitaria nuda.

5.6. Acclimatization

After following the process of hardening and acclimatization, the seedlings transplanted in plastic pots containing the mixture sand, soil and FYM (1:1:1) showed a successful acclimatization rate of 100%. According to Nadha et al. (2013), the Dendrocalamus asper plants shifted to field conditions exhibited 100% survival. Arya et al. (1999) obtained 95% survival rate after transplantation of D. asper plants raised through seeds. Rooted Bambusa vulgaris shoots after acclimatization, showed 100% survival and grew well in greenhouse before planting in the fields; the Oxytenanthera abyssinica plants raised through seeds, showed 75% survival after acclimatization (Ndiaye, 2006, 2014). Sanjay (1990) obtained 80-90% survival achieved through the protocol involving in vitro hardening where humidity around the plants could be controlled at the initial stages.

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Photo 04. 4 months old acclimatized Oxytenanthera abyssinica plants

6. Conclusion

The long and erratic flowering behaviour, low viability and being a preferred food for the rodents, are some of the reasons why studies on enhancing seeds germination are very important for restoration and rejuvenation of Oxytenanthera abyssinica forests. In this work, an efficient and simple protocol for in vitro seed germination has been described. The results present the evidence of coats inhibition effect on germination and the importance of storage at 4°C for increasing the duration of Oxytenanthera abyssinica seeds viability.

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