Journal of Biotechnology & Biomaterials
ISSN: 2155-952X
Derso and Feyissa, J Biotechnol Biomater 2015, 5:2
DOI: 10.4172/2155-952X.1000188
Research Article Open Access

Effect of Seed Treatment on Seed Germination and Seedling Growth Attributes of Yeheb (*Cordeauxia edulis*) with *In-Vitro* Conditions

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Abstract

Yeheb (*Cordeauxia edulis*) is an evergreen shrub in the bean family that produces seeds in semi-arid bush land on sandy soils of Somalia and Ethiopia. It is drought hardy and one of the major sources of food for both animals and humans. It also used as medicine and source of natural dyes for coloring of food and textiles and to build house as it is termite resistance. Even though it is a multipurpose plant and being encouraged by most people, it becomes endangered due to low seed viability and over exploitation. Those problems have to overcome by micro propagation and regeneration process. The pre-condition of those attempts are having adequate seed treatment and germination. Thus, the objective of this study is to know the effect of seed treatment on seed germination and seedling growth Attributes of *C. edulis* in *In-Vitro* conditions. Both coated and de-coated seeds were sterilized using different concentrations of 3%, 5%, 10% and 15% and 2-3 drops of Tween-20 for 5 min and cultured on growth regulators-free solid MS medium. Results in this study revealed that sterilization of de-coated seeds of *C. edulis* with 5% Clorox for 5 min was found to be valuable for both seed germination and seedling growth. As conclusion, it is important for *In Vitro* germinated seedlings which are essential for further experimental process of micro propagation and regeneration. Besides, it is used to as effort for further research activities by reducing time and cost.

Keywords: Contamination; *In-Vitro*; Seed germination; Seedling; Sterilization

Introduction

Yeheb (*Cordeauxia edulis* Hemsl.) is highly branched bush with thick and vertical branches, not taller than 2-3 meters although it can be taller in sheltered spots. Hemsley in 1907 named the yeheb, a bushy Caesalpiniaceae from the Amherstieae tribe, after Captain Cordeaux, who first obtained botanical samples of it in the Ogaden area, Ethiopia, in regions near to Somalia. It is also exotic in Israel, Kenya, Sudan, Tanzania and Yemen [1]. It is an evergreen shrub and produces seeds called yeheb nut [2]. It is leguminous plant in semi-arid bush land on sandy soils [3,4]. Two forms of *C. edulis* are known; the smaller Suley from Northern Somalia and the taller and more common Moqley [5]. Suley is pale green with large leaflets, stem thickness, and the pods contain several smaller seeds and have a bit higher protein and fat contents while Moqley is dark green and have small leaflets, stem thickness, and the pods contain one large seed and less protein and fat contents [6].

Cordeauxia edulis grows in the dry regions where the underground soil is fairly humid in certain seasons of the year. It appears to choose deep, salt-free sandy to loamy-sandy soils with low lime and gypsum contents. The necessities for soil fertility are low, good infiltration of rainwater is very complimentary [7]. Seedlings have been grown without difficulty under moist tropical conditions, but suggested that it may be possible to launch the plant in dry regions where the soil is poor and the conditions are comparable to those of its native country. They recommended that there might be a prospect of increasing establishment and growth of *C. edulis* through fertilizer application [8].

It is a famine food from the dry savannas of Somalia and Ethiopia and relied through recent warfare in the Ogaden desert district to the coverage that it became highly scarce. These are only a few of the potentially economic species awaiting investigation [9]. Similarly, it has been a staple food of nomadic groups [10]. It is grouped among potentially valuable crop plants and is the wild edible plant in Ethiopia [11]. Somalis prefer *C. edulis* to staples such as corn and sorghum. It also has high demand by the urban people among other wild fruit plants. For example, a tin full of nuts is sold for 4000 Somali Shillings (i.e., 0.25 US dollar) in 2001 [12]. On the other hand, *C. edulis* is categorized along with major fodder trees and shrubs. Like other tree and shrub species, it is critical to progress the forest and rangelands and afford further income for pastoral communities [13]. C. *edulis* is known to be termite resistant and therefore used by local people to build houses [14]. It is used for various purposes including firewood, bee forage, mulch, soil conservation, nitrogen fixation, live fence and tannins [15].

It is known to be challenging to insect pest herbivores [16] and has sickening and anti-feedant cause over some insect pest such as *Hylobiusabietis* and *Phyllodectalaticollis* [17]. The leaf extract of *C. edulis* powerfully inhibited some larval growth [18]. There is no any information of fungal, bacterial, viral or physiological diseases in this plant [19]. But the recent study by [20] indicated that the hostile bacteria seem to have potential to influence both seed germination and plant growth. The study indicated that *C. edulis has* difficulties of growing outside its native environment, which may be partly due to scarce of availability of qualified seeds and a low population of beneficial microorganisms.

Seed germination is significantly chief occasion in the plant life cycle and the capability of seed imbibition to start germination can be considered as a vital regulatory step in plant development [21]. *C. edulis* is self-reseeding and the seeds are relatively big and heavy [22].

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Received May 20, 2015; **Accepted** July 10, 2015; **Published** July 17, 2015

Citation: Derso C, Feyissa T (2015) Effect of Seed Treatment on Seed Germination and Seedling Growth Attributes of Yeheb (*Cordeauxia edulis*) with *In-Vitro* Conditions J Biotechnol Biomater 5: 188. doi:10.4172/2155-952X.1000188

Cordeauxia edulis produces seeds, but grows slowly especially in the seedling stage while it is establishing its huge root system. Up to 80% germination can be obtained [23], but seed storage behavior is questionable. Viability is completely lost after four months of open storage at room temperature [12]. Now a day's its incidence has been strictly reduced over most of its novel range and it has disappeared from many localities well known by earlier travelers [24] due to deterioration of the vegetation caused by widespread human intervention, natural states of the plant including biological factors and climate changes that always accompanied the process of deforestation. According to [23], the success of establishing the seedlings in the field has been low. Therefore, the progressive loss of this plant will continue unless there is any appealing response.

So far, there is no research accomplishments reported with regards to the effect of seed treatment on seed germination and seedling growth of *C. edulis*. An *in situ* conservation, management and utilization, biochemical analysis as well as preliminary assessment of the phenology are among research efforts with regard to this species. Some of those results imply the need for further comprehensive protection of the natural stands and expansion of its cultivation.

Therefore, establishment of suitable pre-treatment protocol for seed germination of *C. edulis* with *In Vitro* conditions is crucial. The *In Vitro* germinated seedlings are important to produce large amount of explants within small area under aseptic conditions for the purpose of further micro propagation and regeneration works. According to [25], Sterilization of seeds pass to *In Vitro* proliferation is critical for the development of sanitary *In Vitro* plantlets that make sure the reduction of the contaminants and high survival rate of explants. Plant tissue culture is based on the promise that plants can be separated into their component part, manipulated *In Vitro* and grown to complete plants [26]. Concomitantly, another feature of plant tissue culture greatly served the increased research activity in plant in the near future.

Thus, it is essential to enhance the effectiveness of *In Vitro* seed germination of *C. edulis* by adjusting the seed treatment protocol. Hence, the main objective of this study is to know the effect of seed treatment on seed germination and seedling growth Attributes of *C. edulis* in *In-Vitro* conditions.

Material and Methods

The plant materials and explant source

Matured seeds of *C. edulis* were obtained from Somali Region Pastoral and Agro Pastoralist Research Institute (SORPARI), 630 km South-east of Addis Ababa, which is found in Somali Regional State. The current investigations were carried out at Plant Tissue Culture Laboratory of the Institute of Biotechnology, Addis Ababa University.

Surface sterilization of seeds

Hundred fifty seeds, 30 in each experiment, were washed repeatedly using running tap water and detergent (OMO) following de-coating of them. The seeds were sterilized by 70% ethanol for 1 min and rinsed over again with sterile distilled water followed by sterilization using different concentrations of Clorox (NaOCl, 5.25% of available chlorine) (3%, 5%, 10% and 15% having 0.16%. 0.25%, 0.53% and 0.79% (v/v) hypochlorite, respectively) and 2-3 drops of Tween-20 for 5 min. Finally, it was rinsed four times with sterile distilled water. Seeds without any treatment were used as a control treatment.

Evaluation of germination rate and seedling growth

After sterilization, the germination rate of the seeds was investigated using both coated and de-coated seeds. Those seeds were sterilized and cultured on growth regulators-free MS (Murashige and Skoog, 1962) medium. In each experiment, 30 coated or de-coated seeds were used. The emerged seedlings were evaluated for their respective developmental appearance.

Experimental design, data collection and statistical analysis

In this study, completely Randomized design (CRD) was used. Five seeds per culture vessel were used for each treatment of surface sterilization and seed germination. Each treatment also had six replicates of culture vessel. The effect of Clorox concentration and the germination percentage, seedling states were recorded both in quantitative and qualitative ways. SPSS version 20 statistical software at probability ($\alpha < 0.05$) were used to analyze the whole data.

Results

Seed sterilization

When the seeds were sterilized by using different concentration of Clorox (3%, 5%, 10% and 15%) for 5 min, higher percentage of decontamination (85%) was observed at 15% Clorox (0.79% available chlorine). On the other hand, seed germination was reduced to 10% and even germinated, the growth of hypocotyls become very dwarfed and stunted at this concentration. Among these concentrations, 5% Clorox (0.25% available chlorine) showed 80% decontamination and highest percentage (70%) of seed germination. In this investigation, 3% Clorox showed the least percentage (30%) of decontaminated seeds next to the control treatment.

Increasing the concentration of Clorox resulted in higher decontamination, but decreased the percentage of seed germination (Table 1).

As showed on above table, Clorox concentration significantly affected percentage of decontamination. However, no significant difference was observed among 5%, 10% and 15% Clorox concentration in percentage of disinfected seeds, but 10% and 15% Clorox concentration negatively affected percentage of seed germination than 5% Clorox concentration.

Seed germination

When the seeds were transversally cut at the tip, germination was enhanced. The seeds sown at 45° orientation on MS medium had better germination percentage (Figure 1). Maximum number (73.3%) of de-

Clorox concentration	Decontaminated seeds (%)	Germinated Seeds (%)
3%	30	15
5%	80	70
10%	85	20
15%	85	10

Table 1: Effect of different Clorox concentrations on decontamination and germination percentage of *C. edulis* seeds.

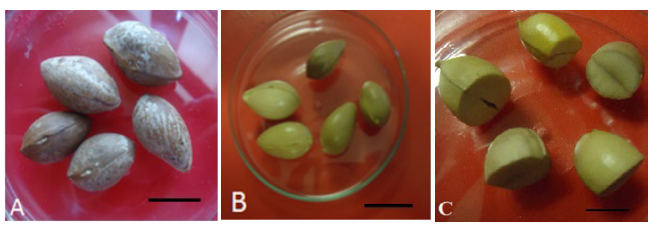

Figure 1: Seeds of *C. edulis* (A) Coated seeds; (B) De-coated seeds and (C) cross-sectional part of seeds of *C. edulis*. Bars represent 3 cm.

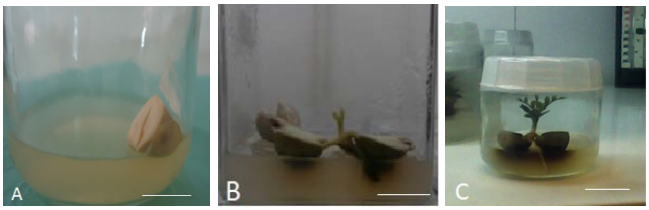

Figure 2: Germination and seedling appearance of *C. edulis* (A) Seed culture on MS medium; (B) New emerging seedling and (C) Two-week-old seedling. Bars represent 2 cm.

coated seeds germinated on MS basal medium in 6-7 days after seed sowing, but intact seeds did not show any sign of germination.

The aerial parts of the seedlings showed stunted growth and the leaves were folded when the concentration of Clorox concentration increases above the recommended. The roots showed rapid growth and it was very long relative to its shoot system at the first stage (Figure 2).

Discussion

Seed sterilization

In the present study, various concentrations of Clorox showed different responses over *C. edulis* seed sterilization. Physical sterilization of seeds by washing with tap water using detergent (OMO) was vital. According to [27], mechanical methods do only remove infectious materials and most pathogenic organisms are from the seed surface.

Among tested concentrations, 5% Clorox for 5 min was found to be the best for *C. edulis* seed sterilization as it resulted in 80% decontaminated seeds. Comparable response was reported as *C. edulis* seeds sterilized in a 5% solution of sodium hypochlorite for 5 min and then rinsed for 5 min in de-ionized water [11]. However, there might be some differences in seed age starting from harvesting, work environment and collection period of seeds. In 5% Clorox solution, 73.3% of aseptic seed germination was obtained and the seedlings were established well. As [28] tested on a medicinal herb, *Andrographis panicuatanee*, no significant correlation was found between contamination and germination percentages. Even though all safety measures were taken with asepsis, microorganism growth in the culture medium can happen and might have affected the germination of seeds [29].

The increasing of Clorox (NaOCl) concentration for *C. edulis* seeds significantly reduced contamination, but germination decreased. Whenever possible, germinated seedlings were stunted with folded leaves. This observation was in agreement [30], the *In Vitro* seed germination of a fiber crop, flax (*Linum usitatissimum)* seedling growth and viability of tissues were negatively affected by sodium hypochlorite (NaOCl) at high concentrations. Similarly on leguminous tree, *Albizia lebbeck*, except the sterilant ($HgCl_2$), increasing in concentration significantly reduced the contamination, but on the other hand seed germination was affected [31].

Seed germination

The seeds germinated better with 45° orientation than those put in horizontal or direct vertical position. This may be important due to the adequate imbibition. As [32], any germination progress is led by imbibition; anything that affects this process can probably influence germination.

In MS medium, 73.3% of de-coated seeds were germinated. In contrast, coated seeds didn't germinate *In Vitro*. This was suggested as hard seed coat may hamper water uptake, resulting in little or no germination in low moisture conditions. According to [33], de-coated seeds of *Phaseolus angularis* were used than coated seeds to obtain better germination (100%) and more consistent etiolated seedling explants. Favorable moisture conditions speed up germination of seeds of different native trees of Ethiopia [34].

When the seeds of *C. edulis* were soaked in water, enhanced its moisture content and arrived at highest germination. As [12], the proportion of germination and viability of *C. edulis* seeds were highly dependent on seed moisture content. Seeds soaking in water enhanced germination [35]. Besides, in plants, DNA injury was repaired in the embryo and restored early in imbibition and can be vital for germination performance [36].

Conclusion

In conclusion, results in this study revealed that pre-treatment of seeds such as soaking in cold water and removing the seed coat facilitated *In Vitro* germination of *C. edulis*. Cutting the de-coated seeds transversally enhanced the *In Vitro* germination. Sterilization of de-coated with 5% Clorox for 5 min was found to be effective. Generally, those treatments are important for *In Vitro* germinated seeds which are essential for further experimental process of micro propagation and regeneration. In addition, it used as effort for further research activities by reducing time and cost for the target plant.

Acknowledgment

Jigjiga University, Addis Ababa University, for financial support, and Somali Region Pastoral and Agro-pastoralist Research Institution, for giving us seed of *C. edulis,* are gratefully acknowledged.

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