Applied Ecology and Forestry Science, 2019, Vol. 4, No. 1, 1-3
Available online at http://pubs.sciepub.com/aefs/4/1/1
Published by Science and Education Publishing
DOI:10.12691/aefs-4-1-1

Liquid Nitrogen as Tree Seeds Dormancy Braking Pre-Treatment

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Received June 10, 2019; Revised July 13, 2019; Accepted July 26, 2019

Abstract Dormancy is complex and seeds need a very specific pretreatment. The common pre- treatment that used to break dormancy is Acid which is dangerous and environment pollutant. So the aim of these experiments is to find substitute pre-treatments. Most of Sudanese seeds have physical dormancy. The coats are hard and need to be rupture or cracked to start germination process. Exposing the seeds to different series of low temperature (Liquid nitrogen) and high temperature (boiling water) may make cracks in the seeds coat. Seeds of Acacia seyal, Acacia nilotica, Cassia fistula, Acacia oerfota and Delonix regia were dipping in liquid nitrogen for 5 minutes and then dipping in hot water for 5 minutes. This were done 1, 2, 3, 4, 5 times and treated with electric burner as upper control and control without treatment. The results showed that the different treatments have not effected the germination percentage of Acacia nilotica, Cassia fistula, Acacia oerfota but the seeds of Acacia seyal and Delonix regia affected the germination percentage significantly. It gives percentage higher than un treated seeds. This result is promising in using the liquid nitrogen as pre-treatment instead of acid.

Keywords: *dormancy, germination percentage, liquid nitrogen, boiling water*

Cite This Article: Mai M. A. Hassan, and Fatima A. Hassan, "Liquid Nitrogen as Tree Seeds Dormancy Braking Pre-Treatment." *Applied Ecology and Forestry Science*, vol. 4, no. 1 (2019): 1-3. doi: 10.12691/aefs-4-1-1.

1. Introduction

The seed phase is the most important stage in the life cycle of higher plants as regards survival; dormancy and germination are natural mechanisms to ensure this. The seed is often well equipped to survive extended periods of unfavorable conditions, and the embryo is protected by one or several layers of other tissues. [1] Information on the dormancy breaking and germination requirements of seeds is critical to understanding not only the population dynamics of a species, but also how to propagate it either in situ or ex situ. The need to germinate seeds of tropical trees increases each year due to the growing demand from silviculture and restoration efforts in various locations throughout tropical regions of the world [2] There various type of dormancy such as Exogenous dormancy, which divided into Physical (impermeability of seed coat or pericarp to water), Chemical (inhibitors in pericarp or seed coat) and Mechanical (Mechanical resistance of pericarp or seed coat to embryo growth), Endogenous dormancy which contain Physiological (germination prevented by physiological inhibiting mechanism) and Combined morpho-physiological dormancy [3] Seed pre-sowing treatments break the impermeable seed coat and allow the embryo to imbibe water. Several artificial methods have been used to break the dormancy of tree seeds with a hard, impermeable seed coat Mechanical or chemical treatments are the method most often used to break seed coat dormancy to obtain uniform and rapid germination. Most of Sudanese seeds have physical dormancy. The coats are hard and need to be rupture or cracked to start germination process The common pre-treatment that used to break dormancy is Acid which is dangerous and environment pollutant. So the aim of these experiments to find substitute pre-treatments. Liquid nitrogen is used as preservation agent for long term storing seeds and it recorded that liquid nitrogen do not affect the seed germination [4], but there were no literature about using it as seed pre-treatment.

2. Materials and Methods

Five tree species were selected *Acacia seyal* (Talah), *Acacia nilotica* (Sunt), Cassia fistula, *Acacia oerfota*(laot) and *Delonix regia* (golde more) (Figure 1, Figure 2, Figure 3, Figure 4, Figure 5).

Figure 1. *Delonix regia* Seeds (golde more)

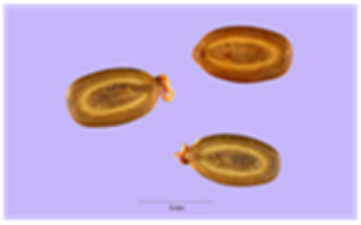

Figure 2. *Acacia seyal* Seeds (Talah)

Figure 3. *Acacia nilotica* seeds (Sunt)

Figure 4. *Acacia oerfota* seeds (laot)

Figure 5. Cassia fistula seeds

According to simple physics law that the materials elongate with heat and shrinkage with cold, this experiments were designed. The seeds exposed different series of low temperature (Liquid nitrogen (-196o c) and high temperature (boiling water (100o c). This were done 1, 2, 3, 4, 5 times and treated with electric burner (Which is suitable for small quantities) as upper control and control without treatment. For each treatment 100 fruits were used. These were divided into 4 replicates of 25 fruits each. Fruits were sown immediately after treatment. fruits were sown in round aluminium dishes filled with moist sand. Dishes were watered daily with a fine shower. Germination was carried out in a controlled germination room at the National Tree Seed Centre –Soba 30°C, light for 12 hours from fluorescent lamps. Germination counts were done at 7 days interval and for a period of 6 weeks.

3. Results and Discussion

Table 1. Seed characteristics of the different species under study

species	MC%	Viability/ cutting test	Purity %
Acacia seyal	5	52	100
Acacia nilotica	4	70	98
Acacia oerfota	4.5	40	100
Delonix regia	4	52	99
Cassia fistula	6	50	99

Table 2. Effect of different treatments on seeds germination percentage

species / Pre- treatments	*Acacia seyal,*	*Acacia nilotica,*	*Acacia oerfota*	*Delonix regia*	*Cassia fistula*
5min liquid N &5 min boiling water(1 time)	226 b	1b	2 b	14 b	3b
5min liquid N &5 min boiling water(2 times)	227 b	0 b	4 b	15 b	4b
5min liquid N &5 min boiling water(3 times)	222 b	2 b	2 b	9 b	2b
5min liquid N &5 min boiling water(4times)	228 b	11b	0 b	/	1b
5min liquid N &5 min boiling water(5 times)	229 b	1 1b	3 b	/	3b
Electric burner (upper control)	50 a	59 a	39 a	47 a	44 a
Control(without pre-treatment)	5 c	1 b	2 b	4 c	0 b
P=	0.001	0.001	0.001	0.001	0.001
SE ±	4	2	3	4	2

Table 3. The seeds parameters of the species under study

Species	Seeds length/ mm	Seeds width/ mm	Seeds thickness/ mm
Acacia nilotica	7.9 c	6.1 b	3.3 c
Delonex regia	19.7 a	6.1 b	4.2 ab
Cassia fistula	9.9 b	7 a	4.3 a
Acacia orefota	5.5 d	4.9 c	3.9 b
Acacia seyal	6.1 d	4 d	1.8 d
P =	0.0001	0.0001	0.0001
SE±	0.3	0.2	0.2
CV =	53	21	29

Present study explored the method to release seed dormancy of three acacia species, Cassia fistula and Delonix regia by exposed to different series of low temperature (Liquid nitrogen (-196°C) and high temperature (boiling water (100°C) tension in the outer cells causes the formation of cracks through which gas and water can penetrate. The effectiveness of tension is normally enhanced by rapid temperature change e.g. by rapidly pouring the seeds into boiling water after dipped in Liquid nitrogen. the results showed that Electric burner treatment was the most effective method to release seed dormancy. Cassia fistul, Acacia Nilotic, Acacia oerfota show no response to pretreatment; germination percentage show no different from the control. In addition to that, the time dipped into liquid nitrogen showed no effects on dormancy breaking..

Cassia fistula is sensitive to temperature [5] reported that Cassia fistula a quick dip in boiling water killed 50% of the seeds, and 68% were killed after 5 minutes' boiling, which agree with this pretreatment ; repeat the treatment duration decrease seed germination percentage The above reference to temperature sensitivity also holds for boiling water some species are sensitive and consequently easily damaged. Both temperature level and duration of exposure are crucial for effect and possible damage .the Acacia nilotica, A. oerfota and A. seyal show different response such differences in seeds response may be attributed to the differences in seeds coat thickness [6] Acacia nilotica, Acacia oerfota very hard coated seed than A. seyal [7] A. nilotica and Acacia oerfota have the thickest seed coat among the acacias of the Sudan imposing seed coat dormancy. [7] Delonix regia and Acacia seyal respond well to pretreatment increased the percentage germination of Acacia seyal seeds from control to treated seed .Referred to liquid nitrogen treatment, five time was the best duration for dormancy release for A. seyal. In addition ,two duration treat time significantly increased germination seed percentage for Delonix regia The effects of liquid nitrogen treatment may closely correlated with seed size; liquid nitrogen effectively released dormancy of large size seed such as Delonix regia.

Some seeds were broken its dormancy in constant periods for example after 6 months of storage and then inter the dormancy phase. So these experiments must repeat in different seasons and different periods but the optimal treatment time varied with species.

4. Conclusion

This result is promising in using the liquid nitrogen as pre-treatment instead of acid.

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