International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Effects of Different Types of Phytohormone over Traditional Seed Pre-Treatments on Germination of Dryland Tree Species

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Abstract: ***The need for more energy, shelter and food has necessitated increased afforestation. However, the problem of seed dormancy has hindered the germination of the several trees species and therefore ways of breaking seed dormancy remains urgent. The main objective of study was therefore to determine the efficacy of different phyto-hormonal over traditional seed pre-treatments on germination of selected dryland tree species. Seeds of four tree species (Balanite aegyptiaca, Melia volkensii, Acacia tortilis and Adansonia digitata) were sterilized by soaking in 1% potassium permanganate ($KMnO_4$) for 10 minutes, rinsed with distilled water and air dried for 2 days before treatment. The four phytohormones were Naphthaleneacetic acid (IAA), 2,4-Dichlorophenoxyacetic acid (2-4,D), Giberrelic acid (GA_3) and N6 Benzyl adenine (BAP). The experimental setup was a Completely Randomized Design (CRD) constituting four hormones with a control (without any pretreatment). The hormonal pre-treatment were also compared with traditional seed pretreatment methods consisting of scarification, H_2SO_4 treatment, warm water at 30°C and hot water at 100°C. Seeds were then be monitored for germination. Mean differences in the germination percentages were analyzed using One-Way ANOVA following arc-sine transformation. Germination in control was always low (≤ 20%) while taking up to 39 days to first germination. Among the traditional seed pre-treatment methods, the highest germination % occurred in H_2SO_4 and but rarely exceeded 45% germination. Phytohormones mainly auxins gave better response over seeds without any pre-treatment and over other traditional pretreatment methods. Amongst all growth hormones, 2,4-D, NAA and BPA gave best response for seed germination (70-90%) and therefore managers of dryland agroforestry species should use phytohormones in enhancing seed germination. However, further studies on the effect different concentrations of hormones and combination of the traditional methods and hormonal treatments should also be conducted.***

Keywords: Dryland afforestation, seed dormancy, seed germination, phytohormones

1. Introduction

Forest especially in the tropics are overexploited at a rate faster than reforestation which competes with other land uses such as food production, livestock grazing, and economic growth [1-2]. Deforestation and forest degradation have been factors that threaten forest productivity and sustainability [3] coupled with impending and increasing demand for forest and forest resources as a result of increasing world population [4]. Africa has the largest drylands areas in the world covering over 40% of the landmass with the largest being Sahara Desert (http://blogs.ei.columbia.edu/2010/09/16/dryland-regions-of- africa). Based on Agenda 21 blueprint for action into the 21st century adopted at the 1992 Rio Earth Summit, new initiatives in agroforestry and farm forestry are seeking to promote poverty alleviation and environmental rehabilitation in the dryland areas through integration of indigenous trees [5-6]. Numerous trees have been identified as fast growing and are categorized as high biomass yielder. Dryland tress that have high biomass and production include: *Melia volkensii, Prosopis juliflora*, *Boscia coriacea*, *Cordia sinensis*, *Maerua subcordata*, *Balanites pedicellaris*, *Zizyphus mauritiana*, *Grewia bicolor*, *Acacia nilotica, Acacia tortilis, Andansonia digitata*, *Tamarandus indica*, *Terminalia brownii*, *Cordia ovalis*, *Croton sylvaticus*, *Balanites pedicellaris*, *Balanites aegyptiaca* [7]. Most of these species are dispersed through seeds.

Seed germination commences with the uptake of water by the dry seed, followed by embryo expansion growth and germination is completed when the radicle has protruded through the surrounding covering layers. Seed germination depends on the interaction of the seed with the environment, and occurs under favourable conditions with the key environmental factors: water availability, appropriate temperature and in some cases light [8]. However, propagation of a number of these dryland tree species are difficult due to problem of low seed germination. The low seed germination is due to seed coat. Most seeds consist of an embryo, surrounded by one or more covering layers. The covering layers consist of a living endosperm of one to several cell layers and a testa, which is mostly dead tissues that cause dormancy leading to poor growth potential [9].

Dormancy is an adaptation that ensures seeds will germinate only when environmental conditions are favorable for survival [10]. Seed dormancy can be due to seed coat, embryo, or a combination of both. Seed coat imposed dormancy may be due to non-permeability to water and/or gases, mechanical prevention of radicle extension, or prevention of inhibitory substances from leaving the embryo. In embryo-imposed dormancy, there is usually a requirement for temperature and/or light treatment that must be satisfied naturally during a period of after-ripening. Seeds of most dryland tree species thus require treatments to overcome seed dormancy and hasten germination. The conditions necessary to allow seeds to break dormancy and germinate can be highly variable among species, within a species, or among seed sources of the same species [11].

Several methods have been used to break seed dormancy. They include socking in water, stratification, leaching, temperature treatment, scarification and light among others [12-15]. Germination stimulators have also been applied to

improve seed germination [9,16-18]. However, the use of growth hormones in breaking seed dormancy of dryland trees species can be affected by many factors such as tree species, type of hormone and climatic factors. Moreover, the information of the response of hormone in breaking dormancy in developed world may not be applicable in other areas due to the different climatic condition and different species of trees. On the basis of the forgoing discussion, this study looked at the use of hormones in germination of dryland forest tree species in Kenya.

2. Materials and Methods

2.1 Study Area

The study was conducted at Kenya Forest Research Institute (KEFRI) Kitui dryland Eco regional centre tree nursery in Kitui, Central within Kitui County in Kenya. The centre is approximately 4 km from County headquarters a long Kitui Prison's road. The area receives bimodal rainfall i.e. long rains in March to May and short rains in October to December. Annual rainfall ranges between 400–800 mm and temperatures between 16–32°C. The area is characterized by sandy clays to clay with top soil of sandy loams with parches of red soils. The main trees species dominating the area are *Acacia species, Terminalia brownii*, *Grevillea robusta*, *Senna seamea*, *Balanite aegypiaca*, *Croton megalocarpus* and *Mangifera indica*.

2.2 Seed source, seed sterilization and preliminary screening

The seeds of four species of the dryland species used in this study were purchased from venders in Kitui, which is one of the dryland areas in Kenya. The seeds included: *Melia volkensii, Balanites aegyptiaca*, *Acacia tortilis* and *Andansonia digitata*. The seeds were sterilized by soaking in 1% potassium permanganate ($KMnO_4$) for 10 min(Plate 1a), rinsed with sterile distilled water thoroughly, air dried for 2 days before experiments. Seeds were stored at 4°C until use.

2.3 Traditional germination experiments

The seeds were soaked in four traditional treatments methods during the study (Plate 1b). Treatments consisted of: 1. mechanical scarification; 2. soaking in H_2SO_4 in for 20 minutes; 3. soaking seeds in hot water (100°C) for 24 hours; 4. Soaking seeds in warm water (30°C) for 24 hours. Three replicates of 45 seeds were used per treatment for each collection.

2.4 Phyto-hormonal Treatments

Seeds were soaked in four main phyto-hormones in this study (Plate 1c) including: 1. auxins: Indoleacetic acid, IAA and 2.4-dichlorophenoxyacetic acid (2-4,D); 2. Giberrelic acids (GA_3); 3. cytokinins (N6-Benzyl adenine). The whole experimental setup was a Completely Randomized Designs (CRD) constituting the hormones applied independently to the seed of each tree species. Water-treated seeds without any hormone served as control. The entire experiment was executed in triplicate. For each hormonal treatment, forty five (45) seeds were soaked in hormones for 24 hours and changes monitored during the experiment.

2.5 Germination Test

The hormone treated samples were each prepared for germination in washed river sand. A total of 30 germination trays (Plate 1d) were used for each of the seed germination tests. All the forty 30 seeds from each of the treatments were monitored for germination. Briefly, the seeds were placed on the top of two layers of filter papers, previously moistened with distilled water in petri dishes. The petri dishes were placed in a germination chamber at a constant temperature of 25°C with a 12-h photoperiod provided by fluorescent lights. Water was added as necessary to maintain moistness. Radicle protrusion of 2 mm was the criterion of germination and the germinated seeds were counted after incubation. Germinated seeds were also monitored for vigour (Plate 1e and f).

Plate 1: See treatment from soaking to germination of the seeds

2.6 Data analysis

Germination data were presented as percentage. They were arcsine transformed to facilitate statistical treatment. To test the efficacious ranges of the hormones on germination, One-Way ANOVA was done. The data were analyzed using GENSTAT 10.0. Significant was declared at $p \leq 0.05$.

3. Results

In absence of any seed-pretreatment, germination % for *Balanite aegyptiaca* was 20%, *Melia volkensii* and *Acacia tortilis* were 13.3% with only 10% of *Adansonia digitata* germinating. The results showing days to first and last germination and seed germination rate of the four species of dryland afforestation trees species are shown in Table 4.1. There were significant differences in the number of days to first germination (χ^2 = 8.912, df = 3, p = 0.005). *B. aegyptiaca* had the least number of days to first germination followed by *A. tortilis*. Also there was a significant differences in the number of days to the last germination of the various forest tree species (χ^2 = 12.988, df = 3, p = 0.002) with *B. aegyptiaca* having the least number of days to last germination followed by *A. tortilis*.

Table 1: Days to first and last germination and seed germination rate of the four species of dryland afforestation tree species

Tree species	*Days to first germination*	*Days to final germination*	*Germination rate*
Melia volkensii	39	64	0.09
Acacia tortilis	33	58	0.10
Balanite aegyptiaca	24	42	0.20
Adansonia digitata	38	61	0.07

Seed germination of four dryland species using four traditional pre-treatment methods are provided in Figure 1. Different pretreatment methods resulted in different germination percentages ($P < 0.05$). For *Melia volkensii*, the highest germination % occurred in H_2SO_4 (36.7%) followed by scarification and hot water (30%) and least in warm water of 30°C (20%). In *A. tortilis*, the highest germination % occurred in H_2SO_4 treatment (42.8%) followed by hot water (42.1%) and least in warm water of 30°C (33%) In *B. aegyptiaca*, H_2SO_4 and hot water at 100°C resulted in highest germination of 46.7% and lowest in warm water at 30°C. In *A. digitata* scarification treatment resulted in highest germination (40%) followed hot water (36.7%), and lowest in warm water at 30°C (23.3%).

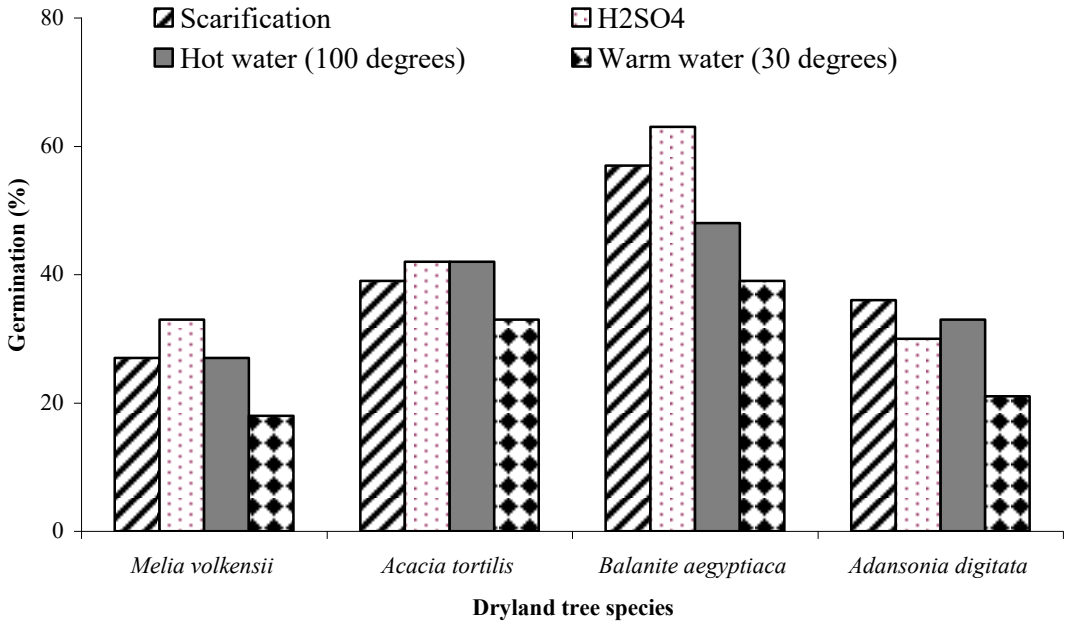

Figure 1: Seed germination of four dryland species using the traditional pre-treatment methods

Results showing the days to first and last germination of the four species of dryland afforestation tree species undergoing the traditional seed pretreatment methods are shown in Table 2. Number of days to first and last germination in for all the tree species was lowest in H_2SO_4 treated seeds followed by scarification and highest in warm water at 30°C.

Table 2: Days to first and last germination of the four species of dryland afforestation tree species undergoing the traditional seed pretreatment methods

	Days to first germination				Days to final germination			
	Scarification	H_2SO_4	Hot water (100°C)	Warm water (30°C)	Scarification	H_2SO_4	Hot water (100°C)	Warm water (30°C)
Melia volkensii	39	37	43	44	43	41	46	50
Acacia tortilis	37	34	38	40	44	42	45	47
Balanite aegyptiaca	25	22	27	31	37	36	39	40
Adansonia digitata	35	29	37	40	51	48	52	54

In *Melia volkensii*, *A. tortilis* and *A. digitata* the germination rates were similar (0.08−0.10 %/day). In *B. aegyptiaca*, highest germination rate occurred in seeds that were scarified (0.27%/day) followed by those treated with H_2SO_4 and hot water at 100°C and lowest in warm water of 30°C (0.15%/day).

Seed germination of four dryland species using phytohormal pre-treatment methods are provided in Figure 2. Different hormonal pretreatment methods resulted in different germination percentages ($P < 0.05$). For *Melia volkensii*, the highest germination % occurred in 2,4-D (70.0%) followed by NAA and BAP (63.3%) and least in GA_3 (60%). In *A. tortilis*, the highest germination % occurred in 2, 4-D and BAP (80%) followed by treatment in NAA (76.8%) and lowest in GA_3 (70%) In *B. aegyptiaca*, NAA and 2,4-D treatment resulted in highest germination of 96.7% followed by germination in BAP treatment and lowest in GA_3. Finally in *A. digitata*, NAA treatment resulted in the highest germination of 66.7% followed treatment in 2,4-D (63.3%), and lowest in BAP and GA_3 (60.0%).

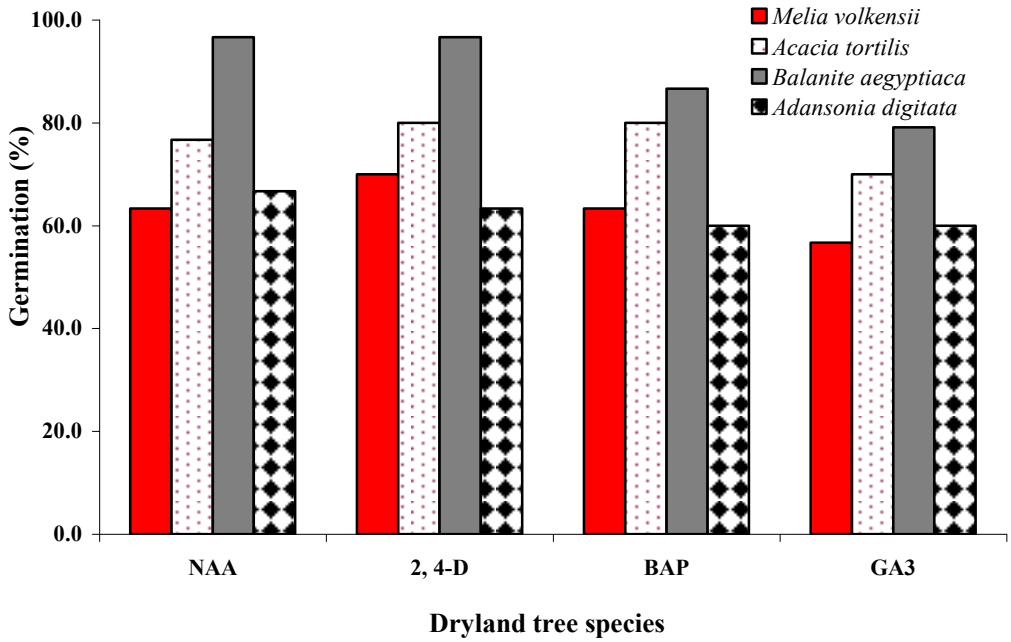

Figure 2: Seed germination of four dryland species pre-treated with phytohormones

Results showing the days to first and last germination of the four species of dryland afforestation tree species undergoing the phytohormonal seed pretreatment methods are shown in Table 3. Number of days to first and last germination in for all the tree species was lowest in 2, 4-D treated seeds followed by NAA and highest in BAP.

Table 3: Days to first and last germination of the four species of dryland afforestation tree species pre-treated with phytohormones

	Days to first germination				Days to final			
	NA	2,	BA	GA	NA	2,	BA	GA
Melia	29	2	33	36	36	3	38	39
Acacia	25	2	28	30	32	3	35	36
Balanite	17	1	19	21	27	2	28	29
Adansoni	23	1	25	27	43	3	40	45

4. Discussion

Dormancy is an adaptation that ensures seeds will germinate only when environmental conditions are favourable [11]. In the current study the germination percentage without any pre-treatment was low (≤ 20%) for all the species tested. Germination of *Balanite aegyptiaca* (20%), *Melia volkensii* and *Acacia tortilis* (13.3%) *Adansonia digitata* (10%) were regarded as low similar to previous studies [9,19-20], which have also described these species to have seed coat dormancy that hinders their germination [14]. Seeds that have hard, thick seed coats that physically prevent water or oxygen movement into seeds have physical dormancy [21]. Indigenous such plants include *Adansonia digitata* [4] which is being threatened of going into extinction because of its inability to regenerate under natural condition. Beside, it took up to 24−39 days to germinate and up to 42−64 days for final germination with low germination normally ≤ 0.20 %/day. These low performance during germination meant that seed pretreatment methods are required to hasten germination.

While various pretreatment methods have been advocated to reduce dormancy and accelerate germination, no single pretreatment technique may be effective for all seed species. In this study traditional pretreatment methods resulted in various germination percentages. The preceding findings of this study show that treatment of the dryland species with H_2SO_4 resulted in the best germination highest germination % over and above the control. The fact that acid treatment gave highest germination values within shortest time indicates that the more rapidly the seed coat is ruptured, the faster the rate of germination. This is more so, since a very widespread cause of seed dormancy is the presence of hard seed coat which prevents the entrance of water, exchange of gases and/or mechanically constrained the embryo [22]. Sulphuric acid is thought to disrupt the seed coat and expose the lumens of the macrosclereids cells, permitting imbibition of water, which triggers germination [23]. Jackson [24] also found that as soon as the seed coat is softened by scarification, the process of hydrolysis would commence to release simple sugars that could be readily utilized in protein synthesis, thereby encouraging germination. However, in *A. digitata* scarification and hot water treatment resulted in the highest germination. This implies that different species have varying rates at which their seed coat is permeable to water and gases [25]. However, the germination percentage using these traditional pre-treatment methods rarely exceeded 60% and took long to germinate suggesting they are not ideal in enhancing seed germination.

The differences observed in the germination percentage of seed subjected to different hormonal treatments shows significant impacts of the various pre-treatments on seed germination. The study determined that the highest germination of 70-96% occurred in auxins (2,4-D followed by NAA), which are similar to previous studies in the tested dryland species: *Alibzia lebbeck and Parkia biglobosa* and to some extent in *Prosopis africana* and *Senne siamea* [26]. Some species are known for which auxins also seem to play a prominent role in the germination process [27-28]. None of

the traditional pretreatment methods attained the high germination levels observed in when treatment was done using hormones. Abscisic Acid (ABA) is an example of a hormone (endogenous) which inhibits seed germination, while gibberellic acid (GA_3) is known to promote seed germination [18]. These two plant hormones are found in many seeds and the concentration ratio of ABA to GA_3 plays a vital role in seed germination. This concentration ratio varies with seed developmental stages. This explains why an external application of GA_3 to the seeds has promoted seed germination as previously determined by Singh *et al*. [29]. Seed germination and seedling growth are known to be regulated by exogenous hormones. Growth regulators used in Pre-sowing seed treatment with growth regulator play an important role in regulating germination and vigour. Out of the four growth regulators auxins had a significant effect on germination.

From the above data it may be concluded that growth hormones gave better response over traditional seed pre-treatment methods. The treatment with hormone and mostly 2,4-D and NAA was effective in breaking seed dormancy and reaching high germination rate and days to germination. The collection of plant hormones, including ABA, IAA, cytokinins, ethylene, gibberellins and brassinosteroids, can positively or adversely affect seed germination, while interacting with each other and therefore such studies should form the basis for the future research.

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