ENHANCING GERMINATION IN THE SEEDS OF AFRICAN EBONY *(Diospyros mespiliformis* HOCHST)

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Abstract
Diospyros mespiliformis is a multipurpose tree species with diverse environmental and ecological significances. However, low germination percentage associated with its seeds has limited its domestication for agroforestry practices. In an attempt to improve its seed germination percentage, nursery experiments were conducted in the College of Forestry Mechanization, Afaka Kaduna. Two separate experiments laid down in a completely randomised design were investigated on the effect of moist air tight or incubation environment and hydropriming on the germination percentage of *D. Mespiliformis* seeds. Mean germination percentages were calculated. Results indicated that *D. mespiliformis* seeds exposed to moist air tight environment for 0, 2, 4, 6 and 8weeks had the germination percentages of 25%, 66.50%, 93.25%, 80% and 93.25% respectively. Highest germination percentage value of 93.25% was recorded for *D. mespiliformis* seeds exposed to four weeks and eight weeks air tight environments. The mean germination times of 9, 8.92, 12.47, 10.70 and 12.47 days were recorded for *D. mespiliformis* seeds exposed to 0, 2, 4, 6 and 8weeks air tight environment respectively. The least mean germination time of 8.92days was recorded for *D. mespiliformis* seeds exposed to 2weeks of air tight environment. Germination percentage values of 25%, 80%, 100%, 80% and 93.25% were recorded for *D. mespiliformis* seeds hydroprimed for 0, 12, 24, 36, and 72 hours respectively. Highest germination percentage value of 100% was recorded for *D. mespiliformis* seeds hydroprimed for 24hours. *D. mespiliformis* seeds hydro primed for 0, 12, 24, 36 and 72 hours had mean germination time of 10.36, 33.95, 44.61, 34.03 and 39.70days. The least mean germination time of 10.36seed/day was recorded for seeds of *D. mespiliformis* that were not hydroprimed.

Keywords: *Diospyros mespiliformis,* Mean germination time, Hydroprimed, Agroforestry practices**.**
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Introduction

Diospyros mespiliformis is a deciduous savanna tree of Africa that belongs to the family of Ebenaceace. It is called Africa ebony, Jackal-berry, Jakkaldessie, Musuma and Mgula kanyaa by English, Africa, Tshivenda Tsonga and Hausa respectively (Thompson and Walter, 2007). The genetic name *Diospyros* means divine and the specific name *mespiliformis* is derived from two words (mesos), meaning half and (pilos) which are bullets. It is extremely widespread, occurring from Senegal east to Eritrea, Ethiopia and Kenya, and south Namibia, northern south Africa and Swaziland, but it is nearly absent in the more humid forest zones of west and central Africa. Trial planting for fruit production

existed in the Israel, and it is also being planted in the southern part of the United States (El-Kamali, 2011).

Its wood is used for posts in house construction flooring, joinery, furniture, ship building, vehicles bodies. Musical instrument, such as drums, house hold utensils such as cups, spoons, pestles, and mortars, tool handles, walking sticks, combs, agricultural implements such as ploughs, boxes, carving and turnery. Its bole is used traditionally for dug-out canoes. Its wood is also used as fire wood, and is value for charcoal production. Its leaves are occasionally eaten as vegetable, and the foliage is browsed by livestock. The gum from the bark is used to mend broken pottery and fruit pulp to glaze and varnish pottery. It is planted for re-afforestation, aesthetics, wind breaks and apiculture (El-Kamali, 2011). Various parts of the trees are used in traditional medicine. Roasted and pulverized roots are taken to treat jaundice, and root decoctions as anthelmintic, to ease childbirth, and to treat malaria pneumonia and syphilis. Its bark preparations are administered to treat cough, bronchial disease, tuberculosis and leprosy, and applied externally to wound, ulcers, bruises and furuncles (El-Kamali, 2011). Its bark is also used in veterinary medicine as vermifuge. Leaf decoctions or infusion are taken to treat fever, diarrhea, dysentery, trypanosomiasis, menorrhagia, whooping cough, hiccough and poisoning. Leaf preparations are externally applied to treat fever, pneumonia, conjunctivitis and otitis, and as antiseptic to wounds. Fruit decoction or infusions are taken to treat dysentery, diarrhoea and menorrhagia. Fruit ash is applied to fungal skin infections and fruit power to ulcers, whereas seed decoctions are administered against headache (Chivandi *et al.*, 2009).

Its twigs are chewed to clean the teeth. Various parts of the tree are used in ritual ceremonies. The composition of fruit per 100g edible portion is water (69g), energy (404kj), protein (1.1g), fat (0.4g), carbohydrate (22g), fiber (6.2), Calcium (96g), mg, iron (1mg), thiamin (0.01mg), riboflavin (0.04g), niaicin (0.24mg) and ascorbic acid (26.6mg). Analysis of the fruits in northern Nigeria showed that only low amount of antinutritional factors such and oxalate, phytate, saponins and tannin are present. The seeds contain about 9% water and their protein contents is 4.9g per 100g fresh weight. *D. mespiliformis* has immense benefits but the low domestication rate has been attributed to inadequate knowledge of breaking dormancy associated with its seed germination. In view of this, investigation was conducted into germination of its seed through presowing treatments.

Materials and Method

Experimental plot

The research was conducted in the nursery of Federal College of Forestry Mechanization, Afaka Kaduna. It is located at the Northern Guinea Savanna ecological zones. It is situated at about 30km along Kaduna Lagos road in Chikun Local Government Area of Kaduna state, Nigeria. The Garmin GPS 72 model was used to determine latitude 10^{o} 35^{1}

and $10^{o}\,34^{1}$ and longitude $7^{o}\,21^{1}$ and $7^{o}\,20^{1}$. Rainfall is approximately 1000 mm annually with the lowest monthly relative humidity averaging 29%. The vegetation is open wood land with tall trees; usually with small boles and broad leaves (Otegbeye *et al.,* 2001).

Fruit collection and materials

The fruits were sourced from the mother tree in Kaduna. The seeds were extracted from the fruits and air dried. The randomly picked samples of seeds were cut open to assess the viability through cutting method (Schmidt, 2000). The sand was collected from the College dam and sterilized in the Biological laboratory at 160^{0}C hours (Adelani *et al.,* 2014). The polypots of 20 x 5 x 5 cm^3 filled with the sterilize sand in the screen were used.

Experiment 1: Effect of moist air tight environment.

The effect of moist air tight environment on the seed germination of *D. mespiliformis* for (0, 2, 4, 6, 8 weeks) was assessed using a completely randomized design with four replications. The seeds planted in 4cm depth of soil at ambient temperature (27^{o}C) without change in orientation served as control. The wet sand (of about 200ml of water to 800g of sand) was packed in 1 liter poly bags and the seeds were planted at 4cm depth. The poly bags containing the seeds and sand were tied at the mouth, put in the sun and their orientation altered daily to ensure uniform distribution of high and low temperature changes during the day and night. Thermometer was used to observe changes in temperature during late evenings and afternoon hours. Each poly bag was replicated four times. 16 poly bags containing four replicates were opened at interval of two weeks. The number of seeds that germinated was recorded. The number of seeds that germinated at every two weeks interval was recorded for eight weeks of the experiment. Seed was considered germinated after development of plumule. Percentage germination was expressed as the total seed germinated per total seeds sown multiplied by 100.

Experiment 2: Effect of hydropriming on germination of *D.mespiliformis* seeds

A total of 960 *D. mespiliformis* seeds were extracted from the fruits, washed and air dried. The initial moisture content of seeds was determined and seeds were dried to the constant weight and final moisture content was determined. Sixty (60) seeds represented a replicate. Four replicates each was soaked in water for 12, 24, 36, and 72 hours. Each of the four replicates was removed from water after each 12 hours and dried back to the initial moisture content before soaking. Each four replicates was planted in 4cm depth of 20 x 5 x $5cm^3$ poly bags filled with sterilized river sand. 200ml of water was applied at two days interval and percentage germination and mean germination time was recorded. Seed was considered germinated after development of plumule. Percentage germination was expressed as the total seed germinated per total seeds sown multiplied by 100. The mean germination time was calculated using the relation:

$$MGT = \frac{\sum(Fx)}{\sum x}$$

Where x is the number of newly germinated seed on each day and F is the number of days, after seeds were set to germinate. *X* is the total number of seeds that germinated at the end of the experiment.

Data Analysis

The results of the germination experiments were analyzed for statistical significance (one way ANOVA) from the statistical software package for personal computer (Statsoft, 1993). All present germination data were arcsine-square root transformed prior to analysis. Multiple comparisons of means were made with 'Least Significant Difference (LSD)" test at the 5% level.

Results and Discussion

Effect of Moist Air Tight Environment or Incubation Weeks

Effect of moist air tight environment or incubation weeks on the germination percentage of *D.mespiliformis* seeds is presented in Table I. The *D.mespiliformis* seeds that were put in air tight environment for 0, 2, 4, 6, 8 weeks had germination percentage of 25%, 66.50%, 93.25%, 80% and 93.25% respectively. Highest germination percentage value of 93.25% was recorded for *D.mespiliformis* seeds exposed to four weeks and eight weeks air tight environment. This is an indication that the range of 4-8 weeks air tight environment enhanced germination of *D. mespiliformis* seeds. The mean germination time of 9, 8.92, 12.47, 10.70 and 12.47 days were recorded for *D. mespiliformis* seeds exposed to 0, 2, 4, 6 and 8 weeks of air tight environment respectively. The least mean germination time of 8.92days was recorded for *D.mespiliformis* seeds exposed to 2 weeks of air tight environment. The seeds responded quickly to the 2 weeks exposure. Germination percentage values of 25%, 80%, 100%, 80% and 93.25% were recorded for *D.mespiliformis* seeds hydroprimed for 0, 12, 24, 36 and 72 hours respectively.

Highest germination percentage value of 100% was recorded for *D. mespiliformis* seeds hydro primed for 24 hours. It can be deduced that 24hours hydropriming of *D. mespiliformis* seeds was appropriate treatment time to achieve optimum germination percentage. Exposing seeds of plant species to appropriate time of hydropriming allowed seeds to imbibe water for a longer time and went through the first stage of germination without protrusion of radicle (Kaya *et al*; 2006). Akinola *et al.* (2006) reported that higher duration of exposure to seed treatment resulted in higher cumulative germination in wild sunflower. Positive effects of seed priming on seed invigoration depend on priming duration (Ashraf and Foolad, 2005).

The efficiency of seed hydropriming for better seedling emergence has been reported (Abdulrahmani *et al;* 2007, Ghassemi-Golezai *et al*; 2008a). Kaya *et al*. (2006) working on germination of sunflower under drought and salt stress reported that hydropriming

improved both rate of germination and mean germination time both under salt and drought stress conditions. Fujikura *et al.* (1993) indicated the beneficial effects of hydropriming on aged or unaged seeds with respect to germination and percentage of normal seedlings in cauliflower. Singh (1995) and Shivankar *et al.* (2003) also concluded that hydroprinming has high potential in improving field emergence and ensures early flowering and harvest under stress conditions especially in dry areas. Lenticels seed by hydropriming resulted in higher seedling emergence in the field, compared to control and seed priming with PEG (Polyethylene glycol) (Ghassemi-Golezani *et al*; 2008b). Hydropriming is a simple and effective method for improving seed germination and seedling emergence of lentil in the field (Ghassemi-Golezani *et al;* 2008b). Caseiro *et al.* (2004) found that hydropriming was the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 hours compared to 48 hours. When seeds imbibe water, the water content reaches a plateau and changes little until radicle emergence (Bradford, 1986). Priming up to their point can have a positive effect, while extended priming duration may negatively affect germination. In other words, duration of seed priming, especially hydropriming, affects seed germination properties. Longer hydropriming duration negatively affects seeds germination properties. Strategies for improving the growth and development of crop species have been investigated for many years (Hamdollah, 2013). Seed priming is a pre-sowing strategy for influencing, seed germination and seedling development by modulating pre-germination metabolic activity prior to emergency of the radicle and generally enhances germination rate and plant performance (Bradford, 1986). During priming, seeds are partially hydrated so that pre-germination metabolic activities proceed, while radicle protrusion is prevented, then are dried back to the original moisture level (McDonald, 2000).

Priming induces a range of biochemical changes in the seed that required initiating germination process through breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibitions and enzymes activation (Ajouri *et al*; 2004). Priming is an effective technique that improves germination of several crops sciences (Singh, 1995). *Maesobotrya barteri* seed emergence was improved by several priming methods and duration. These findings are in line with the work of Mubshar *et al*. (2006) who stated that improvement in priming is affected by some factors such as plant species, priming media, type and concentration and priming duration. Stofella *et al.* (1992) reported also that priming of pepper seeds significantly improved radicle length. It is clear from these results that priming improved germination and growth of *Maesobotrya barteri* (Peter-Onoh *et al*., 2014). During hydroprimng, protein synthesis as well as hormone concentrations are increased and consequently leads to germination and seedling growth in prime seeds (Bailly *et al*., 2000; Ashraf and Foolad, 2005; Pirasteh- Anosheh and Hamidi, 2013).

The *D.mespiliformis* seeds hydroprimed for 0, 12, 24, 36 and 72 hours had mean germination time of 10.36, 33.95, 44.61, 34.03 and 39.70 days respectively. The least

mean germination time of 10.36seed/day was recorded for *D. mespiliformis* seeds not hydroprimed. Untreated seeds did not require time to undergo changes and reaction that took place in hydroprimed ones. On the other hand, the effectiveness of hydropriming was stated by Ghassemi-Golezani (2010b) who reported that the lowest mean germination time, highest germination percentage and seedling dry weight of Pinto bean were achieved with 7 and 14 hours priming duration which was significantly different from 21 hours of hydropriming. The probable reason for early germination of primed seeds may be due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2015). Priming improved the coefficient velocity of emergence of *Maesobotrya barteri*. Early germination of primed seeds over other treatments is probably due to water and gases entering the embryo early through the cracks and causing a series of enzymatic breakdown and resulted in the transformation of the embryo into a seedling early enough than other seed treatment (Peter-Onoh *et al*; 2014). Primed seeds had lower mean emergence time (MET) compared with unprimed seeds (Peter-Onoh *et al*; 2014). These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Sivritepe *et al;*2003).

Significant improvement in the growth of the emerging seedlings (root and shoot) may be attributed to early germination induced by primed over unprimed seeds (Farooq *et al;* 2005), which resulted in vigorous seedlings with more root and shoot length than the seedling from un-primed seeds. Handollah (2013) reported seed priming has been used to improve germination, reduce seedling emergence time and improve stand establishment and yield. During priming, seeds are partially hydrated so that pre-germinative metabolic activities proceed while radicle protrusion is prevented, and then seeds are dried back to the original moisture level.

Table 1: Effect of Moist Airtight Environment Weeks, Seed Hydropriming Hours and Mean Germination Times on the Germination Percentage of *D. mespiliformis*

Airtight Environment	Percentage Germination (%)	Mean Germination Time (Days)	Seed hydro Priming (hours)	Percentage Germination (%)	Mean Germination Time (Days)
0	25.00^{c}	9.00^{c}	0	25.00^{C}	10.36^{d}
2	66.50^{b}	8.92^{c}	12	80.00^{b}	33.95^{c}
4	93.25^{a}	12.47^{a}	24	100.00^{a}	44.61^{a}
6	80.00^{ab}	10.70^{b}	36	80.00^{b}	34.03^{c}
8	93.25^{a}	12.47^{a}	72	93.2^{a}	39.70^{ab}
SE±	11.23	0.03	SE±	6.69	0.03

*Means on the same column having different superscripts are significantly different ($P<0.05$).

Conclusion

The use of moist air tight environment and hydropriming methods laid down in a completely randomized design were employed to overcome dormancy of *D. mespiliform* seeds. The result indicated that the highest germination percentage value of 93.25% was recorded for *D. mespiliformis* seeds exposed to the range of 4-8weeks air tight environments. The least mean germination time of 8.92days was recorded for *D. mespiliformis* seeds exposed to 2weeks of air tight environment. Highest germination percentage value of 100% was recorded for *D. mespiliformis* seeds hydro primed for 24 hours and was recommended for mass seed production of the seedling of this species.

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ANOVA Mean Germination Time of Seed Hydropriming Hours

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	1386.221	4	346.555	277.24424	.000
Within Groups	.006	5	.001		
Total	1386.227	9			

ANOVA for Mean Germination Time of Moist Air tight Environment

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	24.647	4	6.162	4929.392	.000
Within Groups	.006	5	.001		
Total	24.653	9			

ANOVA for the Air tight Environment

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	12822.300	4	3205.575	12.719	.000
Within Groups	3780.500	15	252.033		
Total	16602.800	19			

ANOVA for Seed Hydropriming Hours

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	14023.800	4	3505.950	39.224	.000
Within Groups	1340.750	15	89.383		
Total	15364.550	19			