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EFFECT OF PERIODS OF HYDRO-PRIMING AND SEED WEIGHTS ON THE GERMINATION OF *VITEX DONIANA* SWEET SEEDS

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ABSTRACT

There is paucity of information on breaking the dormancy of Vitex doniana through hydro-priming. Hydro-priming is the method of ensuring uniform and high germination percentage, by soaking seeds in water and follow by drying of seeds, while the emergence of radicle is prevented. There is need to determine the appropriate periods of hydropriming to prevent over or under hydro-priming of seeds. In light of this, investigation was conducted on the effect of periods of hydro-priming and seed weights on the germination of V.doniana seeds. To assess the effect of periods of hydropriming (0, 6, 12, 18, 24 and 36hrs) *and three seed weights (1.2, 1.4 and 3.3g)* ***on the germination of V.doniana seeds, a split-plot experimental design with five replications was employed. Result revealed that periods of hydropriming significantly (P<0.05) enhanced the germination of V.doniana. A significant germination percentage value of 50% was recorded in seeds hydro-primed for 36hours. Germination percentage of seeds increased with the increasing periods of hydro-priming. Highest germination percentage value of 40% was recorded in average seed size of 1.4g A significant germination percentage of 100% was recorded in 1.4g seeds hydro-primed for 36hours. Least mean germination time of 8days was recorded for 1.4 and 3.3g seeds. Hydro-priming of 1.4g for 36hours enhances the germination percentage of V.doniana seeds. The study therefore recommends hydro-primng of 1.4g seeds for 36hours for mass production of its seedlings for agro-forestry systems.***

Key words: Dormancy, Soaking and drying of seeds, Pre-sowing treatment, Germination, Agro-forestry species.

INTRODUCTION

Nigeria is rich in floristic composition and biodiversity with over 4,600 species out of which 205 are endemic, while another 476 species are threatened (FAO, 2000). The forest has productive, protective and social function (Olajide, 2003), however, this important resources are undergoing serious genetic depletion arising from various anthropogenic forces (FAO, 2004).Sale and Olujobi (2014) stated that the increase pressure on Nigeria's forest natural resources owing to population growth and economic pressure has drastically affected available land area for natural resources development. Nigeria is one of the developing countries in Sub-Sharan Africa (Osugiri *et al.*, 2012), which is blessed with a large expanse of land and variable vegetation, that is not sustainably managed (Olajide, 2010).

Oboh and Igharo (2017) reported that the ever increasing rate of forest loss all over the world makes it necessary to plan for and establish plantations and private tree plantings (small scale) of important forest species with a view to meeting the demands for forest products by the people. Okunlola and Akinyele (2017) stated that developing nations including Nigeria are endowed with many indigenous fruits that are of great importance to the rural communities. Such fruits contribute to the diet and economy of rural communities. These forest plants are important and cheap sources of vitamins, minerals, protein,

carbohydrates and fats (Okunlola and Akinyele, 2017). One of such plant is *Vitex doniana*.

Vitex doniana is called Black plum; Dinya; Ucha koro and Oori-nla in English, Hausa, Igbo and Yoruba respectively (Orwa *et al.,* 2009). The blackish pulp of the matured fruits is sweet and edible, and is eaten fresh. This pulp also serves in jam preparation. A beverage is made from the fruit juice, whereas boiled fruits are the basis for an alcoholic liquor and wine (Ky, 2008). The *V. doniana* is an important indigenous fruit or leafy vegetable in Africa (Burkill, 2000; Maundu *et al.,* 2009) for food, medicine and other purposes (Dadjo *et al.,* 2012). Hounkpèvi *et al.* (2018) stated that its leaves are used as fodder for livestock and the young leaves as leafy vegetables in sauces preparation. The blackish pulp of its ripened fruits is edible and used in preparation of some sweet drinks (Hounkpèvi *et al.,* 2018).

In traditional medicine, *V. doniana* have several applications (Ladeji *et al.,* 2005; Ky, 2008; Padmalatha *et al.,* 2009). For instance the leaf, the bark, dried and fresh fruit serve as ingredients in many preparations to treat or heal diseases including conjunctivitis, headache, stiffness, measles, rash, fever, chickenpox, hemiplegia, respiratory diseases, ankylostomiasis, rachitis, gastro-intestinal disorders, jaundice, kidney troubles, leprosy, liver diseases, bleeding after childbirth and diarrhoea. The mature leaves, the bark and the roots have phytotherapeutic properties and are used to heal several diseases (Iwueke *et al.,* 2006, Kilani, 2006, Padmalatha *et al.,* 2009).

V.doniana (Verbenaceae) has numerous utilisations with promising economic potential for poverty alleviation in rural and peri-urban areas in Africa. Mapongmetsem *et al.* (2005) reported that the species contributes to the improvement of soil fertility by litter production and is a good candidate in agroforestry systems. The wood of *V.doniana* is variously used in house construction, furniture, tools and agricultural implements making; it is suitable as firewood and for charcoal production (Arbonnier 2002, Arbonnier, 2004, Ky, 2008, Louppe *et al.,* 2008, Orwa *et al.,* 2009, Dadjo *et al.,* 2012). Hounkpèvi *et al.* (2018) stated based on its socio-economic importance, its integration into the farm production systems could foster domestication strategies and reduce anthropogenic pressures on its natural populations.

The seeds of *V. doniana* are dormant (Oboh and Igharo, 2017). Dormancy is a state where seeds do not germinate when placed under conditions which are normally regarded as favourable for germination (Ajiboye, 2010). Baskin and Baskin (2004) defined a dormant seed (or other germination unit) as one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors (temperature, light/dark, etc.) that otherwise is favourable for its germination, i.e. after the seed becomes non-dormant.

Seed dormancy remains a bottleneck to the propagation of many forest species of economic importance, since about 70% of all major taxonomic groups of seed plants have dormant seeds (Baskin and Baskin, 2003). Oboho and Igharo (2017) stated that dormancy and other seed factors mitigate against easy propagation of many forest tree species. Ease of propagation is intimately tied to seed germination. Oboho (2014) defined germination as the process by which dormant embryo in the seed gets activated grows out of the seed coat and establishes itself as a seedling. Germination is critical to regeneration and dormancy limits the seed germination and seedling availability for afforestation, domestication (Adelani, 2015, Adelani *et al.,* 2018a), reforestation (Aduradola *et al.,* 2005) as well as biodiversity conservation of some important indigenous agroforestry trees species. Oboh and Igharo (2017) reported that even and adequate germination of seeds sometimes requires seed dormancy to be broken either by natural or artificial means in a process known as pre-treatment. There could be physical, physiological or morphological forms of dormancy in a seed (Oboh and Igharo, 2017).

The *V. doniana* seeds present a combination of physical (PY) and physiological dormancy (PD), based on classification by Baskin and Baskin (2004) and Silveira (2013). However, little is known about dormancy breaking requirements and no reliable techniqueis available yet. Imbibition tests revealed that *V. doniana* seeds are physically dormant (N'Danikou *et al.,* 2014) but different treatments

tested so far resulted in germination rates below 60% after six months (Mapongmetsem, 2006; Ky, 2008; Ahoton *et al.*, 2011). Uniform germination is one of the important agronomic requirements for successful domestication of wild harvested economic plants (N'Danikou *et al*., 2015). Priming is one of the methods of ensuring uniform germination. Seed priming is a method to promote rapid and uniform germination of seeds, by controlling imbibitions to an extent where germination is initiated, but insufficient to cause radical emergence (Schmidt, 2000).

Seed priming is an efficient technique for improvement of seed vigor, increasing germination and seedling growth (Dastanpoor *et al.*, 2013). To meet the current demand of forest products through domestication, there is need to embrace cheap, fast and adoptable modern physiological techniques as priming that increase the seed germination percentages, reduce mean germination time and increase seedling growth of agro-forestry tree species (Adelani, 2015). Hydro-priming is a special type of seed priming in which seeds are soaked in water followed by drying of seeds, but the emergence of the radicle is prevented (Farooq *et al.*, 2006). This technique is a common method that can increase rate, percentage and uniformity of germination of seeds (Farooq *et al.*, 2005, 2006; Srivastava *et al.*, 2010). There is dearth of quantified information on the potential of hydro-priming in improving the mean germination time and germination percentage of seeds of forest tree species as *V.doniana.*

Some of the methods as physical, chemical and mechanical scarification only degraded the seed coat for germination (Aliero, 2004; Abubakar and Muhammad, 2013); without rapidly and uniformly influencing the physiology of the seeds (Dewir *et al.*, 2011) and seedlings (Gehlot and Kasera, 2012). Most of methods of breaking seed dormancy do not promote rapid and uniform germination as hydro-priming. Periods of hydro-priming of particular seed weight is essential for successful germination for propagation as well as domestication of indigenous trees species for agroforestry systems. Seed weight is an important factor for successful germination study (Malcolm *et al.*, 2003; Kambizi *et al.*, 2006; Perez-Garcia *et al.*, 2006). Li *et al.* (2015) reported that seed weight had significant influence on seed germination time and total germination fraction. In this light, investigation was conducted to assess the effect of periods of hydro-priming and seed weights on the germination of *V.doniana* seeds.

Overcoming dormancy of *V.doniana* seeds of different weights by hydro-priming will help in meeting the needs of Nigerian without jeopardising environment. New initiatives in agro-forestry are seeking to promote poverty alleviation and environmental rehabilitation through efficient information on overcoming constraints in seed germination and seedling growth (Adelani *et al.*, 2014a) for propagation as well as for domestication purposes (Adelani, 2015).

MATERIALS AND METHODS

Study Area

The pot experiment was carried out at the screen house of Federal College of Forestry Mechanization, Afaka, Kaduna State. The college is located in the Northern Guinea Savannah ecological zone of Nigeria. It is situated in Igabi Local Government Area of Kaduna state, Nigeria. It is located between latitude $10^{o}34'$ and 10^{o} $35'$ and longitude 7^{o} $20'$ and 7^{o} $21'$ (Adelani, 2015). Mean annual rainfall and humidity are approximately 1000 mm and 29% respectively. The vegetation is open woodland with tall trees, usually small boles and broad leaves (Otegbeye *et al.*, 2001).

Fruit collection and Materials

The fruits of *Vitex doniana* were sourced from the mother tree in Buruku Forest Reserve, Kaduna State, Nigeria. The seeds were extracted from the fruits and air dried for 30 minutes. The viability of the randomly selected seed samples was assessed with the cutting method (Schmidt, 2000). The sand was collected from the floor of the college dam, allowed to pass through 2mm sieve and sterilized at 160^{o}C for 24hrs in oven (Adelani and Joseph, 2014). The polypots of 20x10x10cm^3 filled with sterilized sand in the nursery was used. Distilled water was used for the experiment.

For this experiment, germination percentage and mean germination time were calculated using the

following formula (1 and 2) suggested by Schelin *et al*. (2003).
Germination percentage was computed using the formula:

$$\text{Germination Percentage} = \frac{Totalseedgerminated}{Totalseedsown} \text{x } 100 \quad \ldots\ldots (1)$$

Germination count was recorded every two (2) days interval for 12 weeks when no more germination was recorded.
Mean germination time is a measure of the rate and time spread of germination (Soltani *et al.,* 2015). It is denoted as MGT. The unit of mean germination time can be hours, days or other time unit (Ranal and Santana, 2006; Schelin *et al.* (2003)).

$$\text{MGT} = \frac{\sum(f_x)}{\sum X} \quad \ldots\ldots (2)$$

Where: x is the number of newly germinated seeds on each day; f is the numbers of days after seeds were set to germinate; X is the Total number of seeds that germinated at the end of the experiment. Germination percentage and mean germination time were recorded at two days interval for 12weeks.

Experimental design

To investigate the effect of periods of hydro-priming and seed weights on the germination of *Vitex doniana* seeds, a split-plot experimental design with five replications was involved. Six periods of hydro-priming (0, 6, 12, 18, 24 and 36hrs) and three seed weights (1.2, 1.4 and 3.3g) constituted main and sub-plot treatments respectively. The initial moisture content of the samples of extracted ninety (90) seeds was determined by weighing balance (Model-Mettler PM 11-K) before and after drying to constant weight. Five (5) seeds of different weights (1.2, 1.4 and 3.3 g) were soaked in distilled water at periods (0, 6, 12, 18, 24 and 36hrs). Stirring or bubbling was done to ensure uniform treatment and aeration (Adelani, 2015).

After each of duration of the experiment, the seeds were removed, washed, air dried for 30 minutes and treated with fungicide (Vinclozolin). The seeds were sun dried back to the initial moisture content of the seeds. Treated seeds were sown in 4cm depth of the sterilized sand and 200mL / seed of water was applied at two days interval (Adelani *et al*., 2014b). Seeds that were not soaked in the distlled water served as control. A seed was considered germinated if the radical was able to break open the seed coat and plumule emerged. Final germination count was taken when no further germination took place for several days.

Data analysis

The data was collected on the effect of periods of hydro-priming and seed weights on the germination of *V. doniana* seeds. The data were subjected to two way analysis of variance (ANOVA) using SAS (2003) software. Comparisons of significant means were accomplished using Fischer's Least Significant Difference (LSD) at 5% level of significance.

RESULT

Effect of periods of hydro-priming and seed weights on the germination of *V. doniana* seeds

The result of the effect of periods of hydro-priming and seed weights on the germination of *V.doniana* seeds is presented in Table 1. A significant germination percentage value of 50% was recorded in seeds hydro-primed for 36hours. A significant germination percentage was recorded in average seed size of 1.4 g.

Table 1: Effect of periods of hydro-priming and seed weights on the germination of *V.doniana* seeds

P. H. P	P. G (%)	M G T (days)	SW (g)	P.G (%)	M GT (days)
0	0.00^{b}	0.00^{c}	-	-	-
6	7.00^{ab}	13.00^{c}	-	-	-
12	7.00^{ab}	13.00^{c}	-	-	-
18	33.00^{ab}	82.00^{b}	1.2	7.00^{b}	48.00^{b}
24	40.00^{ab}	117.00^{a}	1.4	40.00^{a}	95.00^{a}
36	50.00^{a}	118.00^{a}	3.3	13.00^{b}	27.00^{c}
SE±	15.91	10.8	SE±	12.24	6.00

**Means on the same column having different superscript are significantly different P (<0.05) vertically*
Key: P. H. P- Periods of Hydro-priming, P. G- Percentage germination, M G T- Mean germination time
SW- Seed Weights

Interactive effect of periods of hydro-priming and seed weights on the germination of *V. doniana* seeds

The result of the interactive effect of periods of hydro-priming and seed weights on the germination of *V. doniana* seeds is presented in Table 2. A significant germination percentage of 100% was recorded in 1.4g seeds soaked in water for 36hours.

Table 2: Interactive effect of periods of hydro-priming and seed weights on the germination of *V. doniana* seeds

P. H. P (hrs)	S W(g)		
	1.2	**1.4**	**3.3**
0	0.00[a]	0.00[a]	0.00[a]
6	0.00[a]	0.00[a]	20.00[a]
12	0.00[a]	20.00[a]	0.00[a]
18	80.00[a]	20.00[b]	0.00[c]
24	20.00[b]	80.00[a]	20.00[b]
36	0.00[c]	100.00[a]	40.00[b]
SE±	12.24	12.24	12.24

**Means on the same rows having different superscript are significantly different P (<0.05) vertically*

Key: P.H.P- Periods of Hydro-priming, SW- Seed W eights

Interactive effect of mean germination time of periods of hydro-priming and seed weights on the germination of *V.doniana* seeds

The result of interactive effect of mean germination time of periods of hydro-priming and seed weights on the germination of *V.doniana* seeds is presented in Table 3. A significant least mean germination time of 8days was recorded for seeds of weight 3.3 and 1.4g soaked for 6 and 24hrs as well as 12 and 18hrs respectively.

Table 3: Interactive effect of mean germination time of periods of hydro-priming and seed weights on the germination of *V.doniana* seeds

P.H.P	Seed weights		
	1.2	**1.4**	**3.3**
0	0.00[b]	0.00[b]	0.00[b]
6	0.00[b]	0.00[b]	8.00[b]
12	0.00[b]	8.00[b]	0.00[b]
18	41.00[a]	8.00[b]	0.00[b]
24	17.00[b]	45.00[a]	8.00[b]
36	0.00[b]	53.00[a]	16.00[b]
SE	5.96	5.96	5.96

**Means on the same rows having different superscript are significantly different P (<0.05) vertically*

Key: P.H.P- Periods of Hydro-priming, SW- Seed Weights

Table 4 : ANOVA for effect of periods of hydro-priming and seed weights on the germination of *V. doniana* Seeds

Source	Df	Ss	Ms	Fcal	Ftab
Total	89	155,555.556			
Replication	4	3333.333	833.33	0.66ns	2.87
A	5	30,222.223	6,044.444	4.22*	2.71
Error (a)	20	28,666.667	1433.33		
B	2	9,555.556	4,777.78	6.37*	3.19
AB	10	47,777.777	4,777.78	6.37*	2.03
Error (b)	48	36,000	750		

Table 5: ANOVA for mean germination time of the effect of periods hydro-priming and seed weights on the germination of *V. doniana* seeds

Source	Df	Ss	Ms	Fcal	Ftab
Total	89	46, 940			
Replication	4	1,237.222	309.31	0.53ns	2.87
A	5	8, 756.667	1751.33	2.99*	2.71
Error (a)	20	11,696.778	584.84		
B	2	14, 486.667	7,243.33	36.69*	3.19
AB	10	1,776.667	177.67	0.95ns	2.03
Error (b)	48	8986	187.21		

DISCUSSION

It can be inferred that germination percentage increase with increasing periods of soaking of *V.doniana* seeds in water. Similar observation has been reported by Adelani (2015) and Adelani *et al.* (2018b). Akinola *et al*. (2000) reported that higher duration of exposure to seed treatment resulted in higher cumulative germination in wild sunflower. Positive effect of seed priming on seed invigoration depends on priming duration (Ashraf and Foolad, 2005). Kaya *et al.* (2006) working on germination of sunflower under drought and salt stress reported that hydro-priming improved both rate of germination and mean germination time both under salt and drought stress conditions.

Average seed weight gave highest germination percentage. The better germination exhibited by the heavier seeds could be the result of greater availability of food reserves (Offiong, 2008). Various investigators such as Kolodziejek (2017) and Khan and Shankar (2001) have adduced the highest germination percentage recorded in heavier or heaviest seeds to the presence of more food reserves compare to other weight investigated. Similar observation has been made by Khan (2004). Missanjo *et al.* (2013) stated that larger seeds resulted in higher germination percentage since larger seeds contain more food reserves to support germination.This is in consonance with the reports of Khan *et al.* (2002)., Mosseler *et al.* (2000)., Gholami *et al.*(2009)., Olorunmaiye *et al.* (2010) and Hojjat, (2011). Similar observations have been made by Khurana and Singh (2004), Mwase and Mvula (2011)., Chidumayo (2007). Esen *et al.* (2007) stated that higher performance of larger seeds could be a reflection of the greater amount of nutrients available to the embryo.

A significant germination percentage recorded in seeds hydro-primed for highest periods of time investigated. This shows that water uptake rate in hydro-priming period is slow and seeds had enough time to complete the pre-germination process. Similar observations have been reported by Schmidt (2000)., Varier *et al.(*2010) and Adelani *et al.* (2013). The zero germination percentage recorded for an untreated seed (control) shows that hydro-priming enhances the germination of *V.doinana* seeds. The ability of hydro-priming to enhance seeds germination could be traced to stimulatory effects which emanates from three stages of uptake of water which are the rapid initial uptake due to the seed low water potential and proteins synthesized as well as mitochondria repair, initiation of physiological activities as synthesis of protein by translation of new mRNAs and synthesis of new mitochondria and the completion of process of germination with radicle emergence. Similar observations have been reported by Varier *et al.* (2010). The afore-mentioned reports are in consonance with that of McDonald (2000); Jowkar *et al.* (2012) and Wattanakulpakin *et al.* (2012).

Hydro-priming is beneficial to seed and seedlings by enhances germination as well as seedling growth. Beneficial effects of hydro-priming on grain yield were reported in maize (Murungu *et al.*, 2004), sunflower (Hussain *et al.*, 2006), chickpea (Ghassemi-Golezani *et al.*, 2008; Zarei *et al.*, 2011) and pinto bean (Ghassemi-Golezani *et al.*, 2010). Average seed weight gave higest germination percentage. This statement is corroborated by the reports of Adelani *et al.* (2018b). One can deduce that average seed size coat was not affected by low or high damaging effect of periods of soaking the seeds in water during hydro-priming. Least mean germination time reported in average and a large

seed is an indication that large and average weight seeds germinates early than small seeds. Similar observation has been reported by Adelani *et al.* (2018b). The reason for earlier germination of average and largest seed weight could be traced to the fact that heavier seed weight contains greater amount of food reserves to influence germination most compare to others.

CONCLUSION

Investigation conducted into effect of periods of hydro-priming and different seed weights on the germination of *V.doniana* revealed that highest germination was recorded in seeds soaked in water for 36hours. The result of interactive effect of periods of hydro-priming and seed weights revealed that average weight seeds soaked in water for 36 hours gave highest germination percentage. A hydro-priming of *V.doniana* seeds for 36 hours is recommended for mass production of its seedlings for agroforestry systems. Least mean germination time was recorded in average and largest seeds. Seedlings of high vigour were obtained by selecting the best seed weight and adopting the appropriate pre-sowing treatments to break dormancy of the seeds of indigenous priority species for biodiversity conservation. Breaking the dormancy of the appropriate seed weight will enhance robust germplasm conservation programmes. In the long run, the accessibility of Nigerians to the ample benefits of the high physiological quality tree species will be increased.

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