EFFECT OF PRE-TREATMENTS ON GERMINATION AND EARLY SEEDLING GROWTH OF MAESOBOTRYA BARTERI

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Abstract— Maesobotrya barteri is a neglected indigenous fruit tree in the tropics due to poor cultivation and lack of adequate knowledge of its silviculture. This experiment was conducted to determine the effect of different pre-treatments on germination and early seedling growth of Maesobotrya barteri. The pre germination treatments included soaking seeds in ethanol for 5 minutes(T1), hot water at 100°C for 5 minutes(T2), cold water for 24 hours (T3), hydrogen peroxide for 5 minutes (T4) and untreated (control, T5). Three hundred seeds were used for each treatment. Seeds treated with hydrogen peroxide (H_2O_2) commenced germination after 4 days and achieved 66% germination within 14 days which was significantly different ($P < 0.05$) from other treatments, especially the untreated seeds which had the lowest germination of 20.4%, and commenced first germination after 21 days and 22 days respectively. The results showed significant differences ($P < 0.05$) in growth and biomass parameters between the treatments. Average survival percentages for all the treatments were 100% (H_2O_2), 90% (cold water), 80% (hot water and ethanol) and 60% (control). H_2O_2 was consistently the most effective treatment in terms of the mean value of growth and biomass production with 24.88cm (total seedling length), 2.00mm (collar diameter), 49.27 (seedling vigor index) and 0.58g (total dry weight). Results obtained in this experiment indicate that the pre-germination treatment of Maesobotrya barteri seeds by using H_2O_2 can enhanced germination of the seeds by breaking dormancy. Thus for the production of Maesobotrya barteri fruit, H_2O_2 can be used to promote germination of its seeds.

Index Terms— Maesobotrya barteri, pre-germination treatment, germination, seedling growth

INTRODUCTION

Tropical forests are important reservoir of crop and animal biodiversity that play a fundamental role in giving satisfaction to many needs of humans (Woodward and Susan, 2009). *Maesobotrya barteri* (Pax*et et al.*,) is a tropical forest tree that reaches a height of 10m, it bears fruit from April to June, which is up to 1 cm long, ovoid and often distinctly pointed (Dalziel, 1994). It is a rainforest plant occurring in several parts of the world including Sierra Leone, Southern Nigeria and Western Cameroon (Keay, 1989). It belongs to the family *Euphorbiaceae*, commonly known as bush cherry. The Benin people in Nigeria locally call it "Oruru". The fruits often form a vital part of human nutrition that includes protein (Platt, 2005), vitamins (A, C, E), thiamine, riboflavin, niacin and mineral elements such as phosphorus, calcium, magnesium, potassium and sodium (James, 1995). Some are eaten as a refreshing delicacy, others make up a meal. The species has edible fruits and a potential source of raw material for juice making that can support the economic and industrial development of Nigeria. The Fruits also enhance frequent waste elimination, including bile acids, sterols and fat (Osborne, 2008). The Fiber is of benefit in diverse diseases (Akobundu, 1999) and helps lower cholesterol absorption by preventing the formation of plaques (Scala, 1994). Local communities also use the plant as chewing stick, for the treatment of diarrhea, stomach ache, dysentery, urethral discharge, venereal disease, jaundice, cough, measles and other forms of diseases.

The harvesting, utilization and marketing of indigenous fruit and nuts have been central to the livelihoods of majority of rural communities throughout Africa and can make a difference during period of famine and food scarcity (Akinnifesi *et al.*, 2007). Wild harvesting of fruits from forests and semi domesticated trees growing on-farm and homesteads can substantially boost rural income and employment opportunities in Africa (Leakey *et al.*, 2005). As a result there is need to encourage domestication and large scale cultivation. To achieve this, the knowledge of pre-treatment and germination indices are essential in order for the propagation of forest species to be successful. Seed phase is one of the most important stages in the life cycle of flowering plant in terms of their survival as a result of the impact of seed dormancy and germination. And for successful seed germination, the three fundamental conditions that must be overcome are seed viability, overcoming seed dormancy and favorable environment for germination. Dormancy and germination are often in conflict among tropical and sub-tropical plants. FAO (1994) reported that *Maesobotrya barteri* species is physiologically dormant. In addition,

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the species could become threaten due to habitat loss from anthropogenic factor. The need to enhance rapid, maximum and uniform germination of the species for large scale plantation development is imperative, hence the release of tree seed from dormancy through pre-treatment measure is a critical factor in achieving good seed germination.

This research is designed to investigate the effect of various pre-treatments on the germination and early seedling growth of *Maesobotrya barteri*. It is hoped that the information gathered would help conservation and domestication of the plant species.

Materials and Methods

Source of Plant material

Fruits of *Maesobotrya barteri* were collected from Swamp Forest Station of the Forestry Research Institute of Nigeria (FRIN), Onne, Rivers State, South South Nigeria, which lies on latitude 04^0 51′ N and longitude 007^0 03′ E.

Methods

The fruits were de-pulped to extract the seeds. All the seeds were thoroughly mixed to obtain a seed lot which was used in the study. The processed seeds were subjected to viability test through floatation method. All seeds that floated in water after few minutes of soaking were considered unviable and discarded while the seeds that sank in the pale were regarded as viable and used for the study. The pre-germination treatments used in the experiment were:

T_1: Seeds soaked in 100% ethanol for 5 minutes

T_2: Seeds soaked in hot water (100°C) for 3 minutes

T_3: Seeds soaked in cold water for 24 hours

T_4: Seeds soaked in hydrogen peroxide for 5 minutes and

T_5: Control (Seeds intact without soaking).

300 seeds were sown per treatment, making a total number of 1500 seeds for all treatments. The seeds were sown in plastic germination baskets (30cm in diameter) filled with river sand which was washed and sterilized. Watering was done daily. The germination baskets were kept in a humidified propagator. Each treatment and the control had three replicates and the experiment was laid in Completely Randomized Design (CRD).

Data Collection and Analysis

The germination periods for each treatment were recorded. Percentage germination was calculated for each pre-germination treated seeds. After end of germination and considering time of sprouting of two leaves by the plant, a total of twenty fairly uniform seedlings from each treatment were transplanted into polythene pots (20x 10x6cm), filled with top soil collected from forest floor. And each treatment with three replicates was laid in CRD experiment. Four weeks after transplanting the seedlings, growth attributes such as stem height, stem collar diameter, number of leaves, leave area, survival percentage, root length and total seedling height were measured monthly for six months.. The mean of each growth parameter including seedling vigor index and total dry weight was calculated for each of the treatment. The growth parameters data were subjected to analysis of variance (ANOVA). The means of the significantly different growth parameters were separated using least significant difference (LSD) test.

Results

Germination Indices

The first germination was recorded at 4 days from the seeds soaked in hot water (T_2), cold water (T_3) and hydrogen peroxide (T_4) respectively. The seeds soaked in ethanol for 5 mins (T_1) had its first germination 5 days after sowing while the non-treated seeds control (T_5) recorded the first germination at 21 days after sowing. T_4 had the highest percentage germination value of 66%, followed by T_1 while T_5 had the least of 20.4% (Table 1). The analysis of variance revealed that there was significant difference ($p<0.05$) in germination percentage of *Maesobotrya barteri* seeds as subjected to different treatments (Table 1). The mean germination periods were also computed for *Maesobotrya barteri* seeds as influenced by the various treatments. T_4 had the least germination period of 14 days while T_5 had the highest germination period of 22 days (Table 1).

Table 1: Effectiveness of pre-germination treatments on Germination indices of *Maesobotrya barteri* seeds

Treatments	No of seeds treated and sown	No of seed germinated	Germination %	Days after sowing	Germination Period (days)
T_1	300	168	56.00 ± 1.15^{c}	5.00 ± 0.58^{a}	18.00 ± 0.88^{c}
T_2	300	157	52.33 ± 2.51^{ab}	4.00 ± 1.20^{a}	16.00 ± 0.58^{ab}
T_3	300	95	31.67 ± 3.51^{bc}	4.00 ± 1.15^{a}	17.00 ± 0.58^{ab}
T_4	300	198	66.00 ± 2.00^{a}	4.00 ± 0.58^{a}	14.00 ± 0.58^{a}
T_5	300	60	20.00 ± 2.00^{d}	21.00 ± 0.58^{b}	22.00 ± 0.58^{a}

Means followed by different superscripts are significantly different at 0.05 level of significance.

Growth Indices at Six Months

T_4 treatment had the highest seedling survival percentage (100%), followed by T_3 which is 90%. T_2 and T_1 had 80% and T_5 had the least survival percentage of 60% (Table 2). Analysis of variance showed that there was significant differences ($p<0.05$) among the pre-germination treatments on the survival percentage (Table 2). The mean shoot length of T_4 (18.6cm) was significantly higher at ($p<0.05$) than other treatments (Table 2). T_4 had the highest mean root length of 6.28 cm while T_5 had the least value of 3.16 cm. LSD analysis indicated that there was no difference between mean root length of T_1, T_3, and T_4 (Table 2). Seedlings subjected to T_4 had the highest mean stem-collar diameter of 3.00 mm while those of T_5 had the least mean stem-collar diameter of 1.07 mm. T_1, T_2 and T_3 seedlings had 2.00 mm as mean stem-collar diameter. The ANOVA of the stem-collar diameters shows that there was a significant difference ($P < 0.05$) between the effects of different treatment on the seedlings stem-collar diameters (Table 2).

The seedlings subjected to T_4 had highest mean seedling height of

24.88 cm while T_2 had the least value of 17.05 cm. The analysis of variance (ANOVA) showed that there was significant difference ($P < 0.05$) between the effects of the treatments on the height growth of the seedlings. Seeds soaked in T_1, T_2, T_3 and T_4 produced seedling with a mean number of leaves of 12 respectively, while T_5 gave the lowest number of leaves of 6, which was significantly lower ($P < 0.05$) than other treatments (Table 2). The LSD analysis of leaf area showed that there was no difference between the mean leaf area of T_2 and T_3 seedlings and also between T_1 and T_5 but leaf area T_4 was significantly higher ($P < 0.05$) than other treatments. The T_4 seedlings had the highest mean total dry weight of 0.58 g, while T_3 seedlings had 0.41 g and T_2 seedlings had the least of 0.32 g. The ANOVA of the total dry weight indicated that there was significant difference ($P < 0.05$) in the effects of the treatments on dry matter accumulation (Table 2). Vigor index was highest (49.27) in T_4 which is significantly higher than that of other treatments (Table 2).

Table 2: Effects of pre-germination treatments of *Maesobotrya barteri* seeds on seedlings growth indices at six months after sowing

Treatments	Survival percentage %	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Collar Diameter (cm)	Number of Leaves	Total dry weight (g)	Leaf area (cm^2)	Seedling vigor index
T_1	80.00±2.60^{b}	13.80±0.56^{bc}	4.46±0.57^{abc}	18.27±1.14^{bc}	2.00±0.18^{b}	12±1.73^{b}	0.34±0.01^{c}	45±1.15^{bc}	30.68±0.59^{b}
T_2	80.00±5.77^{b}	12.84±0.58^{b}	4.20±0.57^{ab}	17.05±1.14^{b}	2.00±0.11^{b}	12±0.58^{b}	0.32±0.01^{c}	42±1.15^{b}	26.37±0.59^{c}
T_3	90.00±6.35^{bc}	15.33±0.58^{cd}	5.12±0.57^{ab}	20.45±0.99^{c}	2.00±0.08^{b}	12±1.15^{b}	0.41±0.01^{b}	44±1.15^{b}	19.37±0.66^{d}
T_4	100.00±2.89^{c}	18.60±0.58^{d}	6.28±0.57^{c}	24.88±0.01^{d}	3.00±0.20^{c}	12±0.88^{b}	0.58±0.01^{a}	60±1.15^{a}	49.27±0.58^{a}
T_5	60.00±5.77^{a}	10.14±0.56^{a}	3.16±0.57^{a}	18.31±0.57^{a}	1.07±0.67a	6±1.15^{a}	0.39±0.01^{b}	44±1.15^{c}	8.10±0.59^{e}

Means followed by different superscripts are significantly different at 0.05 level of significance.

Discussion

Germination indices

Some factors like environmental stress (Liheng *et al.,* 2009), chemical inhibitor in seed cotyledon, and hard seed coat, which is impervious to water and aeration required to stimulate germination (Lamprecht, 1989) are main causes of seed dormancy. Most tropical forest trees' seeds often take a long time before germination commences if the seeds of the trees are sown without any pre-germination treatment to break their dormancy (Whitmore, 1998). In this study, germination occurred first at 4 days after sowing from among the seeds soaked in hot water (T_2), cold water (T_3) and hydrogen peroxide (T_4) as against ethanol (T_1) that had first occurrence of germination in the fifth day and untreated seed (T_5) on 21days after sowing (Table 1). A study carried out by Kana *et al.,* (1996) observed that the seeds of some *Albizia* species soaked in cold water for 24 hours started germinating four days ahead of other seeds subjected to different pre- germination treatments. And in the work of Ikojo *et al.,* (2005), it was observed that germination first occurred from the seeds of *Pentaclethra macrophylla* soaked in cold water overnight before sowing among other pre-germination methods used.

T_4 treatment also influenced the first occurrence of germination in *Maesobotrya barteri* seeds. The work of Liheng *et al.,* (2009) buttress the fact that seeds pre-treated with hydrogen peroxide (H_2O_2) can enhanced germination even under environmental stress. The shorter average germination period of 14 days for seedling treated with T_4 when compared with other treatments also indicated that H_2O_2 is beneficial for seed germination. The works of Schopfer *et al.*(2001) and Tamas *et al.* (2004) also shown that oxidants like H_2O_2 can be used in a beneficial way in plant tissues, for example, in a seed. It is observed in this study that the seeds treated with T_4 had the highest germination percentage of 66% (Table 1). The high percentage may be due to the oxidative nature of H_2O_2 that can stimulate growth of sprouts and roots, for example, in barley, wheat, pea, maize and melon (Anonymous, 2002). Jann and Amen (1977), showed that pre-treatment of seeds with oxidants such as H_2O_2 leads to breaking seed dormancy. This result also agrees to those of several other authors who reported a stimulation of germination when seed are treated with H_2O_2 (Muhammad *et al.,* 2006; Dolatabandan and Sanavay, 2008; Yushi *et al.,* 2008). Oxidant treatments such as H_2O_2 appear to improve germination, providing oxygen to the embryo. Similar results were obtained with other oxidants such as sulphuric acid, which was reported by Ibiang *et al.,* (2012) that sulphuric acid had the highest germination percentage (90%) for treatment of *Tetrapleura tetraptera* seeds.

Growth Indices at Six Months

The transplanted seedlings of T_4 had survival percentage of 100% (Table 2). This is an indication that T_4 stimulate the growth of *Maesobotrya barteri* seedlings better when compared with other treatments in this study. Some authors have shown that the production of ROS (reactive oxygen species) like H_2O_2, during seed germination may be a beneficial biological reaction, linked with high germination capacity and seedling development (Cano *et al.,* 1997; Ogawa and Iwabuchi, 2001; Schopfer *et al.*, 2001).

The shoot and root length treated with T_4 where significantly higher than other treatments (Table 2). This present study agrees with the work of Akinnifesi *et al*., (2007), who reported that *Maesobotrya barteri* seeds treated with H_2O_2 had the highest shoot and radicle length than untreated seeds. He further explained that this significant improvement in the growth of the emerging seedlings may be attributed to early germination induced by the chemical over untreated seeds which resulted in vigorous seedlings with more root and shoot length than the seedling from untreated seeds. The mean total height of the seedlings showed that seeds treated with H_2O_2 attained the best initial growth height, followed by cold water, while untreated seeds had the poorest height (Table 2). Previous research buttress the fact that there was increased in seedlings total height of some plant species when seeds are soaked in H_2O_2 (Ogawa and Iwabuchi, 2001; Sarath *et al*., 2007). The improved growth of plant seedlings subjected to H_2O_2 could be attributed to increased oxygen and water uptake (Ching 1959). In addition, it is possible that the oxidation of germination inhibitors present in pericarp by H_2O_2 promotes plant growth (Ogawa and Iwabuchi, 2001).

The present study also revealed that stem collar diameter, number of leaves, and mean total dry weight of *Maesobotrya barteri* seedlings improved significantly when the seeds were soaked in H_2O_2 for 5minutes. The *Maesobotrya barteri* seeds treated with T_4 had highest stem collar diameter while cold water, hot water and ethanol had 2.0mm respectively and control had the lowest stem collar diameter (Table 2). This increase in seedling growth characteristic could be due to chemical nature of H_2O_2 which is further proved by the work of Mabundza *et al* (2010) whose report indicated that sulphuric acid, which is also a chemical, can improve the growth characteristic of passion (*Passiflora edulis*) seedlings. Previous studies have shown that chemical seed treatment improved seed germination and growth under various conditions (Kozlowski, 1972; Anonymous, 2007; Ehiagbanare & Onyibe, 2007; David & Midcap, 2007). The significantly higher mean dry weight of *Maesobotrya barteri* as influenced by T_4 (Table 2) which agrees with the findings of Liheng *et al*., (2009) that seed treatment with H_2O_2 greatly increased all the growth characteristics of wheat seedlings including dry weight. The seedling vigor index as shown in Table 2 indicated that *Maesobotrya barteri* seeds treated with T_4 had the highest mean seedling vigor index while control, T_5 had the lowest value. Anonymous (2007), treated the seeds of *Tamarindus indica* L. with various treatments including sulphuric acid which is an oxidant like H_2O_2 and observed that seeds immersed in sulphuric acid for 10 min produced seedlings with high vigor. This implies that the use of chemical like H_2O_2 as pre treatment is a chemical massagers that act in different manners to stimulate germination and growth in seedlingss.

Conclusion and Recommendation

This study has provided some information on the silvicultural requirement of the *Maesobotrya barteri* species. It was observed that among the pretreatments applied in this experiment, seeds soaked in hydrogen peroxide for 5 minute were found most effective in respect to germination, seedling growth and biomass production in comparison to the control and other pre-treatments. The results indicate that pre-germination treatment of *Maesobotrya barteri* seeds by using H_2O_2 enhanced germination of the seeds and seedling growth. Generally, soaking seeds in hydrogen peroxide will be useful to both the local tree planters and silviculturists to break the dormancy of *Maesobotrya barteri seeds*. This method is simple to apply and requires no special equipment, it can be used to tackle all the different types of dormancy and helps to modify the seed coat, remove inhibitors and soften the seed for easy germination and seedling growth. This will form important element in the quality of seedlings produced for the establishment of *Maesobotrya barteri* plantation.

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