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Optimization of treatments for the germination and establishment of botanical yam (*Dioscorea rotundata)* seeds

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ABSTRACT. Botanical yam (*Dioscorea rotundata*) seeds are currently used by yam breeders to generate and conserve new varieties of yam instead of seed yams (vegetative propagule). Yam seed treatment through scarification is one of the most effective methods of improving seed germination and seedling establishment. This study was carried out under greenhouse condition to determine the best seed treatment for the germination and establishment of botanical yam seeds. The set up involved a soil mixed with carbonized rice husk using five different seed treatments: dry heating (DHS) at 60°C, mechanical scarification + gibberellic acid (MSS + GA), mechanical scarification (MSS), acid scarification with sulfuric acid (ASS) and soaking in hot water at 80°C (SOS). The findings showed that the treatment, MSS + GA and MSS only, had significant ($p<0.05$) positive effect on the seed emergence and seedling establishment compared to the untreated control (COS) and other treatments. ASS caused seed damage resulting to no emergence. The findings showed that carbonized grain waste (rice husk) enhanced soil fertility and seed treatment (MSS + GA) was the best to improve yam seed germination and seedling establishment. These treatments can be efficiently applied to reduce the long gestation periods and increase yield in yam breeding programs.

Keywords: emergence; fertility; gibberellic acid; scarification; seedling.

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Introduction

Yam is a common name for the tuber crop belonging to the family Dioscoreaceae. As perennial herbaceous vines, they are cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America, the Caribbean and Oceania (Anoma & Thamillini, 2016). They are monocots, related to lilies and grasses. Yam as a food plays a significant role by giving carbohydrate, income and dietary to several people. The economic importance species include *Dioscorea rotundata* (white yam), *Dioscorea cayenensis* (yellow yam), *Dioscorea alata* (water yam), *Dioscorea dumetorum* (bitter yam), *Dioscorea bulbifera* (aerial yam) and *Dioscorea esculenta* (Chinese yam). White yam (*D. rotundata*), also called white guinea yam is the most widely cultivated in West Africa.

Global production of yam is around 58.7 million tons, with West Africa producing more than 92 percent. Nigeria and Ghana together produce about 66 percent of the world's yam supply. In any case, these yields are still low in correlation with the expected yield since some plant factors constrained yield capability of yam landraces, which are likewise bothered against a variety of stresses. The production of yam is marred by many hindering factors, the major one being the scarcity of high-quality seed yam of local popular and improved varieties, high degrees of post-harvest losses (almost 40%), high production costs (high cost of seed, labour at land clearing and harvest, and staking which contribute almost 70% of the total production costs).

Despite the large quantity of yam produced in West Africa, there is no formal seed yam production or marketing system to take care of farmers' needs. Traditional method of yam propagation involves the use of seed tubers which are often expensive and prone to pest attack and diseases. Different modern methods have been employed for the production of seed yam. These include the minisett technique developed by National Root Crops Research Institute (NRCRI), Umudike, Nigeria (Aighewi, Asiedu, Maroya, & Balogun, 2015), use of microsetts and microtubes, tissue and organ culture techniques (Balogun & Gueye, 2013), use of vineyard

cuttings (Kikuno, Matsumoto, Shiwachi, Youohara, & Asiedu, 2007; Agele, Ayankanmi, & Kikuno, 2010), use of aeroponics system, use of temporary immersion bioreactor system (Aighewi et al., 2015) and the use of botanic seed/yam seed.

The difference between 'yam seed' (botanical seed) and 'seed yam' (vegetative propagule) can be confusing when the words are used interchangeably. Flowers are produced by yam plants and set seeds in fruits; these seeds which are referred to as 'yam seed' or 'true yam seeds' or 'botanic yam seeds' are used by breeders to generate new varieties. In usual cultivation, yam is propagated vegetatively by sowing the tubers or 'seed yam' which could be little full tubers or pieces (setts) cut from larger tubers. Tubers produced by seed yam are genetically identical to the mother seed material but those produced from yam seeds have an identity different from the parent material.

Preservation of some vegetative propagules, such as seed yam, for next planting season has posed a great challenge to farmers. This is due to its rate of spoilage, bulkiness, storage facilities and environmental hazards. The discovery of propagation of yam through the seeds produced by the flowers instead of the small tubers/seed yam has a promising solution to these challenges. However, even though we select quality seeds, it is advised to go through the seed treatment process for better germination and to prevent seed and soil-borne diseases. Treatment of yam botanic seeds constitutes the major steps taken for the proper germination and establishment of seeds; thus, adequate seed treatment must be adopted to ensure the viability of seeds, healthy germination and development (Balogun, Maroya, & Asiedu, 2014). Treatment of seeds fall into two categories of wet and dry. Wet treatment includes the use of boiling water, acids, organic solvents and alcohol while dry treatment includes use of dry heat, manual or mechanical scarification, impaction, percussion, and microwave energy (Kimura & Islam, 2012). These treatments make some seeds viable and reduce dormancy by softening the hard seed coat, increasing the permeability of water while enhancing enzymatic action (Kimura & Islam, 2012).

Breeders all over the world have been researching on the best treatment that can enhance the germination of seeds without adverse effects on the seed, such as, dormancy, mutation of the genetic makeup of the seed and even seed damage. Seed treatment or priming have been proven to fasten seed germination rate (Pujol et al., 2002). However, before selecting seed for priming aimed at improving the parameters above, it is ideal to look at the simplicity of its completion and applicability to a large number of seeds. This research determined the optimal treatment of botanical yam seeds for enhanced germination and establishment of seedlings through the use of wasteful product (rice husk).

Material and methods

Study location

This research work was carried out inside the green house at Alex Ekwueme Federal University Ndufu-Alike, (AE-FUNAI) in Ebonyi State, Nigeria. The study area was located between Latitude 6˚7'15"N and Longitude 8˚8'58"E.

Sample collection

Yam botanical seeds (*D. rotundata)* were obtained from the yam breeding program of National Root Crops Research Institute (NRCRI), Umudike, Abia State, originating from crossings by open pollination of *D. rotundata* (TD1 09/0002) variety; which are the most cultivated in the region of Federal district and surroundings. Because of natural dehiscence of the fruits, they were packed till they mature into voile bags at equal phenological stage and the seeds were systematically collected after their dispersal.

Sample sorting and processing

Before any treatment, all seeds were sorted as true-seeds or pseudo-seeds by immersion in water at room temperature. The floating seeds were sorted as pseudo-seeds and eliminated while the true-seed (submerged seeds) were removed from water and dried under natural environment according to Ogburia and Adachi (1995).

Experimental design

The experiment was laid out in a Completely Randomized Design (CRD) and was replicated three times with three controls. Two (2) substrates x 6 seed treatments x 3 replicas (4 seeds for each one) in total of 36 polythene bags.

Treatment of yam seeds

A total of four (4) yam seeds each were used for each treatment in the following order:

- Dry heating of seeds (DHS) at 60ºC for 24 hours. The seeds were kept in dry forced air circulation oven at 60ºC for 24 hours (Wetzel, Allen, Cunha, & Salomão, 2006).
- Mechanical scarification of seeds (MSS) by the use of sandpaper followed by seed immersion into a 100 ppm of gibberellic acid (GA) solution for 24 hours (MSS + GA). Mechanical scarification was achieved by manual friction of seeds on sandpaper until removal of a small area of seed coat.
- Mechanical scarification of seeds (MSS) only
- Acid scarification of seeds (ASS) by immersion into 10 ml of concentrated sulfuric acid (95 %) for 5 minutes
- Soaking of seeds (SOS) in hot water at 80ºC for 2 minutes
- Control/untreated seeds (COS) by storage of seeds at room temperature, without any previous treatment

Media preparation

For assessing emergence of seedlings, two different media compositions were used. The media was composed of 75% top soil and 25% carbonized rice husk (CRH) while the control media contained 100% top soil. All these media were packed inside polythene bags (volume of 350 cm^3).

Planting order

In each polythene bags, four seeds were sown at 1cm deep and covered moderately with the media kept under greenhouse condition (25 ± 2ºC) with daily water supply.

Data collection

The following data were collected for analysis:

- No of days it took a particular treatment to germinate/emerge (NDE)
- No of days to 50% emergence (ND 50 % E)
- Leaf length after two and four weeks of emergence
- Leaf width after two and four weeks of emergence
- Leaf area after two and four weeks of emergence (LA 2WAE/LA 4WAE)
- Length of petiole after four weeks of emergence (LP 4WAE)

Daily counts of number of emerged seedlings were performed during 45 days, starting from the sowing. In such count, only seedlings showing potential to keep developing and generate normal plants were considered. The leaf area (LA) was determined as the product of leaf length (LL), leaf width (LW) and a constant A (0.75) (i.e., LA= LL×LW×A).

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) in a CRD experiment using SPSS software version 20. Duncan's multiple range tests at 5% level of significance was used to separate means.

Results

Number of days of emergence (NDE)

The number of days of emergence refers to the time it took for one of the seeds to germinate or emerge. As shown in Figure 1, seeds treated by soaking in hot water (SOS) had the longest days of emergence at the 19^{th} day while MSS + GA for 24 hours had the highest rate of emergence at the 5^{th} day.

Number of days for 50% emergence (ND 50% E)

The number of days of 50 % emergence refers to the time it took two of the seeds to germinate or emerge. Dry heated seeds (DHS) at 600C had the longest days to complete 50% emergence at the 13th day while MSS + GA completed 50% emergence at the 5th day faster than other treatments as shown in Figure 1. Acid scarification (ASS) of seeds caused a negative effect on the seeds which resulted to no germination of seeds even after 45 days while the untreated control seeds (COS) completed 50% emergence on the 15th day.

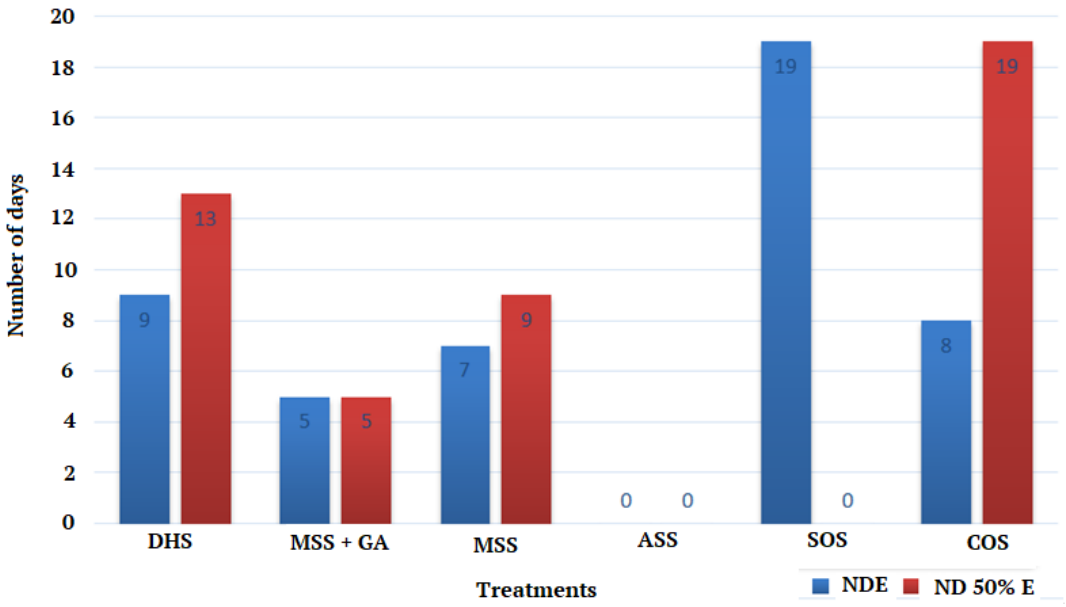

Figure 1. A bar chart of number of days of emergence against treatments. The graph above shows that MSS + GA treatment was more effective than every other treatment since it took five (5) days for the seeds to germinate and also complete 50% emergence.

Leaf area two weeks after emergence (LA 2WAE)

Seeds treated with hot water (SOS) had the smallest leaf area of 0.83 cm^2 after two weeks of emergence while MSS + GA for 24 hours had the largest leaf area of 3.62 cm^2 after two weeks of emergence as shown in Figure 2. Seeds treated with dry heat (DHS) at 60°C had a leaf area of 1.68 cm^2 two weeks after emergence. Mechanical scarification of seeds (MSS) only, recorded leaf area of 2.43 cm^2 in two weeks after emergence and the control had a leaf area of 1.8 cm^2 two weeks after emergence. There was no data record for acid scarification (ASS) treatment due to non-germination of seed.

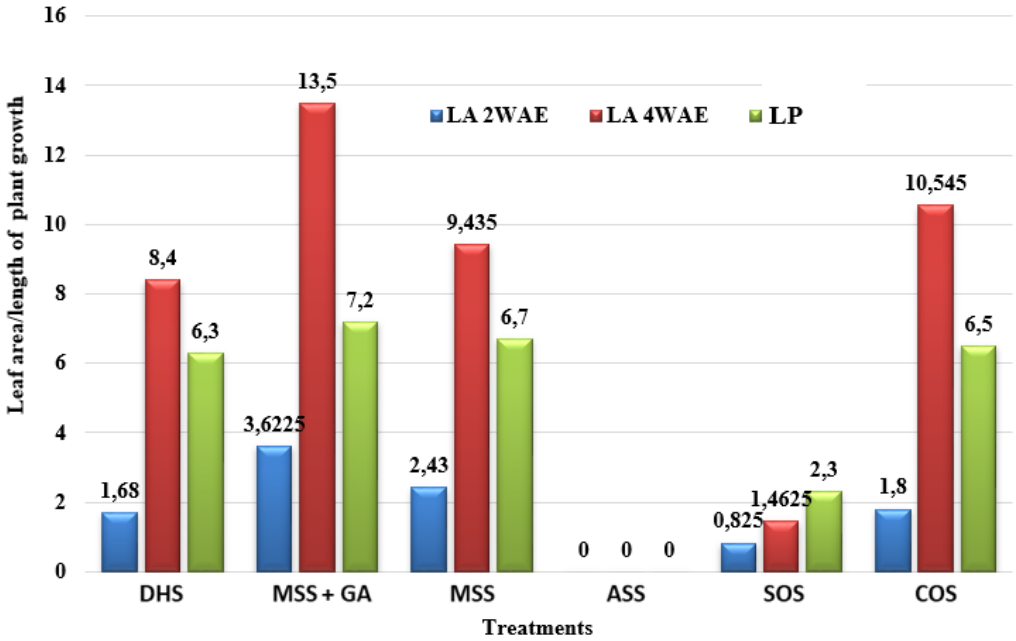

Figure 2. A bar chart showing the leaf area (cm^2) and length (cm) of petiole of yam seed plant against treatments. MSS + GA treatment emerged as the best treatment for the establishment of yam botanical seeds followed by MSS only, while treatment five was the poorest. The 4^{th} treatment damaged the seed and they seeds did not germinate.

Leaf area four weeks after emergence (LA 4WAE)

In the 4^{th} week, seeds treated with hot water (SOS) had the smallest leaf area after emergence while MSS + GA for 24 hours had the largest leaf area of 13.50 cm^2. Seeds treated with dry heat (DHS) at 60°C had a leaf

area of 8.40 cm^2, mechanical scarified seeds recorded a leaf area of 9.44 cm^2 while the control had a leaf area of 10.55 cm^2 four weeks after emergence as shown in Figure 2. The germination of the treated seeds after two and four weeks is shown in Figure 3 (a and b).

(a) **(b)**

Figure 3. (a) Treated botanical yam seedlings after two (2) weeks of germination. (b) Treated botanical yam seedlings after four (4) weeks of germination.

Length of petiole after four weeks of emergence (LP 4WAE)

Seeds treated with hot water (SOS) also had the smallest seedling establishment in terms of length of petiole of 2.30 cm after four weeks of emergence while MSS + GA for 24 hours had the longest petiole of 7.20 cm as shown in Figure 2. Seed treatment with mechanical scarification (MSS) only, had 6.7 cm length of petiole, dry heat treatment (DHS) and the control (COS) recorded 6.3 and 6.5 cm respectively.

Discussion

The treatment analysis showed that all methods of treatments adopted in the study had a significant influence on the germination of the yam seeds. All the treatments were not equal in inducing germination of seeds and establishment of seedlings. As opined by Amanze, Agbo, Eke-Okoro and Njoku (2011), a good viable and properly stored seed before planting may take up to ten (10) days to germinate. In the case of seeds affected by dormancy, it will stay about 21 days after planting before the emergence of seedling. The development of primary leaf, internodes and vines starts a month after seedling emergence. The observed increase or absence in the leaf areas (Figure 2) after 2 and 4 weeks and length of petioles after 4 weeks in all the assessed treatment is a true reflection of the early or late germination/emergence of the yam seeds. Seed treatment using dry heat (DHS) at 60°C for 24 hours showed early germination when compared to the untreated seeds which served as the control (COS). High moisture content of seeds reduces their ability to germinate, and seeds subjected to a high dry heat of about 90°C may lead to damage of the seed. Nakamura (1982) reported that the rate of germination of gourd seeds at 90°C for 5 days reduced to one half of that of untreated seeds. The upper limit of temperature of dry heat for one week may be about 75 - 80°C. Also, dry heat may not have any significant effect in germination of some seeds. There was no reduction of hard seed or improvement of germination (range 87-96%) in seeds of three cultivars (Osjecka 10, Osjecka 88 and Slavonka) of alfalfa during treatment at 40°C for 4 hours (Rutar et al., 2001).

The earliest emergence of seedlings was observed in the treatment using MSS + GA and MSS only. As shown in Figure 1, treatment using MSS + GA emerged and as well completed its 50% germination at the 5th day with a significant difference at $p<0.05$ while MSS treatment emerged at the 7th day and completed 50% emergence at the 9th day with a significant difference at $p<0.05$. Mechanical scarification using sandpaper method as applied in this study has been shown to be effective in seed germination according to Can, Celiktas, Hatipoglu and Avci. (2009). He reported that 3-15% germination rate of *Medicago* species (*Medicago rigidula, M. rotate, M. orbicularis* and *M. scutellata*) was increased to 73-100% using sandpaper mechanical scarification. Also, the effectiveness of mechanical scarification may vary depending on the genus and species of the seeds (Uzun & Aydin, 2004). Treatment with MSS + GA showed a high significant difference in the number of days to 50% emergence compared to the control (COS) untreated seed and others. This suggests that the natural

germination potential of yam seeds is very low (Amanze et al., 2011). The higher germination rate in MSS + GA than MSS only may be attributed to addition of GA which is a plant growth hormone. Research conducted with Arabidopsis gibberellin deficient mutant strains indicated that, seed coat-imposed dormancy can be overridden by application of gibberellin at the stage of emergence (Foley, 2001). It has been found that gibberellin can induce the expressions and repression of RGL2 (gibberellin-response height-regulating factors) which acts as an integrator of environmental and endogenous trigger for germination (Peng & Harberd, 2002). Therefore, by manipulating the concentration of gibberellin during seed pre-treatment it would be possible to change the germination percentage and reduce the time taken for germination.

Acid scarification (ASS) treatment of seed for 5 minutes using 95% concentrated sulfuric acid was found to be unfavorable for seedling emergence as there was no observed emergence and establishment of seedlings. The use of acids especially sulfuric acid in the treatment of seed is to reduce the hardness of seed which affects germination (Uzun & Aydin, 2004; Can et al., 2009). Can et al. (2009) reported that soaking of *Medicago* and *Trifolium* species for 5 minutes in sulfuric acid (95-97%) showed that germination in *Trifolium lappaceum* was increased from 0 in untreated control to 90 % but little or no change was observed in the *Medicago* and other *Trifolium* species. Pandrangi, Elwell, Anantheswaran, and Laborde (2003) used various concentrations (0.1, 0.2, 0.5, 1.0 and 2.0 N) of sulfuric acid under several timings of 2.5, 5, 10, 15, 20, 30, 45 and 60 minutes on alfalfa seeds and reported that all concentrations had little influence on the germination (range 74-76 %). There was reduction of germination when the seeds were soaked longer than 45 minutes. The effectiveness of seed treatment using acid depends on the duration of scarification, concentration of acid and species of seeds (Martin & De La Cuadra, 2004; Alderete-Chavez et al., 2011). Though there was report of improvement using acid scarification, the present study showed that there was seed damage indicating negative response of yam seeds to acid scarification (ASS) treatment.

Soaking of seeds (SOS) in hot water (hot water scarification) was less efficient than the untreated control (COS). Though the seedlings emerged at the 19th day, the same as seen in the untreated control in Figure 1, there was no observed or complete 50% of emergence. Hot-water treatment has been reported to enhance germination by increasing seed coat permeability for water to maximize seed hydration, for gaseous exchange and release of inhibitors such as phenolics (Mohamed-Yaseen, Barringer, Splittstoesser, & Costanza., 1994; Longer & Degago, 1996). Mcdonnell, Grant, and Coons (2012) reported that *Iliamna remota* showed a significantly higher percentage of seeds germination when dipped in water at 70 to 90^{o}C for 60 seconds compared to those exposed at ambient temperature or 100^{o}C. Thus, hot water treatments were effective in breaking dormancy of *I. remota* seeds. Temperatures above 80^{o}C may be too hot thereby, injuring or killing embryos in seeds, and reducing germination percentages.

Furthermore, growth and establishment of the yam seeds were found to vary in all the seed treatments as shown in Figure 2. The growth parameters in leaf area after 2 and 4 weeks and length of secondary leaf petal after four weeks is consistent with the number of days of emergence of the seedling. Better establishment was recorded in both MSS + GA and MSS treatment which were significant at $p<0.05$. DHS and SOS were less effective than the control and did not support better establishment of yam seedlings. It was also observed that seeds with MSS + GA and MSS only, maintained a consistent germination and growth parameters as to the untreated seeds.

Conclusion

Seed treated with MS + GA and MSS only, except for acid scarification, dry heat and hot water scarification, had the potential to improve yam seeds (*D. rotundata)* germination and establishment in a greenhouse condition. Rice husks which are seen as waste product are very useful in the enhancement of seed germination. Commonly, these methods are cost effective, simple, safe, reliable and economically more practicable ways to improve germination rate and establishment of yam seedlings in breeding programs for quality and quantity seed yam delivery to farmers.

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