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Effect of scarification on breaking seed dormancy and germination enhancement in *Annona muricata* L. (Magnoliales: Annonaceae)

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ABSTRACT

The effect of various scarification method on breaking seed dormancy and germination enhancement in *Annona muricata* (Magnoliales: Annonaceae) was examined in this study via: mechanical scarification with sandpaper, file and stone; chemical scarification with 10, 25 and 50% H_2SO_4 for 5 seconds respectively; exposure to wet heat (hot water) for 1, 3 and 5 seconds; exposure to cold treatment by chilling in refrigerator of 4 ºC for 12, 18 and 24 hours; soaking in coconut water for 5, 10 and 15min and untreated seeds as the control. The results of the experiment showed that chemical scarification with H_2SO_4 at 50% for 5 seconds had significantly highest percentage germination (60%). This was followed by the seeds soaked in coconut water for 15 minutes (39.69). Seeds treated with 25% H_2SO_4 for 5 seconds had 30% germination. Other treatment were less or not effective. Untreated seeds had the least percentage germination (6%) with mean germination time of 40.20 and germination index of 0.27. The treatments that gave significantly higher germination percentages also produce low Mean Germination Time (30.01) and increased Germination Index (12.26). These characters shows that chemical scarification with H_2SO_4 at 50% for 5 seconds was the most effective treatment to break dormancy and enhancing seed germination in this species as revealed in this study.

***Keywords*:** Dormancy, germination, scarification, *Annona muricata*, Annonaceae

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1. INTRODUCTION

The seed is a key element in plant production that it exercises a very great influence on the success and failures of both natural and artificial regeneration (Nwoboshi, 1982). According to Gupta and Bandopadhyay (2013), seeds are excellent dispersal unit and means of propagating higher plants which have emerged in the course of plant evolution. Every normal seed contains an embryo (sometimes more than one) which will develop in to the seedling and have a supply of reserve substances which will sustain the seedling in the embryo stages of growth before it becomes self-supporting (Black et al., 2006).

Malcom *et al.* (2003) defined germination as the process by which the dormant embryo grows out of the seed coat and establishes itself as a seedling. However, the seeds of many useful species are unsuitable for direct sowing in the nursery or planting sites on account of its small size, uncertain viability, dormancy, and slow initial growth. Respiration in a germinating seed is very necessary as the active protoplasm requires a constant supply of oxygen to liberate the considerable amount of energy stored in the seed to be made in a form to be utilized by the protoplasm (Amusa, 2010).

Dormancy is the absence of germination in a mature intact seed under favorable condition of light, temperature, water and oxygen within a specific period of time (Hilhorst, 1995). A viable seed (or other germination unit) is said to be dormant when it does not have capacity to germinate in a specified period of time under normal physical environmental factors that otherwise is favourable to its germination (Aghilan *et al*. 2014). According to Baskin and Baskin (2001), seed coats can also impose dormancy because they may contain growth inhibitors or may prevent the leaching of inhibitors from the embryo.

Pre-germination treatments are necessary to speed up germination, seed coat treatments have been used to raise the percentage germination and shorten the period required to reach optimum percentage germination (Willan, 1985).

Scarification involves the process of breaking, scratching or mechanically altering the seed coat to make it permeable to water or gas. Simple methods of carrying this out include rubbing the seed on sand paper, filling or cracking the testa with a hammer or between the jaws of a vice (Aminu, 2012). Generally mechanical scarification is simple and effective, but seeds treated should be planted immediately as scarification renders them highly susceptible to pathogenic attacks. To minimize these hazards it is important also that the scarification should not proceed to the point of injury on the seeds and after the operation the seeds should not be stored for too long (Aminu, 2012). The most hard – coated seeds become permeable to water when the seed coat is broken or punctured by mechanical abrasion or chemical treatment Mayer and Poljakoff-Mayber (1989). Chipping the fruit of *Pterrocarpus anogeisus* at one edge was sufficient to hasten its germination (Owunubi *et al.* 2005).

Annona muricata Linn is one of the highly valued medicinal plants in south west Nigeria. In Nigeria, it is called *sopsop or shawa shawa.* It is a plant that is empirically trusted by societies to have anticancer properties in its leaves (Roesker *et al.* 2007). Germination of its seed under sub-optimal condition has been found to be delayed for 2-3months but can occur in three weeks if condition is okay (Joseph, 2014). Untreated seeds of this species have low germination percentage apparently due to its seed coat-enhancing dormancy. Hence, the objective of this work was to evaluate the effect of different scarification methods on breaking seed dormancy and germination enhancement in *Annona muricata* Linn. (Coria-Téllez, 2018; Liu, 2016).

Photo 1. *Annona muricata* L. (Magnoliales: Annonaceae)

2. MATERIALS AND METHODS

2. 1. Seed Source

Fresh fruits of *Annona muricata* were collected from the parent plant from its natural habitat in Ipoti-Ekiti located in Ijero Local Government Area of Ekiti State and was taken to the herbarium of Plant Science and Biotechnology Department of Ekiti State University, Ado Ekiti for authentication.

2. 2. Viability Test

Seeds were subjected to viability test by using Tetrasolium chloride according to International Seed Testing Association (ISTA, 2004).

2. 3. Dormancy Breaking Treatments and procedure

Before germination experiments, the seeds were subjected to the following pre-sowing treatments;

Mechanical Scarification: Fifty seeds of *Annona muricata* were scarified each with sand paper, stone and file opposite the micropile

Chemical Treatment: Fifty seeds of *Annona muricata* were soaked each in 10%, 25% and 50% sulphuric acid for 5 seconds. The seeds were removed and thoroughly rinsed with distill water to remove acids respectively. The seed were allowed to air dry before planting in Petri dishes for germination.

Cold Treatment (Chilling): Fifty seeds of *Annona muricata* were chilled inside refrigerator (of 4 ºC) and left for 12, 18 and 24 hours after which the seeds were placed inside Petri dishes and moistened for germination.

Hot Water Treatment: Fifty seeds of *Annona muricata* were placed in socks and immersed in hot water (100 ºC) for 1, 3, and 5 seconds. The seeds were removed and immersed in cold distilled water, air dried before planting in Petri dishes.

Coconut water Treatment: Fifty seeds of *Annona muricata* were soaked in coconut water for 5, 10 and 15 min. They were later removed and air dried before planting in petri dishes.

Control Experiment: Fifty untreated seeds of *Annona muricata* were planted in petri dishes as control.

2. 4. Germination Assay

Each Petri-dish was lined with cotton wool and moistened with 6ml distilled water after which the seeds were planted in them and then covered-up with their respective lids (Arowosegbe and Afolayan, 2013). Ten (10) seeds were placed in a sterilized Petri dish.

The experiments was set up in a Randomized Complete Block Design with five replicates. All the Petri dishes were placed on the germination table in the laboratory at room temperature. Germination parameters were determined daily (Ajayi and Fakorede, 2000) until no further germination occurred. The germination percentage was determined using the relation:

$$\text{Germination percentage (GP)} = \frac{\text{Number of germinated seeds}}{\text{Total No. of seeds planted}} \times 100$$

The Mean Germination Time (MGT) was determined as described by Orchad (1977) as follows:

$$\text{Mean Germination Time} = \frac{\sum fx}{\sum x}$$

where f is the number of germinated seed on day x and x is day 1, 2, 3...

The germination index (GI) was calculated according to the Benech Arnold *et al.* (1991) equation:

$$GI = (10 \times n1) + (9 \times n2) + (8 \times n3) \ldots\ldots\ldots\ldots\ldots (1 \times n10)$$

where; n1, n2, n3……n10 = Number of germinated seeds on first, second, third and subsequent days until 10th day; 10, 9 ……..and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively.

2. 5. Statistical Analysis

The data collected were subjected to one-way analysis of variance (ANOVA) and the means were separated at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT). All statistical analyses were done using SAS software, 1999 version.

3. RESULTS

3. 1. Effect of mechanical scarification

The effect of mechanical scarification with sand paper, file and stone on breaking the dormancy and germination enhancement of *A. muricata* was shown in Table 1. Result obtained revealed that mechanical scarification had no effect on the germination percentage of *A. muricata.* However, the seeds scarified with sandpaper had 10.00% germination which was significantly higher than the seeds scarified with stone (6.00%) and the control (6.00%). This was followed by seeds scarified with file which gave 7.00% germination. Significant difference was observed in Mean Germination Time (MGT) of *Annona muricata* seeds subjected to mechanical scarification when compared with the control that had MGT of 40.20 as seeds scarified with sandpaper, file and stone had MGT of 35.14, 36.50 and 36.90 respectively. Sandpaper produced the highest Germination Index (GI) of 1.05 while file and stone had the same GI of 0.54 which was significantly different from the control that had 0.27.

3. 2. Effect of scarification with acid (H_2SO_4)

Table 2 shows the effect of H_2SO_4 treatment on breaking dormancy and enhancing germination of *A. muricata* seeds. The result revealed significant differences in seed germination percentage, MGT and GI among seeds scarified with other methods. Seeds treated with H_2SO_4 (50%) for 5 seconds produced the highest germination percentage (60%), lowest mean germination time (30.01) and highest germination index (12.26). seeds treated with H_2SO_4 (25%) for 5 seconds had no appreciable germination percentage of 30% which was significantly different from the seeds treated with H_2SO_4 (10%) for 5 seconds and the control with germination percentage of 22% and 6% respectively. No significant different abound in MGT of seeds treated with H_2SO_4 (25%) for 5 seconds and those treated with H_2SO_4 (10%) for 5 seconds with mean values of 32.52 and 33.50 respectively. Germination index of seeds differed significantly ($P < 0.05$) with H_2SO_4 at varying concentration as seeds treated with H_2SO_4 (25%) had 8.45, those treated with H_2SO_4 (10%) had 8.00 and the untreated seeds had the lowest of 0.27.

3. 3. Effect of wet heat (Hot water)

Exposing the seeds of *A. muricata* to wet heat irrespective of the duration of exposure at 100^{o}C had no significant effect on germination percentage (Table 3). In fact, germination percentage immersed in 100 ºC wet heat for 3 seconds (5.90%) and seeds immersed in 100^{o}C wet heat for 1 second (6.12) were not significantly different from the control (6.00) while the value obtained in germination percentage of seeds immersed in 100 ºC wet heat for 5 seconds was 7%. Highest mean germination time of 40.20 was observed in the untreated seeds. No significant difference was recorded in MGT of seeds treated with 100 ºC wet heat for 1 second (35.69), 100 ºC wet heat for 3 seconds (37.00) and 100 ºC wet heat for 5 seconds (37.00). highest

GI of 0.42 was recorded in seeds treated with 100 ºC wet heat for 5 seconds and this was not significantly from seeds treated with 100 ºC wet heat for 3 seconds which gave 0.40. Lowest GI of 0.27 was recorded in the control experiment.

3. 4. Effect of cold stratification

Result of this study revealed that exposing seeds of *A. muricata* to cold treatment irrespective of the duration of chilling had no significant effect on the germination percentage when compared with the control (Table 4). Highest MGT (40.20) was observed in the control experiment. This was followed by seeds chilled for 24 hours (39.20). This result also showed that same GI (0.27) was recorded in untreated seeds and those seeds chilled for 12 hours at 4 ºC while those seeds exposed to cold treatment at 4 ºC for 18 and 24 hours had GI of 0.21 and 0.22 respectively.

3. 5. Effect of soaking in coconut water

Soaking seeds of *A. muricata* in coconut water had a little significant effect on the germination percentage, MGT and GI (Table 5). A clear observation of this result revealed that germination of *A. muricata seeds* from 6.00 (untreated seeds) was increased by soaking seeds in coconut water for 5, 10 and 15 minutes to 10.24, 15.17 and 39.69 respectively. Also, highest MGT of 40.20 was recorded in the untreated seeds when compared to the seeds soaked in coconut water for 5, 10 and 15 minutes with MGT of 34.50, 34.00 and 32.00 respectively. Furthermore, highest GI of 12.00 was observed in seeds treated with coconut water for 15 minutes when compared with the control that had 0.27.

Table 1. Effect of mechanical scarification with sandpaper, file and stone on breaking dormancy and germination enhancement in seeds of *Annona muricata*.

Dormancy breaking treatments	Germination (%)	MGT (days)	GI
Control	6.00	40.20	0.27
Sandpaper	10.00	36.14	1.05
File	7.00	36.50	0.54
Stone	6.00	36.90	0.54

Values with the same letter(s) within the column are not significantly different at $P \leq 0.05$
MGT: Mean Germination Time, GI: Germination Index

Table 2. Effect of chemical scarification with H_2SO_4 on breaking dormancy and germination enhancement in seeds of *Annona muricata*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI
Control	6.00	40.20	0.27
H_2SO_4 (10%) for 5 seconds	22.00	33.50	8.00

H_2SO_4 (25%) for 5 seconds	30.00	32.52	8.45
H_2SO_4 (50%) for 5 seconds	60.00	30.01	12.26

Values with the same letter(s) within the column are not significantly different at P ≤ 0.05
MGT: Mean Germination Time, GI: Germination Index

Table 3. Effect of wet heat (hot water) on breaking dormancy and germination enhancement in seeds of *Annona muricata*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI
Control	6.00	40.20	0.27
Wet heat (100 ºC) for 1 seconds	6.12	37.20	0.12
Wet heat (100 ºC) for 3 seconds	5.90	37.00	0.40
Wet heat (100 ºC) for 5 seconds	7.00	35.69	0.42

Values with the same letter(s) within the column are not significantly different at P ≤ 0.05
MGT: Mean Germination Time, GI: Germination Index

Table 4. Effect of cold treatment (chilled inside refrigerator of 4 ºC) on breaking dormancy and germination enhancement in seeds of *Annona muricata.*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI
Control	6.00	40.20	0.27
Chilled for 12 h	5.98	37.00	0.27
Chilled for 18 h	4.00	38.12	0.21
Chilled for 24 h	4.00	39.20	0.22

Values with the same letter(s) within the column are not significantly different at P ≤ 0.05
MGT: Mean Germination Time, GI: Germination Index

Table 5. Effect of soaking in coconut water on breaking dormancy and germination enhancement in seeds of *Annona muricata.*

Dormancy breaking treatments	Germination (%)	MGT (days)	GI
Control	6.00	40.20	0.27
Soaking for 5 min	10.24	34.50	8.00

Soaking for 10 min	15.17	34.00	8.50
Soaking for 15 min	39.69	32.00	12.00

Values with the same letter(s) within the column are not significantly different at $P \leq 0.05$
MGT: Mean Germination Time, GI: Germination Index

4. DISCUSSION

Results of various treatments in the present study revealed that *A. muricata* seeds exhibit dormancy due to hard seed coat. The germination percentage obtained in seeds mechanically scarified and untreated seeds was found to be very low. This further corroborates the report of Mackay et al. (2001) who observed that non-scarified seeds of *L. arboreus* had germination lower than 5%

Results obtained from the present studies revealed that scarification of *A. muricata* seed with H_2SO_4 to break its dormancy was effective as it resulted to a higher germination percentage when compared with the control. It was also observed that the higher the concentration of the acid, the higher the percentage germination of A. muricata seeds as the highest germination percentage was found in the seed lots soaked in 50% H_2SO_4 for 5 seconds. Therefore, soaking the seeds in a higher concentration with the same duration might lead to more germination percentage of this species. Ibrahim and Otegbeye (2004) reported that Soaking seeds in chemical tried in India and Indonesia showed that Acacia albida, Acacia senegal and Acacia nilotica seeds require soaking in concentrated sulphuric acid for 20, 40 and 80 minutes respectively, in order to modify their seeds coats.

Previous work on Parkia biglobosa (Aliero, 2004), Enterolobium contortisiliquum (Malavasi and Malavasi, 2004), Rhynchosia capitata (Ali et al., 2011) also showed that soaking of seeds of these plants in H_2SO_4 can break dormancy and increase germination percentage. The mechanism involved in the possible seed germination influenced by H_2SO_4 could be due to the capacity of the acid to break the seed coat (Ali et al., 2011) by softening it thereby allowing water imbibition and oxygen penetration into the seed. According to Nadjafi et al. (2006), scarification of seed coat with acids such as H_2SO_4 usually leads to elimination of exogenous dormancy. Soaking in concentrated sulphuric acid has also proved useful for Albizia lebbeck, Cassia nodasa and Delonix regia in Nigeria (Nwoboshi, 1982). Also, Lula et al (2000) obtained 44% seed germination through chemical scarification of Paspalum paniculatum for 20 minutes. Meneghell et al. (2000) reported that chemical scarification of Enterolobium contortisiliguum seeds with sulfuric acid for 10 min increased germination percentage and gave best uniformity of seedling emergence. Verma et al (2001) reported that acid treatment was effective in breaking *Glycyrrhiza glabra* dormancy and obtained 88 % germination. Hartmann et al., (2001) also reported that Seed scarification by immersion in H_2SO_4 is often used to eliminate barriers delaying germination.

Seeds exposure to wet heat irrespective of temperature and duration of exposure used in this study did not improve the seed germination of *A. muricata*. Reason for this could be due to the short period of exposure. This finding agreed with the previous assertion of Mawahib (2000) that Soaking seed in boiling water for 3, 10, or 15 minutes gave the highest germination percentage, while soaking in 1 and 5 minutes gave significantly lower germination percentage in *Delonix regia*.

Cold treatment (chilling) irrespective of its duration failed to encourage *Annona muricata* seed germination as no appreciable improvement was recorded in *A. muricata* seeds subjected to cold treatment, percentage germination with this treatment was not significantly different from the control, longer MGT was observed and very low GI was recorded. Failure by the seeds to germinate after chilling for different hours may indicate that cold stratification is not a precondition of germination of *Annona muricata* seeds. Most of the chilled seeds were observed intact with their seed coats still very hard after 30 days of planting which, if persist could lead to the total death of the embryo. These results are similar to the findings of Whyman (1993) who reported that subjection of seeds to cold treatment may damage embryo.

Little effectiveness of coconut water method is due to limited time of soaking as percentage germination of *A. muricata* seeds increases with increasing in the time of soaking. However, it was revealed from the results of this work that coconut water encouraged seed germination of *A. muricata* to some extent. This might be attributed to the fact that coconut water possesses some elements that favours plant improvement. This finding agrees with the previous assertion of Nasib, *et al*. (2007) who reported that coconut water has been used as a supplement in many laboratories to improve regeneration of plant cells.

5. CONCLUSION

The results obtained from this study revealed that seeds of *A. muricata* exhibits dormancy imposed by hard seed coat. Although, soaking in coconut water encourage the germination of the seed of this species, scarifying the seed with concentrated H_2SO_4 produced significantly higher germination percentage, reduced the MGT and increased the GI. Improvement of the effect of H_2SO_4 on breaking seed dormancy in *A. muricata* is therefore recommended.

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