

Scarification of Exotic and Indigenous Plant Seeds in Nigeria: Effect on Dormancy and Germination

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ABSTRACT

Dormancy is exhibited in many seed producing plants. It could be endogenous or exogenous, depending on the plant and the type of seed the plant produce. A survival strategy, plant use to conserve their genetic materials during unfavourable conditions. Scarification treatments has been used in this work to break the dormancy of *Anacardium occidentale*, *Annona muricata*, *Jatropha curcas*, *Tamarindus indica* and *Artocarpus heterophyllus* using 65% Nitric acid (HNO₃), 65% Sulphuric acid (H₂SO₄), 0.5% Potassium tetraoxosulphate(VI) (K₂SO₄), 0.5% Urea (CH₄N₂O), 43% Ethanol (C₂H₆O) and Distilled water. Nitric acid (65% HNO₃) produced the best result for *Anacardium occidentale* with high numbers of seedlings and a germination period of 15 days. *Jatropha curcas* did not produce a favourable result from the treatments. *Tamarindus indica*, water treatment produced the best result with six days of germination shorter than the controlled value (16 days). Nitric acid (65% HNO₃) and water favor *Annona muricata* with germination period of 19 days as against 24 days for control experiment. Water and Potassium sulphate are the best treatments for *Artocarpus heterophyllus* as they produce viable seedlings with short germination period of 14 and 15 days which give a good result better than the 18 days of the control experiment.

INTRODUCTION

Dormancy indicates a temporal retardation in growth of an organism. Dormant organism's shows periods of arrested growth, this is highly pronounced in some animals and plants. In plants, dormancy can be observed in seed and seedling germination. According to Vleeshouwers *et al.* (1995), Thompson (2000), Fenner and Thompson (2005), dormancy is part of the characteristic requirement for the growth of plant seeds and its survival. Dormancy is exhibited by many plants as a survival strategy, this enable them to survive in harsh or extreme climates. The term dormancy is used to describe a seed that fails to germinate under favourable condition at a specified time. Seed germination process in some plants are temporarily delayed due to dormancy, this serves as an advantage for seed dispersal due to seasonal changes in different parts of the world (Hilhorst, 1995; Baskin & Baskin, 1998). Accessing the part of the seed from which dormancy can be classified, exogenous and endogenous dormancy are the two types of dormancy recognized. Exogenous dormancy is imposed by the seed coat on the matured embryo, it is caused when the seed coat is too hard, limiting the free expansion of the embryo for the protrusion of the plumule and radicle during germination. The seed coat becomes impermeable during seed maturation and drying. Impermeable seed coats and other layers prevent seeds from water up take and gaseous absorption consequently preventing seed germination (Li & Foley, 1997). High concentration of growth inhibitors such as abscisic acid ($C_{15}H_{20}O_4$) on the seed coat and its surrounding layers can delay the growth of the embryo. Endogenous dormancy is also known as embryo dormancy, this is due to some conditions embedded in the embryo such as the presence of growth inhibitors and absence of growth promoters. This will prevent embryo growth and seed germination until chemical change takes place.

Seed development of dormancy, desiccation tolerance and accumulation of food reserves occur when seed have undergo embryogenesis and seed maturation phase also known as the growth phase (Bentsink & Koornneef, 2008).

Seed dormancy may be primary or secondary. Seeds exhibiting primary state of dormancy are released from their parent plant in a dormant state. Examples of families with such seeds are Anacardiaceae, Sapindaceae and Cannaceae. Seeds with secondary dormancy are released in non-dormant state but become dormant if the condition for germination becomes unfavorable. There are various causes of dormancy some of which are: Hard seed coat, Immature embryo, Germination inhibitors, Period after ripening: Some seeds have a period of ripening. Those seeds germinate only after the completion of that period. Dormancy mechanism has been diversified due to diversity of

habitat and the climates of the habitat which those seeds operate. Naturally, the seed coats of physically dormant seeds become permeable through repeated heating and cooling over long periods of time in the soil seed bank (Finch-Savage & Leubner-Metzger, 2006; Offord *et al.*, 2009). For example, temperature fluctuations during dry seasons in northern Australia facilitate dormancy breaking in impermeable seeds of *Stylosanthes humilis* and *Stylosanthes hamata* (Baskin, 2003).

Nikolaeva (1969) devised a dormancy classification system stressing that dormancy is determined by both morphological and physiological properties of the seed (Nikolaeva, 2004). Light and gibberellins can both break dormancy and promote germination in seeds with coat dormancy according to Casal and Sánchez (1998), Sanchez and Mella (2004), and Kucera *et al.* (2005).

The abundant form of dormancy is physiological dormancy which is found in seeds of gymnosperms and most angiosperms. It is the most prevalent form of dormancy in temperate seed banks and abundant dormancy class in the field. There are three levels of physiological dormancy; deep, intermediate and non-deep (Baskin & Baskin, 2004). Embryos that are differentiated into cotyledons and hypocotyl-radicle undergo morphological dormancy when the seeds are underdeveloped in terms of embryo size. These embryos need time to grow and germinate e.g. *Apium graveolens* (Jacobsen & Pressman, 1979). Morphophysiological dormancy is common in seeds with underdeveloped embryos, but in addition they have a physiological component to their dormancy (Baskin & Baskin, 2004). These seeds therefore require a combination of warm or cold stratification and other forms of dormancy-breaking treatments which can be replaced by gibberellic acid application. Examples of plants and families with morphophysiological dormancy are *Trollius ledebouri*, *Fraxinus excelsior*, *Ranunculaceae*, *Oleaceae* (Finch-Savage & Clay, 1997). Mechanical or chemical scarification can break physical dormancy caused by water-impermeable layers of palisade cells in the seed coat (Baskin, 2003).

It has been discovered that after most artificial treatments under natural conditions, the first site of water penetration in seeds is the strophiole (Baskin & Baskin, 1998). Several techniques have been used to soften seed coats and make seeds permeable, some of which are: Acid scarification (soaking seeds in conc. Sulphuric or Nitric acid). Which is very effective in treating Acacia seeds. Physical dormancy has been identified in the seeds of these plants across 15 angiosperm families (Rooser, 2017).

Water temperature of about 40 °C or less has been proven to be effective in promoting germination when seeds are soaked in it, but majorly in seeds with permeable seed coat. However, boiling some

seed species in water removes cuticle and sometimes part of the palisade layers of the seed coat which effectively break dormancy. Soaking in water within the range 60-90 °C is often as effective as soaking at 100 °C. lower temperatures creates less chances of damage (Baskin & Baskin, 1998). According to Bewley and Black (1994), Secondary dormancy occurs in some non-dormant and post dormant seeds that are exposed to unfavorable condition for germination. Though the mechanisms of secondary dormancy are not fully understood yet, but it might involve the loss of sensitivity in receptors of the plasma membrane. Our study therefore sets out to determine the best chemical treatment in breaking seed dormancy, determine the germination period of some seeds and evaluate the effect of some chemical treatments on seed dormancy and germination.

MATERIALS AND METHODS

The materials for this study are nursery bags, 65% Nitric acid (HNO₃), 65% Sulphuric acid (H₂SO₄), 0.5% Potassium tetraoxosulphate VI (K₂SO₄), 0.5% Urea (CH₄N₂O), 43% Ethanol (C₂H₆O), Distilled water (H₂O), 110 seeds of *Anacardium occidentales* Linn, 110 seeds of *Annona muricata* Linn, 110 seeds of *Jatropha curcas* Linn, 110 seeds of *Tamarindus indica* Linn, 110 seeds of *Artocarpus heterophyllus* Lam, specimen bottles, loamy soil, hand gloves and hoe.

METHODS

The prepared chemicals were collected from the laboratory. 100 seeds each of *Anacardium occidentale* Linn, *Annona muricata* Linn, *Jatropha curcas* Linn, *Tamarindus indica* Linn, *Artocarpus heterophyllus* Lam were soaked in various chemicals (HNO₃, H₂SO₄, H₂O, K₂SO₄, CH₄N₂O and C₂H₆O) as shown in plate 1 and 2. The seeds in HNO₃ and H₂SO₄ acid were removed at an interval of 10 minutes (i.e. 10, 20, and 30 minutes respectively for the various seeds) according to the method used by El-Siddig *et al.* (2001). This method was modified by rinsing and soaking the seeds in distilled water for 24hours. The various, seeds in equal numbers were soaked in 0.5% urea, 43% ethanol, 0.5% K₂SO₄, and distilled water separately for 24 hours (Yücel, 2000; Purohit *et al.*, 2015). The seeds in these treatments were rinsed with water and planted in nursery bags as shown in plate 3. The nursery bags were labeled according to the type of seeds, treatment used and time variation. The planted seeds were observed for a period of 30 days and records were taken daily.



Plate 1 & 2: Seeds soaked in different treatments of water Urea, alcohol and potassium sulphate



Plate 3: Seeds planted in polythene bags

RESULT

White arrows indicate seeds of *Jatropha curcas*, *Tamarindus indica*, *Anona muricata* and *Anacardium occidentale* treated with Nitric acid having seedlings with luxuriant growth after 30 days, *Artocarpus heterophyllus* did not survive in it (plate 4 and table 1).

Yellow arrows indicate seeds of *Jatropha curcas*, *Artocarpus heterophyllus*, *Annona muricata* and *Tamarindus indica* treated with water having seedling with variations in growth and survival after 30 days. *Anacardium occidentale* did not survive well in this treatment, because most of the seeds started fermenting after 24 hours thus it produced only one seedling after planting the seeds (plate 4 and table 1).

Blue arrow indicates seeds of *Anacardium occidentale*, *Jatropha curcas*, *Artocarpus heterophyllus*, *Annona muricata* and *Tamarindus indica* treated with Nitric acid, Sulphuric acid, Urea and Ethanol. *Anacardium occidentale* survived in nitric acid, sulphuric acid and urea but it produced only 2 seedlings in ethanol treatment. *Jatropha curcas* only survive in nitric and sulphuric acid at limited time of 10-20 minutes treatment but did not survive in urea and ethanol. *Artocarpus heterophyllus* did not survive in nitric acid, sulphuric acid and ethanol due to its tin seed coat that could be rapidly degraded by acids and alcohol but 2 seedlings was produced in the treatment with urea. *Annona muricata* and *Tamarindus indica* survived in nitric acid, sulphuric acid and urea. *Tamarindus indica* produced one seedling in ethanol treatment but *Annona muricata* did not survived in it (plate 4 and table 1).



Plate 4: Seedlings after thirty days (30) of planting

Table 1: Result Effect of the treatments on seeds used

Seeds	65% HNO ₃ 10 mins	65% HNO ₃ 20 mins	65% HNO ₃ 30 mins	65% H ₂ SO ₄ 10 mins	65% H ₂ SO ₄ 20 mins	65% H ₂ SO ₄ 30 mins	H ₂ O 24 hrs	0.5% K ₂ SO ₄ 24 hrs	0.5% CH ₄ N ₂ O 24 hrs	43% C ₂ H ₆ O 24 hrs	Control
<i>Anacardium occidentale</i>											
No of seeds used	10	10	10	10	10	10	10	10	10	10	10
No of germinated seeds	10	5	7	8	7	7	1	5	9	2	8
Days taken to germinate	15	17	13	16	16	15	19	15	16	17	19
<i>Jatropha curcas</i>											
No of seeds used	10	10	10	10	10	10	10	10	10	10	10
No of germinated seeds	8	0	0	5	3	0	8	10	0	0	9
Days taken to germinate	6	0	0	7	6	0	9	9	0	0	6
<i>Tamarindus indica</i>											
No of seeds used	10	10	10	10	10	10	10	10	10	10	10
No of germinated seeds	10	9	10	8	4	0	10	10	9	1	10
Days taken to germinate	12	17	14	19	19	0	6	11	14	17	16
<i>Annona muricata</i>											
No of seeds used	10	10	10	10	10	10	10	10	10	10	10
No of germinated seeds	8	9	7	5	8	7	6	6	2	0	7
Days taken to germinate	19	22	23	20	22	22	19	23	24	0	24
<i>Artocarpus heterophyllus</i>											
No of seeds used	10	10	10	10	10	10	10	10	10	10	10
No of germinated seeds	0	0	0	0	0	0	7	9	2	0	9
Days taken to germinate	0	0	0	0	0	0	14	15	24	0	18



Plate 5 Anacardium



Plate 6 Jathropha



Plate 7 Tamarindus



Plate 8 Anona



Plate 9 Artocarpus

Plate 5: Seedlings of *Anacardium occidentale*

The whole chemical treatments process for *Anacardium occidentales* produced viable seedlings, although water produced only one viable seedling and two for ethanol (43% C₂H₆O for 24 hours), which produced two viable seedlings (as seen in Table 1) with germination period of 19 and 17 respectively. The set germination period for the controlled experiment was 19 days. Water has same germination period with the set value of the controlled experiment. The reason for decrease in number of seedling survival in water is due to the formation of foam (lather) which might be as a result of fermentation causing decay of the embryo. The best treatment for *Anacardium occidentales* is Nitric acid (65% HNO₃ for 10 minutes) which produced high numbers of seedlings or viable seeds (10) and a germination period of 15 days as supported by the work of Yücel (2000). Oyewole & Koffa, (2010) work on the response of sprouting in cashew nut due to storage length, nut size, nut soaking and their interactions. They discovered

that nuts presoaked in water for 24 or 36 hours before sowing was observed to give the best result for soaking treatment and therefore recommend it to farmers involved in large scale cashew farming. This result does not fit into our experiments as fermentation was noticed after 24 hours of soaking cashew seed.

Plate 6: Seedlings of *Jatropha curcas*

The seeds of *Jatropha curcas* did not survive in Urea and ethanol (0.5% $\text{CH}_4\text{N}_2\text{O}$ and 43% $\text{C}_2\text{H}_6\text{O}$ soaked for 24 hours). Though they survive the Nitric and sulphuric acid treatments at 10 minutes but not above it as supported by the work done by Davis *et al.* (1991) for the improvement of seedling emergence of *Lupinus texensis* through acid scarification. Seed survival decreases as the time increases for the acid treatments (20-30 minutes) as seen in Table 1. Many of the survived acid treated seeds had delay germination as against the control. There was no significant difference between acid treated seeds (Nitric acid) and the control seeds germination time. The luxuriant growth of *Jathropha curcas* might be due to the presence of microorganism as reported in the work of Jha & Saraf, (2011), on the effect of plant growth promoting rhizobacteria on seed germination, behaviour and seedling vigor of *Jatropha curcas* seed inoculated with five strains of bacteria isolates; *Brevibacillus brevis* MS1, *Enterobacter aerogenes* MS2, *Bacillus licheniformis* MS3, *Micrococcus* sp. MS4 and *Acinetobacter calcoaceticus* MS5. The highest germination percentage was observed in MS5 (63.33 %) and the germination capacity (80 %) followed by MS3 (60 %) with germination capacity (73.33 %) while maximum seedling vigor index (929.05) was reported in MS5 followed by MS3 (895.8). The seedling vigor reported in our work in treatment with Nitric acid for 10 minute may be due to the presence of residual nitrogen compound from the treated seed couple with the activities of soil microorganism which naturally fix nitrogen compound to plant root in which the five strains of microorganism mentioned in the work of Jha & Saraf, (2011) above falls into that category.

Plate 7: Seedlings of *Tamarindus indica*

The whole chemical treatments for *Tamarindus indica* was favourable except ethanol (43% $\text{C}_2\text{H}_6\text{O}$ for 24 hours) which produce only one seedling and took one extra day (17 day) to germinate above the set control value (16 days) and also sulphuric acid (65% H_2SO_4 for 30 minutes) which does not produce any seedling. Nitric acid (65% HNO_3 for 10 minutes) and

potassium sulphate (0.5% K_2SO_4 for 24 hours) produce seedlings at periods with close range (12 and 11 days) but the best treatment for *Tamarindus indica* is water which recorded no seed mortality with six days of germination which is better than the control value (16) as seen in Table 1. Information available on breaking seed coat dormancy in *Tamarindus indica* is very meager. Naikawadi, (2016) soaked seed of *Tephrosia purpurea* with 75% H_2SO_4 for 2.5 minutes, 75% HCl for 5 minutes, Indole-3-acetic (IAA) and Gibberellic acid (GA3), 5.0 mM GA3 for 24 hours and discovered that seeds soaked in IAA and GA3, 5.0 mM GA3 for 24 hours treatment gives better germination and other treatments produced fairly good seedlings. Though *Tephrosia purpurea* and *Tamarindus indica* are not closely related in terms of their generic name but they are closely related at their family level. This may suggest the reason why their seeds have similar responses to acids and water.

Similar work done by Tigabu & Odén, (2001) on the Effect of scarification, gibberellic acid and temperature on seed germination of *Albizia grandibracteata* and *Albizia gummifera* from Ethiopia, which belongs to the same family and subfamily with *Tamarindus*. They soaked these two species of *Albizia* in sulphuric acid, gibberellic acid and hot water. The whole treatment improves the germination and vigour of the plant species which shares specific pod and seed characteristics with *Tamarindus indica*.

Plate 8: Seedlings of *Annona muricata*

The seeds of *Annona muricata* did not survive in ethanol (0.5% CH_4N_2O and 43%) and only 2 seeds survived in urea (0.5% CH_4N_2O) but other treatments produced viable seedlings. Nitric acid (65% HNO_3 for 10 minutes) and water has favourable germination period of 19 days against the set period of the control experiment which is 24 days (See Table 1). Similar result was obtained in work done by Chagas *et al.*, (2013) which was geared at breaking the dormancy in sugar apple (*Annona muricata*) seeds using physical and chemical method. They found out that scarification and soaking in water for 24 h is economical and has a lot of practical applications which concur with the 19 days germination period in our experiment.

Plate 9: Seedlings of *Artocarpus heterophyllus*

The seeds of *Artocarpus heterophyllus* only survived in Urea, potassium sulphate and water (0.5% CH₄N₂O, 0.5% K₂SO₄ and H₂O). It does not survive in high concentration of the acids used in this experiment due to its thin seed coat. Water and potassium sulphate are the best treatments for *Artocarpus heterophyllus* as they produce viable seedlings with short germination period of 14 and 15 days against the 18 days of the control experiment as seen in Table 1. The results of this finding are in conformity with the findings of LAL, (2015), in the area of reduction of germination days by soaking seeds of *Artocarpus heterophyllus* in growth regulators and thiourea.

CONCLUSION

The best treatment for *Anacardium occidentale* is Nitric acid which produced high numbers of seedlings or viable seeds and low germination period. It is better to plant *Jathropha curcas* in the cultural way of planting it than using chemical treatments. Nitric acid and water are the best treatments for *Annona muricata* and *Tamarindus indica*. Water and potassium sulphate are the best treatments for *Artocarpus heterophyllus*, Seeds treated with Nitric acid produced healthy seedlings after 30 days than the rest treatments.

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