

## Breaking Dormancy In *Gmelina arborea* Roxb. Through Treatment Of Seeds With Chemical Substances And Alternating Temperature

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### Abstract

Seeds of *Gmelina arborea* Roxb. were subjected to different treatment [chemical scarification with concentrated sulphuric acid ( $H_2SO_4$ ), potassium nitrate ( $KNO_3$ ), potassium nitrite ( $KNO_2$ ) and alternating temperature regimes] to ascertain the best treatment for terminating dormancy in the seeds. Initial viability and germination tests showed that the seeds of *G. arborea* are viable but dormant. Seed viability percentage of 96 was recorded. Dormancy in the seeds was found to be the physical type caused by hard seed coat. Chemical scarification for 10 minutes with concentrated  $H_2SO_4$  was very effective in breaking seed dormancy in the species. This increased water uptake in the seeds to 68% compared with the 15% in unscarified seeds after 7 days. Scarification gave a germination percentage of 80 over 43% for the unscarified after 26 days. 1mM and 10mM  $KNO_3$  and  $KNO_2$  terminated dormancy and highly enhanced germination in the scarified than unscarified seeds. Alternating temperatures promoted germination of *G. arborea* seeds with the best results recorded at 9 cycles of alternating temperature at 30 °C for 16hours and 45 °C for 8 hours.

**Key words:** Dormancy, *Gmelina arborea*, Chemicals, Alternating temperature

### Introduction

*Gmelina arborea* Roxb. (Verbenaceae) is an important tree, which produces sound veneers that make good second-class plywood and good pulpwood in Nigeria (Chittenden, 1964). According to Oduwaiye (1981), the unbleached pulp of this species produces lower grades of wrapping paper, while the bleached product is suitable for making high quality writing and printing papers. High-grade papers can be made by mixing *Gmelina* pulp with small quantities of long fibre pulp from conifers such as *Pinus caribaea*.

Resumption of metabolic activity in seeds leading to germination only takes place when such conditions, as adequate oxygen, temperature and moisture, necessary for germination are available. However, problems of dormancy and particularly innate dormancy inhibits germination as observed by Odetola and Kozlowski (1979), Onochie (1990), Agboola (1995), Agboola and Adedire (1998), and Mensah and Agbagwa (2001). This is the situation in seeds of *G. arborea*. Most tree seeds are short-lived under natural conditions especially in the tropics where ambient temperatures are high (Agboola and Adedire, 1998). Species like *G.*

*arborea* have alternated heavy and light seed years and may produce poor quality seeds especially in the light seed years (Okoro, 1983). In some tree species seeds are usually dormant thereby delaying germination (Olofinboba, 1979 and Agboola 1995). Thus, a precursor to germination in such situation is the removal of dormancy, which could be either physical or chemical in nature. Physical dormancy results from hard seed coats or pericarp, which are impermeable to water and gases (Esashi and Leopold, 1968), while chemical dormancy may be caused by inhibitory chemicals present in fruits and seed covering (Fasidi and Olofinboba, 1975). Vincent and Roberts (1977) reported the promotory effects of light, alternating temperature and nitrate ions on the germination of some species with dormancy problems. They stated that these factors (light, alternating temperature and nitrate ions) had little effect on their own, but positive first-order interactions were common between all possible combinations of any two of the factors. Agboola (1998) and Agboola and Adedire (1998) by exposure of dormant seeds to concentrated  $H_2SO_4$  for 10 to 15 minutes eliminated the dormancy condition in some tropical weed and some tree species respectively. Mensah and Agbagwa (2001) eliminated innate dormancy in *Capsicum frutescens* by exposing freshly harvested seeds of the species to different concentrations of  $KNO_3$  and  $KNO_2$  after an initial 15 days period of drying at a controlled temperature of  $30^\circ C$ .

Seeds of *G. arborea*, commonly referred to as 'stones' are innately dormant due to the hard and tough seed coat characteristics resulting in partial or total impermeability to water and gases. The seeds require special

treatment before resumption of metabolic activity leading to germination. The study aims at finding the method of eliminating dormancy in the seeds of *G. arborea*. This is with the view to raising more seedlings for the successful establishment of their stands for commercial agro-forestry uses.

## Materials and Methods

Fresh ripe seeds of fruits of *G. arborea* were collected directly from the ground under growing stands along the road to the old Botanic Garden, University of Port Harcourt. These were immediately depulped by hand peeling using sharp knife to avoid decay. The depulped seeds were cleaned, washed several times under running tap water, and finally air-dried by spreading out on the laboratory bench.

A batch of the air-dried seeds was subjected to an initial chemical scarification for 10 minutes using concentrated  $H_2SO_4$ . The seeds were subsequently washed several times under tap water and later with de-ionized water. A second batch was left unscarified. Fifty seeds from each batch were halved longitudinally using a sharp stainless knife. The seed halves were dipped into solution of a 5% triphenyl tetrazolium chloride (TTC) in order to test their viability. Seeds whose embryos turned pink or reddish were regarded as viable according to Lakon (1949) and the ISTA (1976) rules on seed testing.

The germination experiments were carried out using 20 seeds per 9cm petri dish on white sand. The white sand, which was sterilized by autoclaving at  $121^\circ C$  for 15 minutes, was chosen over Whatman thick seed test paper to discourage microbial growth. Three replicates were used in each test. The treatments (scarified and un-scarified) were moistened at

intervals with 5ml of de-ionized water, or where standard nitrate treatment was given, with 5ml of 1mM, 10mM or 100mM of  $\text{KNO}_3$  or  $\text{KNO}_2$ . Another batch of unscarified seeds were subjected to alternating temperature regimes of 30°C for 16 hours (lower temperature) and 45°C for 8 hours (upper temperature) for different number of cycles (1, 3, 6, 9, and 12 cycles). The experiments were monitored daily and germination deemed to have occurred when the radicle extrudes by 1mm.

## Results

Initial viability and germination tests showed that the seeds of *G. arborea* are viable but dormant. Mean viability of 96% was recorded.

**Rate of water uptake:** Within the first three days of treatment, the water content of the scarified seeds reached 64% of the initial dry weight. This sharp increase levelled off when the percentage water uptake of the seeds reached 68% on the fifth day (Fig. 1).

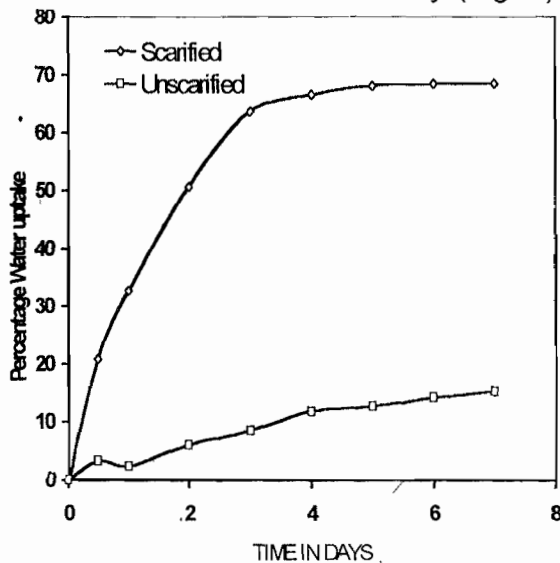


FIG. 1: Percentage water uptake by scarified and unscarified seeds

On the other hand, water uptake in unscarified seeds was just slight reaching 15% when compared to 68% reached by the scarified seeds after 7 days. This difference was significant even at 0.1% probability level ( $p > 0.0001$  t value 8.16).

**Concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ):** The percentage germination for scarified and unscarified seeds over 26 days of incubation was 69% and 31% respectively. At the end of the experiment (i.e. 36 days of incubation), percentage germination was 80% for scarified and 43% unscarified (Fig. 2). The difference between the scarified and un-scarified was significant at 0.1% level of probability ( $p > 0.001$  t value 7.53). While the scarified seeds started germinating on the 10<sup>th</sup> day, the control (unscarified) commenced on the 18<sup>th</sup> day. There was therefore a decrease in germination time with respect to scarified seeds.

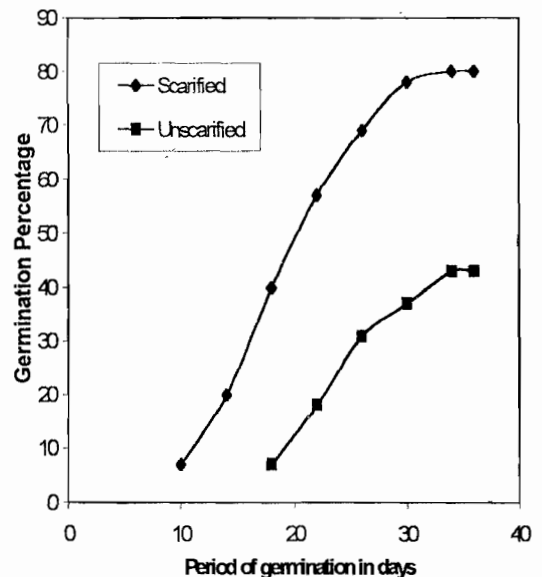


Fig. 2 Response of seeds of *G. arborea* to  $\text{H}_2\text{SO}_4$  scarification with subsequent germination at 30°C

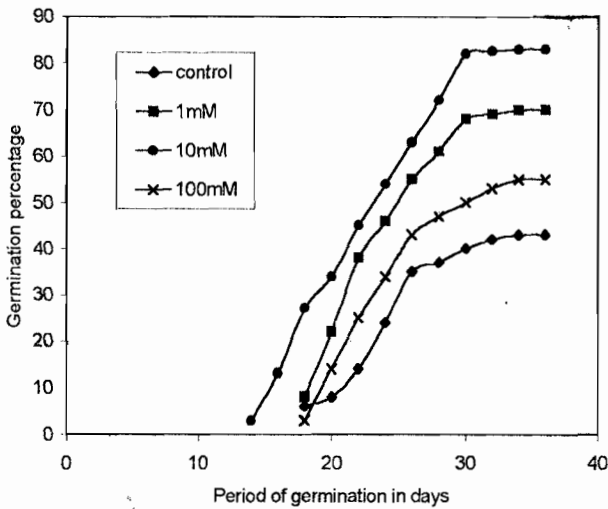


Fig. 3: Germination responses of unscarified seeds of *G. arborea* to different concentrations of  $KNO_3$  at 30 C

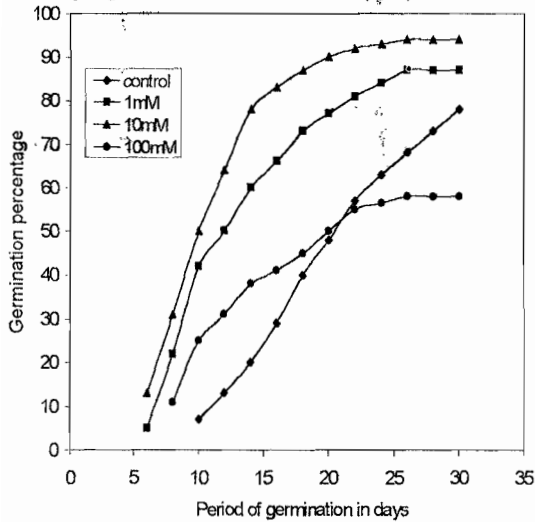


Fig. 4: Germination responses of scarified seeds of *G. arborea* to different concentrations of  $KNO_3$  at 30 C

**Effects of potassium nitrate ( $KNO_3$ ) on un-scarified seeds:**

Un-scarified seeds treated with 1mM, 10mM and 100mM  $KNO_3$  promoted germination over the control (Fig. 3). Germinations percentages for 1mM and 10mM treatments were 68 and 82 respectively after 30 days of incubation. 100mM treatment recorded 46% germination and enhanced germination over the control.

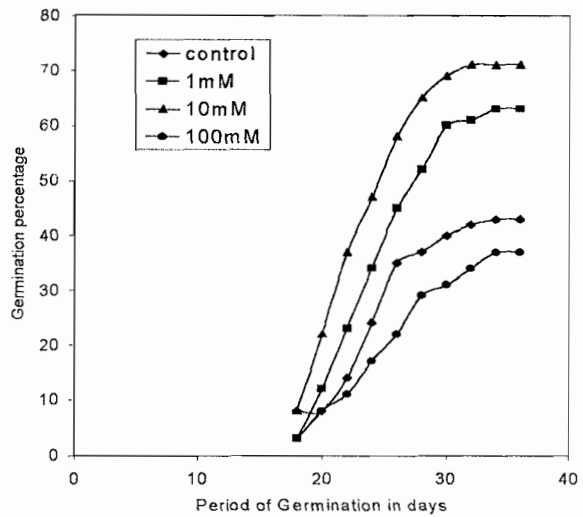


Fig. 5: Germination responses of unscarified seeds of *G. arborea* to different concentrations of  $KNO_2$  at 30 C

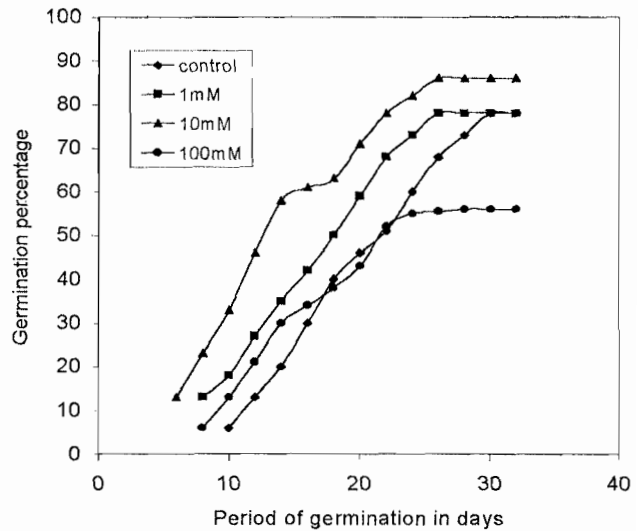


Fig.6: Germination responses of scarified seeds of *G. arborea* to different concentrations of  $KNO_2$  at 30 C

Significant difference in germination percentage was observed between the 1mM and 10mM treatments and the control at 5% and 1% levels of probabilities respectively ( $p < 0.01$  t value 5.04 and 5.04 and  $p > 0.05$  t value 3.87). However, differences in percentage germination between the control and 100mM treatments ( $p < 0.05$  t value 2.70) were not significant. A decrease in germination time of about.

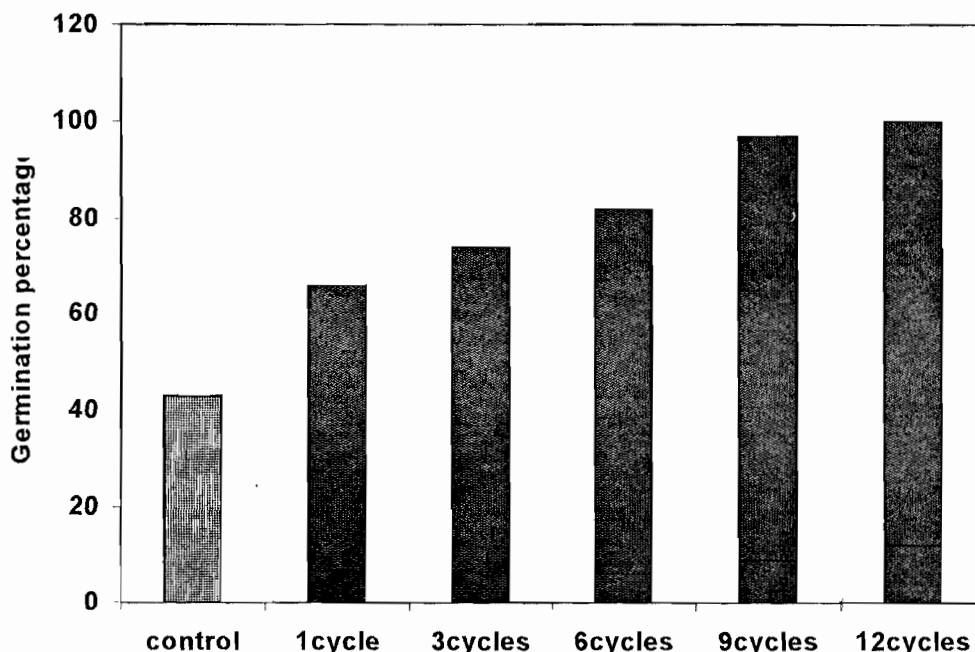


Fig. 7: Mean percentage germination of unscarified *G.arborea* seeds subject alternating temperature treatment at different number of diurnal cycles

4 days with respect to 10mM treatment was observed.

#### Effects of potassium nitrate ( $KNO_3$ ) on scarified seeds:

Subjection of scarified seeds to  $KNO_3$  treatments further enhanced germination (Fig. 4). 1mM and 10mM treatments resulted in germination percentages of 87 and 93 compared with control with 69% after 26 days of incubation. Seeds treated with 100mM  $KNO_3$  had a germination percentage of 58 after 26 days. The differences between 1mM, 10mM and 100mM treatments and the control were significant at levels of probabilities ( $p > 0.01$  t values 4.58, and 4.49). There was a decrease in germination time with respect to 1mM and 10mM treatments (Fig. 4).

#### Effects of potassium nitrite ( $KNO_2$ ) on un-scarified seeds:

Within a 30-day period treatment of un-scarified seeds with 100mM  $KNO_2$  resulted in lower germination percentage (37%) than the control. However, 10mM and

1mM  $KNO_2$  treatments resulted in 72 and 60 percent germination respectively. These were higher than the values for the control (Fig. 5). 10mM treatment enhanced germination over the other treatments. The differences between 1mM and 10mM treatments and the control were significant at 5% level of probabilities each after 35 days of incubation ( $p > 0.05$  t values 4.17 and 3.89). The difference between 100mM treatment and the control was not significant even at 5% level of probability ( $p < 0.05$  t value 2.26).

#### Effects of potassium nitrite ( $KNO_2$ ) on scarified seeds:

1mM  $KNO_2$  treatment of scarified seeds gave a slight improvement of germination percentage after 10, 18 and 26 days of incubation but there was no difference in the percentage germination between the 1mM treatment and the control after 30 days of incubation (Fig. 6), each having 78% germination. 10mM treatment enhanced germination over

the control given 86% germination after 30 days of incubation compared to the control that gave 78%. The difference was significant at 1% level of probability ( $p > 0.01$  t value 5.61). There was a fall in the mean percentage germination compared to the control following 100mM treatment of scarified stones. 100mM  $KNO_2$  treatment gave only 56% germination after 30 days of incubation while the control gave 78%. The difference between the control and 100mM treatment was significant at 1% level of probability.

#### **Effects of alternating temperature:**

There was promotion of percentage germination of seeds treated with alternating temperatures 30 °C (16h) to 45 °C (8h) over the control. The promotion depends on the number of cycles (Fig. 7). 1 cycle treatment gave the least promotion of germination of 65% compared to control with 53% germination. 9 cycles gave the highest promotory effects of 88%. When the number of cycles was increased to 12, there was no difference between the 9 cycles and the 12 cycles treatments, both having 88% germination. The 9 cycles treatment was significantly different from the control at 1% probability level ( $p > 0.01$  t value 4.22).

#### **Discussion**

Initial viability tests on the seeds of *G. arborea* resulted in a high percentage mean viability of 96 with a corresponding low germination rate. High viability percentage with low germinability implies that seeds of *G. arborea* exhibit dormancy. Chemical scarification of these seeds with concentrated  $H_2SO_4$  for 10 minutes eliminated dormancy in the seeds with a concomitant increase in the germination of the scarified seeds over the control. Seed dormancy in this

species appears to be the physical type caused by hard seed coat (Esashi and Leopold, 1968; Fasidi and Olofinboba, 1975; Agboola and Adedire, 1998), which is a hindrance to entry of water and gases. The seeds therefore germinated immediately the barrier was removed by scarification. The seed coat of certain species has been reported as a barrier to germination by preventing the entry of adequate amount of water and gases (Esashi and Leopold, 1968; Agboola and Adedire, 1998) and restraining the enlargement of the embryo (Esashi and Leopold, 1968;

Odetola and Kozlowski, 1979). The water uptake results (Fig. 1), which indicated a more rapid rate of water uptake by scarified seeds than the unscarified, is a further confirmation that the seed coat of the species serves as barrier to water uptake and subsequent germination. This agrees with the findings of Agboola (1998) who reported that the elimination of the restrictions on seed covering resumes the supply of water and germination.

There was enhancement of germination by 1mM and 10mM concentrations of  $KNO_3$  and  $KNO_2$  over the control in unscarified and scarified seeds of the species. However, 100mM treatments performed below the control in each case except for unscarified  $KNO_3$  treatment (Figs. 3 – 6). It is noteworthy that promotion of germination by 1mM and 10mM concentrations of these nitrogenous compounds was highest with scarified seeds. This is expected following the water uptake results above, which linked the germination difficulties in this species to the seed coat. That these substances eliminated dormancy even in the unscarified seeds is explainable based on the fact that they have been previously implicated in the promotion

of germination (Kozlowski, 1972; Mayer and Poljakoff-Mayber, 1975; Vincent and Roberts, 1979; Mensah and Agbagwa, 2001). These nitrogenous compounds including thiourea, ethylene, gibberellin and kinetin (Kozlowski, 1972), function cumulatively via activation of enzymes, mobilization of food materials leading to successful embryo growth and hence germination in viable seeds especially when the barrier effects have been overcome (Roberts, 1969; Agboola and Adedire, 1998). The results show that  $KNO_3$  enhanced germination fairly better than  $KNO_2$ . This fact, which is associated with the mobility of the nitrate ions over the nitrite ions have been reported by Mensah and Agbagwa (2001).

Most tree seeds are short-lived under natural conditions especially in the tropics where ambient temperatures are high (Agboola and Adedire, 1998). Plants like *G. arborea* have alternated heavy and light seed years and may produce poor quality seeds especially in the light seed years (Okoro, 1983). Diurnal cycles of alternating temperature regime of 30C (16h) to 45C (8h) and subsequent germination at 30C promoted germination of unscarified stones of *G. arborea* (Fig. 7). There was progressive increase in mean percentage germinations as the number of cycles increased. Stimulation of germination by alternating temperature regimes have been reported by Vincent and Roberts (1977), These results seem to agree with the findings of Vincent and Roberts (1977), Totterdell and Roberts (1979) and Esenewo and Adebona (1990). Alternating temperatures promote germination probably by causing expansion and contraction of the seed coat leading to their being cracked. This is obtained in nature by

high temperature during the day, followed by low temperature at night.

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