

# **RESEARCH PAPER**

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# The effect of seed coat removal on seed germination of *Terminalia superba* Engl. & Diels

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# Abstract

The influence of four constant temperatures: 20°C, 25°C, 30°C, 35°C and three germination media: 1% water agar, heat sterilized river sand and seed testing paper (STP) on the germination of decoated seeds of *Terminalia superba* Engl. & Diels. were investigated. The germination media were placed in 90 mm diameter plastic Petri dishes with seventy five decoated seeds in 3 replicates of 25 seeds. The statistical design used in the investigation was a completely randomized design in a 3 x 4 factorial (germination media × incubation temperatures). Decoated seeds of T. superba germinated at all the four temperatures investigated. The optimum temperatures were determined as 25°C, 30°C and 35°C. All the three media can be considered ideal for the reason that these temperatures interacted with the germination media to record germination percentages ranging from 73 to 89% in the study. Mean germination time (MGT) was significantly (p < 0.001) shorter when agar was used as germination media compared to when germination was carried out on STP and soil. The shapes of germination curves describing the cumulative germination of decoated seeds of *T. superba* at all temperatures and on all the germination media investigated are S-shaped.

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### Introduction

Seed germination is controlled by several environmental factors, such as seed moisture content, temperature, and light. Seed condition also affects germination; for example, the seed coat may be water impermeable, or the mature seed may contain an underdeveloped embryo that only grows to full size after imbibition (Geneve, 2003). The seed coat represents a first line of defense against adverse external factors (helps protect the embryo from mechanical injury and from drying out) and also acts as channel for transmitting environmental cues to the interior of the seed (Radchuk and Borisjuk, 2014). Integrity of seed coat surface is extremely important for seed quality and fitness during seed storage or germination, and diverse technologies are available for preserving and enhancing of seed surface (Black and Halmer, 2006; Brooker et al., 2007).

That notwithstanding, an impermeable seed or fruit coat may impose physical dormancy which must be broken before water and air can reach the embryo and initiate germination (Baskin and Baskin, 2014). Most species have a seed coat which is impervious to water. This causes seed dormancy so that germination may extend over months or years. Example are the acacias and hence for their efficient germination at the nursery, it is necessary to apply some form of presowing treatment to ensure not only a high final germination percentage but rapid and uniform germination (Doran et al., 1983). The seeds of Prunus yedoensis also have slow and poor germination when intact. Decoating their seeds shortens the mean germination time (MGT) and improves seed germination percentage significantly. (Hyun Kim, 2019). Decoated seeds of Syzygium cumini germinated faster than coated seeds under nursery conditions, with high significant germination percentages, dry matter production rates and vigor indices (Sivasubramaniam and Selvarani, 2012).

Saeed and Thanos (2006) found seed coat to be inhibitory to the germination of *Pinus gerardiana* as removal of seed coat promoted both rate and final germination. Chika *et al.*, 2020 reported that decoating seeds of *Mansonia altissima* improved their germination and resulted in the highest germination percentage. Bedada *et al.* (2018) also reported that de-coating improved germination and early nursery performance of *Olea europaea*.

*Terminalia superba* is one of the most heavily exploited African timber species, and locally, supplies have dwindled, with reports of declining populations in Côte d'Ivoire, Ghana, Nigeria, Cameroon and Congo (FAO,1984; N'Sosso, 1990). In Ghana, *T. superba* was one of the priority species earmarked for planting during the National Forest Plantation Development Programme launched in the year 2002. It is also on the list of priority species being planted under the on-going Ghana Forest Investment Programme. These developments have resulted in the need for sufficient quantities of good quality seeds to meet planting targets.

Cobbinah *et al.* (2001) and NTSC (2008) have reported days to first germination as 16 and 23 days respectively for seeds of *T. superba* sown intact. Unlike *T. ivorensis*, no serious dormancy problem has been reported about *T. superba*. However, pretreatment methods such as nicking or soaking in water have been recommended for faster and even germination (TTSA, 2010).

This study investigated the response of decoated seeds (seed with testa or seed coat removed) of *T. superba* to different temperatures and various germination media.

### Materials and methods

### Seed collection, processing and drying:

Seeds were harvested from a natural forest located at a small town called Abofour (Latitute 06. 19430N Longitude 00. 05090E) in the Offinso Municipal District of the Ashanti Region of Ghana in November 2017. Fruits were collected from the crowns of 3 trees by spreading cotton mats under the trees and a climber climbing up to cut branches to release the fruits. Collections from the 3 trees were bulked and quickly transported to the National Tree Seed Centre in Kumasi. These were spread on mats under shade and cleaning done by hand sorting. One kilogram of seed was sampled from the processed bulk and placed in a cotton bag. The bag was placed in a blue drum filled with silica gel to further dry the seeds to lower moisture content of 10%. Seed samples were kept in an aluminum foil, sealed and kept for 2 weeks.

#### Seed moisture content determination:

Moisture content of seeds was determined gravimetrically during the study. 5 grams of seeds in four replications were used for these tests. Fresh weights of seed samples were recorded after which they were dried in the laboratory oven at 103°C for 17 hours (ISTA, 1999), and cooled after drying for 45 minutes in a desiccator over silica gel. Dried samples were weighed again and moisture content expressed as a percentage of fresh weight. The moisture content was then calculated using the formulae:

$$\frac{\mathrm{IW}-\mathrm{FW}}{\mathrm{IW}}\times 100$$

Where, IW =initial weight and FW=final weight

#### Decoating of seeds:

Seeds were decoated with the aid of laboratory forceps and tweezers. Prior to the seed-coat removal, seeds were rehydrated on moist germination towel at 25°C for 24 hours (Masetto *et al.*, 2014).

The essence of the rehydration process was to allow the seed coat imbibe some water and make their removal from the seeds easier.

#### Germination of decoated seeds:

Three germination media were used. These were 1% water agar, heat sterilized river sand and seed testing paper (STP) in 90 mm diameter plastic Petri dishes. 75 decoated seeds were placed on the germination media in 3 replicates of 25 seeds. These were placed in incubators set at four constant temperatures, namely 20, 25, 30 and 35°C with 12- hour light and 12-hour darkness regimes. Germination was scored daily. Those with protruded radicle were considered to have germinated.

Germination counts were used to calculate for the total germination percent and mean germination time.

Germination percentage =

Total number of decoated seeds which germinated Total number of decoated seeds in all replicates x 100

Mean Germination Time (MGT) was calculated by the formula given by Ellis and Roberts (1981)

$$MGT = \frac{n_1 x \ d_1 + n_2 x \ d_2 + n_3 x \ d_3 + \dots}{Total number of days}$$

Where, n= number of germinated seeds, and d = number of days





Left Figs. i and ii. Whole (L) and decoated (R) seeds of *Terminalia superba*. Right

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## Statistical Analysis:

The data gathered was analysed using the Analysis of Variance for the Completely Randomised Design in factorial. The statistical software used was the SPSS version 20.

# Results

Effect of germination media on the germination of decoated seeds of *T. superba*: Among the three germination media, Agar supported the germination of decoated seeds of *T. superba* significantly higher (p < 0.001) than germination on STP. STP also supported germination of decoated seeds of *T. superba* significantly better than Sand (soil) (Fig. 1).



**Fig. 1.** Effect of germination media on the germination of decoated seeds of *T. superba*. Bars represent two standard error of the difference.

# Effect of temperature on the germination of decoated seeds of T. superba

Germination percentage of decoated seeds of *T*. *superba* was significantly higher at 35°C and 30 °C (p < 0.001). This was followed by germination percentage 25°C. Germination percentage at 20°C was significantly (p < 0.001) the least among the four temperature regimes (Fig. 2).



**Fig. 2.** Effect of temperature on the germination of decoated seeds of *T. superba*. Bars represent two standard error of the difference.

Effect of temperature and media on the germination of decoated seeds of *T. superba.*: Germination percentages on agar and STP at  $35^{\circ}$ C and  $30^{\circ}$ C, as well as on agar and soil at 25 and  $35^{\circ}$ C was significantly higher than the rest (p < 0.001). The lowest germination percentages were recorded at  $20^{\circ}$ C on all the media (Fig. 3).



**Fig. 3.** Effect of temperature and media on the germination of decoated seeds of *T. superba*. Bars represent two standard error of the difference.

# Effect of germination media on mean germination time of decoated seeds of T. superba

Mean germination time (MGT) was significantly (p < 0.001) shorter when agar was used as germination media (12 days) compare to when germination was carried out on STP and soil (16 days) as shown in Fig. 4.



**Fig. 4.** Effect of germination media on mean germination time decoated seeds of *T. superba*. Bars represent two standard error of the difference.

# Effect of temperature on mean germination time of decoated seeds of T. superba

The shortest MGT was recorded at germination temperatures 30 and  $35^{\circ}$ C (12 days). This was significantly shorter than (p < 0.001) MGT of 14 days recorded at 25 °C. The longest MGT of 16 days was recorded at 20°C (Fig. 5).



**Fig. 5.** Effect of temperature on mean germination time of decoated seeds of *T. superba*. Bars represent two standard error of the difference.

Effect of temperature and media on mean germination time of decoated seeds of *T*. superba Agar as germination media and germination temperatures of 30 and 35°C interacted to give the shortest MGT of 11 days. This was significantly shorter (p < 0.001) than the 12 days and above recorded when media and temperatures interacted in other cases. The longest MGTs of 16 days were recorded at 20°C on all the three germination media (Fig. 6).



**Fig. 6.** Effect of temperature and media on mean germination time of decoated seeds of *T. superba*. Bars represent two standard error of the difference.

# The germination time course of decoated seeds of Terminalia superba

The shapes of germination curves describing the cumulative germination of decoated seeds of *T*. *superba* at all temperatures studied and on all germination media over time are s-shaped. Curves generated at 20, 30 and  $35^{\circ}$ C exhibited steeper slopes compared to curves generated at 20°C (Fig.s 7a to 7d).



**Fig.** 7a. The germination time course of decoated seeds of *T. superba* at 20 °C. Vertical bars represent  $\pm$  standard error values.



**Fig.** 7b. The germination time course of decoated seeds of *T. superba* at 25 °C. Vertical bars represent  $\pm$  standard error values.



**Fig.** 7c. The germination time course of decoated seeds of *T. superba* at 30°C. Vertical bars represent  $\pm$  standard error values.



**Fig.** 7**d.** The germination time course of decoated seeds of *T. superba* at 20 °C. Vertical bars represent  $\pm$  standard error values.

# Discussions

According to (AOSA, 1992), seed germination papers, blotter papers, pleated paper, sand, agar or soil are recommended media for seed germination testing. However, each germination medium has its own property and suitability for different species (Smith et al., 2002). In this study, germination of decoated seeds of the T. superba was highest on agar (79%). This was followed by germination on STP (73%) and then germination on soil (70%). This observation is supported by the findings of Asomaning (2009), on the germination of intact seeds of T. superba. Bahuguna (1987), also reported that T. myriocarpa performed better on germination paper than on sand. These findings indicate that the Terminalia species germinate poorly on sand compared to their performance on agar and STP.

The study has further shown that the best temperature for the germination of decoated seeds of *T. superba* is 35 °C. This is closely followed by temperature 30°C. This observation is exactly in line with the report of Asomaning (2009), in a similar work with intact seeds of *T. superba*. This has reiterated the fact that *T. superba* is a typical tropical species and therefore requires a relatively higher temperature for germination as reported by Daws *et al.* (2004).

A very important observation in this study is that decoated seeds of the species germinated at 20 and 25°C. This was not the case when intact seeds were tested at temperatures 20, 25, 30 and 35°C. No germination was recorded when intact seeds of *T. superba* were incubated at 20 and 25°C (Asomaning, 2009). Germination of intact seeds occurred at 25°C only when seeds were pre-treated with chemicals. There was no germination at 20°C even after seeds were pre-treated with chemicals (Asomaning, 2009). It is evident from this study that decoating seeds of *T. superba* widens the temperature range over which the species will germinate.

In the same way as germination percentage of decoated seeds of *T. superba* on agar was the highest among the other media, and germination percentages

were highest at temperatures 35 and 30°C, agar as a media and temperatures 35 and 30°C interacted to give the highest germination for the germination materials. This is clear indication that agar as a media and higher germination temperatures of 30 and 35°C are the best germination conditions for the decoated seeds of *T. superba*. It must be also noted that at temperatures 25, 30 and 35 germination percentages of decoated seeds of *T. superba* on the STP and sand were also very impressive (above 70%)

Mean germination time (MGT) or average time to germination is an indication of the spread of germination of a seed lot. A shorter MGT indicates rapid germination of a seed lot. The study has shown that shorter MGTs have been recorded when decoated seeds of T. superba were germinated on agar, STP and on sand at temperatures 20, 25 30 and 35°C. Asomaning et al. (2011) reported of MGTs of 30 and 24 days when intact seeds of T. superba were placed on agar at constant temperatures 30/30 and 35/35°C on the thermogradient plate. Thus there is a remarkable reduction in MGT when T. superba seeds are decoated before they are germinated. The study has further indicated that time to first germination of decoated seeds was 3 and 4 days for samples on agar which were set at 35 and 30 °C respectively (results not shown). Time to first germination of 15 and 25 days had earlier been reported by Asomaning et al. (2011) for intact seeds of the species incubated at constant temperatures 35/35 and 30/30 °C respectively on the thermogradient plate.

Germination curves are used to describe differences in germination characteristics of seed lots. Cumulative germination percentage curves of seeds nurtured at different temperatures may be used to monitor differences in increases in germination percentage over time, periods of biggest seed germinability, continuousness in seed germination and periods at which germination remains constant (Silveira and Fernandes, 2006). The shapes of germination curves describing the cumulative germination of decoated seeds of *T. superba* at all temperatures and on all the germination media investigated are S-shaped. This observation reflects typical cumulative germination of a population of seeds over time as reported by Palazzo and Brar (1997); Shafii and Price (2001). The gradient of a cumulative germination curve indicates how fast or otherwise the germination of the particular species proceeded. In the present investigations curves generated at 25, 30 and 35°C exhibited steeper slopes compared to curves generated at 20°C because these temperatures were more favourable for the germination of the species and thus increases in germination percentage over time, than at 20°C. Shorter mean germination times, faster increases in germination over time and final germination percentages were recorded at these favourable temperatures compared to germination at 20°C. Germination curves generated for intact T. superba seeds incubated on the thermogradient plate also appeared as S-shaped similar to those generated using decoated seeds in this study (Asomaning et al. 2011).

### Conclusions

This study has shown that decoated seeds of Terminalia superba can be germinated successfully on agar, seed testing paper (STP) or sand. The temperature requirements for germinating decoated seeds of T. superba are 20, 25, 30 and 35°C. Thus, the range of temperatures required for germinating decoated seeds is broadened compared to the germination of intact or whole seeds of the species. In addition, mean germination time is also significantly reduced when decoated seeds are used as germination materials compared to mean germination time when intact seeds are germinated. Decoating seeds of Terminalia superba seeds prior to their sowing is highly recommended as the practice can reduce the average time of germination and therefore lead to the production of more uniform seedlings. The practice will also ensure the use of relatively lower temperature incubators (20 and 25°C) for germination of seeds compared to the use of intact seeds.

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